Microangiopathic Haemolytic Anaemia Diagnosis and Management in Thrombotic Thrombocytopenic Purpura and Haemolytic Uraemic Syndrome: A Review

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ABSRTRACT

Microangiopathic haemolytic anaemia (MAHA) describes non-immune haemolysis by intravascular fragmentation of red blood cells, resulting from microvascular thrombosis characteristic of thrombotic microangiopathy (TMA). TMA-associated MAHAs include several diseases but are mostly associated with thrombotic thrombocytopenic purpura (TTP) and haemolytic-uremic syndrome (HUS). TTP is caused by a severe deficiency in ADAMTS13 proteinase, responsible for regulating coagulation, either due to presence of anti-ADAMTS13 (acquired iTTP; immune-mediated) or mutations in ADAMTS13 itself (congenital cTTP). HUS is caused by abnormal and uncontrolled complement activation, either by bacterial toxin activity (typical dHUS) or lack of normal regulatory proteins (atypical aHUS). This review focuses on TTP and HUS in relation to MAHA aetiology, pathogenesis, diagnosis and treatment.

The overlap in clinical presentation between TTP and HUS emphasise the importance of specific diagnostic assays in differential diagnosis. Therapeutic plasma exchange (TPE) and renal replacement therapy (RRT) are reported as relatively effective standard treatment methods. However, novel therapies for TTP (Caplacizumab) and HUS (complement blockade therapy or Eculizumab) currently undergoing clinical trials should be reviewed for future use once approved and validated, to further improve patient prognosis, as both TTP and HUS mortality rates remain significantly high (5-16% and 15-33% respectively).

INTRODUCTION

Microangiopathic haemolytic anaemia (MAHA) describes non-immune haemolysis by intravascular fragmentation (mechanical injury) of red blood cells into fragments called schistocytes. As a microangiopathic subclass of anaemia it is characteristic of thrombotic microangiopathy (TMA), a specific pathologic lesion in vessel walls of arterioles and capillaries, resulting in microvascular thrombosis. Not all MAHA is caused by a TMA, but nearly all TMAs cause MAHA and thrombocytopenia (George and Nester, 2020). TMAs include several diseases but are most associated with thrombotic thrombocytopenic purpura (TTP) and haemolytic-uremic syndrome (HUS). TMA related MAHA is also associated with some infections (e.g. HIV), pregnancy (e.g. HELLP syndrome), bone marrow transplants, systemic vasculitis, and particular drugs (e.g. immunosuppressants). Severe thrombocytopenia and organ failure also accompany MAHA in TMAs (Shenkman and Einav, 2014). MAHA is also associated with disseminated intravascular coagulation (DIC), malignant hypertension and cancers (Vincent et al., 2018). MAHAs not related to TMAs are usually associated with intravascular mechanical devices (e.g. prosthetic heart valves) that cause mechanical injury to the red cells, resulting in non-immune haemolysis (Tsai, 2014). This review focuses on TTP and HUS in relation to MAHA aetiology, pathogenesis, diagnosis and treatment.

AETIOLOGY

TTP and HUS are the most common causes of TMA, where arterioles and capillaries become occluded by disseminated microthrombi formed from agglutinated platelets. Platelets are consumed which eventually results in severe thrombocytopenia. The occlusion causes increased shear force acting on red cells passing through the microvasculature resulting in haemolytic anaemia (MAHA) and organ ischaemia, which may progress to organ failure (Shenkman and Einav, 2014).

TTP is caused by a severe deficiency in ADAMTS13 protease enzyme responsible for cleaving von Willebrand factor (vWF) multimers and is divided into two types: acquired (immune-mediated; iTTP) and congenital (cTTP or Upshaw-Schulman syndrome). Acquired TTP is most frequent and is caused by the presence of autoantibodies against ADAMTS13, whereas congenital TTP is associated with mutations in gene coding for the enzyme (Joly et al., 2017). vWF is essential for platelet and sub-endothelial adhesion and plays a role in platelet-platelet cohesion and aggregation inside blood vessels. VWF is stored as high molecular weight multimers, which are more haemostatically competent than monomers and require homeostasis control by ADAMTS13. The absence of ADAMTS13 results in ultra-large vWF (ULVWF) multimers persisting in circulation after their release is stimulated, leading to spontaneous platelet aggregation and the formation of vWF-rich microthrombi. This process uses up platelets (thrombocytopenia) and occludes vessel flow, causing microangiopathic haemolytic anaemia (MAHA) and organ ischaemia (Kremer Hovinga et al., 2017).

