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Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies.

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Running title: Terminology in TTP and TMAs

Key words: ADAMTS-13 protein, human; thrombocytopenia; diagnosis, differential; thrombotic microangiopathy; thrombotic thrombocytopenic purpura

Essentials

- An international collaboration provides a consensus for clinical definitions.
- This concerns thrombotic microangiopathies and thrombotic thrombocytopenic purpura (TTP).
- The consensus defines diagnosis, disease monitoring and response to treatment.
- Requirements for ADAMTS-13 are given.

Abstract:

Background Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are two important acute conditions to diagnose. Thrombotic Microangiopathy is a broad pathophysiological process that leads to microangiopathic hemolytic anemia, thrombocytopenia and involves capillary and small vessel platelet aggregates. The most common cause being disseminated intravascular coagulation (DIC), which may be differentiated by abnormal coagulation. Clinically, a number of conditions present with microangiopathic hemolytic anemia and thrombocytopenia (MAHAT), including cancer, infection, transplantation, drugs, autoimmune disease and pre-eclampsia and HELLP (Hemolysis, Elevated Liver enzymes, Low Platelet count) syndrome in pregnancy. Despite overlapping clinical presentations, TTP and HUS have distinct pathophysiology and treatment pathways. **Objectives:** Presented is a consensus document from an international working group on TTP and associated TMAs (thrombotic microangiopathies). **Methods:** The international working group has proposed definitions and terminology based on published information and consensus based recommendations. **Conclusion:** The consensus aims to aid clinical decisions but also future studies and trials, utilizing standardized definitions. It presents classification of the causes of TMA, and criteria for clinical response, remission and relapse of congenital and immune mediated TTP.

Introduction

The elucidation of the pathophysiology of TTP and HUS, in the past 20 years, has transformed our understanding of the phenotypes, genotypes and therapies for these life-threatening conditions. Work on standardization has been addressed [1], but this document aims to develop robust criteria for future clinical use, studies and trials. Clinical and pathophysiologic features of TTP, atypical Hemolytic Uremic Syndrome (aHUS) and disorders with similar presentations, their investigation and subsequent management vary. This consensus document aims to rationalize and standardize definitions. Conditions often included in the initial differential diagnosis of TTP are discussed. Definitions for remission, refractory and relapsing disease are defined. ADAMTS-13 (*a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13*) assays are central to diagnosis and are discussed. We therefore also describe the minimum requirements for validation of current and future assays.

Methods:

The development of this document involved key international, primarily clinical, experts in TTP and related TMAs. The concept was supported by the European Hematology Association, scientific working group (EHA-SWG), platelet group, who have endorsed this collaboration. Members of the group have met at key international meetings, including EHA, International Society of Thrombosis and

Hemostasis (ISTH) and American Society of Hematology (ASH) and all versions of the document have been reviewed and edited by the authors. Articles were identified by a computer-assisted search of the literature published in English using the National Library of Medicine PubMed database. The authors also undertook a focused review of the available literature. Where there was a lack of robust evidence, the international working group concluded a consensus-based approach was preferable. The conclusions are relevant to both children and adults.

Thrombotic Microangiopathy

The term TMA is a pathological term used to describe occlusive microvascular or macrovascular disease, often with intraluminal thrombus formation, but is also defined clinically by microangiopathic hemolytic anemia and thrombocytopenia (MAHAT). This does not specifically define a condition; therefore further investigations are required to identify the underlying cause for the presentation of a TMA, including TTP (Figure 1).

Pathological Features of TMAs

A number of different pathological entities have been identified, including:
(i) von Willebrand factor (VWF)-platelet thrombosis; seen with severe ADAMTS-13 deficiency related to anti-ADAMTS-13 antibodies or a congenital absence of ADAMTS-13, the hallmark of TTP. The characteristic pathology of TTP is the presence of ultra large VWF multimers and VWF and platelet rich thrombi in arterioles and capillaries[2, 3]. Presentation with large vessel thrombosis (stroke or myocardial infarction) is believed to result from vascular injury caused by thrombosis of the vasa vasorum

(ii) Fibrin-platelet thrombosis; commonly seen in disseminated intravascular coagulation (DIC) [4] but occasionally seen in catastrophic antiphospholipid syndrome (CAPS), heparin induced thrombocytopenia (HIT), and HELLP syndrome.

