Thrombotic Microangiopathies

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Contents

M. Harber (ed.), *Primer on Nephrology*, https://doi.org/10.1007/978-3-030-76419-7_51

Learning Objectives

1. The combination of thrombocytopenia and microangiopathic haemolysis with a normal clotting screen is highly suggestive of a TMA.
2. TMAs can present with a wide range of clinical features including neurological involvement and acute kidney injury.
3. In patients with a suspected TMA, it is important to investigate thoroughly to identify the cause of the TMA.
4. TMAs should be treated as an emergency as they can be rapidly fatal.
5. The treatment required will depend on the clinical features at presentation, but urgent plasma exchange should be considered until TTP has been excluded.

In a patient with thrombotic microangiopathy endothelial dysfunction leads to the formation of thrombi in small vessels, platelet consumption, and mechanical damage to erythrocytes (microangiopathic haemolysis).

Haemolytic uraemic syndrome (HUS) is a form of TMA that is caused by Shiga toxin in 90% of cases with the remaining cases (atypical HUS) most commonly due to excessive complement activation. Kidney injury is the most common clinical feature.

Thrombotic thrombocytopenic purpura (TTP) is a form of TMA due to an inherited or acquired deficiency in ADAMTS13 which leads to the accumulation high molecular weight multimers of von Willebrand factor on the surface of endothelial cells and the formation of platelet-rich thrombi. Neurological disease is the most common clinical feature.

51.1 Introduction

Thrombotic microangiopathies (TMAs) are a group of rare diseases characterised by thrombocytopenia, microangiopathic haemolytic anaemia (MAHA), and occlusion of small vessels by thrombi, the site, and severity which determines the clinical presentation. A diagnosis of TMA should be considered in all patients presenting with a combination of thrombocytopenia and microangiopathic haemolytic anaemia as TMA can rapidly progress to organ failure and death. TMAs have been divided into two broad diseases, thrombotic thrombocytopenia purpura (TTP), and haemolytic uraemia syndrome (HUS), based on their clinical manifestations. TTP typically causes neurological disease whereas in HUS acute kidney, injury predominates. Although in many cases the diagnosis is clear, in other cases it is not possible to reliably distinguish between these diseases purely on clinical criteria as significant overlap can exist. As a better understanding of the molecular basis of TTP and HUS has developed, it is now possible to diagnose and differentiate between these diseases with greater accuracy, and classification of TMAs is now based on aetiology rather than clinical features. The early recognition of the clinical and laboratory features of a TMA by clinicians remains critically important to allow appropriate investigation and early initiation of treatment.

51.2 Clinical Features

The clinical features depend on the site of vascular occlusion with predominant involvement of the central nervous system in TTP and the renal vasculature in HUS. However, there is significant clinical overlap, and a classification based on aetiology rather than clinical features provides a better guide to prognosis and a rationale for therapy. TMA can also occur in a range of other clinical scenarios where features of the original disease may be evident. These are listed in ◘ Table 51.1.

51.2.1 Thrombotic Thrombocytopenic Purpura.

With the exception of the inherited form of TTP (Upshaw-Schulman syndrome) which usually occurs early in childhood TTP occurs predominantly in adults (90%), the features of TMA are present, and the thrombocytopenia is often profound, with platelet counts lower than in HUS. Neurological symptoms and signs are usually present and often severe and can include headache, focal neurological deficit, seizures, and reduced level of consciousness. Cardiac involvement can also occur in up to 40% of patients. Fever is frequently present, and renal impairment, including an abnormal

Table 51.1 Classification of HUS, TTP, and other TMA associated diseases

Reduced ADAMTS13 activity (TTP). Genetic (homozygotic or compound heterozygotic) Acquired (inhibitory autoantibody)
Shiga toxin-induced HUS Shiga toxin-producing *E. coli* and *Shigella dysenteriae* type 1
Atypical HUS due to disorders of complement regulation Genetic disorders of complement regulation Acquired disorders of complement regulation
Atypical HUS due to other genetic causes DGKε Defective cobalamin (B12) metabolism
TMA associated with pregnancy Preeclampsia/HELLP syndrome aHUS and TTP
TMA associated with other infections *Streptococcus pneumoniae* HIV Influenza Herpes viruses (CMV, EBV, HHV8) Hepatitis A and C
Drug-related TMA Chemotherapy (mitomycin, cisplatin, gemcitabine) VEGF inhibitors (bevacizumab, aflibercept) Anti-platelet drugs (ticlopidine, clopidogrel) Immunosuppressants (ciclosporin, tacrolimus, sirolimus) Interferons (α and β) Tyrosine kinase inhibitors (sunitinib, sorafenib) Antibiotics (penicillins, ciprofloxacin)
Malignancy-related TMA Epithelial malignancies (stomach, bowel, prostate, breast) Haematological malignancy
TMA related to malignant hypertension
TMA following bone marrow transplantation
TMA following solid organ transplantation Recurrent aHUS De novo TMA (drugs, ischaemia reperfusion injury) Antibody-mediated rejection

urinary sediment, can be present. The disease can progress rapidly with a high mortality associated with TTP without appropriate treatment and even with treatment a mortality of >10% is reported.

