Thrombotic microangiopathies and the kidney

Paul Warwicker

Abstract

Thrombotic microangiopathies are rare but carry a high morbidity and mortality. Recent research has helped to clarify their aetiology. A high index of suspicion is required in patients presenting with renal impairment and features of microangiopathy such as a low platelet count and Coomb's test-negative haemolytic anaemia. In Shiga toxin-mediated haemolytic–uraemic syndrome, bacteria such as *Escherichia coli* O157 and O104 adhere to the intestinal mucosa and secrete a highly potent Shiga cytotoxin that binds to the glomerular endothelium and causes an endotheliopathy. In atypical haemolytic–uraemic syndrome, the alternative pathway of complement, in the absence of an intact regulatory system, runs out of control. The endothelium is the predominant target, in a variety of organs including the kidneys, brain and heart. In thrombotic thrombocytopenic purpura, dysfunction of an enzyme (ADAMTS13) that cleaves and inactivates ultralarge multimers of von Willebrand factor leads to the formation of platelet-rich thrombi. Most thrombotic microangiopathies are treatable if identified early.

Keywords Atypical haemolytic–uraemic syndrome; complement system; MRCP; pneumococcal haemolytic–uraemic syndrome; Shiga toxin-mediated haemolytic–uraemic syndrome; thrombotic microangiopathy; thrombotic thrombocytopenic purpura; von Willebrand factor

Key points

- Renal thrombotic microangiopathies (TMAs) are rare, carry a high morbidity and mortality, but are treatable
- Diagnosis requires a high index of suspicion because TMAs present in widely diverse ways
- The mainstay of diagnosis rests on finding microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury
- Clinical presentation, serum creatinine concentration, platelet count, ADAMT13 activity and Shiga toxin assays help to differentiate the causes of renal TMAs

Introduction

Thrombotic microangiopathy (TMA) describes small vessel narrowing and occlusions caused by microthrombi. The pathological consequences and clinical features are:

- microangiopathic haemolytic anaemia (MAHA) – a non-immune red cell mechanical destruction occurring in small vessels
- consumptive thrombocytopenia (platelet-rich thrombi)
- occlusion of the blood vessels causing downstream ischaemic lesions, including acute kidney injury (AKI) and cerebral impairment.

The diseases that cause TMA are often rare (Table 1), and a high index of suspicion is vital in order to make the diagnosis. Laboratory features indicating a TMA are listed in Table 2.

Paul Warwicker *MD FRCP is Consultant Nephrologist at the Lancashire Teaching Hospitals NHS Trust, Preston, UK. Competing interests: Dr Warwicker has received speaker's fees and travel expenses from Alexion Pharmaceuticals (eculizumab).*

In the last 15 years, the aetiology of the main forms of TMA has been clarified; resulting in new and effective targeted treatments (Figure 1).

Shiga toxin-mediated haemolytic–uraemic syndrome (HUS)

TMA complicating infective diarrhoea caused by Shiga toxin-secreting bacteria used to be known as diarrhoeal-associated HUS but is now generally classified as Shiga toxin-mediated HUS (Stx HUS) or Shiga-like toxin-associated HUS (STEC HUS). Implicated bacteria include *Escherichia coli* serotypes O157 and O104 and *Shigella dysenteriae* type 1. This condition was first described in the 1980s, since when there has been a dramatic rise in the incidence of infections and outbreaks. It is now one of the most common forms of renal TMA.

Once ingested, the bacteria adhere to the intestinal mucosa and secrete a highly potent Shiga cytotoxin, a ribosomal inactivating protein, which binds with high affinity to receptors expressed on the glomerular endothelium. This causes an endotheliopathy characterized by activation of platelets, leucocytes, the coagulation cascade and the alternative complement pathway. The classical features on renal biopsy (which is in fact rarely indicated or safe in Stx HUS) are therefore predominantly glomerular microthrombi.

Clinical features include an acute diarrhoeal prodrome, often becoming bloody. Most individuals recover without major complications, but a proportion (5–15%) develop Stx HUS.

Investigations to confirm the diagnosis should include:

- stool culture for STEC O157 or a rectal swab if there is no stool
- serology for non-O157 STEC
- stool polymerase chain reaction (PCR) for *Stx1* and *Stx2* genes (in the UK, serology and PCR are performed at the Public Health England Reference Laboratory).

Management is predominantly supportive, including close monitoring of electrolytes, fluid status and renal replacement therapies. Drugs to reduce gut motility can paradoxically worsen the condition, and, unless the patient is septicaemic, antibiotics are also contraindicated.