(iii) Thrombotic Microangiopathy; characterized by endothelial swelling or disruption associated with hyalinosis or fibrinoid necrosis, usually without an inflammatory cell infiltrate. Chronic TMA is associated with myointimal proliferation resulting in concentric rings of cells and matrix surrounding small vessels (sometimes termed onion skinning), which can be indistinguishable from the effects of chronic hypertension. Intraluminal thrombosis is frequently present, but is not required for the diagnosis [5]. Examples include MAHAT caused by shiga toxin, complement dysregulation and drugs (eg gemcitabine).

(iv) Vasculopathy/vasculitis; changes involve not only endothelial cells and intima, may be autoimmune (e.g. lupus vasculitis [6], scleroderma renal crisis [7]) or infectious (e.g. Rocky Mountain spotted fever [8] , viraemia, fungemia). An inflammatory component may be evident.

(v) Intravascular clusters of cancer cells (tumor cell embolism) may occur in patients with advanced cancer [9].

Clinical features of TMAs

Peripheral blood film features consistent with MAHA include fragmented red blood cells (schistocytes), polychromasia and anemia. Quantification of fragmentation/schistocytes is not reliably available and remains subjective. Laboratory evidence of hemolysis includes elevated lactate dehydrogenase (LDH), reticulocytosis, low/absent haptoglobin, and a negative direct antiglobulin test. Thrombocytopenia will be evident in the complete blood count (CBC) and on the blood film [10].

Thrombotic thrombocytopenic purpura (TTP)

Definition of TTP: **MAHA with moderate or severe thrombocytopenia, with associated organ dysfunction, including neurologic, cardiac, gastrointestinal and renal involvement, although oliguria or anuric renal failure requiring renal replacement therapy is not typically a feature [10, 11]. TTP is confirmed by a severe deficiency (<10%) of ADAMTS-13 activity [2, 3]**

TTP is recognized as a multi-organ process with variable clinical features. Anemia may not be immediately obvious. Thrombocytopenia is generally severe (platelet count <30 $x10^9$/L), but higher platelet numbers do not exclude the diagnosis.

Presentation with renal failure requiring renal replacement is not a common feature of TTP. However, occasionally patients with multi organ failure may develop an acute kidney injury requiring renal support. Congenital TTP (cTTP) patients may present with acute renal failure [12]. cTTP cases may previously have been misclassified as HUS [13]

Acute TTP presentations may include bleeding symptoms such as bruising, hematuria or thrombotic symptoms associated with neurological or cardiac involvement. In practice, TTP is suspected in:

- Isolated MAHAT
- New focal neurological symptoms, seizures or myocardial infarction (MI), with unexplained MAHAT
- Prior history of TTP

Confirming a diagnosis of TTP:

Current ADAMTS-13 activity assays provide levels of sensitivity <5% or 1%. However, for the diagnosis of TTP, ADAMTS-13 activity levels < 10% are diagnostic. This is to include antibody mediated cases and cTTP, including late onset cTTP.

The diagnosis of TTP should be confirmed with ADAMTS-13 activity performed on a sample taken prior to initiation of therapy, specifically plasma-based. Samples taken immediately following plasma therapy may give a falsely raised ADAMTS-13 activity. The presence of anti-ADAMTS-13 IgG antibodies may help confirm TTP in these situations. However, in autoimmune TTP, >75% of patients exhibit <10% ADAMTS-13 activity immediately before the next therapeutic plasma exchange session for several days after starting daily exchange therapy [14].

Assays at diagnosis should include: ADAMTS-13 activity, functional inhibitor based on mixing studies, and/or an anti-ADAMTS-13 IgG. This panel should correctly identify TTP patients in the majority of cases.