51.2.2 Haemolytic Uraemic Syndrome

Haemolytic uraemic syndrome is a group of diseases usually presenting with evidence of thrombocytopenia, microangiopathic haemolysis, and acute kidney injury. Although often thought of as a disease predominantly affecting children, it is clear that HUS can affect any age group, and this diagnosis should be considered in any patient presenting with a TMA and renal impairment.

51.2.3 Shiga Toxin-Associated HUS

This is the commonest form of HUS and is caused by gastrointestinal infection with bacteria that produce Shiga toxin, most frequently Shiga toxin-producing *Escherichia coli* (STEC). Farm animals are the natural reservoir for STEC, and infection occurs after direct contact or after consumption of undercooked meat or contaminated food products. Symptoms typically begin after a 4–7-day incubation period with the abrupt onset of diarrhoea, which is usually bloody (60%), and abdominal pain. The features of HUS develop in approximately 10% of people infected with STEC with development of thrombocytopenia, microangiopathic haemolysis, and acute kidney injury 2–10 days after the onset of diarrhoea, which may have resolved. Importantly 5–10% of patients with STEC HUS report no preceding gastrointestinal symptoms. In the acute phase, 50% of patients require dialysis, and there is a reported mortality of 1–2%. Neurological symptoms and signs are common and may be present in approximately 20–30% of patients. Although renal recovery is usual after the initial presentation, 40% of patients subsequently develop CKD or hypertension. In patients who progress to ESKD, kidney transplantation is an option as recurrence is very rare.

51.2.4 Atypical HUS

The clinical presentation of aHUS can be indistinguishable from other causes of TMA, with renal involvement predominating. Other organ involvement, including neurological and cardiac disease, can be present. Traditionally thought of as a disease primarily affecting children, all age groups can be affected. Preceding gastroenteritic symptoms are reported by 25% of patients with aHUS; therefore, the presence of diarrhoea is not a robust criteria to distinguish between atypical and STEC forms of HUS. A family history of aHUS may be present as may a history of previous episodes of TMA as aHUS can run a remitting, relapsing course.

Without treatment the prognosis is poor with 50% of patients progressing to renal failure or dying within 1 year of presentation. The severity of aHUS is influenced by the underlying genetic abnormality in complement regulation responsible for disease development (Table 51.2) [1]. Once a patient develops end-stage renal failure, the other features of the disease usually remit.

Table 51.2 Complement defects associated with TMA

Complement defect	Function of protein	Frequency	Rate of End-stage renal disease (ESRD)
Factor H mutations	Dissociation of convertases and cofactor for factor I-mediated cleavage and inactivation of C3b	15–30%	70–80%
CD46 mutations	Cofactor for factor I-mediated cleavage and inactivation of C3b	10–15%	20%
Factor I mutations	Serine protease degrading C3b to inactive smaller fragments	5–10%	50–80%
Factor B mutations[a]	Binds to C3b to form the alternative pathway C3 convertase	1–2%	70–80%
C3 mutations[a]	Pivotal complement protein at the convergence of the three activation pathways	5–10%	50–60%
Anti-factor H autoantibodies	Inhibit function of factor H	6–11%	40–60%

ESRD End-stage renal disease
[a]Denotes gain-of-function mutation

Recurrence after a kidney transplant is common and occurs in 80–90% of patients with certain mutations (Factor H, C3, Factor B), and a high rate of graft loss is reported if the disease recurs and is not treated appropriately [2]. Patients with no identifiable mutation have a lower but significant risk of relapse. The exception is patients with CD46 mutations who rarely develop recurrent disease. Previously, because of the risk of relapse, patients with aHUS were not considered for kidney transplant alone. The availability of therapeutic complement inhibition means that transplantation is now a viable option.

51.3 Epidemiology

TTP has an annual incidence of approximately 2–5 cases per million population, with over 90% of cases occurring in adults. The majority of cases (>95% in adults) are caused by an autoantibody that inhibits the function of ADAMTS13. The highest incidence is after the age of 40 years, and females are affected more commonly than males (2:1). Inherited deficiency in ADAMTS13 activity is rare (5% of cases) and usually presents in childhood.

