Thrombotic Thrombocytopenic Purpura and Atypical Hemolytic Uremic Syndrome: Syndromes or Diseases?

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Abstract

Many diseases are first recognized as a syndrome, which is a collection of symptoms and signs that are presumed to be the consequence of a particular etiology or disease mechanism. This process works for many syndromes but may turn out to be confusing or misleading when the syndrome can result from more than one with vastly different etiology or mechanism. This review discusses how the syndrome of microangiopathic hemolytic anemia (MAHA) and thrombocytopenia has evolved from vaguely-defined 'TTP' (thrombotic thrombocytopenic purpura), 'TTP/HUS' (hemolytic uremic syndrome) or 'TTP/TMA' (thrombotic microangiopathy) to a collection of diseases that, among others, include mechanistically-defined TTP and atypical hemolytic uremic syndrome (AHUS). These two diseases account for the majority of patients presenting with the syndrome of MAHA and thrombocytopenia; yet they but may also present without either MAHA or thrombocytopenia. The advances highlight the need for a new scheme of disease classification and provide the basis for a more rational approach to the diagnosis and management of TTP and AHUS.

Keywords: Thrombotic thrombocytopenic purpura; Atypical hemolytic uremic syndrome; Microangiopathic hemolytic anemia; Complement; ADAMTS[1]3

Introduction

Down observed in 1866 that 'Those who have any given attention to congenital mental lesions, must have been frequently puzzled how to arrange, in any satisfactory way, the different classes of this defect which may have come under their observation. Nor will the difficulty be lessened by an appeal to what has been written on the subject. The systems of classification are generally so vague and artificial, that, not only do they assist but feebly, in any mental arrange of the phenomenon which are presented, but they completely fail in exerting any practical influence on the subject' [1].

Such frustration applies just as fittingly to the syndrome of microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. Patients presenting with the syndrome of MAHA and thrombocytopenia (MAHA/T) have been given the diagnosis of thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), TTP/HUS or TTP/TMA (thrombotic microangiopathy). Almost invariably the criteria for these diagnostic terms are vague, artificial or both.

A serious consequence of indiscriminate usage of diagnostic terms is that all patients presenting with the syndrome of MAHA/T are treated as one disease, 'TTP', with plasma exchange, supplemented with anti-platelet agents, corticosteroids, immunosuppressive drugs, and even splenectomy. On the other hand, the correct diagnosis is missed for patients who do not have MAHA, thrombocytopenia or both.

Difference between Syndromes and Diseases

Many diseases are first recognized as a syndrome, which is simply a particular collection of symptoms and/or signs. It is intuitively assumed that a syndrome is the consequence of a particular disease etiology or pathogenesis. When the etiology of the syndrome is identified, it becomes a disease, although often under the same or similar term. The discovery of the etiology allows exclusion of patients with different etiology etiology or pathogenesis, and identification of patients with atypical features of the disease. Almost invariably the disease is found to be more variable in manifestations than previously recognized. Down syndrome took this path after the discovery of trisomy 21 in 1959 as its cause [2].

Unfortunately, not all syndromes are due to one particular etiology or pathogenesis. Defining 'TTP' or 'TTP/HUS' as a syndrome of MAHA and thrombocytopenia is a conspicuous example of how syndrome-based disease definition may go seriously astray. Until recently, 'TTP' or 'TTP/HUS' has been a source of much confusion and uncertainty. Some have tried to rectify the confusion by adding criteria such as young age and severe renal dysfunction for atypical HUS, and fever and neurologic deficits for TTP. Such conditions are artificial and do not resolve the intrinsic uncertainty of epiphenomenon criteria.

From Syndromes of TTP or TTP/HUS to Diseases of STX-HUS, TTP, AHUS and Others

Among the patients presenting with the syndrome of MAHA/T, a subset, first recognized in young children, have a prodrome of hemorrhagic diarrhea and are often afflicted with prominent renal failure [3]. This subset of patients, often given the diagnosis of typical or diarrhea+ hemolytic uremic syndrome (D+HUS), are found at autopsy to have thrombotic microangiopathy (TMA), which is characterized with endothelial injury and thrombosis in the small arteries, arterioles and glomerular capillaries in the kidney [4].

