Review Article

Consensus opinion on diagnosis and management of thrombotic microangiopathy in Australia and New Zealand

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SUMMARY AT A GLANCE

The management of thrombotic microangiopathy (TMA) is challenging; this consensus approach from Australia and New Zealand provides a practical strategy for diagnosing and treating patients with TMA.

ABSTRACT:

Thrombotic microangiopathy (TMA) arises in a variety of clinical circumstances with the potential to cause significant dysfunction of the kidneys, brain, gastrointestinal tract and heart. TMA should be considered in all patients with thrombocytopenia and anaemia, with an immediate request to the haematology laboratory to look for red cell fragments on a blood film. While TMA of any aetiology generally demands prompt treatment, this is especially so in thrombotic thrombocytopenic purpura (TTP) and atypical haemolytic uraemic syndrome (aHUS), where organ failure may be precipitous, irreversible and fatal. In all adults, urgent, empirical plasma exchange (PE) should be started within 4–8 h of presentation for a possible diagnosis of TTP, pending a result for ADAMTS13 activity (a disintegrin and metalloprotease thrombospondin, number 13). A sodium citrate plasma sample should be collected for ADAMTS13 testing prior to any plasma therapy. In children, Shiga toxin-associated haemolytic uraemic syndrome due to infection with *Escherichia coli* (STEC-HUS) is the commonest cause of TMA, and is managed supportively. If TTP and STEC-HUS have been excluded, a diagnosis of aHUS should be considered, for which treatment is with the monoclonal complement C5 inhibitor, eculizumab. While early confirmation of aHUS is often not possible, except in the minority of patients in whom autoantibodies against factor H are identified, genetic testing ultimately reveals a complement-related mutation in a significant proportion of aHUS cases. The presence of other TMA-associated conditions (e.g. infection, pregnancy/postpartum and malignant hypertension) does not exclude TTP or aHUS as the underlying cause of TMA.

Thrombotic microangiopathy (TMA) describes a pathological process in which platelet aggregation and thrombus formation in small blood vessels cause luminal narrowing or occlusion, producing end-organ ischaemia and infarction. TMA results from endothelial injury in the microcirculation, with activation of the complement and/or coagulation systems. One or more organs may be affected, most commonly the kidneys, brain, heart, and gastrointestinal tract. TMA is recognized by: (i) thrombocytopenia; (ii) microangiopathic haemolytic anaemia (MAHA) with red cell fragments (schistocytes); and (iii) the clinical and laboratory abnormalities attributable to organ-specific dysfunction.

For decades, the approach to TMA focussed on two poorly understood syndromes of TMA, distinguished on the basis of gross clinical and laboratory features: thrombotic thrombocytopenic purpura (TTP)[1] and haemolytic uraemic syndrome

(HUS).[2] Advances in genetics and molecular biology have enabled classification of TMA according to distinct causes, in turn directing the clinician to specific treatments. Essential diagnostic tools include: DNA amplification from stool or rectal swab of Shiga toxin produced by *Escherichia coli* (STEC) in STEC-HUS; serological assessment for severely reduced ADAMTS13 (a disintegrin and metalloprotease thrombospondin, number 13) activity in TTP; and mutational analysis of complement-related genes in atypical HUS (aHUS). As numerous other conditions can be associated with TMA, including infection, malignancy, drug exposure, transplantation, malignant hypertension, pregnancy/postpartum and disseminated intravascular coagulation (DIC), these are included within the broad 'differential diagnosis' of TMA. These TMA-associated conditions sometimes arise in patients with a previously unrecognized genetic or acquired predisposition to TMA. Thus, initial management of TMA may need to address more than one potential aetiological factor, and TTP and aHUS in particular should not be discounted simply because another condition (e.g. malignant hypertension) is present. Diagnostic and treatment dilemmas frequently arise, exemplified in pregnancy/postpartum, where potential causes of TMA include HELLP (haemolysis, elevated liver enzymes, and low platelets) syndrome, severe postpartum haemorrhage, autoimmune diseases, aHUS and TTP.[3,4]

Patients with TMA may present with a variety of nonspecific symptoms to general practitioners and the emergency department, or on referral to haematologists and renal physicians. More extreme cases may be admitted to intensive care with multi-organ dysfunction from an undiagnosed but life-threatening illness. The potential for rapid progression to end-stage organ failure or death necessitates urgent therapy, undertaken concurrently with investigations for the underlying cause(s) of TMA.[5] Published data from the Australian TMA registry[6] reveal a heterogeneous approach to management of TMA, highlighting potential knowledge gaps and the need for a guidance document relevant to a broad medical audience.

Table 1 Laboratory features of TMA[†]

Platelets <150 × 10⁹/L or >25% fall from baseline
Haemoglobin <100 g/L
Red cell fragments (schistocytes) on a peripheral blood film
Reticulocyte count elevated
Lactate dehydrogenase elevated
Haptoglobin reduced
Bilirubin elevated
Direct antiglobulin (Coombs') test negative (with a few exceptions[‡])
Coagulation profile (APTT, INR, fibrinogen) normal (except in DIC, lupus anticoagulant, therapeutic anticoagulation)

[†]Not all features may be present, especially early in a TMA episode. [‡]Direct antiglobulin test is sometimes positive in pneumococcal HUS, autoimmune diseases, or with prior blood product administration.