Measurement of ADAMTS-13 at regular intervals during treatment and in remission (e.g. weekly during treatment, monthly, then 3 monthly during follow-up period, extending to 6-12 monthly) may provide data in regard to the risk of relapse and persistence of subclinical disease activity [15]. ADAMTS-13 activity <10% with or without a detectable inhibitor is associated with a significant risk of relapse and can guide elective, prophylactic therapy preventing further relapse [16-18] in iTTP.

Congenital TTP **(Upshaw-Schulman syndrome) (cTTP): persistent severe deficiency (<10%) of ADAMTS-13 activity, with no evidence of anti-ADAMTS-13 autoantibodies, confirmed by molecular analysis of the *ADAMTS-13* gene confirming a pathogenic homozygous or compound heterozygous mutational defect.**

Persistent ADAMTS-13 activity <10% with no detectable anti-ADAMTS-13 IgG antibodies (confirmed in the acute phase and in remission after treatment) suggests a diagnosis of cTTP, especially in the following circumstances:

- Neonates presenting with thrombocytopenia, red cell fragmentation on blood film, often with hyperbilirubinemia.
- Children/ adults if there is a familial antecedent of TTP, especially in siblings from consanguineous parents.
- First TTP episode in adulthood, in particular, during pregnancy.

The diagnosis of cTTP is confirmed following mutational analysis [19-21]. Patients can present in the neonatal period, childhood or adulthood, with presentations typically associated with a trigger, such as infection, vaccination, or dehydration [22]. Pregnancy may precipitate acute manifestations in women with cTTP. Presentation of TTP in pregnancy, often associated with fetal loss, particularly in the 2nd trimester, requires exclusion of late onset cTTP [23-25].

The diagnosis is confirmed if pathogenic *ADAMTS-13* mutations are identified. However, many genetic variants are of unknown significance and require expression studies. Infusion of plasma resulting in recovery of ADAMTS-13 activity and the disappearance of ADAMTS-13 activity with a half life of 2-3 days, supports the diagnosis of cTTP [26].

Immune mediated TTP (iTTP):

This defines acquired TTP and can be divided into those cases in which a precipitating cause can or cannot be confirmed. All cases require immediate therapy (TPE and steroids), but differentiating subgroups can help to tailor therapy.

Primary iTTP: This describes acquired autoimmune TTP for which there is no obvious underlying precipitating cause/disease, ADAMTS-13 activity <10% with the presence of ADAMTS-13 autoantibodies.

Primary iTTP accounts for the majority of cases of TTP. The precipitating factor or trigger for primary immune TTP has not been identified, although an underlying genetic risk in Caucasian patients has reported the presence of HLA DQ-7, HLA DRB1*11 & HLA DRB3* [27]

Secondary iTTP: This describes acquired autoimmune TTP for which a defined underlying disorder or trigger can be identified including connective tissue disease (such as systemic lupus erythematosis (SLE)), human immunodeficiency virus infection, cytomegalovirus (CMV) infection and/or a specific precipitating factor (e.g. pregnancy, drugs). Treatment of the underlying disorder and/or removing the underlying precipitant may be required as well as standard TTP therapy. The presence of severe deficiency (<10%) of ADAMTS-13 activity and ADAMTS-13 autoantibodies confirms a diagnosis of TTP.

Secondary causes include infectious agents such as HIV [28] , drugs, such as ticlodipine, quinine, simvastatin [29, 30], trimethoprim [31] , pegylated interferon [32], as examples. The association is typically rare and idiosyncratic. Pregnancy or certain autoimmune diseases, such as SLE [33], Sjogren's syndrome [34] and rheumatoid arthritis (RA) maybe associated with iTTP. Treatment of the underlying precipitant or stopping implicated drugs in conjunction with acute standard therapy (plasma exchange and immunosuppression eg steroids) may be necessary. In HIV associated TTP, plasma exchange is of value, in conjunction with HAART (highly active antiretroviral therapy) [28, 35]. Secondary iTTP constitutes only a small proportion of the overall cases of TTP.

