Citations for ADAMTS13 function and inhibitor screen LDT comment:

Scully M et al. *JTH.* 2017. 15:312

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

- TTP: multiorgan disease, clinical features variable. Confirmed by ADAMTS-13 activity < 10%
- For the diagnosis of TTP, ADAMTS-13 activity levels of < 10% are diagnostic. This is to include antibody-mediated cases and cTTP, including late-onset cTTP.
- Congenital TTP(cTTP) (Upshaw–Schulman syndrome): This is defined as persistent severe deficiency (< 10%) of ADAMTS-13 activity, with no evidence of anti-ADAMTS-13 inhibitory autoantibodies, confirmed by molecular analysis of the ADAMTS-13 gene confirming a pathogenic homozygous or compound heterozygous mutational defect
- Severe hemolysis with marked hyperbilirubinemia or severe lipemia may cause falsely low ADAMTS-13 activity results, particularly when fluorogenic detection, e.g. FRETS, is used; free hemoglobin in plasma from patients with intravascular hemolysis can also inhibit ADAMTS-13 activity

Bennett ST ed et al. Laboratory Hemostasis, 2nd ed. Springer, 2015.

- TTP is associated with ADAMTS13 <5-10%
- HUS has normal to mildly decreased ADAMTS13 activity

Williams LA et al. *AJCP*. 2016. 145:158.

- ADAMTS13 deficiency (below the laboratory reference range) can be seen in other conditions such as sepsis, DIC, and severe liver dysfunction. However, activity less than 10% is extremely rare, except in TTP.
- In a patient with a preliminary differential diagnosis of TTP-HUS, an ADAMTS13 result of more than 10% and no other explanation for the TMA findings should prompt presumptive treatment for aHUS. Although

Scully M et al. *BJH*. 2014. 164:759

- Atypical HUS, and other TMAs by definition, will not have low (<10%) ADAMTS13 activity or the presence of significant Anti-ADAMTS13 immunoglobulin G (IgG) autoantibodies. In contrast, idiopathic TTP will have low ADAMTS13 activity (<10%) and, in the majority of cases (excluding congenital TTP), antibody can be detected
- Atypical haemolytic uraemic syndrome may have a normal ADAMTS13 at presentation or it can be reduced, usually to about 30–40%. Similar results can be seen in other TMAs, such as malignant hypertension, scleroderma, autoimmunemediated TMA and transplant-associated TMA. The reason some secondary cases have a normal ADAMTS13 at presentation and some are

reduced is unclear. It may be because of the raised VWF/thrombin levels, which result in consumption of ADAMTS13, but this does not explain the full picture.

Cataland SR et al. *Blood.* 2014. 123:2478.

- Receiver operating characteristic (ROC) analysis was performed and showed that using a threshold for determining ADAMTS13 deficiency of 10% yielded a sensitivity and specificity of 100% in differentiating acquired TTP from other types of TMAs,

Shelat SG et al. *JTH.* 2006. 4:1707

- 52% of idiopathic TTP had inhibitory autoantibodies, 29% have non-neutralizing anti-ADAMTS13 Ab

Coppo P et al. *PLOS One*. 2010. 5:e10208

- Idiopathic TMA with detectable ADAMTS13 activity shares features of atypical HUS and antiphospholipid syndrome

