**INTRODUCTION**

The term thrombotic microangiopathy (TMA) refers to rare multisystem diseases sharing massive occlusion of small vessels (arterioles and capillaries) due to microvascular endothelial damage leading to thrombosis, with platelet-rich thrombi and microangiopathic hemolytic anemia (MAHA). TMA is not itself a clinical diagnosis and is not an etiology for a specific disorder: it is just a pathologic diagnosis made by tissue biopsy (1,2). TMAs are medical emergencies requiring rapid diagnosis and appropriate treatment.

The term MAHA refers to a non-immune hemolytic anemia caused by intravascular red blood cell (RBC) fragmentation, resulting in:

- schistocytosis, with a confident threshold of 1% in peripheral blood (PB) to support a clinical diagnosis of TMA(3,4).
- consumption thrombocytopenia with platelets (PLT) < 150x10^9 or decrease from the baseline >25%)
- negative direct antiglobulin test (DAT)
- hemolysis signs such as increased lactate dehydrogenase (LDH) due to red cell hemolysis, and/or decreased hemoglobin (HB) and/or haptoglobin,
- fever and organ involvement, including renal impairment and/or neurological symptoms, gastrointestinal, cardiovascular, pulmonary, visual symptoms.
Not all MAHAs are caused by a TMA, but all TMAs cause MAHA and thrombocytopenia.

**History**

Moschowitz in 1924 described for the first time the case of abrupt onset and progression of petechial bleeding, pallor, fever, paralysis, hematuria and coma (5), with disseminated microvascular hyaline thrombi in arterioles and capillaries. In 1947 Singer et al. first introduced the term Thrombotic Thrombocytopenic Purpura (TTP) (6). In 1952 Symmers introduced the term Thrombotic Micro-Angiopathy (TMA) to describe the vascular lesions observed in TTP (7). In 1955 Gasser (8) described the symptoms of a child with hemolytic anemia, thrombocytopenia and renal failure with diffuse bilateral cortical necrosis: this was called Hemolytic Uremic Syndrome (HUS). In 1982 Moake (9) suggested a defective processing of ultralarge von Willebrand factor (ULVWF) multimers produced by endothelial cells. In 1985, Kamali (10) associated HUS with infections with Escherichia coli producing Shiga toxin (ST). Furlan et al. (11) reported that proteolytic cleavage of vWF is increased in some patients with von Willebrand disease type 2A. The hemostatically active large vWF multimers are degraded to smaller less active forms and it has been suggested that the polypeptide subunit of vWF is cleaved at the peptide bond 842Tyr-843Met. A deficiency of this protease predisposes patients with TTP to platelet thrombosis (12,13). Based on partial amino acid sequence, vWF-cleaving Protease is identified as a new member of the ADAMTS family metalloproteinase and designated ADAMTS13 (Adisintegrin AND Metalloproteinase with Thrombospondin type 1 domain, no. 13) (14,15)

**Epidemiology and pathogenesis of TMA**

TMAs are rare diseases: five to ten cases/year/million of TTPs are acquired, with a male:female ratio of 1:2 and a peak of incidence during the 4th decade of life. Hereditary TTP represents one or less cases/year/million (19, 20).

The most prominent diagnoses associated with TMA are thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS). They usually occur, respectively, in adults and in children. As discussed below, their pathogenesis is different: TTP results from a severe ADAMTS13 deficiency, which can be caused by circulating autoantibodies or ADAMTS13 mutations, while HUS is correlated to infection with ST producing bacteria or gene mutations causing an excess of activation of the alternative pathway (16). According to recent observation in TTP/HUS registries, emerging features of these disorders are diagnostic value of ADAMST13 measurement, efficacy of plasma exchange (PEX) and frequency of relapses after remission. (17,18).

Many different disorders can cause TMA (i.e., secondary TMA, see below)

Other clinical TMA presentations are:
- HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelet count) in 0.5 to 0.9% of pregnancies and in 10-20% of severe preeclampsia (21);
- catastrophic antiphospholipid syndrome (CAPS), very rare in less than 1% of patients with acute multiorgan thrombosis;
- malignant hypertension, in about 2.6 cases/year/100,000 with a higher incidence among blacks;
- cancer: about 5% of patients with disseminated malignancy;
- transplant-associated TMA following a) non-renal solid organ transplantation (incidence 5%, 4.0% in liver, 2.3% in lung (22, 23), b) renal transplantation, with 5.6/1000/year with a 50% mortality rate at three years (24). c) following hematopoietic progenitor cell transplantation with variable ranges, from 0% to 74% and median incidence of 7.9% (2, 25).