Classification of TMAs affecting the kidneys

Infection associated
- Shiga toxin associated (*Escherichia coli*, *Shigella dysenteriae*) — **Stx HUS**
- *Streptococcus pneumoniae* sepsis — **pHUS**
- HIV

Complement dysregulation — aHUS
- Genetic
- Acquired

ADAMTS13 protease deficiency — TTP
- Genetic
- Acquired

Drug-related — including calcineurin inhibitors, oral contraceptive pill, VEGF-inhibitors, chemotherapy especially cisplatin, quinine, ticlopidine

Accelerated hypertension

Disseminated intravascular coagulation

Connective tissue disorders — including SLE and antiphospholipid syndrome

Pregnancy including pre-eclampsia and HELLP syndrome

Paraneoplastic

Transplantation

Inborn abnormalities of metabolism — including cobalamin C deficiency and mutations in DGKE

Pancreatitis

aHUS, atypical haemolytic—uraemic syndrome; pHUS, pneumococcal haemolytic—uraemic syndrome; SLE, systemic lupus erythematosus; Stx HUS, Shiga toxin-mediated haemolytic—uraemic syndrome; TTP, thrombotic thrombocytopenic purpura; VEGF, vascular endothelial growth factor; DGKE, diacylglycerol kinase E.

Source: Adapted from Barbour et al.,[1] and Besbas et al.[2].

Table 1

In 2011, an outbreak in Germany caused by bean sprouts contaminated with *E. coli* O104 led to >900 cases of STEC HUS and 54 deaths. In general, however, the vast majority of patients recover, although some are left with renal impairment. For those with established, irreversible renal failure, transplantation is usually safe with little chance of recurrence.

Laboratory features of a renal TMA

Full blood count
- Anaemia
- Thrombocytopenia

Blood film
- Red cell fragmentation ('schistocytosis' >1%)
- Polychromasia (reticulocytosis)
- Low, absent or giant platelets

Haemolysis screen
- Elevated lactate dehydrogenase
- Hyperbilirubinaemia (unconjugated)
- Reticulocytosis
- Low haptoglobulins
- Free haemoglobin in serum and urine

Renal impairment

Normal coagulation screen

(except disseminated intravascular coagulation)

Negative direct antiglobulin (Coombs') test

Adapted from Barbour et al.[1].

Table 2

Atypical haemolytic—uraemic syndrome

Atypical HUS (aHUS) is a much rarer, non-infective and more severe form of renal TMA. It has an incidence of 2 per million population, and a prevalence of 7 per million in children.[3] It is characterized by mortality rates as high as 25%, and results in established renal failure in >50% of patients.

aHUS is caused predominantly by dysregulation of the complement system (complement-associated gene mutations or pathological polymorphisms being found in 60—70% of patients). In the late 1990s, the first gene mutations were described in the gene for a serum-bound complement regulatory protein called complement factor H. Since then, several complement-related genes and acquired autoantibodies have been implicated (including factors I, B, C3, membrane cofactor protein (MCP) or CD46 and thrombomodulin), as well as, more recently, a gene for an endothelial kinase, *DGKE* (non-complement-related).

It is thought that a number of precipitating stimuli, including infections, trauma and pregnancy, stimulate the alternative complement pathway, which, in the absence of an intact regulatory system, runs out of control. The predominant target is the endothelium, in a variety of organs including the kidneys, brain and heart. In the kidneys, the smaller arterioles are mainly affected, causing the characteristic histological features of arteriolar intimal thickening and luminal narrowing. It is not a surprise that these patients, with multiple small renal arteriolar stenoses, frequently present with severe hypertension, presumably driven by intense activation of the renin—angiotensin system. Rarely, atypical HUS causes peripheral gangrene.

The diagnosis of aHUS is frequently missed or delayed. The condition should be suspected in patients presenting with AKI