The hemorrhagic diarrhea of D+HUS was demonstrated in most cases to result from colitis due to infection of shiga toxin producing *E. coli* [5]. Subsequent analysis of stools for shiga toxins or shiga toxin-producing *E. coli* show that not all cases with the infection have obvious hemorrhagic diarrhea before they go on to develop the complications of TMA. Furthermore, some patients who develop TMA following such

infections do not have MAHA, thrombocytopenia or both; and the renal insufficiency may be mild. To encompass these forme frusta cases, it is necessary to replace the syndrome of D+HUS with the disease of shiga toxin associated HUS (STX-HUS), which is TMA following infection with shiga toxin-producing microorganisms, most commonly *E. coli* serotype O157:H7. This etiologically defined disease includes most of the cases previously given the diagnosis of typical or D+HUS, but also includes the forme fruste cases that do not have MAHA, thrombocytopenia, or a prodrome of hemorrhagic diarrhea.

In children, the syndrome of MAHA/T is also often associated with prominent renal dysfunction even when it is not preceded by a prodrome of hemorrhagic diarrhea or shiga toxin associated colitis. Such cases have been given the diagnosis of atypical hemolytic uremic syndrome (AHUS). On the other hand, most adult cases presenting with the syndrome of MAHA/T do not have a hemorrhagic prodrome or evidence of stool shiga toxins, and have normal or mildly abnormal renal dysfunction. These adult patients are given the diagnosis of TTP. Many adult hematologists believe, incorrectly, that AHUS only occurs in children and the small numbers of adult cases that do develop severe renal dysfunction merely have TTP with exceptionally severe renal injury.

In recent years, ADAMTS13, a circulating metalloprotease, was identified and cloned during research to understand how the von Willebrand factor (VWF) multimers in normal plasma are generated [6]. In the circulation, ADAMTS13 prevents the activation of VWF by cleaving the large polymeric protein whenever its conformation is being unfolded by shear stress. Deficiency of ADAMTS13, due to inhibitory antibodies or genetic mutations, is found in essentially all patients who are given the diagnosis of 'TTP' but do not have serious renal dysfunction (maximal serum creatinine <2.5 mg/dl) or a potential cause such as pneumococcal sepsis, pregnancy, autoimmunity with positive ANA tests, hematopoietic stem cell therapy or chemotherapeutic drugs.

Separately, defective regulation of the alternative complement pathway has been found in many children and adults given the diagnosis of 'AHUS' [7]. These findings show that 'TTP' with ADAMTS13 deficiency and 'AHUS' with defective regulation of the alternative complement pathway, although similar in often presenting with MAHA and thrombocytopenia, are indeed etiologically distinct.

Comparison of the pathological features of patients with ADAMTS13 deficiency and those with defective regulation of the alternative complement pathway further reveals that these two disorders are also quite different pathologically [8].

A review of the literature shows that clinicians have often used the term of 'TMA' almost synonymously with the syndrome of MAHA/T. This usage implies, incorrectly, that a patient presenting with MAHA/T have endothelial injury (microangiopathy) and thrombosis in small vessels and vice versa. On the other hand, pathologists often use the 'TMA' term for any lesions with micro vascular thrombosis, even in patients without MAHA and thrombocytopenia.

To avoid confusion, TMA should be used only for the pathological syndrome of endothelial injury (microangiopathy) and thrombosis, as is found in STX-HUS. AHUS associated with defective regulation of the alternative complement pathway is also associated with the pathological features of TMA. In 'TTP' with ADAMTS13 deficiency, the cardinal feature of pathology is VWF-rich platelet thrombi in arterioles and capillaries, accompanied with no evidence of endothelial injury. Thus, not only TTP with ADAMTS13 deficiency and AHUS with defective complement regulation are different in pathogenesis, but they are also quite different pathologically.

The molecular tests developed in research have been translated into clinical practice. Unsurprisingly, these tests identify patients that have severe deficiency of ADAMTS13 activity (<10% of the activity in normal plasma) or mutations affecting the regulation of alternative complement pathway but do not have MAHA, thrombocytopenia, or both, although many of these patients do have MAHA and thrombocytopenia on other occasions. The tests also identify individuals with ADAMTS13 deficiency or defective regulation of the alternative complement pathway but have never been ill. These cases would be excluded from the conventional syndrome-based diagnosis of 'TTP' or 'AHUS' until they present with the complications later in life. However, this practice would be analogous to excluding the diagnosis of sickle cell anemia for a patient who has the hemoglobin $\beta^s\beta^s$ genotype but has not experienced a painful crisis.

Asymptomatic individuals found in family study to have genetic deficiency of ADAMTS13 should be considered to have TTP because the individuals are at risk of developing micro vascular thrombosis upon exposure to stresses of infection, trauma, surgery or pregnancy. These stresses may trigger intravascular VWF-platelet aggregation because they may increase the release of VWF from endothelial cells, augment the shear stress profile in the circulation, and/or further decrease the plasma ADAMTS13 activity.