Finally, TMAs are also part of the pathology of disseminated intravascular coagulation (DIC), in which it results from the deposition of fibrin or platelets within the microvasculature. (26) and scleroderma renal crisis (27). In Table 1 the TMAs are listed according to the cause).

This review mainly deals with diagnostic aspects of MAHA and TMAs. A number of clinical problems await solution in TMA, such as positioning of rituximab in the treatment sequence of primary TTP, management of ST-producing Escherichia coli-HUS complicated by encephalopathy,
efficacy and long-term safety of eculizumab in atypical HUS, and elucidating the pathogenesis of secondary TMA (28-30).

Clinical forms of TMA

TTP is a clinical emergency with a mortality rate up to 90% if not promptly treated (31). African-Caribbean ancestry (32) and obesity (33) are risk factors. It is caused by lack or deficiency of ADAMTS13. In normal individuals endothelial cells produce VWF multimers from the Weibel-Palade bodies and the metalloprotease enzyme ADAMTS 13 cleaves the unusually large multimers avoiding platelet adhesion (34). When the VWF multimers are not cleaved, platelets adhere and the endothelial layer of small vessels are damaged causing platelet aggregation and fibrin deposition in microcirculation. Infections, drugs, pregnancy/delivery (35, 36) may act as triggers in predisposed individuals. ADAMTS13 activity may result absent or highly inhibited by circulating autoantibodies (acquired TTP) or in less than 5% of TTP due to ADAMTS13 gene mutation (congenital TTP, Upshaw-Schulman syndrome, or USS), an autosomal recessive disease presenting with early onset in childhood (37, 38). ADAMTS13 deficiency is most frequently acquired via ADAMTS13 autoantibodies, but rarely it is via recessively inherited mutations of ADAMTS13 gene (USS). Up to 75% of patients in the acute phase show anti-ADAMTS13 IgG that inhibit the proteolytic activity towards VWF and circulate as immuno-complex (IC) determining ADAMTS13 deficiency: 20-25% of patients may not have detectable IgG: different, not fully clarified mechanisms underlie ADAMTS13 deficiency. Less than 2% of all cases are USS, either homozygous or heterozygous. At least 150 distinct mutations of the ADAMTS13 gene are worldwide reported, out of these 70% are missense, and 30% truncating (39). In the suspect of a congenital form of HUS. ADAMTS13 level should be evaluated by measuring both its activity with a fluorogenic assay (40) and its antigen level to differentiate type 1 (both activity and antigen decreased) and type 2 deficiency (severe activity defect associated with subnormal antigen level).

ST-mediated HUS is associated with the microbiological finding of *Escherichia Coli*, mainly O157:H7 and O104:H4 serotypes, and/or *Shigella dysenteriae Type 1* infection: the production of the ST leads to endothelial and glomerular damage with an acute clinical picture. It is usually caused by food, with a seasonal distribution with summer peak: it represents the main cause of acute renal impairment in children less than 3 years old. An entero-hemorrhagic diarrhea self-resolves in most cases, but in 5-7% of them, HUS develops a few days after. ST causes endothelial cell damage: it is a pentamer of B subunits that binds to a globotriasylceramide receptor expressed by endothelial cells, is internalized by endocytosis and inhibits protein synthesis, causing cell death by apoptosis (41) and exposure of the extracellular matrix with platelet aggregation, fibrin deposition and mechanical hemolysis: kidney, gastrointestinal tract and central nervous system (CNS) are key target organs. It can be nearly as severe as aHUS, with mortality of up to 5% (42).

Complement-mediated TMA presents with thrombocytopenia, mechanical hemolysis, acute renal failure, with severe arterial hypertension and ischemic damage due to activation and/or abnormal regulation of the alternative pathway of complement on cell surfaces: mutations in C3 and Factor B, autoantibodies against factor H interfering with regulation, disturb recognition of C3b by factor H, factor I, or CD46; disturb recognition of self-cell surface molecules, such as sialic acid or glycosaminoglycans, by factor H (43). About 20% of cases show a subclinical onset, with slow disease progression (44).