METHODS

In 2014, a TMA working group (the authors of this document) was formed to provide up to date information for Australian and New Zealander clinicians without specialist knowledge in the field. Following a review of both published and unpublished evidence including several international guidelines and findings from the Australian TMA registry,[6] a 61-point questionnaire was distributed. Responses from the members of the working group formed the basis for this consensus document (using the modified Delphi method).

RECOGNITION OF TMA

Unexplained thrombocytopenia (platelets <150 × 10⁹/L or >25% fall from baseline) and MAHA (haemoglobin <100 g/L with red cell fragments on a peripheral blood film) are considered sufficient for TMA. As fragments are sometimes rare or absent, early TMA should still be suspected with an elevated lactate dehydrogenase (LDH) or other markers of intravascular haemolysis (Table 1), although non-microangiopathic causes of anaemia must also be considered. The clotting profile is normal in TMA except in disseminated intravascular coagulation (DIC). Evidence of end-organ dysfunction should be sought (Table 2). Historically, TTP was characterized by the 'pentad' of fever, MAHA, thrombocytopenia, neurological sequelae and renal impairment,[7] but the pentad is not required for diagnosis and is now relatively uncommon.[8] Biopsy of affected organs is generally unnecessary, but may indicate chronicity (Table 3).

CAUSES OF TMA

STEC-HUS

Shiga toxin secreted by *Escherichia coli* or other bacteria is the commonest cause of childhood TMA,[9,10] typically with a diarrhoeal prodrome and renal failure, and sometimes neurologic impairment.[11] Although bloody diarrhoea is common, this does not reliably distinguish STEC-HUS from other causes of TMA, notably aHUS.[12,13] Shiga toxin in contaminated foods traverses the gut lumen, entering the circulation and causing direct endothelial injury, primarily within the glomerular and cerebral microvasculature. In 1995, contaminated meat products led to an outbreak in South Australia, and in 2011 an epidemic in Germany was traced to contaminated sprouts, resulting in 34 deaths (with adverse outcomes especially common in adults[14]).

Pneumococcal HUS

Haemolytic uraemic syndrome due to systemic *Streptococcus pneumoniae* infection mainly affects children.[15] Release of

Table 2 TMA-induced organ dysfunction

Cardiac
Acute myocardial infarction, cardiomyopathy (heart failure)
Neurological
Headache, visual disturbance, hyperreflexia, encephalopathy, syncope, focal deficit, seizure, coma
Gastrointestinal
(Bloody) diarrhoea, vomiting, abdominal pain, melaena, pancreatitis, cholecystitis
Renal
Oliguria, acute kidney injury, hypertension, haematuria, proteinuria
Skin
Purpura, petechiae, digital ischaemia
Lungs
Dyspnoea, pulmonary embolism
Adrenal
Hypotension
Pregnancy complications
Placental insufficiency, fetal distress

bacterial neuraminidase A is thought to expose the Thomsen-Friedenreich antigen on host red cells and glomerular endothelium, to which specific IgM autoantibodies bind, causing agglutination and TMA.

TTP

Although rare, TTP is an important cause of relapsing TMA, with the capacity to progress rapidly (acute mortality of 10% despite optimal care).[16] Involvement of the central nervous system is typical, often with non-specific symptoms (e.g. delirium, seizures). It has been suggested that more severe thrombocytopenia (platelet count $<30 \times 10^9$/L) and milder renal impairment (serum creatinine <200 μmol/L) may help to distinguish TTP from aHUS[17,18] but these criteria are neither sensitive nor specific.[19,20] Rather, TTP is defined by severely reduced (<10%) activity of the von

Table 3 Renal biopsy features of TMA[†]

1. Light microscopy
Acute
Thrombi and fragmented red blood cells in glomerular capillaries, arterioles and small arteries
Endothelial cell swelling
Ischaemic wrinkling of the glomerular basement membrane, collapsed glomerular tuft
Mesangiolysis
Chronic
Double contours of the glomerular basement membrane
Arteriolar or small arterial 'onion skin' appearance of intimal fibrosis
2. Immunohistochemistry
Sparse, nonspecific glomerular complement C3 and immunoglobulin M
Fibrin staining of thrombi
3. Electron microscopy
Endothelial swelling and subendothelial electron-lucent material (acute)
Duplication of glomerular basement membrane (chronic)

[†]due to any cause (not specific for aHUS).