There remain, however, a number of cases that meet the clinical criteria for TTP, but with ADAMTS-13 activity levels that are not severely deficient and in whom an alternative diagnosis cannot be determined. In these patients treatment with plasma exchange, needs to be individualized and continued only if a clear clinical benefit is obvious. Such cases should be discussed with expert centres in TTP/HUS.

Differential diagnosis of TMA

There are a number of conditions that have presenting features (both clinical and laboratory) similar to TTP. ADAMTS-13 assays are essential in their differentiation **(Table 1).**

Hemolytic Uremic Syndrome (HUS): HUS is defined by MAHAT and renal injury, which is the predominant feature. Thrombocytopenia may not be as severe as in TTP, and anemia at presentation can be variable (Figure 1).

Infection Associated HUS (IA- HUS), often referred to as STEC-HUS describes an infectious etiology associated typically with E. coli 0157:H7, that express Shiga toxin. The TMA presents 5-7 days after the infection with hemorrhagic colitis. E coli 0157: H7 is not the only subtype associated with infection associated HUS. Indeed other subtypes of E coli [36], Salmonella, Shigella, and Campylobacter associated HUS can present similarly.

Complement Mediated HUS (CM-HUS) usually results from defective regulation of the alternative complement pathway [37]. CM-HUS can be triggered by infection (and diarrhea may be evident at presentation), vaccinations, or pregnancy. Heterozygous mutations may be identified, affecting complement regulators including complement factors H (CFH) and I (CFI), complement factor H related protein (CFHR1, 3 and 5), CD46 (MCP), or gain of function mutations in C3 and complement factor B (CFB). CM-HUS associated with anti-complement factor H antibodies (associated with a polymorphic deletion of CFHR1) can also occur, particularly in children, and is responsive to immunosuppressive therapy [38]. CM-HUS is also described associated with defects in other genes, including *THBD*, which encodes thrombomodulin and with bi-allelic mutation of the *DGKE* gene that encodes diacycl glycerol kinase ε, which regulates diacyl glycerol activated Protein

Kinase C signaling in endothelial cells. While the pathophysiology of *DGKE*-associated HUS is not completely understood it is not thought to directly involve disruption of complement alternative pathway regulation [39].

Mutational analysis may take weeks to be completed and in 40-50% of cases no mutations are identified [40]. Therefore, treatment decisions must be made on the basis of the clinical presentation and absence of severe ADAMTS-13 deficiency. CM-HUS may respond to plasma exchange but eculizumab, a humanized monoclonal antibody, which binds to and inhibit C5, is more efficacious, except in DGKE-associated disease [41]. In addition, new complement inhibitors are being developed. A C5 polymorphism, described in the Japanese population, is associated with a reduced response to eculizumab [42].

The clinical presentation of CM-HUS can be variable and includes:

- renal failure, MAHAT;
- renal failure with MAHA but a normal platelet count;
- chronic or progressive renal failure, without a history of MAHA;
- severe hypertension, MAHA or thrombocytopenia, with or without renal impairment, often diagnosed by renal biopsy.

In addition to molecular testing, a response to anti-complement therapy may provide confirmation of the diagnosis.

Rare secondary causes have been identified causing HUS [43](Table 1).

Definition of hematological response to treatment

Clinical Response: Sustained normalization of platelet counts above the lower limit of the established reference range (eg >150x10^{9}/L) and LDH (<1.5 upper limit of normal (ULN)) after cessation of plasma exchange.

Clinical Remission: Clinical response after cessation of plasma exchange and maintained for greater than 30 days.

Clinical response and clinical remission would be associated with stabilization of parameters if end organ damage is severe or improvement in function with normalization of laboratory parameters.

Exacerbation: reduction in platelet count to below the lower limit of the established reference range (eg<150 x 10^9/L), increased LDH, and the need to restart plasma exchange within 30 days of the last plasma exchange after a clinical response to plasma exchange.