HUS is divided into typical (Shiga toxin-associated) and atypical (aHUS) subtypes. Shiga toxin-associated HUS is caused by a Shiga toxin-producing bacterium but is mostly associated with *Escherichia coli* O157:H7 or Shigella infections. It is also referred to as diarrhoea-associated haemolytic–uraemic syndrome (dHUS), as it causes bloody diarrhoea. Atypical HUS does not present with bloody diarrhoea and is caused by activating mutations (inherited) or autoantibody-mediated (acquired) defects in the complement regulatory proteins. These proteins regulate deposition/activation of complement on cell surfaces, predominantly the endothelium. All HUS subtypes result in abnormal and uncontrolled complement activation, either by bacterial toxin activity (dHUS) or lack of normal regulatory proteins (aHUS). The resulting endothelial damage leads to platelet activation and thrombosis (Firth, 2019). dHUS is usually limited to the renal endothelium and hence associated with acute kidney injury (AKI), whereas aHUS is related to systemic multi-organ complications. Drug-induced HUS has also been reported and is commonly associated with the antimalarial drug Quinine. It is speculated to involve immune injury to endothelial cells, especially glomerular endothelium (Al-Nouri et al., 2015).

PATHOGENESIS

TTP is defined as a severe deficiency in ADAMTS13 (enzymatic activity <10%), which is the only biologic marker specific for this disorder (Joly et al., 2017). ADAMTS13 is primarily synthesized by hepatic stellate cells in the liver, as well as platelets, renal podocytes, renal tubular epithelial cells, and endothelial cells. It is released into circulation as an active enzyme, where it circulates free or bound to soluble von Willebrand Factor (3-5%). vWF is a multimeric plasma glycoprotein and the only known substrate for ADAMTS13. Storage of vWF is as ultra-large vWF (ULVWF) multimers in endothelial cells (Weibel–Palade bodies) or α-granules of megakaryocytes and platelets (Kremer Hovinga et al., 2017). vWF is essential for haemostasis, where its main function is supporting platelet adhesion and aggregation at sites of vessel injury, as well as storing and protecting FVIII from proteolytic degradation in the circulation. In its native conformation, it is inert for adhesive function and resists proteolysis by its regulator ADAMTS13. On exposure to repeated cycles of high shear stress levels, in capillaries and arterioles, it changes its conformation to an unfolded state in which it can support platelet adhesion and activation. The conformational responsiveness of vWF to shear stress is directly related to its size. ULVWF multimers are more responsive than smaller multimers, created by repetitive proteolysis by ADAMTS13, and therefore are more haemostatically competent (Tsai, 2014). In the absence of or at

substantially reduced activity of ADAMTS13, ULVWF multimers accumulate and become activated by shear stress in the circulation, resulting in platelet aggregation and microvascular thrombosis (Shenkman and Einav, 2014). Thrombocytopenia and MAHA ensue due to platelet consumption and mechanical destruction of circulating red cells as they pass through the occluded microvasculature see Figure 1 (Kalpatthi and Kiss, 2020).

Acquired TTP (immune-mediated; iTTP) involves the formation of polyclonal anti-ADAMTS13 (mostly IgG) autoantibodies against the cysteine-rich/spacer domain of ADAMTS13, which inhibit its proteolytic activity towards vWF. Significant amounts of ADAMTS13-specific immune complexes (ICs) have also been reported, which also contribute to severe deficiency of ADAMTS13 (Joly et al., 2017). Various causative factors of iTTP have been identified, including HIV infection, pregnancy and anti-platelet or immunosuppressive drugs. These trigger the formation of autoantibodies against ADAMTS13 or stimulate the secretion of large quantities of ULVWF multimers from endothelial cells (Shenkman and Einav, 2014).