STEC infection, typically serotype O157, is the commonest cause of HUS and accounts for 90% of cases of HUS. STEC HUS has an annual incidence of seven cases per million population. The incidence of STEC HUS is highest in the summer months. Other *E. coli* serotypes also cause HUS, including the serotypes O26, O111, O103, and O145, as can infection with other Shiga toxin-producing bacteria, particularly *Shigella dysenteriae* type 1, which is common in parts of Asia. The largest recorded outbreak of STEC HUS occurred in continental Europe, mainly Germany, in 2011 and was caused by *E. coli* O104 [3]. This outbreak was notable because of the high proportion of adults affected, the high mortality rate (4.3%), and the high proportion of patients with neurological sequelae.

Atypical HUS is less common and accounts for the remaining 10% of cases. Complement-mediated aHUS is the most common form and has an annual incidence of 0.4 per million population. It most commonly presents in childhood, but any age can be affected. Females are more frequently affected (approximately 60% of cases). Other inherited or secondary forms of aHUS are rare in the population as a whole.

51.4 Aetiopathology of the Thrombotic Microangiopathies

The vascular endothelium has a critical role in maintaining normal haemostasis. Activation or injury to the endothelium results in a reduction in endothelial anticoagulant activity and release of pro-thrombotic molecules. Activation of the coagulation cascade results in platelet aggregation and trapping of erythrocytes in a fibrin mesh, finally leading to thrombus formation. In the context of vascular injury, this will bridge any defect in the endothelium and vessel wall and, because coagulation is usually localised, will not result in detectable changes in coagulation tests or other haematological parameters.

In contrast in a TMA, there is widespread activation of the endothelium. There is no breach of the endothelium to bridge, but instead small vessels are occluded by thrombi causing ischaemic tissue injury. Because of the more extensive endothelial activation, haematological abnormalities are evident. Platelets are consumed within the thrombi, and a low platelet count ($<150x10^9$/l) will usually be present. Platelets fall early in the disease, and although a normal platelet count can be present, significant TMA is unusual in the absence of thrombocytopenia. Erythrocytes are trapped within the thrombi and

are also damaged as they pass over activated endothelium and through partially occluded vessels leading to microangiopathic haemolysis.

51.4.1 Thrombotic Thrombocytopenic Purpura

TTP is caused by a severe deficiency in the protease enzyme 'a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13' (ADAMTS13) which is responsible for cleaving von Willebrand factor (VWF). VWF is involved in haemostasis, and the surface expression of VWF on endothelial cells induces platelet aggregation and thrombus formation. VWF is initially produced as ultra-large multimers which are gradually broken down by ADAMTS13. If ADAMTS13 activity is reduced, multimers of VWF accumulate on the endothelial surface leading to platelet aggregation and thrombus formation [4]. Inherited TTP is rare accounting for 5% of all cases of TTP. It is due to a homozygous (or compound heterozygous) mutation in the *ADAMTS13* gene, and there is a high degree of penetrance. Inherited TTP accounts for a higher proportion of childhood TTP. More commonly (>90% of cases), TTP is due to an autoantibody that inhibits the function of ADAMTS13 [5].

TTP has also been linked to the platelet inhibitors ticlopidine and clopidogrel. This is a rare side effect which usually develops in the first few weeks after starting treatment. Autoantibodies to ADAMTS13 have been reported in some cases and plasma exchange may improve outcome.

51.4.2 Shiga Toxin-Associated HUS

Once ingested, STEC adheres to the intestinal epithelium and releases toxin. Shiga toxin is absorbed across the intestinal epithelium and transported to the target organ bound to erythrocytes and leukocytes. It exerts a cytotoxic effect by binding to globotriaosylceramide 3 (Gb3), which leads to endocytosis of toxin and retrograde transport to the endoplasmic reticulum where the toxin inhibits protein synthesis. This disrupts cell function, finally leading to cell death [6]. Gb3 receptors are highly expressed on glomerular endothelial cells, podocytes, and tubular cells; therefore, explaining why the kidney is the primary target of the disease. Shiga toxin-mediated injury and activation of endothelial cells produces a pro-thrombotic state with activation of the coagulation cascade, platelet and erythrocyte consumption, and the vaso-occlusive disease which typifies a TMA. HUS only develops in a minority of people infected with STEC suggesting that other genetic or environmental factors are important in determining whether HUS develops.