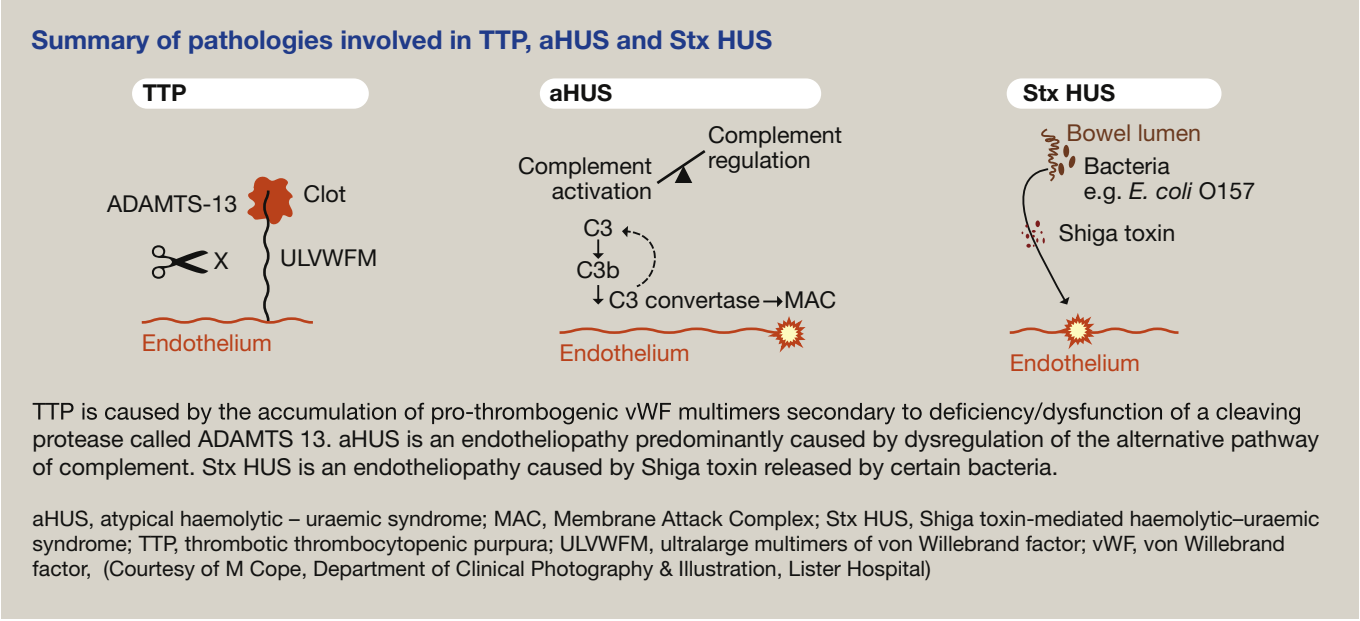

Figure 1

complicated by anaemia and thrombocytopenia, or AKI and unexplained severe hypertension, especially if there is evidence of microangiopathy including raised lactate dehydrogenase concentration and schistocytes (red cell fragments). The concentration of C3 complement can be reduced but is frequently normal. There is usually no time to wait for the results of genetic mutation analysis. Stool cultures and Shiga toxin assays should be considered to rule out STEC HUS, and ADAMTS13 (ADAM metallopeptidase with thrombospondin type 1 motif, 13) assays sent to rule out thrombotic thrombocytopenic purpura (TTP).

Initial management involves tight control of hypertension (frequently requiring several agents, including angiotensin-converting enzyme inhibitors) and supportive measures including dialysis. Plasma exchange with fresh frozen plasma removes dysfunctional complement factors and is a surrogate form of delivery of functional complement regulators. Plasma exchanges/infusions have been reported to reduce the mortality of aHUS from around 50%–25%.[1]

Eculizumab, a humanized monoclonal antibody that inhibits C5 activation, has been licensed as first-line therapy for aHUS in Europe and the USA. It induces a fast, effective and sustained remission in >80% of patients.

Renal transplantation in aHUS is associated with a 60% chance of recurrence, with subsequent graft loss occurring in >90% of patients. Genetic analysis allows identification of rarer subtypes carrying a lower risk of recurrence, such as mutations in the membrane-bound MCP gene. Combined liver–kidney transplantation has been tried with varying success (the liver is a source of wild-type functional complement proteins). Eculizumab-supported transplantation appears promising. Living related donation is best avoided in light of a series of cases where the donor has gone on to develop aHUS because of an unrecognized genetic predisposition.

A useful site with both patient and clinician advice is at http://www.atypicalhus.co.uk.

Thrombotic thrombocytopenic purpura

TTP is classically defined by the pentad of thrombocytopenia, MAHA, renal impairment, fever and neurological signs. However, all five features are not always present in the early stages, and the diagnosis should be considered in patients with thrombocytopenia and MAHA alone. It is also rare (incidence of 6 per million) and, if undiagnosed and untreated, carries a high mortality (15–20%).[1]

Early scientific literature frequently confused TTP with aHUS, often considering the diseases as a single entity. Research in the late 1990s, however, defined a distinct aetiology for TTP, establishing that the condition resulted from dysfunction of an enzyme (ADAMTS13) that cleaves and inactivates ultralarge multimers of von Willebrand factor. von Willebrand factor is a glycoprotein involved in platelet aggregation and thrombus formation, and the ultralarge multimers of von Willebrand factor are particularly thrombogenic.

Dysfunction of ADAMTS13 is occasionally inherited/familial (approximately 5% of cases) but is usually acquired and related to an inhibitory antibody (immunoglobulin). Treatment includes prompt plasma exchange (using solvent/detergent-treated plasma) to remove the offending antibody and replace the dysfunctional ADAMTS13 (fresh ADAMTS13 is found in the cryosupernatant fraction of plasma). Therapies to reduce the production of autoantibody, including high-dose corticosteroids and rituximab (anti-CD20), are also used. Newer therapies such as recombinant ADAMTS13 and nanobodies that target the interaction between von Willebrand factor and platelets, are currently being assessed.