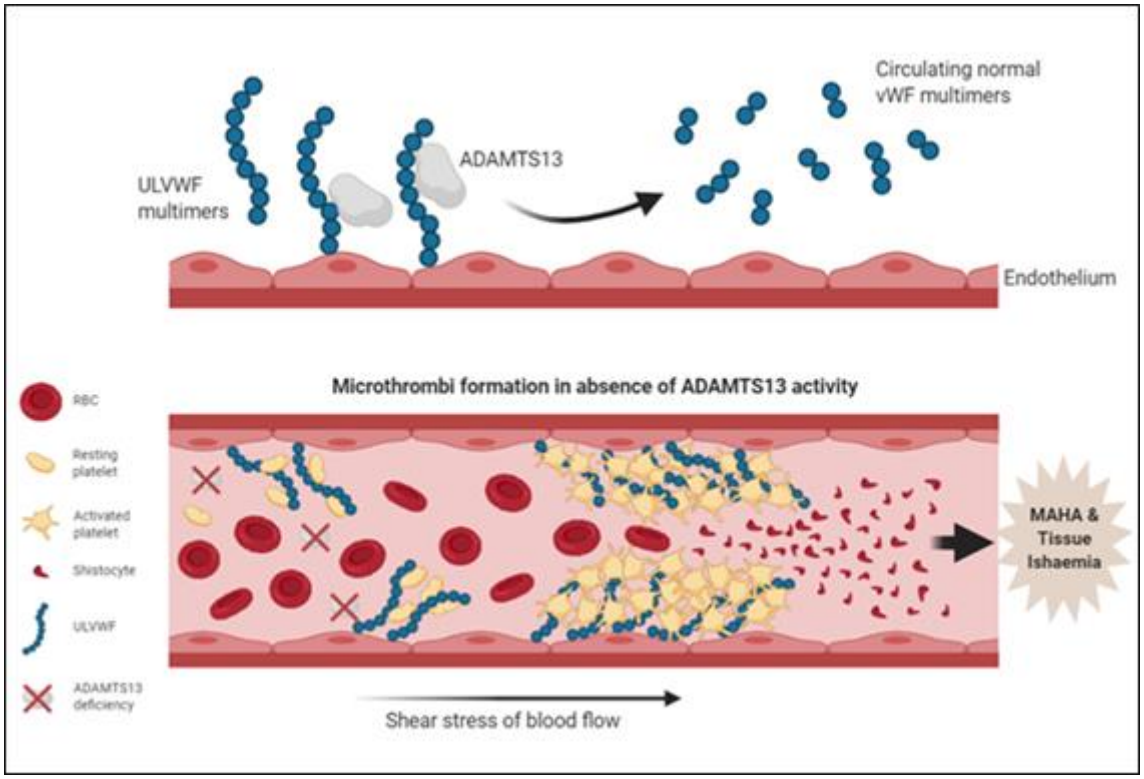

Figure 1: Pathophysiology of TTP. Under normal physiological conditions ULVWF multimers are cleaved into smaller multimers, which are less haemostatically competent (less adhesive to platelets). In the absence or reduced functional activity of ADAMTS13 (iTTP or cTTP), the accumulation of ULVWF multimers leads to widespread spontaneous platelet binding and thrombosis (microthrombi and thrombocytopenia). The resulting narrowing of the vessel lumen increases mechanical stress on the RBCs, leading to MAHA (schistocytes) and tissue ischaemia (figure adapted and redrawn from Joly et al., 2017).

Congenital TTP (cTTP or Upshaw-Schulman syndrome) is associated with rare recessive biallelic mutations in ADAMTS13, which are mostly responsible for quantitative ADAMTS13 defects. Approximately 150 distinct mutations have been identified spanning the entire ADAMTS13 gene, which mainly affect the N-terminal region (Joly et al., 2017). The most common mutations include the missense mutation c.3178C>T (p.R1060W) in exon 24, and the frameshift mutation c.4143_4144dupA in exon 29 (Kremer Hovinga et al., 2017).