51.4.3 Atypical HUS

Atypical HUS was used to describe cases of HUS not caused by STEC infection. It is now clear that this represents a group of diseases with similar clinical features but different aetiologies. Most cases of aHUS are due to a genetic or acquired defect in the regulation of the complement cascade. Mutations in the complement control protein Factor H were first described in 1998 [7]. Since then loss-of-function mutations, polymorphisms, or antibodies that interfere with the function of complement inhibitors and gain-of-function mutations in complement activators have been identified in approximately 70% of patients with aHUS.

The complement cascade is a system of over 30 proteins and is a pivotal component of the innate immune system. It is activated by three distinct pathways, but it is the alternative pathway, with its continuous, low level of activation, that is critical in the development of aHUS. In the alternative pathway, activation of C3 occurs by spontaneous hydrolysis. Binding of Factor B to activated C3 results in further cleavage of C3 to produce C3a (an anaphylatoxin) and C3b which generates the C3 convertase of the alternative pathway (C3bBb). This cleaves more C3 with amplification by a system of positive feedback and generation of a C5 convertase. C5 is cleaved into C5a, a potent anaphylatoxin, and C5b which leads to the assembly of the membrane attack complex (MAC, C5b-9). The MAC forms a membrane-spanning lytic pore which when deposited in sub-lytic concentrations can alter cell phenotype. MAC deposited on endothelial cells induces a pro-thrombotic change resulting in a TMA.

In normal circumstances activation of complement is controlled by a series of cell membrane-bound and soluble inhibitors to prevent injury to autologous cells. If there is loss of function in one of these inhibitors, increased activation of complement occurs leading to endothelial cell damage and development of TMA. Loss of function is usually due to a mutation in one of the control proteins, most frequently Factor H, but also membrane cofactor protein (MCP, CD46) and Factor I. In addition gain-of-function mutations have been described in proteins involved in complement activation (Table 51.2) [8]. The disease usually follows an autosomal dominant pattern of inheritance with incomplete penetrance. Approximately 50–60% of people who carry a disease-associated mutation develop the disease. Other genetic and environmental factors influence disease

development (a multi-hit hypothesis) including commonly occurring genetic variants in complement genes that predispose to disease in the presence of a pathogenic mutation [9]. Environmental triggers, including infection, pregnancy, transplantation, and drug exposure, are involved in triggering TMA in a patient with a genetic susceptibility. Another recognised cause of aHUS is autoantibodies that interfere with the function of complement regulators in particular anti-Factor H.

51.5 Diagnosis

The initial step is to diagnose the presence of a TMA. This is based on the clinical presentation and the presence of the characteristic biochemical and haematological changes which are summarised in ◘ Table 51.3. Once this has been established, the next step is to identify the cause of the TMA.

51.5.1 Thrombotic Thrombocytopenic Purpura

The diagnosis is made by testing ADAMTS13 activity, with severe deficiency (<5–10% activity), being associated with the disease. ADAMTS13 activity can also be low in TMA associated with autoimmune disease, pregnancy, and drugs and can be low in patients with malignancy or chronic infection without features of TMA. If low ADAMTS13 activity is found, the patient should be screened for inhibitory autoantibodies to ADAMTS13 or mutations in the *ADAMTS13* gene (in the absence of an inhibitor). It is critical to perform these tests before starting plasma-based therapies.

◘ **Table 51.3** Laboratory investigation in suspected TMA

Test	Result in TMA
Full blood count	Anemia and thrombocytopenia
Blood film	Red cell fragmentation (schistocytes)
Reticulocyte count	Elevated
Lactate dehydrogenase	Raised due to release from damaged red cells
Liver function tests	Isolated raise in bilirubin due to haemoglobin degradation
Haptoglobin	Reduced due to trafficking of free haemoglobin
Creatinine	Elevated due to renal dysfunction
Coagulation screen	Normal (differentiating TMA from disseminated intravascular coagulation)
Direct antiglobulin test	Negative (differentiating TMA from immune hemolysis)
Urinalysis	Haemoglobinuria

51.5.2 Shiga Toxin-Associated HUS

STEC O157 can be identified after culture from the stool or from a rectal swab (which is a useful technique to obtain cultures in children or after diarrhoea has stopped). It is possible to test for the presence of Shiga toxin, and the gene encoding Shiga toxin can be detected by polymerase chain reaction. Infection can be confirmed by measuring the serological response to the O-serotype of Shiga toxin-producing *E. coli*; however, it is not possible to test for all serotypes. Renal biopsy is rarely necessary to confirm the diagnosis, but when performed arteriolar and glomerular capillary thrombosis is seen, with glomerular capillaries congested with fragmented erythrocytes. Acute tubular injury and mesangiolysis are commonly seen.