Willebrand factor (vWF)-cleaving protease, ADAMTS13. Ultra-large vWF multimers are normally released from endothelial cells, and then cleaved by ADAMTS13 into smaller fragments which are important in primary haemostasis. In TTP, severely reduced ADAMTS13 activity allows uncleaved, ultra-large vWF multimers to accumulate in the microcirculation, where they bind and activate platelets causing TMA. TTP may be subdivided according to whether reduced enzyme activity is acquired or congenital:

1. Acquired TTP results from the production of anti-ADAMTS13 autoantibodies,[21,22] and affects predominantly young women, although it can present at any age.[6] Relapse rates have been estimated at 20–50%.[16]
2. Congenital TTP (Upshaw-Schulman syndrome) accounts for <5% of TTP cases and is due to *ADAMTS13* mutations. Onset occurs at any age,[16,23] with episodes often triggered by pregnancy/postpartum or infection.[16,24] Renal failure is occasionally prominent (presenting similarly to aHUS).

aHUS

Atypical haemolytic uraemic syndrome is a rare, relapsing disease in which TMA causes prominent renal failure,[9,25] and sometimes potentially fatal cardiac and neurological complications. aHUS arises primarily from uncontrolled activation of the alternative pathway (AP) of complement,[5,26,27] culminating in complement C5 activation and formation of the membrane attack complex (MAC) on microvascular endothelial cells. Genetic and/or acquired abnormalities that impair AP regulators including Factor H (FH), or otherwise enhance AP activation, are identified in 40–60% of aHUS patients.[28–30] Onset of TMA often follows an environmental factor that further disturbs the balance between stimulation and inhibition of the complement system. Indeed, international registries report TMA-precipitating conditions such as infection, drug exposure or pregnancy (mainly postpartum) in 70% of aHUS patients.[28] While aHUS has often been considered a paediatric diagnosis, onset is nearly as common in adulthood,[29,31] and the diagnosis should not be discounted even in older patients with a first episode of TMA. Although a family history increases the likelihood of aHUS as the cause of TMA, sporadic cases are more common than familial ones.

Conditions associated with TMA

Thrombotic microangiopathy occurs in a wide variety of clinical settings including pregnancy/postpartum, infection and following exposure to certain drugs[32,33] (Tables 4,5). Any of these may independently induce endothelial injury, platelet aggregation, or activation of the complement and/or coagulation systems. The term 'secondary' TMA has been used in such cases, to distinguish them from the 'primary'

Table 4 Causes of TMA

1. STEC-HUS – Shiga toxin from *E. coli* (STEC) and other bacteria
2. Pneumococcal HUS (especially in children)
3. TTP – acquired or congenital ADAMTS13 deficiency
4. aHUS – genetic or acquired complement abnormalities
5. TMA-associated conditions
- infection - viral (HIV, HCV, CMV, EBV, HSV, influenza A H1N1, parvovirus), bacterial or fungal
- pregnancy and postpartum
- malignant hypertension
- autoimmune – systemic lupus erythematosus, scleroderma, antiphospholipid syndrome (APS) including catastrophic APS (CAPS)
- DIC
- transplantation (solid organ and haematopoietic stem cell)
- drugs (see Table 5)
- malignancy (solid organ and haematopoietic)
- pancreatitis
- metabolic disorders (mainly in infants) – cobalamin C defect, methionine synthase deficiency, coenzyme Q deficiency
- glomerular disorders (IgA nephropathy, ANCA-associated vasculitis, C3 glomerulopathy)

TMA disorders of TTP and aHUS. However, separating secondary TMA from a primary disorder can be difficult or even impossible, as one or more TMA-associated conditions/drugs may be present in patients with TTP or aHUS (including those with complement-related mutations[28,34]). It is therefore important in all patients presenting with TMA to consider both precipitants for the current episode (e.g. infection, drug toxicity) and the possibility of an underlying diagnosis of TTP or aHUS.

INVESTIGATION OF TMA

Appropriate investigations to determine the cause(s) and contributors to TMA are listed in Table 6, including measurement of ADAMTS13 activity, which is critical for determining the cause of TMA and directing appropriate treatment. A practical suggestion is to collect a 5 mL plasma citrate sample in all patients presenting with TMA (for either immediate ADAMTS13 testing or storage). The sample must

Table 5 Examples of drugs associated with TMA

Drug class	Examples
Antiplatelet	Ticlopidine
Antibacterial	Quinine, penicillin
Antiviral	Valacyclovir
Interferon	
Antitumour	Gemcitabine, sunitinib, bortezomib, carfilzomib
Calcineurin inhibitors	Cyclosporin
Mammalian target of rapamycin (mTOR) inhibitors	Sirolimus
VEGF inhibitors	Bevacizumab
Oral contraceptives	
Illicit	Cocaine, intravenous oxycodone

Table 6 Investigations for cause of TMA

Stool or rectal swab PCR for Shiga toxins 1 and 2
ADAMTS13 activity
- if ADAMTS13 activity <10%, then also test for presence of ADAMTS13 autoantibodies
- If ADAMTS13 activity level <10% and ADAMTS13 autoantibodies absent, test for *ADAMTS13* gene mutation
FH autoantibodies in suspected aHUS (especially children)
Complement protein assays in suspected aHUS e.g. plasma C3, C4, sC5b-9, AP WIESLAB®, FH levels
Mutational analysis in suspected aHUS (*CFH, C3, CFB, CFI, CD46, CFHR1–5, THBD, DGKE*)
Fundoscopy (for malignant hypertension)
Blood cultures, urinary pneumococcal antigen (especially in children)
Viral serologies including HIV
Nasopharyngeal swab for influenza A H1N1
Coagulation screen (APTT, INR, fibrinogen), D-dimer
Plasma B12 and homocysteine levels, plasma and urine methylmalonic acid levels, mutational analysis of *MMACHC* and *MTR* genes
β-hCG (pregnancy)
Autoimmune serologies (ANA, dsDNA, ENAs, antiphospholipid)