Qu L et al. *Seminars Thromb Hemostasis*. 2005.31:691

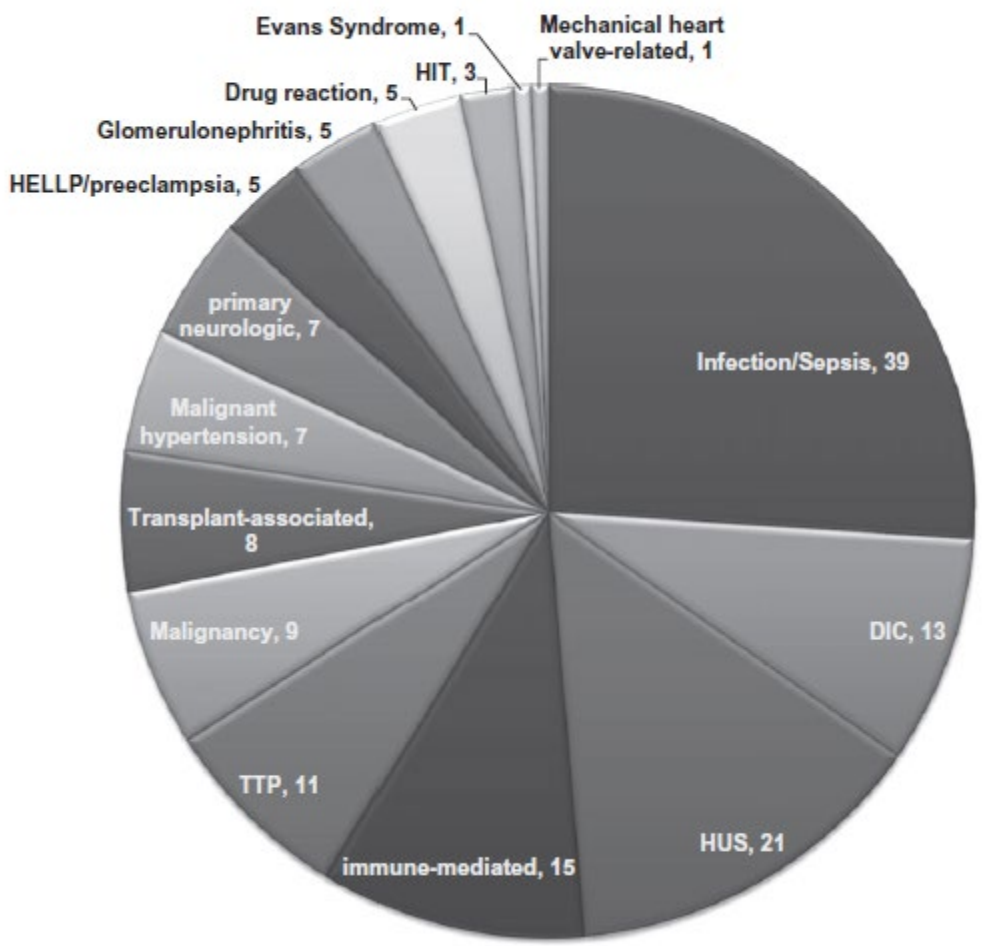
- TMA post-HSCT has not been associated with marked decrease in ADAMTS13 level or inhibitors to ADAMTS13