51.5.3 Atypical HUS

Initially aHUS is a diagnosis of exclusion in patients with a TMA, negative tests for STEC infection, and preserved ADAMTS13 protease activity. A low plasma C3 level is suggestive of aHUS, although C3 levels can be normal particularly in patients with CD46 mutations. Low concentrations of circulating Factor H or Factor I can be detected if the protein is not synthesised as a consequence of a mutation; however, mutations may result in normal quantities of non-functioning protein. Low cell surface expression of CD46 can be detected on peripheral blood mononuclear cells.

Genetic and immunological testing is usually required to identify the exact aetiology. Because of the time required to perform these tests, they are rarely useful in guiding initial diagnosis and treatment but should be performed in all patients with suspected aHUS. Screening for genetic or immune defects will identify a cause in approximately 70% of cases [1]. The remaining 30% of patients may have mutations in other genes, have a ‘secondary TMA’ (see below) or a disease without an identifiable cause. In some of these situations, patients may still respond to complement inhibition.

51.6 Treatment

51.6.1 Thrombotic Thrombocytopenic Purpura

Without treatment mortality in patients with TTP is high (over 90%). Plasma-based therapy, either exchange or infusion, is the mainstay of treatment, with plasma exchange generally being preferred in adults because of its ability to remove inhibitory autoantibodies [10]. Despite treatment mortality rate remains high (>10%). Early treatment (within hours) is important, and treatment should begin based on clinical and laboratory features before ADAMTS13 activity is known. It is less important to exclude aHUS as plasma therapy is an appropriate treatment for this condition. Plasma exchange should be with fresh frozen plasma (or equivalent product) and should be at least 1.5 plasma volume on a daily basis until a response is seen. Increasing the volume of exchange or twice-daily exchanges should be considered if no response is seen. Immunosuppression should be considered in patients with TTP. High-dose intravenous methylprednisolone followed by oral steroids should be started in patients with suspected TTP. Relapse after remission occurs in up to 30% of patients. A beneficial effect of rituximab (anti-CD20) has been reported, particularly in reducing the risk of relapse. Other drugs, including vincristine, cyclophosphamide, and cyclosporine A, have been used to treat TTP, but rituximab is now preferred. A monoclonal antibody that blocks the interaction between VWF and platelet glycoprotein 1b, caplacizumab, has shown promising results in recent trials [11].

51.6.2 Shiga Toxin-Associated HUS

In most cases this is a self-limiting disease, and treatment is supportive until resolution of the acute episode. In severe cases, this will include renal replacement therapy and when necessary other organ support. The use of antibiotics in STEC HUS is controversial. Some authors have reported a worse outcome with the use of antibiotics, possibly due to the increased release of Shiga toxin [12]. However, more recent reports from the German outbreak with STEC O104 suggest a better outcome after antibiotic treatment [13, 14]. Reports following the German outbreak and other studies have failed to show any beneficial effect of plasma exchange or other plasma-based therapy in the treatment of STEC HUS an observation supported by a Cochrane systematic review in 2009 [15].

A role for complement activation has been suggested in the pathogenesis of STEC HUS. This is supported by a report in 2011 of three patients with severe STEC HUS who responded to treatment with the complement inhibitor, eculizumab [16]. Future studies may define a role for complement inhibition in this disease, but at present the routine use of eculizumab in STEC HUS cannot be recommended.

51.6.3 Atypical HUS

Plasma therapy has until recently been the main therapy for aHUS. This is usually plasma exchange with fresh frozen plasma or equivalent. Plasma exchange, as opposed to plasma infusion, not only avoids problems of volume overload and hyperviscosity but will remove proteins with abnormal function and autoantibodies that may interfere with the function of normal proteins. As many as 40% of patients are resistant to plasma therapy and show signs of ongoing TMA and progressive organ damage despite treatment. Of the patients who respond in some cases, treatment can be withdrawn while others become dependent on plasma therapy to maintain remission.

Eculizumab is a monoclonal antibody that inhibits activation of C5 and is licenced for the treatment of aHUS. The first beneficial effects of eculizumab in aHUS were reported in 2009 [17, 18], and these reports have been confirmed in clinical trials [19]. Adolescent or adults patients with aHUS were enrolled in the initial trials with either plasma-resistant or plasma-sensitive aHUS, and eculizumab was very effective at inducing and maintaining remission. Kidney function also improved after eculizumab treatment. In these trials approximately 30% of patients did not have an identifiable defect in complement regulation but responded to treatment with eculizumab. Inhibition of C5 activity with eculizumab increases susceptibility to meningococcal infection. Vaccination with a tetravalent (ACWY) and anti-B serotype vaccine is mandatory prior to eculizumab use, and some centres recommend long-term antibiotic prophylaxis. Although life-long treatment with eculizumab was recommended in the initial licence, it is now clear that a significant proportion of patients can withdraw from treatment without relapse [20, 21].