In some acute TTP cases, severe ADAMTS13 deficiency may result from different and currently unclear mechanisms, as anti-ADAMTS13 IgG may not be detectable in 20-25% of these patients. The

following have been hypothesised: low sensitivity of the anti-ADAMTS13 IgG assays, unrecognised Ig isotypes, acute liver failure (low ADAMTS13 synthesis/secretion), degradation of ADAMTS13 by sepsis enzymes, and ADAMTS13 inhibition by free haemoglobin or interleukins (Joly et al., 2017). Although the deficiency in ADAMTS13 is the prime cause of microvascular thrombosis in TTP, the tendency to form vWF-platelet aggregation and thrombosis is also affected by responsiveness of vWF to shear stress (modified by plasma proteins such as FVIII, thrombospondin-1 and beta 2-glycoprotein 1) and the shear stress profile itself (Tsai, 2014).

HUS is a distinct type of TMA which has a pathology mainly associated with the kidneys (AKI). It is divided into two categories: typical/diarrhoea-associated HUS (dHUS) and atypical HUS (aHUS) (Afshar-Kharghan, 2016). Diarrhoea-associated HUS (dHUS) is most commonly a result of a Shiga toxin-producing E. coli (STEC) infection, O157:H7 being the most frequent serotype isolated. STEC are highly infectious organisms and the infection occurs through ingestion of contaminated food or water. In the stomach, intrinsic acid resistance allows them to survive the acidic environment and move on to colonise the intestinal mucosa. These bacteria use specific proteins to attach and efface the enterocytes, leading to loss of microvilli and formation of lesions (haemorrhagic colitis). Actin is accumulated within the host cells to further anchor the bacteria. The STEC then begin to produce the Shiga toxins (Stx), which are ribosome inactivating proteins. Two structurally similar types of Stx can be produced (Stx1 or Stx2), but Stx2 is more likely to cause HUS (Walsh and Johnson, 2018). These exotoxins are directly responsible for cell damage of the microvascular endothelium, due to expression of the toxin-specific receptors on these cells. Once endocytosed and transported inside the cell ribosomal peptide elongation is inhibited by the enzymatically active toxin subunit cleaving a single adenine base from the human rRNA. Protein synthesis inhibition then leads to cell death via an apoptotic pathway (Ibama et al., 2019).

Once the epithelium and endothelium are breached, the Stx toxins cross the intestinal wall into the bloodstream where they bind circulating platelets/leukocytes, activating them. These travel to distal sites such as the kidneys, where the microvascular endothelium expresses the primary cellular target: the globotriaosylceramide (Gb3) receptor. In the kidney, this receptor is also found on tubular cells, mesangial cells and podocytes. These cells are infiltrated by the same mechanism as above. Binding Gb3 and toxin endocytosis eventually leads to inhibition of protein transcription, apoptosis, induction of inflammatory cytokines (TNF-α, GM-CSF, IL-8) and cellular necrosis (Walsh and Johnson, 2018).

At higher Stx concentrations, endothelial apoptosis leads to cell detachment, exposing the subendothelial bed rich with prothrombogenic tissue factor and collagen (Joseph et al., 2020). Endothelial cell injury also inhibits prostacyclins and prostaglandins, activating thromboxanes, which induce platelet aggregation and microthrombi formation (Ibama et al., 2019). Stx toxin is also capable of inducing a prothrombogenic phenotype of the epithelial cells, which begin producing increased quantities of tissue factor and vWF (Joseph et al., 2020). Renal capillary lumen becomes partly occluded by the microthrombosis, increasing shear stress on the red cells and causing fragmentation into schistocytes (MAHA). Thrombocytopenia develops as platelets are used up in the formation of microthrombi (Ibama et al., 2019). Finally, recent studies have shown that Stx is also capable of activating the alternative complement pathway, by prompting the formation of platelet/red cell derived microvesicles coated with C3 and/or C9. Activated complement fraction C3a is then believed to trigger microvascular thrombosis by mobilizing P-selectin on the surface of endothelial cells, which plays a role in initial platelet adhesion (Joseph et al., 2020).