When the platelet count has recovered to 50×10^9/litre, low-molecular-weight heparin should be considered as thromboprophylaxis.

In cases where it is clinically difficult to distinguish TTP from HUS, and while awaiting the results of ADAMTS13 studies, a simple analysis of platelet count and serum creatinine is remarkably effective in differentiating the two conditions. aHUS

usually presents with more significant renal impairment and less pronounced thrombocytopenia. A platelet count >30 × 10^9/litre and/or a serum creatinine >200 micromol/litre makes a diagnosis of classical TTP unlikely.[4,5]

Other thrombotic microangiopathies

A number of other TMAs can affect the kidneys (Table 1). HIV-associated TMA was common in the AIDS era, but with the advent of highly active antiretroviral therapy it is thankfully now rarer. TMA associated with pneumococcal infection (pHUS) is caused by unmasking of the T blood group (Thomsen—Friedenreich) antigen through the action of the pneumococcal neuraminidase; this leaves cell surfaces subject to the binding of naturally occurring anti-T antibodies, with subsequent complement-mediated injury. Administration of whole blood or plasma/plasma exchange (containing anti-T antibodies) can worsen the haemolysis and is contraindicated in this specific form of HUS. ◆

KEY REFERENCES

1 Barbour T, Johnson S, Cohney S, Hughes P. Thrombotic microangiopathy and associated renal disorders. *Nephrol Dial Transplant* 2012; **27:** 2673—85.

2 Besbas N, Karpman D, Landau D, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. *Kidney Int* 2006; **70:** 423—31.

3 Taylor CM, Machin S, Wigmore SJ, et al. Working party from the Renal Association, the British Committee for Standards in Haematology and the British Transplantation Society. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. *Br J Haematol* 2010; **148:** 37—47.

4 Coppo P, Schwarzinger M, Buffet M, et al. Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. *PLoS One* 2010; **5**: e10208.

5 Zuber J, Fakhouri F, Roumenina LT, et al. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8:** 643—57.

TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 30-year-old man presented with headaches and blurred vision. He had no previous medical history. His father had died prematurely at the age of 35, but the patient did not know the cause. On clinical examination, his blood pressure was 240/120 mmHg. The fundi showed hypertensive changes.

Investigations

- Haemoglobin 98 g/litre (130—180)
- Platelets 106 × 10^9/litre (150—400)
- Blood film showed red cell fragments
- Creatinine 550 micromol/litre (60—110)
- Serum complement C3 74 mg/dl (65—190)
- Serum complement C4 55 mg/dl (15—50)
- Renal ultrasonography showed normal-sized kidneys

What is the most likely underlying diagnosis?

A. Thrombotic thrombocytopenic purpura
B. Shiga toxin-mediated haemolytic—uraemic syndrome
C. Chronic pyelonephritis
D. Atypical haemolytic—uraemic syndrome
E. Atherosclerotic renal artery stenosis

Question 2

A 40-year-old man was found unconscious.
On clinical examination, his temperature was 39°C, and blood pressure 150/80 mmHg.

Investigations

- Haemoglobin 86 g/litre (130—180)
- Platelets 4 × 10^9/litre (150—400)
- Blood film showed red cell fragments
- C-reactive protein 5 mg/litre (<10)
- Creatinine 146 micromol/litre (60—110)
- International normalized ratio 1.2 (<1.4)
- CT scan of the brain was normal

What is the next appropriate treatment?

A. Platelet infusion and then a lumbar puncture
B. Plasma exchange with fresh frozen plasma replacement
C. Eculizumab infusion
D. Haemodialysis
E. Aggressive treatment of hypertension

Question 3

An 80-year-old man presented with vomiting, fever and persistent diarrhoea after a meal out.
On clinical examination, he appeared dehydrated, with a blood pressure of 90/68 mmHg. He had not passed urine

Investigations

- Haemoglobin 112 g/litre (130–180)
- Platelets 86 $\times$ 10^9/litre (150–400)
- Blood film showed red cell fragments
- C-reactive protein 89 mg/litre (<10)
- Potassium 7.2 mmol/litre (3.5–4.9)
- Bicarbonate 12 mmol/litre (20–28)
- Urea 54 mmol/litre (2.5–7.0)
- Creatinine 800 micromol/litre (60–110),

Which of the following treatments should be considered first:

A. Broad-spectrum antibiotics
B. Regular loperamide to treat the diarrhoea
C. Intravenous calcium gluconate, fluid challenge and, if required, haemodialysis
D. Eculizumab infusion
E. Plasma exchange