Relapse: Fall in platelet count to below the lower limit of the established reference range (eg <150 x $10^{\wedge 9}$/L), +/- clinical symptoms >30 days after stopping plasma exchange for an acute TTP episode, requiring re-initiation of therapy. This is usually associated with a new increase in LDH.

In patients achieving a normal platelet count, a >10% reduction within 24 hours may represent impending exacerbation (during a period of daily plasma exchange therapy or within 30 days of stopping plasma exchange) or relapse and requires close monitoring. Exacerbation or relapse may not demonstrate the full spectrum of clinical features seen in the initial acute TTP presentation. ADAMTS-13 activity will be <10% at exacerbation/relapse, either as a persisting deficiency or a de novo deficiency after transient normalisation.

Refractory TTP: Persistent thrombocytopenia, lack of sustained platelet count increment or platelet counts <50 x109/L and persistently raised LDH (>1.5 X ULN) despite 5 plasma exchange [44] and steroid treatment. This would be defined as SEVERE if the platelet count remained < 30 x 10^9/L.

Normalization of a platelet count is typically above the lower limit of the established reference range (eg >150x10^9/L), however, country/regional variability relating to the 'normal range' should be noted. LDH may not be in the normal laboratory range with clinical response or remission, but decreasing levels with increasing platelet counts is in keeping with clinical response.

The definition of refractory TTP can be challenging. Cases with an initial response, but then a reduced platelet count that is difficult to increase, may be considered refractory and this may occur after 5 plasma exchange procedures. However, the clinical condition is likely to be more stable at this point.

Intensive plasma exchange refers to increased volumes per plasma exchange eg a single volume to 1.5 plasma volume or increased frequency of plasma exchange eg twice daily. Protocols will be institution specific.

During an acute presentation, the definition of refractory disease may require an escalation in therapy. The median time to achieve a clinical response, with daily plasma exchange, is approximately 10-15 days [45]. Patients admitted to an ICU setting with neurological and /or cardiac features may require longer plasma exchange therapy [46]. Patients failing to achieve remission or whose platelet count and LDH initially improve but worsen despite on-going treatment would also be considered to have refractory disease.

Relapsing TTP:

There are a number of identified risk factors that may be associated with or precipitate a relapse of iTTP,

- **ADAMTS-13 deficiency (particularly <10% activity)**
- **Persistent severe ADAMTS-13 deficiency in remission**
- **Persistent anti-ADAMTS-13 antibodies in remission**

or congenital disease

- **Pregnancy**
- **Infection/live vaccinations**
- **Drugs including drugs of abuse eg. cocaine, alcohol**

In iTTP, a severely reduced ADAMTS-13 activity persisting during remission is associated with relapse, particularly when associated with inhibitory autoantibodies [47]. Therapy should be considered to clear antibodies and normalize ADAMTS-13 activity, in patients felt to be at high risk for relapse [16, 18]. Pregnancy [48] , infections and live vaccinations in particular may precipitate an acute episode of cTTP. Often, an initial acute TTP episode, even though occurring only in adulthood, is followed by recurrent episodes [49].

Factors relating to severity/prognosis of TTP

- **Presence of Anti-ADAMTS-13 IgG antibody associated with severe ADAMTS-13 deficiency**
- **Raised troponin at presentation**
- **Presence of neurological features**
- **Older age**
- **Low ADAMTS-13 antigen at presentation**

There are few predictors of disease severity, but necessity of intubation and ventilation, older age, neurological features [50]and cardiac symptoms and/or raised troponin,[51] with high inhibitor levels/IgG autoantibodies to ADAMTS-13 are key predictors. Ethnicity and ADAMTS-13 activity <10% are associated with exacerbation and relapse respectively. Non Caucasians have increased exacerbation and relapse, but reduced mortality. Inhibitor or anti-ADAMTS-13 IgG levels predicting the severity of disease have not been defined [52], but levels (anti-ADAMTS-13 IgG >50% or > 2 BU/ml) are associated with a poorer outcome [51]. Low ADAMTS-13 antigen level at presentation may be associated with worse clinical outcome in iTTP [53]. Routine laboratory parameters such as platelet count, LDH, and hemoglobin are not predictive of severity, although failure to improve with therapy and hence refractory disease is associated with a worse outcome.