51.7 Other Genetic Causes of HUS

51.7.1 HUS Due to Defective Cobalamin (B12) Metabolism

Methylmalonic aciduria and homocystinuria type C protein is involved in the metabolism of vitamin B12 (cobalamin). Homozygotic or compound heterozygotic mutations in the gene encoding this protein, *MMACHC*,

causes an aHUS-like disease in addition to a wide range of other clinical features including neurological, cardiac, and pulmonary abnormalities. Biochemically patients have elevated plasma homocysteine, low methionine, and methylmalonic aciduria. Biochemical and genetic analysis is vital in patients presenting with a TMA, particularly if there are other clinical abnormalities. Patients usually present in childhood, and without treatment the prognosis is poor, with approaching a 100% mortality without treatment. Patients respond to treatment with hydroxocobalamin (B12) and betaine, and treatment can be initiated before the results of biochemical and genetic analyses are available.

51.7.2 HUS Due to Mutations in Diacylglycerol Kinase ε

Homozygous or compound heterozygous mutations in the lipid kinase diacylglycerol kinase ε can lead to atypical HUS. Patients usually present in childhood, significant proteinuria is common, there may be histological features of membranoproliferative glomerulonephritis, and the disease may follow a relapsing remitting course. There is no effective treatment, and patients commonly progress to CKD and ESRD at which stage transplant is an option as disease recurrence has not been reported.

51.7.3 HUS Due to Mutations in Other Genes

Genetic variants in thrombomodulin (*THBD*) have been reported in patients with atypical HUS [22]. Thrombomodulin is part of the coagulation pathway and enhances clot formation. It may also activate complement and possibly links these two pathways. New genes associated with atypical HUS are being identified, for example, *INF2* [23], which may provide insights into the pathophysiology of TMA as well as explaining the disease in some patients in whom no complement defect is identified.

51.8 HUS Occurring in the Context of Other Infections

51.8.1 HUS Following Streptococcal Infection

This is a rare form of HUS complicating infection with *Streptococcus pneumonia* (septicaemia, pneumonia with empyema and meningitis) accounting for 5% of childhood HUS [24]. Patients are usually young (<2 years), and the disease is associated with a high mortality (approximately 25%). The enzyme neuraminidase is produced by the bacteria and released into the plasma where it strips neuraminic acid residues from the glycocalyx of many cells including erythrocytes, platelets, and endothelial cells. This exposes the Thomsen-Friedenreich (T-) antigen which is recognised by naturally occurring antibodies that bind to the exposed antigen leading to endothelial and platelet activation and TMA. The presence of the antigen on erythrocytes explains why in this type of HUS, uniquely, the direct antiglobulin test (DAT, Coombes test) is positive. Treatment is supportive with the eradication of *Streptococcus pneumoniae* infection.

51.8.2 HUS Following HIV Infection

HUS was common in patients with HIV infection (2–7% of patients) before the introduction of highly active antiretroviral therapy (now <1%). Although other forms of HUS (e.g., STEC related) can occur in patients infected with HIV there appears to be a specific HIV-related form of HUS associated with high viral load, low CD4 counts, and opportunistic infection. The mechanism by which HIV induces a TMA is not known but is hypothesised that direct virus infection causes endothelial activation inducing a pro-thrombotic state.

51.9 TMAs Occurring in Association with Other Conditions

51.9.1 Pregnancy

There are a number of causes of a TMA either during pregnancy or in the post-partum period including pre-eclampsia, HELLP syndrome, TTP, and aHUS. Differentiating between these can be difficult because of significant clinical overlap.

Pregnancy can also unmask inherited ADAMTS13 deficiency, in which case disease occurs early in pregnancy. Approximately 20% of cases of aHUS occur in association with pregnancy, and these most commonly occur in the post-partum period. A significant proportion of women developing atypical HUS in association with pregnancy have a genetic defect in a complement regulator, suggesting pregnancy is the trigger for disease in women with a genetic predisposition.