Abbreviations: CFH, CFI, CFB, complement factors H, I, B; CFHR, complement factor H-related protein; DGKE, diacylglycerol kinase epsilon; MMACHC, methylmalonic aciduria (cobalamin C deficiency) with homocystei-nuria; MTR, methionine synthase (deficiency); THBD, thrombomodulin.

be obtained prior to commencement of any plasma therapy, which can produce false negative results.[16]

DIAGNOSIS OF TTP

ADAMTS13 activity <10% is considered diagnostic of TTP.[6,35] Several Australian laboratories measure ADAMTS13 activity by ELISA, and can be contacted regarding urgent requests (Table 7). ADAMTS13 activity below 10% with detection of ADAMTS13 autoantibodies indicates acquired TTP, with the autoantibody titre being potentially useful in assessing response to treatment and prognosis.[36,37] By contrast, negative ADAMTS13 autoantibody testing in a patient with ADAMTS13 activity below 10% suggests congenital TTP, which unlike acquired TTP is treatable with plasma infusion alone (to replace deficient enzyme). Infrequent testing of ADAMTS13 autoantibodies in Australia (16% of patients) has been noted as an area for improved clinical practice.[6]

DIAGNOSIS OF AHUS

aHUS as a diagnosis of exclusion

The initial diagnosis of aHUS[9,25,38–40] (or 'complement-mediated TMA'[32]) is currently based on exclusion of STEC-HUS and TTP, hence requiring confirmation of ADAMTS13 activity ≥10% (Fig. 1). Exclusion of STEC-HUS and TTP may in fact be sufficient for a diagnosis of aHUS,[38] although it has been argued that either of aHUS or secondary TMA remains possible.[25] As noted, differentiating aHUS and secondary TMA can be challenging, with numerous reports of

Table 7 Australian pathology services that offer specialized tests in TMA

ADAMTS13 activity testing
Monash Medical Centre, Clayton, Victoria (ph. 03 9594 3490)
NSW Health Pathology - Hunter, Lambton Heights, NSW (ph. 02 4921 4415)
South Eastern Area Laboratory Services, Prince of Wales Hospital, NSW (ph. 02 9382 9003)
Royal Brisbane and Women's Hospital, Herston, Queensland (ph. 07 3636 8074)
Fiona Stanley Hospital, Murdoch, WA (ph. 08 6152 2684)
ADAMTS13 autoantibody testing
Monash Medical Centre, Clayton, Victoria (ph. 03 9594 3490)
NSW Health Pathology - Hunter, Lambton Heights, NSW (ph. 02 4921 4415)
FH autoantibody testing
The Royal Children's Hospital, Parkville, Victoria (ph. 03 9345 5725)
NSW Health Pathology - Hunter, Lambton Heights, NSW (ph. 02 4921 4018)
aHUS mutational analysis and *ADAMTS13* gene testing
Children's Hospital, Westmead, NSW (ph. 02 9845 3244)
NSW Health Pathology - Hunter, Lambton Heights, NSW (ph. 02 4921 4312)

patients with recognized TMA-associated conditions who are subsequently found to have mutations in complement-related genes (consistent with a diagnosis of aHUS).

FH autoantibodies

In some patients with aHUS, autoantibodies are detected targeting FH (typically in conjunction with genetic abnormalities in FH-related proteins 1 and 3). FH autoantibody-associated aHUS presents most commonly in children, but can occur in adults.[41] An ELISA for FH autoantibodies is available (Table 7) and may also be useful in monitoring response to therapy.

Role of genetic testing in aHUS

Confirmation of genetically determined aHUS is neither possible nor necessary for initial treatment. However, genetic testing may ultimately confirm a diagnosis of aHUS and guide prognosis, including in some patients previously thought to have secondary TMA (e.g. due to malignant hypertension). Clearly, genetic testing is not required in all cases, and can be considered depending on the clinical circumstances. These may include specific treatment decisions regarding duration of eculizumab therapy or planning for renal transplantation in those reaching end-stage kidney disease. Several laboratories offer genetic testing in Australia (Table 7), but interpretation of results is complex, with an ever-increasing number of gene variants identified, some of which are not pathogenic.[42] New genetic associations continue to be reported in familial aHUS, including mutations affecting coagulation-related proteins (e.g. diacylglycerol kinase epsilon, DGKε[43]) and other metabolic pathways ostensibly unrelated to complement (e.g. inverted formin 2, INF2[44]). Yet even with comprehensive testing, a mutation is currently not found in a significant number of patients deemed to have aHUS.[45]