Odronic S et al. *J Clin Apher*. 2014. 29:284

- Cocaine induced TMA had ADAMTS13 of 63%, reference range >66%

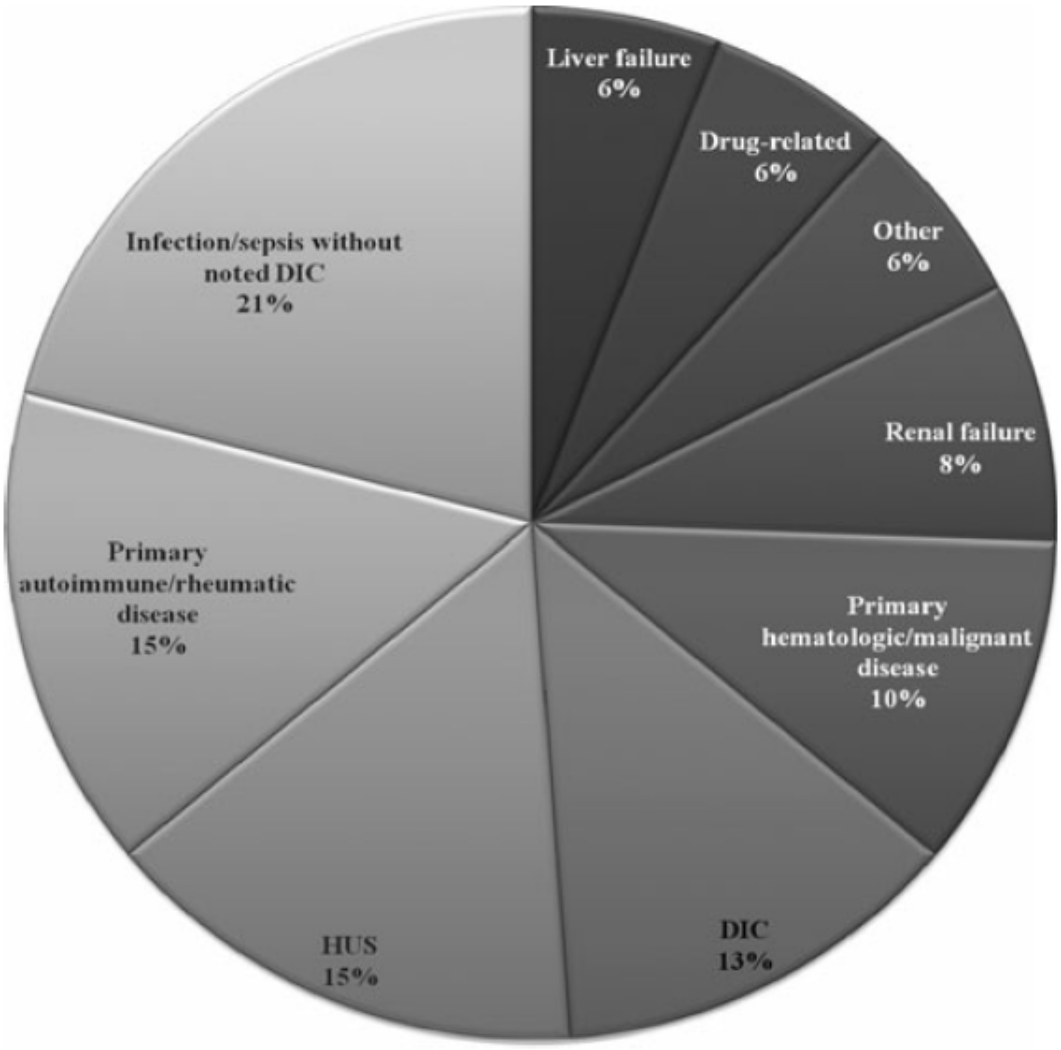
Bentley MJ et al. *Transfusion*. 2010. 50:1654

- Using recursive partitioning, an ADAMTS13 level of <15% successfully predicted severe ADAMTS13 activity in all 10 of our patients with TTP who were responsive to plasma exchange
- ADAMTS13 level of <15% “was a generous value chosen because it seemed at the upper bounds of that used elsewhere. Others may argue for a more stringent cut-off”
- Final diagnoses in patients with clinically suspected TTP (regardless of ADAMTS13 level)



Bentley MJ et al. *Vox Sang*. 2013. 105:313

- ADAMTS13 level of <10% was used to define TTP
- "As ADAMTS 13 testing has become more widespread, it has become evident that mild deficiency can occur in disorders other than TTP, while patients with TTP have more severe deficiency"
- "Other common final diagnoses for patients in our study with clinically suspected TTP include disseminated intravascular coagulation (DIC) (N = 23 (13%)) and infection or sepsis without specifically noted DIC (N = 40 (21%)). Timely institution of appropriate anti-infective therapy in the case of sepsis, and determining and correcting the underlying cause of DIC, infectious or otherwise, is critical. While infusion of plasma or blood products and additional resuscitative, supportive care may be indicated, plasma exchange is not required for these patients and may delay appropriate diagnosis and treatment, as well as expose them to unnecessary risks."
- Final diagnoses in patients clinically suspected of having TTP and with ADAMTS13 >10%.