Similarly, asymptomatic individuals found in family study to have defective regulation of the alternative complement pathway should be considered to have AHUS because the individuals are at risk of developing TMA upon exposure to the stresses of infection, surgery, pregnancy or intravenous radiographic contrast media. These stresses may trigger uncontrolled complement activation, resulting in TMA with micro vascular endothelial injury and thrombosis in the kidney.

Both groups of asymptomatic individuals likely have milder forms of their diseases and thus require stronger triggers to precipitate intravascular VWF-platelet aggregation in the case of ADAMTS13 deficiency or uncontrolled complement activation and endothelial injury in the case of defective alternative complement regulation. Diagnosis of TTP or AHUS before complications occur provides the best opportunity to save the individuals from serious and sometimes fatal consequences of the diseases.

To encompass such forme fruste cases, it is necessary to re-define TTP and AHUS as etiology/pathogenesis-based diseases (Table 1). The difference between mechanistically defined TTP and AHUS is further delineated in table 2.

The complications of TTP include thrombocytopenia, due to platelet consumption in thrombosis; MAHA, due to mechanical injury of the red blood cells in the stenotic microvasculature; and ischemic dysfunctions of organs such as brain, heart and kidney that are affected with micro vascular thrombosis.

In contrast, the complications of AHUS include, in addition to thrombocytopenia due to platelet consumption in thrombosis and MAHA due to mechanical injury of red cells by abnormal levels of shear stress, renal insufficiency, hypertension and extra-renal manifestations. Renal failure in AHUS may result from direct glomerular injury by C5b9 (also known as membrane attack complex, MAC) or ischemic injury due to microvascular stenosis, which may be caused by thrombosis, non-thrombotic endothelial swelling and sub endothelial expansion, or both. Hypertension results from dysregulated renin release due to pre-glomerular hemodynamic disruption.

Extra-renal manifestations of AHUS such as headache, visual defects, dyspnea, chest pain, abdominal pain, anorexia, nausea, vomiting, soft tissue swelling and sudden death are associated with posterior reversible encephalopathy syndrome (PRES) of the brain; edema of brain, retina, bronchial wall, alveoli, intestinal wall, mesentery, pancreas and/or cutaneous soft tissues; and fluids in pleural, pericardial and/or peritoneal cavities. These abnormalities are believed to result from

Syndrome-based definitions	
•	TTP: MAHA/T (diad); MAHA/T plus neurological deficits(triad); or triad pus renal abnormalities and fever (pentad)
•	Typical or D+ HUS: renal failure + MAHA/T following a prodrome of hemorrhagic diarrhea
•	Atypical or D- HUS: renal failure +MAHA/T without a prodrome of hemorrhagic diarrhea
Pros:	
−	The diagnostic criteria are readily available in clinical practice.
Cons:	
−	The severity of renal failure for diagnosis of AHUS is uncertain.
−	The criteria do not clearly distinguish TTP, D+HUS or D-HUS from one another or other causes of MAHA/T
−	The criteria do not encompass patients without MAHA, thrombocytopenia or both.
Etiology/mechanism-based definitions	
•	TTP: a disorder with predisposition to arteriolar and capillary thrombosis due to inhibitory antibodies or mutations of ADAMTS13
•	STX-HUS: a disorder with TMA due to infection with shiga toxin producing microorganisms
•	AHUS: a disorder with predisposition to TMA due to defective regulation of the alternative complement pathway
Pros:	
−	Each disease is conceptually distinct.
−	Do not rely on arbitrary thresholds of anemia, thrombocytopenia or renal failure
−	Include patients without MAHA, thrombocytopenia, neurological deficits, or renal failure
Cons:	
−	Laboratory tests for diagnosis of TTP (ADAMTS13 activity and inhibitor) and AHUS (gene sequencing of **CFH, CD46, CFI, CFB, C3** and **THBD**; and ELISA for CFH antibodies) are not readily available.

Table 1: Two different approaches to defining TTP and HUS

Abbreviations: ADAMTS13: Adisintegrin and Metalloprotease with thrombospondin type 1 repeats member 13; AHUS: Atypical hemolytic uremic syndrome; CFB: Complement factor B; CFH: Complement factor H; CFI: Complement factor I; D+: Diarrhea positive; D-: Diarrhea negative; HUS: Hemolytic uremic syndrome; MAHA/T: Microangiopathic hemolytic anemia and thrombocytopenia; STX: Shiga toxins; THBD: Thrombomodulin.

abnormal vascular permeability induced by anaphylatoxins C3a and C5a of complement activation.