ADAMTS-13 assays:

ADAMTS-13 activity: determines the amount of functional ADAMTS-13 via direct or indirect measurement of VWF cleavage. Current methods include FRETS-VWF73, chromogenic VWF-73 [54], chromogenic VWF-73 [55], FRETS-rVWF71 [56] FRET-VWF86 and SELDI-TOF mass spectrometry [57]. A standard curve using pooled normal plasma (PNP) for each assay plus control samples (e.g. plasma of a cTTP patient for an low range sample; the WHO 1st International Standard ADAMTS-13 [58]), which have undergone in-house calibration.

ADAMTS-13 antigen: determines the amount of ADAMTS-13 protein in plasma. Low levels appear to be associated with poor prognosis [53].

Anti-ADAMTS-13 antibodies: usually IgG but IgM and IgA may be also relevant. Anti-ADAMTS-13 antibodies are measured by ELISA, Western blot or immunoprecipitation. ADAMTS-13 antibodies by ELISA may be seen in 5%-10% of healthy individuals however, this may be manufacturer/assay dependent [59]. ELISA or Western blotting using recombinant ADAMTS-13 to detect anti-ADAMTS-13 in patient's plasma can assess the presence of anti-ADAMTS-13 antibodies. In-house or commercially available assays should have appropriate validation and controls to include low, medium and high antibody titres.

ADAMTS-13 inhibitor assays: identifies and quantitates anti-ADAMTS-13 antibodies that functionally inhibit ADAMTS-13 in vitro. Functional ADAMTS-13 inhibitors are assessed in classical Bethesda-like mixing studies. Samples are heat-inactivated for 30 minutes at 56°C to abolish endogenous ADAMTS-13, before mixing, at various dilutions, with PNP. After incubation at 37°C for 2 hours, residual ADAMTS-13 activity is measured and the residual activity transformed in Bethesda units,(1BU/ml of inhibitor inhibits 50% of normal plasma ADAMTS-13). For routine purposes it is usually sufficient to titrate up to 2 Bethesda units/ml.

Factors affecting ADAMTS-13 levels

Severe hemolysis with marked hyperbilirubinemia [60]or severe lipemia may give falsely low ADAMTS-13 activity results, particularly when using fluorogenic detection e.g. FRETS [54]; Free hemoglobin in plasma from intravascular hemolysis can also inhibit ADAMTS-13 activity [61].

There is good concordance between assays in the range of severe deficiency of ADAMTS-13 (defined as either <5% or <10%). However assay conditions can affect results. There are numerous variables in quantification of ADAMTS-13 activity levels among different platforms or when performed in different laboratories. Further studies involving large series of plasma samples are needed to evaluate the newly developed commercial kits.

Pre-analytic aspects: specimen type and acceptability criteria

ADAMTS-13 activity can be assessed in citrated plasma, serum or lithium-heparin plasma. While extensive comparisons between these are missing, small series demonstrate equality [62]. EDTA plasma is not suitable for ADAMTS-13 activity testing, as it is an irreversible inhibitor of ADAMTS-13 activity.

Evaluation of assays

- Detection (sensitivity) limit of the method:

. For ADAMTS-13 activity measurement (defined as the lowest activity that is distinguishable from buffer or heat inactivated plasma respectively): this should be < 5%, but preferably < 1%.

. For the detection of inhibitors: difficult to standardize, but a negative control and at least 2 positive antibody controls, which have demonstrated reproducibility with the technique, should be considered. The detection limit is approximately 0.4-0.6 BU/ml.

- Precision of the method: intra-assay reproducibility (within series), inter-assay reproducibility (between run in one given laboratory and also inter-laboratory precision): should have CVs of <10%.