51.9.2 Malignancy and its Treatment

HUS- and TTP-like syndromes are associated with disseminated adenocarcinoma (gastric, colonic, breast, and prostate) and haematological malignancies, and the

TMA can pre-date the diagnosis of malignancy. In addition drugs used in the treatment of these cancers can cause a TMA including mitomycin, gemcitabine, platinum-based drugs, tyrosine kinase inhibitors, and VEGF inhibitors (Table 51.1). It can be difficult to determine whether the TMA is due to malignancy or its treatment. There is some evidence of a response to steroids and plasma exchange.

51.9.3 Drug-Induced TMA

The development of a TMA has been reported in association with the use of a range of drugs in addition to those used for the treatment of cancer (Table 51.1). For some drugs this appears to be a direct effect on the endothelium as is the case for Interferon-β [25] and bevacizumab [26], while in the case of quinine, the TMA is due to the development of autoantibodies against platelet glycoproteins. The TMA induced by clopidogrel [27] and ticlopidine [28] leads to the production of antibodies against ADAMTS13 and a TTP-like disease which responds to plasma exchange. In some drugs the exact mechanism of TMA development is unknown, and management is supportive with the withdrawal of the causative drug.

51.9.4 Malignant Hypertension

Severe hypertension causing a TMA and atypical HUS due to an inherited defect in complement regulation can present with identical clinical features. Pre-existing hypertension and other features of hypertensive end-organ damage in this patient group make a secondary TMA more likely. However, if there is no improvement in laboratory parameters with control of blood pressure, treatment with eculizumab should be considered. This strategy avoids missing patients with a defect in complement regulation.

51.9.5 Solid Organ Transplantation

A TMA can occur after any solid organ transplant, most frequently after kidney transplantation. It can be due to a number of factors including calcineurin inhibitor toxicity, ischaemia reperfusion injury, antibody-mediated rejection, and infection. Complement mutations have been reported, in up to 30% from one series [29], possibly due to recurrence of previously undiagnosed atypical HUS. Complement inhibition should be considered particularly if there is uncertainty about the primary diagnosis or the TMA does not resolve with measures such as calcineurin inhibitor withdrawal.

51.9.6 Bone Marrow Transplantation

The development of a TMA has been reported in 10–40% of patients following allogeneic bone marrow transplantation. There are several factors that could contribute to this including graft versus host response, calcineurin inhibitor use, chemotherapy, and infection. Defects in complement regulation have also been reported and complement activation can be demonstrated in some cases. These observations have led to the use of eculizumab in this situation although evidence that this should be part of the management of this condition is still lacking.

51.9.7 Autoimmune Disease

TMA is seen in patients with systemic lupus erythematosus (SLE) and scleroderma renal crisis. In patients with anti-phospholipid antibody syndrome (APS), particularly catastrophic APS, antibodies bind to and activate platelets and endothelial cells leading to a pro-thrombotic state and a TMA. TMAs have also been described in patients with primary glomerular diseases including IgA nephropathy, FSGS, and ANCA-associated vasculitis.

Tips, Tricks, and Pitfalls

1. Consider a thrombotic microangiopathy in any patient presenting with unexplained thrombocytopenia.
2. In the presence of thrombocytopenia, check for haemolysis by requesting a blood film and measuring serum lactate dehydrogenase and haptoglobin. Also check the clotting profile and fibrinogen concentration as this will be abnormal if the patient has disseminated intravascular coagulation.
3. Make sure that you send off blood for ADAMTS13 activity and complement protein levels before you start plasma-based therapies. Once plasma infusion has been given, it is difficult to interpret the results, and diagnosis may be delayed.
4. Always send stool samples for STEC culture and for toxin PCR even if diarrhoea has stopped or the patient did not report diarrhoea at all. Diarrhoea will often stop before HUS develops, and 5–10% of cases STEC HUS have no diarrhoea. This may require discussion with your

microbiologist to arrange culture in the absence of diarrhoea.
5. Plasma exchange should be started as quickly as possible, ideally within 4 hours of presentation with suspected TTP. This may require transfer to a specialist centre where this treatment is available out of hours.
6. Patients with TMA are at relatively low risk of bleeding, and platelet transfusions should be avoided if possible as this may increase the risk of thrombosis and a worsening of disease severity.

Questions

1. Should plasma exchange be delayed until ADAMTS activity is known in patients with suspected TTP?
2. Why should stool be sent for testing in patients with suspected STEC HUS even if diarrhoea has resolved?
3. Does a negative stool culture exclude STEC HUS?
4. Can complement-mediated TMAs be identified by a low circulating C3 level?
5. What infections are more common in patients treated with eculizumab?