In AHUS, the pathology of TMA is found at autopsy primarily in the kidney. It is assumed that the microenvironment of varying pH and ionic strength in the kidney is conducive to complement activation; anaphylatoxins C3a and C5a are likely released in the circulation from the kidney. This may explain why extra-renal manifestations often abate when a patient develops end stage renal disease and relapse after kidney transplantation.

STX-HUS, TTP and AHUS are not the sole causes of MAHA and thrombocytopenia. Overall, five different types of pathology have been associated with MAHA and thrombocytopenia (Table 3). The pathological lesions share the common feature of arteriolar stenosis, which generates abnormal levels of shear stress and cause mechanical injury of the red blood cells. Thrombocytopenia often accompanies MAHA because thrombosis is the most common cause of arteriolar stenosis. In diseases with non-thrombotic arteriolar stenosis, thrombocytopenia may occur via other mechanisms, e.g. immune thrombocytopenia in vasculitis or decreased megakaryopoiesis due to bone marrow metastasis in patients with tumor cell embolism.

TTP and AHUS together account for the majority of cases presenting with MAHA/T. In the author's series, TTP and AHUS each account for 60% and 20% respectively of the cases presenting with MAHA and thrombocytopenia [9]. However, these figures are not intended to be taken literally. Firstly, the case series includes many referrals whose diagnosis the clinicians were uncertain. Secondly, the incidence of STX-HUS is likely to vary widely, depending on geographic locations and occurrence of outbreaks. The HELLP syndrome, the most cause of MAHA and thrombocytopenia during pregnancy, may account for a larger fraction of the cases encountered at institutions with busy obstetric services. The incidence of MAHA/T also varies widely following hematopoietic stem cell therapy [10,11].

Not included in table 3 are intravascular devices such as ventricular assist devices, prosthetic heart valves and extracorporeal membrane oxygenators that are also commonly associated with hemolysis due to mechanical injury of red blood cells.

How the New Scheme affects the Diagnosis of TTP and AHUS

Thrombotic thrombocytopenic purpura

Most cases of TTP are diagnosed when a patient presents with thrombocytopenia and MAHA, especially at its very first presentation. Neurological abnormalities such as altered mental status or focal deficits are common but not invariably present. Fever occurs when the disease is advanced. The kidney function is normal or only mildly impaired in most cases. Nevertheless, advanced renal failure does not exclude TTP because it may result from a concurrent disorder such as STX-HUS [12], complement factor H mutation [13], or anti-glomerular basement membrane nephropathy (a personal unpublished case).

Since most TTP patients are closely monitored after they achieve remission, increasing numbers of relapses of TTP are diagnosed at the stage of thrombocytopenia, before MAHA ensues. Occasionally, TTP may present *de novo* as 'idiopathic thrombocytopenia', without MAHA. TTP should be suspected when a patient who appears to be a case of 'idiopathic thrombocytopenic purpura (ITP)' also has symptoms such as fatigue, headache or dizziness that is not otherwise explainable (Table 4). TTP should also be suspected when a patient presenting with stroke or myocardial infarction has history of TTP or a decrease of the platelet count from baseline levels that cannot be attributed to other causes. Uncommonly, smoldering TTP may present with thrombocytosis due to compensatory thrombocytopoiesis.

Confirming the diagnosis of *TTP*

When TTP is suspected, the diagnosis can be confirmed by ADAMTS13 analysis. The analysis includes plasma ADAMTS13 activity and inhibitors of ADAMTS13. With a reliable assay, the plasma ADAMTS13 activity is ≤ 10% in patients who have active platelet consumption. On the other hand, a patient can be in clinical remission with ADAMTS13 ≤ 10%. When