Coagulation-mediated TMA is caused by mutations of genes encoding for thrombomodulin (*THBD*), plasminogen (*PLG*) and diacylglycerol kinase epsilon (*DGKE*), inducing upregulation of prothrombotic factors (45, 46).

Metabolism-mediated TMA, usually seen in infants, is caused by mutations in genes encoding for Methylmalonic Aciduria and Homocystinuria type C (*MMACHC*) (47).

Drug-mediated TMA (48) can be caused by:
- immune-mediated mechanisms with antibodies formation (quinine) (49)
- dose-dependent/toxicity mechanisms (cyclosporine, tacrolimus, clopidogrel, interferon, vascular endothelial growth factor inhibitor, mitomycin C)
- induction of drug-independent antibodies (ticlopidine).

New observation are not rare, such as TMA associated with the i.v. injection of adulterated Opana ER tablets (50)

**Secondary TMAs** are caused by a coexisting disease or condition: systemic infections (51), especially those caused by Streptococcus pneumoniae and the influenza virus are considered as causes not just triggers for the disease. Other conditions are transplantation (solid organ or bone marrow) (52), autoimmune disease (53), cancer (54), pregnancy (55), cytotoxic drugs, disseminated intravascular coagulation (DIC), severe vitamin B12 deficiency (56), pancreatitis. Common feature is that they may cause direct cell damage, promote activation of the complement system in general, or enhance activation of complement on self-cells (43).

**Diagnostic tests.**

TMA is nearly always accompanied by MAHA: a very careful clinical evaluation is needed to exclude other causes underlying MAHA other than a TMA such as the presence of intravascular and/or heart devices, those rare cases of paroxysmal nocturnal hemoglobinuria, heparin-induced thrombocytopenia presenting with MAHA and systemic disorders associated with MAHA with or without TMA, such as systemic infections. The main causes of **secondary TMAs** are mentioned above; patient’s history and physical examination are fundamental steps for the most appropriate diagnostic pathway. Diagnosis of MAHA is confirmed by negativity of direct antiglobulin test (DAT), increased LDH and/or decreased haptoglobin. Organ involvement should be investigated. Complete blood count (CBC) in MAHA shows normocytic anemia, reticulocytosis, severe thrombocytopenia, while in the peripheral blood smear schistocytes, microspherocytes and polychromatophilic RBCs, identifiable as immature reticulocytes by vital stains, are detected. Schistocytes are fragmented red cells appearing in a variety of shapes, rectangular, crescent or helmet shaped. Traditionally are identified and counted by microscopic observation by trained laboratory scientists, with a large margin of error (3). In TMA RBCs are physically sheared by fibrin networks in the peripheral circulation: the appearance of schistocytes may be one of the earliest signs of a TMA and its detection and quantitation are of primary importance. In 2012 the International Council for Standardization in Haematology (ICSH) has published specific recommendations to standardize schistocyte identification, enumeration, and reporting (3), including morphological criteria for the identification of specific schistocyte types. Reference values ≤ 0.1% in adult, 0.3-1.9% in newborn and ≤ 5.5% in preterm. Schistocytes should be evaluated on smears at microscope medium magnification, as a percentage after counting at least 1000 red blood cells (Fig. 1). Schistocyte count has definite clinical value for diagnosis of TMA in absence of additional severe red cell shape abnormalities, with confident threshold value of 1%. Fragmented RBC enumeration by automated counters is a complement to microscope, providing rapid results with high predictive value for negative samples (3, 4). Increased megakaryocytes in bone marrow (Fig. 2) usually with left shift, associated to thrombocytopenia testifies presence of peripheral platelet consumption: bone marrow aspiration is not mandatory but can facilitate the differential diagnosis (versus promyelocytic leukemias with DIC or other hypoplastic/aplastic marrow diseases, including hemophagocytic syndrome).