In atypical HUS (aHUS), the lack of normal regulatory proteins leads to abnormal activation of the complement cascade, which damages the renal endothelium and hence causes platelet activation and thrombosis. The defects in these complement regulatory proteins are either inherited or acquired (antibody-mediated) (Firth, 2019). Inherited/primary aHUS is associated with the dysregulation of the alternative complement pathway, mainly by mutations affecting proteins that regulate complement activation; FH (Factor H), FI (Factor I) and MCP (membrane cofactor protein). Some gain-of function mutations also occur in the alternative complement activators C3 and FB (Factor B) (Afshar-Kharghan, 2016). These mutations cause unregulated complement activation, and thus excessive generation of

activation proteins such as C3a, C5a and C5b-9 (membrane attack complex, MAC). C3a and C5a cause abnormal vascular permeability (oedema), while MAC causes endothelial cell lysis, resulting in endothelial swelling and injury. The resulting oedema and cellular proliferation cause subendothelial expansion. This, combined with endothelial cell swelling, may cause luminal stenosis and eventually lead to ischaemic organ injury, where initially MAHA occurs without thrombocytopenia (no thrombosis). Once the endothelium is damaged and prothrombotic components in the subendothelial matrix (collagen, vWF, fibrinogen, fibronectin and laminin) are exposed, the coagulation system is activated. Microvascular thrombosis ensues, leading to tissue ischaemia, thrombocytopenia and high shear stress, which fragments red cells into schistocytes (MAHA) (Tsai, 2014).

Acquired/secondary aHUS is associated with the presence of antibodies directed against complement Factor H (FH), and is typically detected in 5–10% of aHUS cases (Tsai, 2014). Anti-FH has been found in pregnancy, cancer, chemotherapy patients, post solid-organ/hematopoietic stem cell transplant and autoimmune disorders (Afshar-Kharghan, 2016). Factor H is the main regulator of the alterative complement pathway, functioning both as a cofactor for Factor I (FI; regulates complement activation) and in degradation of C3 convertase (contributes to formation of MAC). FH also has host recognition properties, allowing it to protect host cells by preventing complement activation on their surface. Anti-FH antibodies are mostly directed against the C-terminal responsible for host cell recognition, thus preventing the protective effect and causing complement activation with endothelial cell lysis (MAC) (Karpman et al., 2016). Endothelial injury leads to thrombosis, MAHA and thrombocytopenia.