DIAGNOSIS OF STEC-HUS

In patients presenting with TMA and diarrhoea, a stool sample or rectal swab should be obtained for PCR-based detection of Shiga toxins 1 and 2. A negative test should prompt retesting if clinical suspicion is high. STEC-HUS is a notifiable disease.
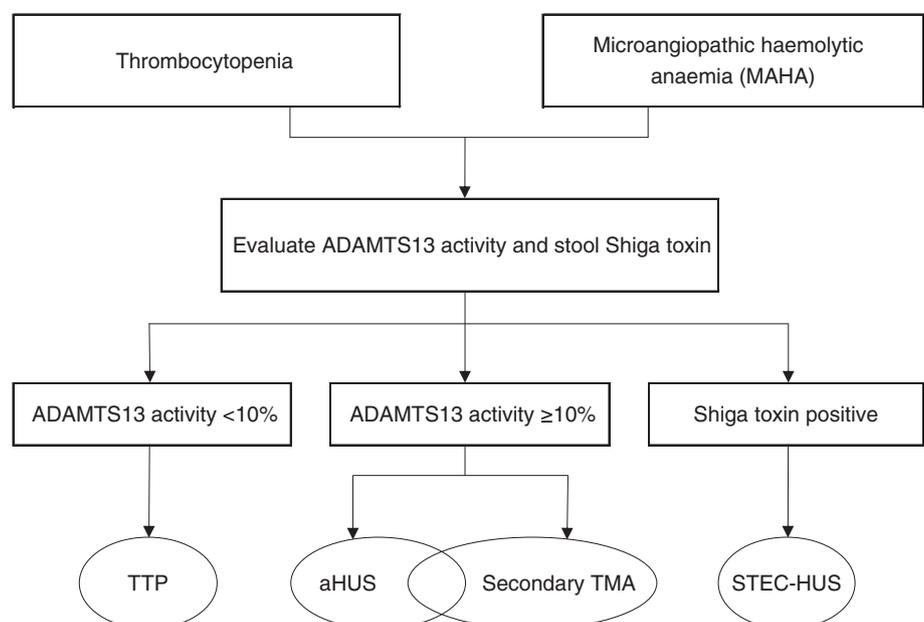

Fig. 1 Diagnostic algorithm for thrombotic microangiopathy (TMA) including thrombotic thrombocytopenic purpura (TTP) and atypical haemolytic uraemic syndrome (aHUS).

INITIAL TREATMENT OF TMA

Thrombotic microangiopathy is a medical emergency with the potential for rapid and possibly fatal decline in some patients (e.g. TTP, aHUS, sepsis). Early treatment is often empiric (Fig. 2), undertaken concurrently with diagnostic testing. Five key steps in initial treatment of TMA are summarized below, with evidence presented in the sections that follow.

Urgent plasma exchange for TTP

In patients with acquired TTP, plasma exchange (PE) is the quickest and most effective means of removing pathogenic ADAMTS13 autoantibodies. As delays in commencement of PE are associated with increased mortality, all adults presenting with TMA should receive PE urgently as empiric therapy, until TTP has been excluded (based on ADAMTS13 activity ≥10%). Ideally, PE should be commenced within 4–8 h of presentation, and this may necessitate urgent transfer to a centre offering PE. Plasma infusion without plasmapheresis is indicated only for patients known to have the congenital form of TTP, or where PE is delayed more than a few hours (provided plasma infusion does not further delay transfer for PE). In patients receiving empirical PE in whom demonstration of ADAMTS13 activity ≥10% (on a pre-treatment sample) leads

to a revised diagnosis of aHUS, eculizumab should replace PE (even if a haematological response to PE has been observed).