Diseases	TTP		AHUS	
	Genetic	**Autoimmune**	**Genetic**	**Autoimmune**
Age of presentation	Infancy - early childhood; less commonly thereafter	Adolescence - adulthood; <10 years uncommon	Infancy to adulthood	
Pathogenesis	Severe deficiency of ADAMTS13 (<10%)		Defective regulation of the alternative complement pathway	
	Genetic mutations	Inhibitory antibodies	Genetic mutations	Antibodies of CFH
Pathology	VWF-rich platelet thrombi in arterioles and capillaries		Thrombotic microangiopathy (Endothelial injury and thrombosis)	
Pathophysiology of complications	- Thrombotic ischemia o Brain: altered mental status, focal deficits, visual defects, seizures, coma o Kidney: hematuria, renal insufficiency (maximal Cr<2.5 mg/dl)[1] o Heart: myocardial injury, electrical-mechanical dissociation, arrhythmia o Pancreatitis - Thrombocytopenia: platelet consumption - MAHA: mechanical injury of RBC		- Renal failure: o Direct glomerular injury by MAC o Ischemic glomerular injury ▪ Thrombotic ischemia ▪ Non thrombotic ischemia - Hypertension: dysregulated renin release - Extra-renal complications: abnormal vascular permeability[5] - Thrombocytopenia: platelet consumption - MAHA: mechanical injury of RBC	
Laboratory diagnosis	Plasma ADAMTS13 activity <10%		Gene sequencing: *CFH, CD46, CFI, CFB, C3, THBD*	Antibodies of CFH
	No inhibitors[2]	Inhibitors[2]		
Acute treatment	Plasma infusion[3]	Plasma exchange	Anti-complement therapy with eculizumab	
	Recombinant ADAMTS13[4]			
Long-term treatment	Plasma infusion every 2-3 weeks for most cases	ADAMTS13-guided rituximab	- Anti-complement therapy - Monitoring of early symptoms	
Prognosis	>90% fatal if not treated; Good if treated promptly and appropriately		>50% ESRD or death if not treated or treated with plasma exchange; Good with prompt anti-complement therapy	

Table 2: Comparisons between TTP and AHUS

[1]Renal insufficiency can be more serious in genetic ADAMTS13 deficiency.

[2]ADAMTS13 inhibitors are only detected in 80%-90% of patients with the inhibitors. When inhibitors are not detected, onset during infancy or early childhood and partial deficiency in first degree relatives favor genetic ADAMTS13 deficiency; Less-than-expected ADAMTS13 increase following plasma exchange, increase of ADAMTS13 above 15% during remission and normal ADAMTS13 levels in first degree relatives favor autoimmune deficiency.

[3]Plasma exchange if the patient has renal failure with impaired urine output.

[4]Under development.

[5]Abbnormal vascular permeability may present as posterior reversible encephalopathy syndrome (PRES) of the brain; edema of brain, retina, bronchial wall, alveoli, intestinal wall, mesentery, pancreas and/or cutaneous soft tissues; and fluids in pleural, pericardial and/or peritoneal cavities.

Abbreviations: ADAMTS13: Adisintegrin and Metalloprotease with thrombospondin type 1 repeat, member 13; AHUS: Atypical hemolytic uremic syndrome; CFB, CFH, and CFI: complement factors B, H and I; ESRD: End stage renal disease; MAC: Membrane attack complex; THBD: Thrombomodulin; TTP: Thrombotic thrombocytopenic purpura.

ADAMTS13 activity is greater than 10%, VWF-platelet aggregation and micro vascular thrombosis do not occur. A plasma ADAMTS13 activity >10% during periods of persisting thrombocytopenia or declining platelet counts excludes TTP as the cause of the thrombocytopenia or declining platelet counts.

A plasma ADAMTS13 activity >10% but <20% (based on the mean level of cases of MAHA and thrombocytopenia due to other causes, minus 3 standard deviations) is also likely to signify TTP. Such levels are most commonly observed in TTP patient who have received transfusion of blood products before the testing, but also occasionally in TTP patients undergoing spontaneous remission.

Detection of ADAMTS13 inhibitors support the diagnosis of acquired TTP due to autoimmunity of ADAMTS13. However, since the sensitivity of assay is in the range of 80%-90%, a negative inhibitor assay result does not exclude the diagnosis of autoimmune TTP. When inhibitors are not detected, further investigation is needed to distinguish between hereditary and acquired TTP. Findings that favor the diagnosis of hereditary TTP include age of initial presentation less than 5 years and partial deficiency of ADAMTS13 in first degree relatives. Conversely, plasma ADAMTS13

levels that increase less than expected after plasma therapy, or recover to greater than 15% during remission, favor the diagnosis of acquired TTP due to autoimmunity of ADAMTS13.

Plasma ADAMTS13 activity may be decreased in a variety of pathological conditions such as sepsis, DIC, cirrhosis and multi organ failures. The plasma ADAMTS13 activity level also decreases during pregnancy, especially when it is complicated with the HELLP (hemolysis, elevated liver enzymes and low platelet counts) syndrome [14,15]. However, the plasma ADAMTS13 level in these pathological and physiological conditions does not decrease below 10% to cause microvascular VWF-platelet thrombosis. It should be noted that the plasma ADAMTS13 activity level may be 10% or lower, artifactually, if the blood sample is properly handled and processed.