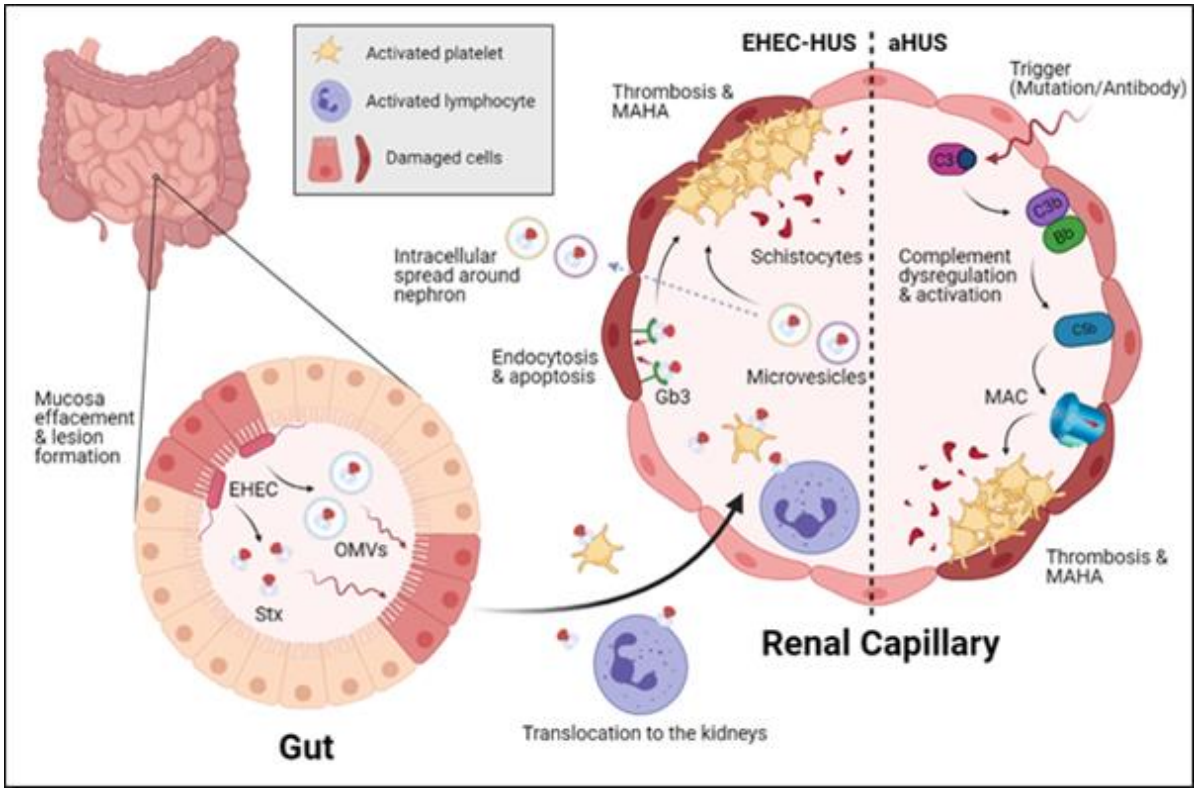

Figure 2: Pathophysiology of EHEC-HUS (STEC-HUS) and aHUS. Shiga toxin-producing Escherichia coli (STEC) ingestion results in gut colonisation and formation of lesions in the mucosa. Shiga toxins (Stx) are then produced and released, either as free particles or secreted through outer membrane vesicles (OMVs). Once the intestinal epithelium and endothelium is breached by the injury, the toxins enter the bloodstream and bind blood cells, which carry them to the kidneys. The toxins bind their target receptor globotriaosylceramide (Gb3) on the endothelial cells (glomerular and peritubular capillary) and undergo endocytosis. Infection leads to inhibition of protein synthesis and apoptotic cell death. The combination of damaged endothelium and activated platelets results in thrombosis, MAHA (schistocytes) and thrombocytopenia. Microvesicles also contribute to microvascular thrombosis, by triggering further platelet adhesion and allowing toxin transfer between cells and across basement membrane. This spreads the toxin around the nephron, eventually leading to acute kidney injury (AKI). In atypical HUS (aHUS), the uninhibited complement activation, either caused by autoantibodies or mutations in complement regulatory proteins, results in endothelial injury and thrombosis, leading to MAHA (figure adapted and redrawn from Karpman et al., 2016).

DIAGNOSIS

Clinical presentation of TTP

TTP is typically characterised by a severe disease course of acute onset. Although the disease onset is usually sudden, some flu-like early signs are frequently reported during the preceding days or at the time of diagnosis. These symptoms include fatigue and pains in the joints, muscles, abdomen or lumbar region (Kremer Hovinga et al., 2017). Clinical signs at onset include fever, signs of haemolytic anaemia (fatigue, dyspnoea, pallor and jaundice) and signs of thrombocytopenia (purpura, petechiae and skin/mucosal haemorrhaging). The renal and central nervous systems are most frequently affected. Neurological manifestations are often transient and range from a mild headache or mental changes/confusion to focal neurological deficits, seizures and coma. Variable degrees of renal dysfunction are observed but are generally mild (Kalpatthi and Kiss, 2020). The absence of bloody diarrhoea and severe renal dysfunction (oliguria or anuria) helps to differentiate TTP from HUS, where these symptoms are common (Chiasakul and Cuker, 2018). Other less common symptoms associated with TTP include cardiac arrhythmias (electrolyte imbalance), myocardial infarction (microthrombi) and gastrointestinal problems (nausea, vomiting, diarrhoea) (Kalpatthi and Kiss, 2020).

As the above clinical signs are not specific for TTP, a differential diagnosis should be carried out, where other TMAs with similar manifestations must be considered (HUS or other TMA syndromes associated with pregnancy, cancer, sepsis or organ transplantation). ADAMTS13 activity assays are pivotal in the differential diagnosis, but the results may not always be readily available. As TTP is classified a medical emergency requiring rapid diagnosis and treatment, a presumptive diagnosis must often be made and treatment initiated based on clinical presentation, history of conditions/comorbidities and routine laboratory assays (Kremer Hovinga et al., 2017). In the long term, the differential diagnosis is crucial, as patients with severe ADAMTS13 deficiency are more likely to respond to therapeutic plasma exchange (TPE) when compared with other TMAs (Joly et al., 2017).