Eculizumab for aHUS

In patients diagnosed with aHUS, eculizumab should be commenced as soon as possible. Under the Australian Pharmaceutical Benefits Scheme (PBS) Section 100, approval for eculizumab requires tests for TTP (ADAMTS13 activity) and, in patients with diarrhoea, STEC-HUS (Shiga toxin) to have been sent, although initial doses can be approved before the results become available (https://www.humanservices.gov.au/health-professionals/enablers/atypical-haemolytic-uraemic-syndrome). There may be specific circumstances in which eculizumab is considered preferable to PE whilst, the results of ADAMTS13 activity testing are awaited. These could include patients with relapsing aHUS in whom TTP has previously been excluded, those with a family history of aHUS, women with post-partum onset of TMA and significant renal impairment, and children with TMA and significant renal impairment in whom STEC-HUS and pneumococcal HUS have been excluded. However, as noted above, any delay in commencing PE in adults in whom TTP has not been excluded carries significant risk. Eculizumab is not publicly funded in New Zealand. In children with FH
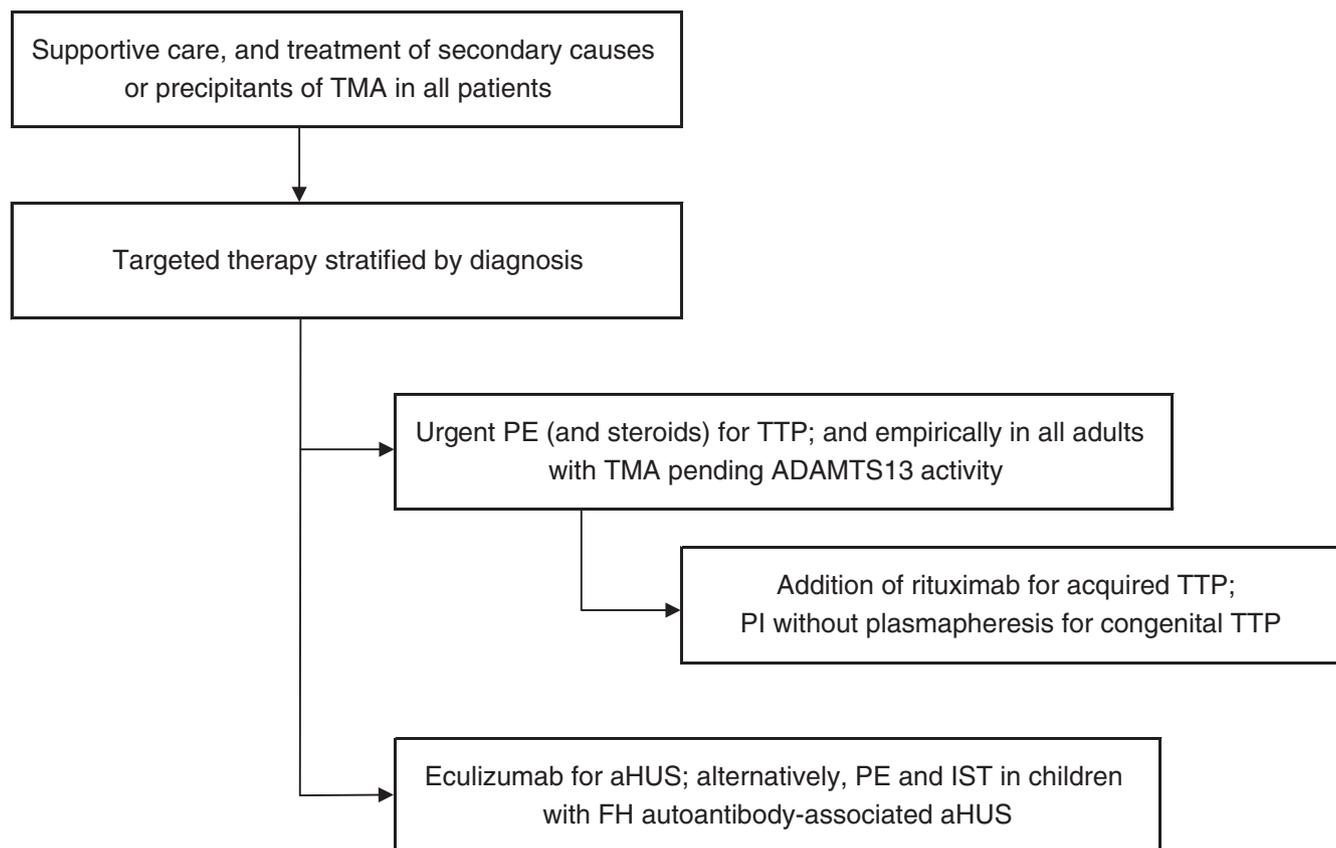
Supportive care, and treatment of secondary causes or precipitants of TMA in all patients

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Targeted therapy stratified by diagnosis

→ Urgent PE (and steroids) for TTP; and empirically in all adults with TMA pending ADAMTS13 activity

→ Addition of rituximab for acquired TTP; PI without plasmapheresis for congenital TTP

→ Eculizumab for aHUS; alternatively, PE and IST in children with FH autoantibody-associated aHUS

Fig. 2 Treatment algorithm for thrombotic microangiopathy (TMA) including thrombotic thrombocytopenic purpura (TTP) and atypical haemolytic uraemic syndrome (aHUS).

autoantibody-associated aHUS, PE and immunosuppressive therapy (IST) may be considered as an alternative to eculizumab.

Steroids and rituximab for acquired TTP

High dose steroids should be used in conjunction with PE for acquired TTP, and in all patients treated empirically with PE pending the results of ADAMTS13 testing (e.g. oral prednisolone 1 mg/kg per day or, for the most severe cases, pulse IV methylprednisolone 1 g daily for the first 3 days). Rituximab (off-label) may be appropriate once acquired TTP has been confirmed, especially in severe or relapsing cases. Steroids and rituximab are not beneficial in congenital TTP.

Management of any potential secondary causes or precipitants of TMA

Steps to modify secondary causes or precipitating factors should be undertaken in all patients with TMA. In certain situations, these may be sufficient for resolution of TMA, for example, antibiotics for pneumococcal HUS, antihypertensive therapy in malignant hypertension or scleroderma renal crisis, cessation of specific drugs (IV oxycodone, VEGF inhibitors or gemcitabine), fetal and placental delivery in HELLP, and B12 replacement in cobalamin C defect or methionine synthase deficiency. However, in adults in whom TTP has not been excluded, PE should not be postponed in the hope for clinical improvement. In patients diagnosed with aHUS, eculizumab should also not be delayed, and in those patients in whom a diagnosis of secondary TMA was initially preferred, evidence of persisting TMA despite modification of secondary causes should again prompt consideration of aHUS and treatment with eculizumab. In some contexts (e.g. active malignancy), use of PE or eculizumab is futile.