Atypical hemolytic uremic syndrome

Most cases of AHUS are suspected when the patients present with the triad of renal insufficiency, MAHA and thrombocytopenia. The renal insufficiency is often severe; yet it can also be mild, especially initially. Thus the severity of renal function impairment does not reliably distinguish

MAHA/T is a common mode of presentation	MAHA/T is not a common mode of presentation
1. Thrombotic microangiopathy	
− STX-HUS − AHUS	− Neuraminidase-HUS due to Pneumococcal sepsis − Anti-VEGF drugs − Chemotherapeutic and other drugs − DGKE nephropathy − Cobalamin C disease − Malignant hypertension (?)
2. VWF-platelet thrombosis	
− TTP	(None)
3. Fibrin-platelet thrombosis	
− HELLP syndrome of pregnancy	− DIC − Anti-phospholipid antibody syndrome − Heparin induced thrombocytopenia − Paroxysmal nocturnal hemoglobinuria
4. Vasculitis/vasculopathy	
(None)	− Scleroderma with renal crisis − Vasculitis, immune complex or ANCA+ − Rocky mountain spotted fever − Fungemia, viremia − Malignant hypertension
5. Tumor cell embolism (intravascular clusters of tumor cells)	
(None)	− Metastatic neoplastic diseases

Table 3: Classification of MAHA/T based on pathological features in arterioles and capillaries

Abbreviations: AHUS: Atypical hemolytic uremic syndrome; ANCA: Anti-neutrophil cytoplasmic antibody; DGKE: Diacylglycerol kinase epsilon; DIC: Disseminated intravascular coagulopathy; HELLP: Hemolysis, elevated liver enzymes and low platelet count; MAHA/T: Microangiopathic hemolytic anemia and thrombocytopenia; VEGF: Vascular endothelial growth factor.

AHUS from TTP. Other conditions that should raise the possibility of AHUS are listed in table 4.

In patients without severe ADAMTS13 deficiency and comorbid conditions, AHUS is the presumptive diagnosis for those presenting with renal insufficiency of any severity accompanied with MAHA, thrombocytopenia and/or systemic abnormal vascular permeability. TMA in kidney biopsy further supports the diagnosis.

However, AHUS is not the only cause of TMA. Other causes of TMA should be excluded. These include STX-HUS, neuraminidase-HUS in association with pneumococcal or other sepsis, anti-vascular endothelial growth factor drugs, chemotherapeutic drugs, DGKE (diacyl glycerol kinase epsilon) nephropathy and cobalamin C disease (Table 3). Nephropathy due to mutations of *DGKE* gene and, rarely, cobalamin C disease may present as idiopathic TMA [16-18]. *DGKE* nephropathy typically presents during infancy and its diagnosis can be confirmed by mutation analysis of DGKE. Cobalamin C disease, due to mutation of *MMACHC* (methylmalonic aciduria cblC type with homocystinuria) gene, is characterized by elevated serum homocysteine and methylcobalamine levels but normal folate and B12 levels [19]. Only rarely does it present as idiopathic TMA.

Severe or malignant hypertension is often believed to cause the syndrome of MAHA and thrombocytopenia and the pathology of TMA [20-22]. Nevertheless, some of the cases are found in retrospect to have AHUS [7]. AHUS may even present as severe or malignant hypertension without MAHA, thrombocytopenia or renal failure [23]. AHUS should be suspected in a case of hypertension that is severe but labile or associated with progressive deterioration of renal function or extra-renal complications of abnormal vascular permeability, with or without concurrent MAHA and/or thrombocytopenia.

When a patient with end stage renal disease due to AHUS undergoes kidney transplantation, the risk of recurrent TMA and graft loss is very high. The graft loss can be prevented with anticomplement therapy. Therefore, the possibility of AHUS should be considered when the cause of renal failure is unknown for a candidate of kidney transplantation.

AHUS in patients with co morbid conditions

Certain conditions such as febrile illnesses, inflammation, surgery, trauma, or intravenous radiographic contrast agents are not known to directly link to micro vascular stenosis or thrombosis but may trigger complement activation and the development of TMA in patients with defective regulation of the alternative complement pathway.

In other co morbid conditions such as hematopoietic stem cell therapy, pregnancy, or autoimmunity with positive antinuclear antibodies, antineutrophil cytoplasmic antibodies or antiphospholipid antibodies, renal insufficiency with MAHA and/or thrombocytopenia may result from AHUS or other mechanisms. AHUS is the presumptive diagnosis after other potential causes are excluded (Table 4).