In conclusion, we have developed definitions for TTP and associated TMAs that we hope will promote uniformity in publications, associated studies and clinical trials. The field is ever expanding and the group will be required to undertake a review again in future years.

Acknowledgements

The development of this document involved key international, primarily clinical, experts involved with TTP, HUS and related disorders. The concept was supported by the EHA-SWG platelet group, chaired by the late Dr Roberto Stasi (Rome, Italy and London, UK). Thanks to Professor HM Tsai, Taiwan, for his valuable contribution and review of the manuscript.

Addendum

M. Scully was responsible for the concept and design.

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The members of the International Working group have all contributed to this work, and are listed in the Appendix.

Disclosure of Conflict of Interests

K. Friedman reports personal fees from Ablynx and Baxalta, outside the submitted work. P. Coppo reports grants, personal fees and non-financial support from Alexion Inc.; grants from Roche; and personal fees from Ablynx, during the conduct of the study. K. Pavenski reports honoraria from Alexion Inc., outside the submitted work. B. Lämmle reports grants from Baxter Biosciences and personal fees from Baxalta, Alexion Inc., Siemens, and Baxalta, outside the submitted work. In addition, B. Lämmle has a patent von Willebrand factor protease issued. J. Kremer Hovinga reports grants, personal fees and non-financial support from Baxalta; personal fees from Alexion; and she sat on advisory boards for Ablynx, during the conduct of the study. E. Sadler reports personal fees from Ablybx, 23andMe, XO1 Limited, Baxter HealthCare, and BioMarin, outside the submitted work. In addition, E. Sadler has a patent Fluorogenic substrate for ADAMTS13, US 8,663,912 issued to Washington University. M. Scully reports grants from Baxter Global and personal fees from Novartis, Ablynx, and Alexion, outside the submitted work. M. Matsumoto reports grants from the Ministry of Health, Labour, and Welfare of Japan and the Ministry of Education, Culture, Sports, Science and Technology of Japan, during the conduct of the study. In addition, M. Matsumoto has a patent ADAMTS13 activity assay with royalties paid. In addition to having been a member of a scientific advisory board for Ablynx, F. Peyvandi reports grants from Alexion, Ablynx, and Biokit; grants and personal fees from Biotest, Bayer, Kedrion Biopharma, and Novo Nordisk; and personal fees from Octapharma, Sobi, CSL Behring, LFB, and Grifols outside the submitted work. Y. Fujimura has a patent ADAMTS13 activity assay from Alfresa pending. D. Gale reports personal fees from Alexion, outside the submitted work

Appendix

International Working Group for Thrombotic Thrombocytopenic Purpura

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Disease	Summary of Disease	Comment
TTP	Acute haematological emergency, requiring prompt diagnosis and treatment. More likely to have neurological/cardiac features-but a multi system disorder.	ADAMTS-13 measured for definitive diagnosis. Plasma exchange is the mainstay of treatment, typically additional immunomodulating therapy required in iTTP.
HUS		
A. IA-HUS	The TMA presents 5-7 days after the infection with haemorrhagic colitis.	Confirmation of infection is serological and/PCR.
B CM-HUS	CM-HUS can be triggered by infection (and diarrhoea maybe present at presentation), vaccinations, or pregnancy. .	Diagnosis may be confirmed by complement mutations eg CFH, CFI, MCP, CFB, C3 or anti CFH antibodies. Homozygous or compound heterozygous diacylglycerol kinase (DGKE) mutations typically do not respond to current complement

		inhibitors [39] Treatment decisions must be made on the basis of the clinical presentation and absence of severe ADAMTS-13 deficiency. A response to anti-complement therapy may provide confirmation of the diagnosis.
C. Streptococcus pneumoniae	This is seen almost exclusively in young children. Typically present with pneumonia. Incidence reduced since polyvalent vaccine (in some countries). Have a DAT positive haemolytic anaemia	Thomsen-Friedenreich cryptoantigen, present on red cells, platelet, glomeruli and hepatocytes are exposed by neuramindase, produced by all subtypes of Streptococcus pneumoniae. The TF antigen interacts with IgM antibodies, resulting in agglutination. Treatment is supportive, but still washed blood products may be requested [63]