Supportive therapy alone for STEC-HUS

Retrospective studies have not shown a benefit to eculizumab, PE or steroids in STEC-HUS,[46,47] for which supportive therapy alone is recommended (e.g. dialysis and other treatment for organ failure). Nevertheless, some authors do favour PE or eculizumab (off-label) for severe extra-renal manifestations of STEC-HUS including seizures, gastrointestinal haemorrhage or cardiac complications. Prospective trials of eculizumab (NCT02205541) and azithromycin (NCT02205541) are underway in children with STEC-HUS.

EVIDENCE FOR TREATMENT OF AHUS

Eculizumab

In the absence of randomized, controlled trials (RCTs), four prospective, open-label, uncontrolled trials in over 100 patients provide evidence in support of eculizumab as first-line therapy in aHUS.[25,40] The initial two trials enrolled adolescents and adults with persistent aHUS unresponsive to plasma therapy (trial 1) or longstanding aHUS with chronic kidney disease (trial 2).[48] Clinical benefit (including marked improvement in renal function) was maintained on therapy at 2 years.[49] Eculizumab was effective in patients with and without complement-related mutations or FH autoantibodies (as in both later trials in children[50] and adults[51]). Response was sometimes dramatic, including attainment of dialysis independence,[49] as observed in a retrospective Australian cohort[52] and subsequent reports in which dialysis had preceded eculizumab therapy for up to several months.[39,53,54]

Risks associated with eculizumab

Eculizumab is well-tolerated but the associated systemic complement inhibition increases susceptibility to *Neisseria meningitidis*. Even with mandatory prior vaccination in Australia against serotypes A, C, W and Y and long-term antibiotic prophylaxis (a consideration in all patients for the duration of eculizumab therapy and for 2–3 months after cessation[27]), there is a risk of meningococcal sepsis or meningitis.[55] Safe use in pregnancy has been described in aHUS[34] and paroxysmal nocturnal haemoglobinuria (PNH).[56]

Duration of eculizumab

In patients with aHUS responsive to eculizumab, drug discontinuation or increased dosing interval is associated with a risk of relapse.[57] However, lifelong therapy and the associated burden of ongoing hospital visits, risk of meningococcal infection, and cost make drug cessation desirable where possible/appropriate. Limited data are available to guide drug discontinuation, but it appears a history of recurrent disease or the presence of a complement-related mutation increases the likelihood of relapse.[39,57,58] Monitoring of drug levels and complement biomarkers may ultimately facilitate extended dosing intervals in some patients. One group reported success using patient-performed dipstick testing for haemoglobinuria as a means to timely detection of relapse and reinstitution of eculizumab.[58,59] In FH autoantibody-associated aHUS, adjustment of eculizumab dose based on the autoantibody titre has also been reported.[57]

Failure of eculizumab

Occasional therapeutic failure has been observed with eculizumab. Some patients may require higher doses,[39,60] whereas others are unresponsive because of TMA due to STEC-HUS, cobalamin C defect,[61] methionine synthase deficiency,[62] or *DGKE*[43] or *INF2*[44] mutations. Drug resistance has been reported in Japanese patients with PNH resulting from a polymorphism in complement C5 that impairs eculizumab binding.[63]

Plasma therapy in aHUS

Use of PE/plasma infusion in aHUS can be considered as second-line therapy where eculizumab is unavailable or proves ineffective. Retrospective aHUS case series (prior to the availability of eculizumab) reported improved haematological parameters with PE, but long-term outcomes were often poor.[28,29] Thus, we do not support a recent US guideline recommending PE for 'complement-mediated TMA', with substitution of eculizumab only in patients 'refractory to or dependent on' PE.[32]

Treatment of FH autoantibody-associated aHUS in children

In children with FH autoantibody-associated aHUS, either eculizumab or PE may be used.[40] IST (high dose steroids together with cyclophosphamide, rituximab or mycophenolate) used in conjunction with PE suppresses autoantibody production and shortens the duration of treatment,[64] with retrospective series suggesting superiority to PE alone.[29,65] Mycophenolate or azathioprine have also been used as maintenance therapy, which is generally given for 12 months, and can be adjusted based on autoantibody titre.