Laboratory Investigation of TTP

Routine FBC (full blood count) detects evidence of anaemia and severe thrombocytopenia (typically <30 x10^9/L). Investigation of the blood smear reveals schistocytes (>1%). In some cases, delayed appearance of schistocytes may occur after onset of clinical signs and symptoms. In rare cases, especially relapse, they do not appear throughout the whole course of the disease (Chiasakul and Cuker, 2018). Other markers of MAHA include reticulocytosis (>120 x10^9/L), indirect hyperbilirubinemia, elevated LDH (lactate dehydrogenase) and decreased haptoglobin (Joly et al., 2017; Kalpatthi and Kiss, 2020). To aid differential diagnosis, other TMA-associated conditions should be ruled out. A basic metabolic panel (serum creatinine and blood urea nitrogen) can rule out severe renal impairment. A coagulation screen (PT, APTT), fibrinogen and D-dimers levels can rule out DIC. A pregnancy screen is performed on women of childbearing age. Cardiac involvement is associated with higher mortality rates and refractoriness to therapy; therefore, troponin-I, electrocardiogram and echocardiogram should be performed. HIV and antinuclear antibodies should also be investigated (Kalpatthi and Kiss, 2020).

As schistocytes are the morphological hallmark of TTP, they are instrumental in the diagnostic screen and require strict standardisation of microscopical identification criteria by the ICSH (International Council for Standardisation in Haematology). Schistocytes are always smaller than intact RBCs and the term encompasses all irregular triangular/crescent-shaped cells, including helmet cells, keratocytes (cells with pointed projections/horns), and microspherocytes (cells lacking central pallor; which are only included in count in the presence of other shapes mentioned) (Joly et al., 2017; Zini et al., 2011). A schistocyte count of >1% is a robust indicator of a TMA. If schistocytes are absent, but TMA is highly suspected, blood smear screening should be repeated daily, as in some cases their appearance can be delayed. Automated blood cell counters should only be used to complement microscopy or follow-up on true-positive samples (Zini et al., 2011).

Confirmatory assays for diagnosis of TTP include ADAMTS13 activity assay, ADAMTS13 functional inhibitor assay, and anti-ADAMTS13 antibody assay. These assays are used to differentiate between TTP and other TMAs, or between iTTP and cTTP. The ADAMTS13 activity assay uses fluorescence resonance energy transfer (FRET) methodology to assess the ability of the patient's enzyme to cleave vWF, where the resulting cleavage product is proportional to the level of ADAMTS13 activity (Chiasakul and Cuker, 2018). The antibody assays determine the presence of anti-ADAMTS13 autoantibodies and/or their inhibitory potential, while antigen assays measure the plasma concentration of ADAMTS13 (Kremer Hovinga et al., 2017). Undetectable ADAMTS13 activity (<10%), with a positive anti-ADAMTS13 antibody assay, confirms the diagnosis of iTTP (Kalpatthi and Kiss, 2020). ADAMTS13 activity should recover during remission. If severely reduced ADAMT13 activity persists during remission and the anti-ADAMTS13 antibody assay is negative, cTTP is suspected. Molecular analysis is then required to confirm that diagnosis (Kremer Hovinga et al., 2017). The diagnostic algorithm for TTP is presented in Figure 3.