Once primary TMA is confirmed, the type should be determined to provide the patient with the specific treatment, plasma exchange (PEX) in TTP and eculizumab in complement-mediated TMA. Patient’s sample for assay of ADAMTS13 functional levels should be investigated.
ADAMTS13 activity measurements (degradation of a VWF substrate) are currently based on different methods (57); fluorescence resonance energy transfer (FRET) (58), chromogenic enzyme-linked immunosorbent assay (ELISA) (59), mass spectrometry (60), simplified methods based on coagulation analyzers (61). Results of ADAMTS13 measurements are expressed as a percentage of the ADAMTS13 activity in normal pooled plasma, with a threshold of $<10\%$. An international WHO standard plasma for the measurement of ADAMTS13 has recently become available (62). DNA testing for ADAMTS13 genes has also been developed (63). Clinical interpretation is fundamental because of possible false low results due to hemolysis or increased bilirubin, especially in FRET-based assays. Moreover, unfortunately results of the diagnostic tests are not immediately available, while a critically ill patient with MAHA and thrombocytopenia should be immediately treated. In this scenario the PLASMIC score (64) does represent an immediate help in calculating the diagnostic probability for TTP evaluating very simple parameters/information. One point is assigned to:
i) platelet count $<30 \times 10^9$/L

ii) indirect bilirubin $>2$mg/dL or reticulocyte count $>2.5\%$ or undetectable haptoglobin

iii) no active cancer

iv) no history of solid organ or stem cell transplant

v) MCV $<90$fL

vi) INR $<1.5$

vii) serum creatinine $<2.0$ mg/L.

The PLASMIC Risk Score for severe ADAMTS13 deficiency can be low ($<5$), intermediate (5) or high ($>5$). In ST-HUS acute onset, abdominal pain, vomiting and bloody diarrhea precede by several days sign and symptoms of a MAHA associated with thrombocytopenia: stool cultures for enteric pathogens do confirm the correct diagnosis. In complement-mediated TMA, symptoms are less typical, more insidious and generic (acute renal failure, edema): up to 20% of cases present with multi-organ failure (CNS, cardiac, pulmonary, intestinal). It is reported as familial and sporadic, presenting in up to 80% of children and 50% of adults (65, 66). Quantitative, genetic and functional complement assessment will lead to the diagnosis: waiting for lab test results it is mandatory to start treatment with PEX, moving to anti-complement therapy after results. In Drug-mediated TMA supportive therapy and drug discontinuation are indicated while in metabolism-mediated TMA and coagulation-mediated TMA the role of molecular testing is fundamental. Figure 4 displays an algorithm for the differential laboratory diagnosis in patients with a clinical suspect of TMA.

Conclusions.
The differential diagnosis of TTP, HUS forms, and TMA from other etiologies can be challenging. The most important step to making a correct diagnosis is clinical history (underlying disease, medications). In a patient who has been in an intensive care unit for a long period a TMA may be more likely associated with their underlying illness. A clinical history of hypertension, metastatic malignancy, concurrent polychemotherapy or immunosuppressive therapy, HELLP syndrome, and allogeneic stem cell transplant, which presents with TMA signs, should be considered as possible causes for the TMA presentation. In the vast majority of cases a level of ADAMTS13 activity $<10\%$ are a useful element in the differential diagnosis. Finally, not infrequently a diagnostic evaluation should continue after the patient has recovered with therapy, especially where biochemical and molecular biology studies, including mutation analysis of complement factors, may add useful elements.

1) It is possible, in these Authors’ opinion, that a lower threshold should be considered, given the increased sensitivity of last generation methodologies.
References


Table 1.

Thrombotic microangiopathies listed according to causes

- Thrombotic thrombocytopenic purpura, ADAMTS13 deficiency mediated:
  - Genetic: <10% ADAMTS13 activity
  - Acquired due to antibodies to ADAMTS13

- Shiga toxin-mediated hemolytic uremic syndrome, sustained by enteropathogen microrganisms (*Shigella dysenteriae* and some serotypes of *Escherichia coli*, such as *O157:H7* and *O104:H4*)

- Complement mediated TMA, due to mutations in complement regulatory genes and/or antibodies blocking the complement functions

- Coagulation mediated TMA, due to mutations involving *DGKE, PLG, THBD* genes

- Metabolism-mediated TMA due to mutations in *MMACHC* gene (Methylmalonic Aciduria and Homocystinuria type C)

- Drug-mediated TMA via immunologic pathway (antibodies) and/or toxicity (quinine, ticlopidine, clopidogrel, interferon, contraceptives, etc.)

- Secondary TMAs: are initiated by a coexisting disease or condition such as infection (Streptococcic pneumoniae infection, influenza virus), transplantation (solid organ or bone marrow), autoimmune disease, cancer, pregnancy, certain cytotoxic drugs (anticancer drugs, immunosuppressive), radiotherapy, malignant hypertension, disseminated intravascular coagulation (DIC), severe vitamin B12 deficiency, pancreatitis