In renal transplantation

Recurrence of aHUS following renal transplantation has been a dreaded and relatively common occurrence, with high rates of graft loss.[28,66] Certain complement-related mutations (e.g. of FH) carry a higher risk for post-transplant aHUS recurrence. Treatment with PE as rescue therapy for recurrent or *de novo* post-transplant aHUS has not improved graft survival. However, some authors favour prophylactic PE at time of transplantation in aHUS recipients, to reduce the risk of recurrence (with small, retrospective series in support[66]). Eculizumab initiated at time of transplantation is effective in preventing post-transplant aHUS recurrence[27,67] and is being considered for PBS funding in Australia (at present, funded only as rescue therapy once aHUS recurs). Living related-donor transplantation carries a significant risk of familial aHUS triggered in the donor by nephrectomy,[68] and should be considered only if a complement-related mutation can first be identified in the recipient with aHUS, and then excluded in the related donor.[27]

PLASMA THERAPY IN TTP

Benefit, timing and duration of PE

In 1991, a small RCT reported acute survival exceeding 90% for patients treated with PE for TTP, a condition previously associated with near 100% mortality.[69] Benefit in acquired TTP is attributed to removal of pathogenic ADAMTS13 autoantibodies and UL-vWF multimers, and most likely also to replenishment of ADAMTS13 in infused plasma. As noted, PE should be initiated as soon as possible,[16,70] as delays are associated with increased mortality and treatment failure.[71,72] PE should be continued daily until at least 2 days after the platelet count has normalized ($>150 \times 10^9$/L), bearing in mind other potential causes for any persisting thrombocytopenia.[16,70] Limited data indicate that weaning of PE prior to its cessation is of no value.[73]

Choice of fluid replacement for PE

Cryoprecipitate-deplete plasma (CDP) lacks UL-vWF multimers, making it a logical replacement fluid in TTP, although superiority to fresh frozen plasma (FFP) has not been demonstrated,[69,74] and both are used for treatment of TTP in Australia. Current UK guidelines recommend the use of solvent detergent-treated plasma (SDP) based on lower risks of both allergic/anaphylactic reaction to plasma and transfusion-transmitted infection.[16,75] SDP is not funded in Australia and New Zealand under national blood supply plans. In pneumococcal HUS, plasma and blood products are generally avoided as these may contain antibodies against the Thomsen-Friedenreich antigen.

Platelet transfusions

As thrombocytopenia in TTP is a result of platelet aggregation and consumption within microvascular thrombi, platelet transfusion should be avoided except for life-threatening bleeding,[76,77] and administered only in conjunction with central venous catheter insertion for immediate PE.[78]

Use of antiplatelet agents and anticoagulants

Data are lacking with regard to the efficacy of low-dose aspirin in TTP, but its use appears to be safe and reasonable provided the platelet count is greater than 50×10^9/L.[16] Prophylactic low molecular weight heparin is also routinely commenced at this platelet count.

STEROIDS AND RITUXIMAB IN ACQUIRED TTP

Initial treatment (added to PE)

Corticosteroids have been used historically in acquired TTP and are a logical choice given the autoimmune basis of disease. A small RCT supports the use of steroids[79] but there is no uniformly accepted regimen for dose and duration. Initial treatment with rituximab reduces relapse rates based on prospective studies,[80,81] and is recommended for patients presenting with poor prognostic features including neurological or cardiac involvement, and for relapsing disease.[16]

Table 8 Summary of key recommendations

TMA should be considered in all patients with thrombocytopenia and anaemia
• immediate request for a peripheral blood film for red cell fragments
Urgent, empirical PE (and steroids) should be commenced in all adults with TMA
• ideally, within 4–8 h of presentation
• a blood sample for ADAMTS13 testing must be collected beforehand
• confirmation of TTP is based on ADAMTS13 activity <10%
If TTP and STEC-HUS have been excluded, aHUS should be considered
• aHUS is treated with the complement C5 inhibitor, eculizumab
The presence of other TMA-associated conditions does not exclude TTP or aHUS

Refractory disease

In patients with unresponsive acquired TTP, intensification of treatment may include: twice daily PE, substitution of CDP for FFP, addition of rituximab, or of IST. Rituximab has been successfully used as adjunctive treatment in refractory and relapsing acquired TTP with a number need to treat of 2 compared to historical controls.[16,81] Historically, chemotherapeutic agents such as vincristine and cyclophosphamide were used without proven efficacy and with the potential for severe toxicity.[16]

Prevention of relapse

Some patients have persistently low ADAMTS13 activity despite resolution of an acute episode of acquired TTP, and a proportion of these patients will subsequently relapse. Rituximab may prevent relapse in this setting and has long been advocated in U.K. guidelines. Splenectomy is associated with significant mortality and its use in prevention of relapse has therefore been restricted to severe, refractory cases when other options have been exhausted.[82]

NOVEL THERAPIES IN TTP

Caplacizumab

Caplacizumab is a humanized nanobody that inhibits the interaction between UL-vWF and platelets. Following publication of a small placebo-controlled RCT,[83] the results of a multicentre phase 3 RCT (HERCULES) showed quicker resolution of acquired TTP, with improved clinical outcomes and a favourable safety profile.

Recombinant ADAMTS13

In vitro, this agent restores the VWF-cleaving capacity of congenitally ADAMTS13-deficient plasma, and neutralises ADAMTS13 antibodies in plasma of patients with acquired TTP.[84,85] Safety was demonstrated in a study of 15 patients with congenital TTP.[86]

Bortezomib

Successful use of bortezomib for refractory acquired TTP was first reported in 2013 in an Australian patient.[87] In a recent UK series, 5 of 6 refractory TTP patients treated with bortezomib achieved complete remission, although they also received other therapies and hence the role of bortezomib remains unclear.[88]

Key recommendations are summarized in Table 8.

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