Answers

1. No. It is important to take blood for ADAMTS13 activity before starting plasma exchange, but treatment should be started as soon as possible as TTP can progress rapidly.
2. The diarrhoeal illness may have resolved by the time HUS develops, and some patients with STEC HUS report no diarrhoea at all. It is therefore important to send stool for culture and PCR in all cases of TMA. These cases should be discussed with a microbiologist.
3. No. As HUS can develop some time after the initial infection STEC culture can be negative by the time patients present. PCR can improve diagnosis rates, and serology can be informative in some cases.
4. No. C3 levels can be low in aHUS due to excessive activation of complement, but a normal C3 level does not exclude this. Also low C3 levels have been reported in some patients with STEC HUS.
5. The complement membrane attack complex is particularly important in defence against *Neisseria meningitidis* infection; therefore, patients on eculizumab are particularly susceptible to meningococcal infection. Patients on eculizumab should be vaccinated against meningococcus and long-term prophylactic antibiotics considered. Infection with other *Neisseria* species can also occur.

Case Study

Case 1

A 27-year-old man presented with severe abdominal pain. A diagnosis of pancreatitis was made was based on a raised amylase and pancreatic inflammation evident on imaging. No gall stones were identified and the patient did not drink alcohol. On admission he was thrombocytopenic with a platelet count of 62 x 10^9/l, his haemoglobin was low (109 g/l), and he had evidence of fragmentation on his blood film. His coagulation screen was normal. He was managed conservatively and the symptoms and laboratory abnormalities resolved. He presented again 9 months later again with pancreatitis and laboratory findings of thrombocytopenia and microangiopathic haemolytic anaemia.

Screening was performed for a genetic abnormality in complement regulation, and a pathogenic variant in *CD46* was identified making a diagnosis of complement-mediated aHUS most likely. This case demonstrates that TMAs can affect and organ and should be considered in patients presenting with thrombocytopenia and haemolysis. Although disseminated intravascular haemolysis can occur during the severe inflammatory response associated with pancreatitis, the normal clotting screen in this case makes it unlikely.

Case 2

A 58-year-woman presented to her local hospital with a 2-hour history of drowsiness and slurring of her speech. She was initially diagnosed as having had a CVA, but her admission blood demonstrated severe thrombocytopenia (platelets 9 x 10^9/ml) and evidence of haemolysis with anaemia (haemoglobin 97 g/l, LDH unmeasurable due to haemolysis, and fragmentation on the blood film). A diagnosis of TTP was made, and she was transferred to the closest hospital where plasma exchange was available. By the time she arrived, her level of consciousness had fallen, and she required ventilation to protect her airway. Blood for ADAMTS13 activity was taken and plasma exchange (1.5 x plasma volume initiated). She was given pulsed methylprednisolone. On day 3 she remained ventilator dependent. The frequency of plasma exchange was increased to twice daily, and she started a course of rituximab. After 7 days her platelet count started to improve; plasma exchange frequency was reduced to daily and finally stopped on day 18 after admission following normalisation of her platelet count and weaning from the ventilator. She was finally discharged from the hospital for further rehabilitation.

This case demonstrates how rapidly TTP can cause severe neurological disease, which can be fatal without treat-

ment, and highlights the importance of rapid initiation of plasma exchange. It also demonstrates that after the acute phase, patients can be left with residual neurocognitive impairment.

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Case 3

A 5-year-old boy developed abdominal pain and bloody diarrhoea 5 days after visiting a petting farm. The diarrhoea resolved, but 9 days after the onset of diarrhoea, he presented with pallor and reduced consciousness. He has a seizure in the emergency room and required ventilation. He was thrombocytopenic (platelets 43 x 10^9/l) and anaemic (haemoglobin 76 g/l) with evidence of haemolysis. He had acute kidney injury with a serum creatinine of 340 μmol/l and oligoanuria. He was started on peritoneal dialysis. STEC culture was negative but Shiga toxin PCR was positive. He remained dialysis dependent for 6 days and required blood transfusion on 2 occasions. Twelve days after admission, he was discharged from the hospital with no neurological deficit and improving renal function. This case is characteristic of STEC infection with a diarrhoeal illness preceding the onset of HUS. It emphasises the importance of STEC testing in all cases and potential for neurological involvement. The good outcome is normal after the acute phase, but these children require long-term monitoring because of the risk of CKD and hypertension.

Conclusion

A thrombotic microangiopathy can develop in a range of diseases and presents with a characteristic pattern of laboratory findings. The main diseases that cause a TMA, TTP and HUS, are rare diseases that can progress rapidly and without treatment are associated with a high morbidity and mortality. Therefore, early recognition that a TMA is present, and appropriate investigation to determine its cause is essential to make the diagnosis and to institute correct treatment, possibly with support from specialist centres.

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