In patients who do not require immunosuppression for graft versus host disease after hematopoietic stem cell therapy, deranged recovery of the immune system may lead to the generation of antibodies of complement factor H (CFH) and hence AHUS. The complications of AHUS may appear a few weeks to several months after myeloablation or discontinuation of immunosuppressive drugs [11,24]. Such post-immunosuppression autoimmunity can also lead to inhibitory autoantibodies of ADAMTS13 and acquired TTP.

For most patients presenting with renal insufficiency, MAHA and thrombocytopenia and are found to have positive antinuclear antibodies, antineutrophil cytoplasmic antibodies, or antiphospholipid antibodies but no severe ADAMTS13 deficiency, kidney biopsy is often needed to

TTP
− Diad (MAHA/T), triad (diad + neurological deficits) or pentad (triad + renal abnormality and fever)
− Thrombocytopenia with fatigue or non-specific CNS symptoms (e.g. headache, dizziness)
− Stroke or myocardial infarction with thrombocytopenia of no apparent causes
− Stroke or myocardial infarction with a history of TTP
− Thrombocytosis with MAHA
AHUS
− Renal insufficiency with MAHA, thrombocytopenia, with or without systemic abnormal vascular permeability
− Severe but labile idiopathic hypertension, particularly when it is associated with renal insufficiency, MAHA, thrombocytopenia, and/or systemic abnormal vascular permeability
− TMA in kidney biopsy
• Other causes of TMA (Table 3) should be excluded
− Renal failure of unknown etiology, before renal transplantation
Comorbid conditions in which MAHA/T may result from AHUS
− Febrile illnesses, inflammation, surgery, trauma, or intravenous radiographic contrast media
− Hematopoietic stem cell therapy
• Exclusion of viremia, fungemia
• AHUS may result from antibodies of complement factor H, or less commonly, genetic mutations
• May require therapeutic diagnosis to distinguish from drug induced TMA
− Autoimmunity with positive ANA, ANCA or antiphospholipid antibodies
• Kidney biopsy is often necessarily to distinguish TMA from fibrin-platelet thrombosis, vasculopathy and vasculitis
− Pregnancy
• Preeclampsia/HELLP syndrome is the most common cause of hypertension, MAHA and thrombocytopenia during pregnancy
• Preeclampsia/HELLP may transition to AHUS in patients with defects in complement regulation
• AHUS should be suspected when renal failure is more than mild or persists, worsens or occurs after delivery

Table 4: Conditions in which TTP or AHUS should be suspected

Abbreviations: ANA: anti-nuclear antibodies; ANCA: Anti-neutrophil cytoplasmic antibodies; CNS: Central nervous system; HELLP: Hemolysis, elevated liver enzymes and low platelet counts; MAHA/T: Microangiopathic hemolytic anemia and thrombocytopenia; TMA: Thrombotic microangiopathy; STX-HUS: Shiga toxin associated hemolytic uremic syndrome; TTP: thrombotic thrombocytopenic purpura; VEGF: vascular endothelial growth factor.

distinguish TMA from other types of pathology such as vasculopathy (*e.g.* renal scleroderma), vasculitis, and microvascular fibrin-platelet thrombosis (*e.g.* catastrophic antiphospholipid antibody syndrome) .

During pregnancy, the HELLP syndrome is the most common cause of MAHA and thrombocytopenia. On the other hand, activation of the complement system during normal pregnancy [25], can trigger the development of TMA in patients with preexisting defective regulation of the alternative complement pathway. If such women happen to have preeclampsia or the HELLP syndrome, the transition to AHUS may not be easily recognized. Such cases of AHUS are often assumed instead to be worsening of preeclampsia/HELLP. Misdiagnosis of AHUS as preeclampsia/HELLP may account for the high prevalence (~10%) of AHUS mutations in clinical series of preeclampsia/HELLP [26]. AHUS should be suspected when renal failure is severe or presumed 'preeclampsia/HELLP' persists, worsens or occurs after delivery.

Confirming the diagnosis of AHUS

The diagnosis of AHUS is confirmed by mutation analysis of regulators of the alternative complement pathway, which includes complement factor H (CFH), CD46 (membrane cofactor protein, MCP), complement factor I (CFI), and thrombomodulin (THBD) and ELISA for antibodies of CFH. Mutation analysis also includes complement factor B (CFB) or C3, since gain of function mutations of either protein may disrupt the regulation of the alternative complement pathway.