D. Cobalamin C deficiency	A rare cause of HUS that is caused by recessive mutations of the methylmalonic aciduria (cobalamin deficiency) cblC type, with homocystinuria (MMACHC) gene. Presentation is typically within the first year of life [64].	
Connective tissue disease	MAHAT may be a primary feature. Further systemic features/laboratory testing confirm the diagnosis	Autoimmune screen, tissue biopsy eg SLE, scleroderma, antiphospholipid syndrome, vasculitis (eg ANCA)

Transplantation, either solid organ or bone marrow/ hematopoietic stem cell (HSC):	May present with MAHAT with or without renal involvement. It is typically associated with immunosuppressive therapy, such as Tacrolimus, Ciclosporin A, underlying opportunistic infections (eg CMV, Adenovirus, Aspergillus) or graft-versus-host disease [65],	HSC transplant associated TMAs do not have severe ADAMTS-13 deficiency (ie <10%). Complement alternative pathway dysregulation may be involved in the pathogenesis of HSCT-TMA . In patients developing TMA post renal transplantation, consideration should be given to possible CM-HUS, which may have been the cause of the initial renal failure and the failed transplanted kidney [66]
Disseminated intravascular Coagulation (DIC)	DIC is associated with abnormalities in routine coagulation and CBC screens [67].	DIC is typically due to an underlying precipitant such as sepsis, malignancy, haematological disorders, obstetric complications or trauma.

Pregnancy associated TMAS	Pre-eclampsia and HELLP occur in the second half of pregnancy or early post partum.	Complement activation in HELLP or pre-eclampsia may trigger the development of TMA. Complement abnormalities have been identified in some cases of HELLP/pre-eclampsia [68],
A. HELLP syndrome	There are no specific diagnostic clinical or laboratory factors. ADAMTS-13 activity is typically normal. HELLP can be divided into type 1, 2 and 3 based on the platelet count (<50 $x10^9/L$, 50-100 $x10^9/L$, 100-150 $x10^9/L$) [69].	May be associated with complications such as DIC, and placental abruption.

B. Pre-eclampsia	Defined as de novo hypertension (>140/90 mm Hg) and proteinuria (>0.3 g per 24 hours) after 20 weeks of gestation .	Control of BP. Delivery will improve the situation.
Drugs	Ticlodipine [70] may be associated with autoantibodies to ADAMTS-13, case reports with quinine [71], simvastatin, trimethoprim or interferon. Clopidogrel is not associated with TTP. TMA may occur with gemcitabine, bleomycin, and mitomycin. VEGF inhibitors were associated with features of TMA reminiscent of HUS [72].	Stopping the offending drug maybe associated with improvement.

Pancreatitis	Caused by alcohol excess, bile duct obstruction or possibly an autoimmune precipitant [73].	ADAMTS-13 levels may not be severely reduced [74]. Identifying the underlying condition reduces the risk of further relapses.
Malignancy	In patients with MAHAT for whom a severe deficiency of ADAMTS-13 activity is not confirmed, tumour markers and radiological investigations should be considered [75].	ADAMTS-13 activity is normal or mildly decreased.

Table 1: Summary of characteristics associated with conditions presenting with microangiopathic haemolytic anaemia and thrombocytopenia (MAHAT)

IR-HUS: Infection related haemolytic uraemic syndrome; PEX: plasma exchange, BP: blood pressure, HELLP: haemolysis, elevated liver enzymes & low platelets, DIC: disseminated intravascular coagulation, TMA: thrombotic microangiopathy, CM-HUS: . Complement mediated-HUS

Figure 1: Differential diagnosis of thrombotic Microangiopathies presenting with MAHA

Key: MAHA: microangiopathic haemolytic anaemia; MAHAT: MAHA and thrombocytopenia-TTP: thrombotic thrombocytopenic purpura; IA-HUS: Infection associated HUS, PCR: polymerase chain reaction