Liver failure
6%
Drug-related
6%
Other
6%
Renal failure
8%
Primary
hematologic/malignant
disease
10%
DIC
13%
HUS
15%
Primary
autoimmune/rheumatic
disease
15%
Infection/sepsis without
noted DIC
21%

Tsai H-M. Hematol Oncol Clin N Am. 2013. 27:565

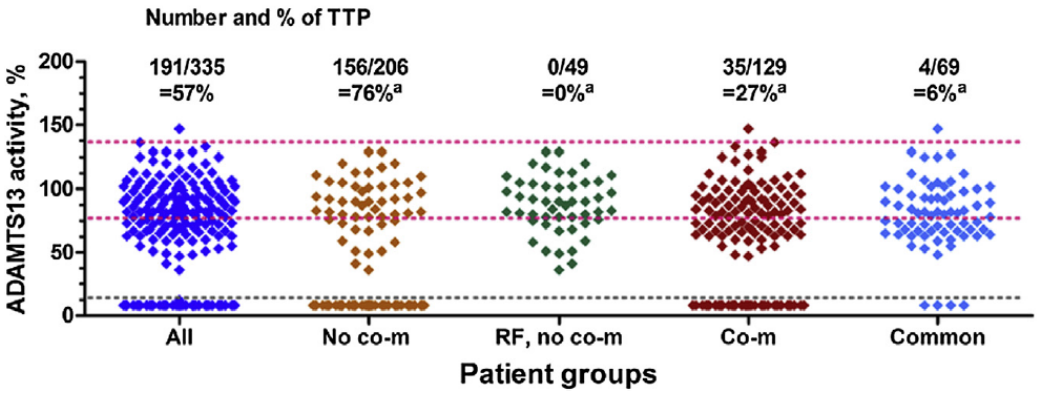


Fig. 5. The plasma ADAMTS13 level segregates TTP from other types of MAHA. The patients without severe ADAMTS13 deficiency in the group without comorbidity are considered to have aHUS. Diagnosis of aHUS in the group of patients with comorbidity is further elaborated in **Table 1**. All, all patients investigated for microangiopathic hemolysis unrelated to vascular devices. Hereditary TTP is also excluded from this analysis, as some of the patients did develop acute or chronic renal failure; No co-m, all patients without comorbidity; RF, no co-m, patients with renal failure (maximum creatinine >2.5 mg/dL) in the no co-m group; Co-m, all patients with comorbidity; Common, all patients with common comorbid conditions, including autoimmune connective tissue disease, hematopoietic stem cell therapy, drugs other than ticlopidine, metastatic cancers, and pregnancy. [a] *P*<.001 compared with the group of all patients.

TTP Registry (http://www.ttpregistry.net/html/symptoms), accessed 6/9/2017

Usually USS patients have a severely deficient ADAMTS13 activity of <10% of the normal [6]. In this low range there may be residual ADAMTS13 activity, depending on the underlying mutations [8][9][13]

A severe ADAMTS13 deficiency <5% or <10% of the normal (depending on the definitions)[1][24] is highly specific for the diagnosis of TTP [18][25]. ADAMTS13 activity assays are based on the direct or indirect measurement of VWF-cleavage products. Its activity should be measured in blood samples taken before therapy has started, to prevent false high ADAMTS13 activity [2]. If a severe ADAMTS13 deficiency is present an ADAMTS13 inhibitor assay is needed to distinguish between the acquired, autoantibody-mediated and the congenital form of TTP (USS) [1]. The presence of antibodies can be tested by ELISA or functional inhibitor assays. The level of ADAMTS13 inhibitor may be fluctuating over the course of disease and depends on free circulatory antibodies, therefore an onetime negative test result does not always exclude the presence of ADAMTS13 inhibitors and thereby an autoimmune origin of TTP [2]. A severe ADAMTS13 deficiency in the absence of an inhibitor, confirmed on a second time point in a healthy episode of a possible USS patient, usually sets the trigger to perform a molecular analysis of the ADAMTS13 gene to confirm a mutation. In unclear cases a plasma infusion trial can be

done, showing an USS in the absence of anti-ADAMTS13-antibodies a full recovery of infused plasma-ADAMTS13 activity as well as a plasma half-life of infused ADAMTS13 activity of 2–4 days. A deficiency of ADAMTS13 activity in first-degree relatives is also a very strong indicator for an Upshaw-Schulman Syndrome [1][2][6].

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