The laboratory tests for confirmation of AHUS are not yet optimal. The tests only identify 40% -75% of patients that are known to have defective regulation of the alternative complement pathway. Negative test results do not exclude the diagnosis of AHUS. Furthermore these tests may have turnaround times of weeks to months. Therefore, for patients presenting with acute complications, therapeutic decisions are made in most cases when the diagnosis of AHUS is only presumptive.

New concepts in the management of TTP and AHUS

TTP

The conventional treatment for acquired TTP is plasma exchange. While this treatment decreases the risk of death from greater than 90% to less than 10%, it does not address the high risk of subsequent relapses [6]. Corticosteroids, cyclophosphamide or azathioprine are not very effective in decreasing the risk of relapse and not infrequently are associated with potentially serious complications.

Preemptive rituximab therapy, immediately after the diagnosis of acquired TTP is confirmed, may decrease the risk of relapse for 1-3 years [27]. Nevertheless, relapses continue to occur thereafter. Late relapses may be prevented by a strategy of repeated rituximab therapy guided by serial plasma ADAMTS13 activity levels [6,28].

Hereditary TTP responds to plasma infusion at approximately 5-7.5 ml/kg every 2-3 weeks. Most patients require maintenance therapy to prevent unpredictable but potentially serious complications such as strokes and progressive deterioration of renal and occasionally mental functions that may occur in some patients who are not treated.

AHUS

Historically, AHUS has been treated as TTP with plasma exchange, supplemented, without good basis for most cases in retrospect, with corticosteroids, immunosuppressive drugs such as cyclophosphamide and rituximab and even splenectomy. With such treatment, more than 50% of the cases die or develop end stage renal disease by one year [7,29].

Anticomplement therapy with eculizumab has been shown to be highly effective in suppressing complement activation, thereby preventing relapse of TMA and progressive deterioration of the kidney function [30]. Many patients gladly find their kidney function improving with anticomplement therapy.

In patients with thrombocytopenia or extra-renal complications of abnormal vascular permeability, anticomplement therapy is followed by resolution of thrombocytopenia and steady alleviation of the extra-renal complications by one week [31]. For patients with labile hypertension, the blood pressure often stabilizes by two weeks of treatment. Thus, when eculizumab is instituted for presumptive AHUS, lack of expected responses practically excludes the diagnosis of AHUS and nullify the indication of continuing anticomplement therapy, unless there is a reason for the lack of response. Examples for lack of response include pseudo-thrombocytopenia due to *in vitro* platelet clumping, concurrent plasma exchange, and inadequate suppression of complement activation. Adequacy of complement suppression can be assessed by total complement test (CH50) [32]. In contrast, improvement of the kidney function may be quite slow, spanning over the course of many months. Because kidney injury may be irreversible, lack of renal function improvement with anticomplement therapy does not exclude the diagnosis of AHUS.

Patients who have evidence of disease activity such as headache, anorexia, nausea, abdominal pain or increasingly unstable blood pressures before each biweekly dose of treatment obviously require the treatment more frequently and the treatment needs to continue. Anticomplement therapy should also be continued indefinitely for patients who have frequent relapses of disease activity or progressive renal function deterioration without the treatment. Nevertheless, historically, it is known that some patients have stable kidney function and no relapses for years after one episode. For such patients, indefinite long-term maintenance treatment would unnecessarily expose the patients to the risk of fulminant meningococcal sepsis in association of anticomplement therapy for uncertain benefit. Nevertheless, identifying these patients *a* priori remains a challenge.

One way to overcome this challenge is a strategy of trial and error. For patients who are asymptomatic and have stable renal functions with anti-complement therapy, the treatment may be tapered off by gradually increasing the length of interval before each successive dose of treatment. During periods of tapering and treatment discontinuation, the patients should be closely monitored. Any recurrence of symptoms, hemolysis, decrease in platelet count, or increase in blood pressure, LDH or serum creatinine should be promptly evaluated for the possibility of relapses and the need of reinstitution of anticomplement therapy.

Conclusions

Since the discovery of ADMTS13 deficiency in TTP and defective regulation of the alternative complement pathway in AHUS, it has become apparent that the syndrome of MAHA and thrombocytopenia may result from a group of etiologically or mechanistically diverse disorders. With a new scheme of disease classification, it is now possible to approach the diagnosis of patients presenting with MAHA and thrombocytopenia in a rational manner and make the diagnosis of TTP or AHUS in patients of without MAHA and thrombocytopenia. Correct diagnosis is an essential first step for proper management of these potentially serious diseases.

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