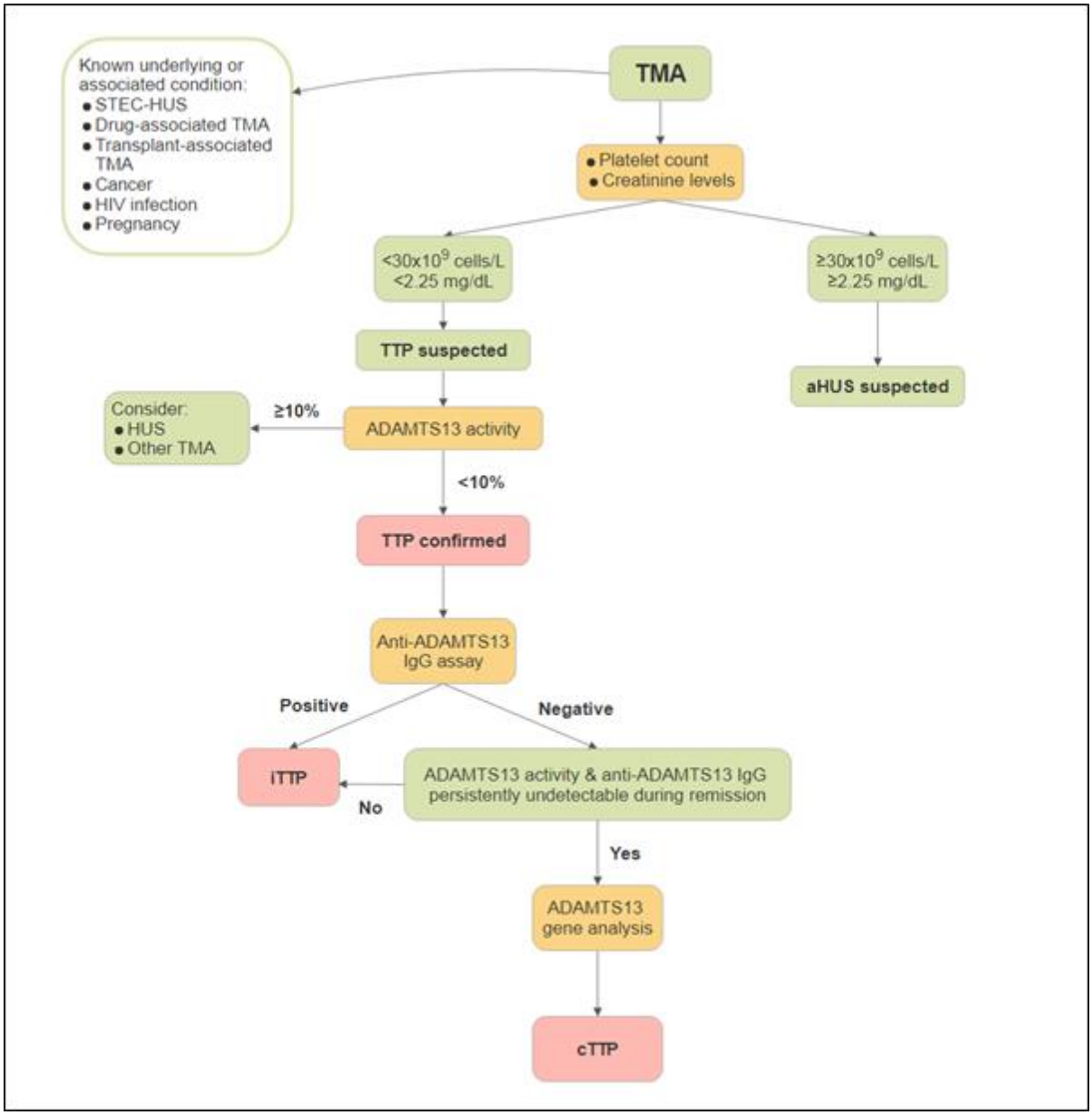

Figure 3: Diagnostic algorithm for diagnosis of TTP in a patient presenting with TMA, consisting of MAHA and thrombocytopenia, with or without organ failure (figure adapted and redrawn from Kremer Hovinga et al., 2017).

Clinical presentation of HUS

STEC-related disease exhibits a broad range of severity, from asymptomatic carriage to lethal dHUS. Rapid diagnosis is essential both for timely treatment and epidemic control. Symptoms at presentation can differ greatly with age (children <5 years old and elderly are more prone to developing HUS). However, patients generally present with severe abdominal cramps and painful diarrhoea. Nausea, vomiting and fever are less common, as the infection is usually limited to the colon (bacteraemia is rare) (Joseph et al., 2020). Severe gastroenteritis is associated with rectal prolapse, colonic gangrene or perforation. Haemorrhagic colitis manifests as bloody diarrhoea due to perforation of the intestinal wall. Haemolytic anaemia (MAHA), thrombocytopenia and AKI usually develop within 2-12 days after diarrhoea onset (Karpman et al., 2016). Bloody diarrhoea occurs 1-5 days after symptom onset but is not considered a defining diagnostic feature of dHUS, as it only occurs in 70-80% cases (Joseph et al., 2020). Signs of anaemia (acute pallor with or without jaundice) present within 3-14 days after the onset of bloody diarrhoea. Other manifestations include cardiac failure (fluid overload, oedema, and hypertension), CNS involvement (ranging from lethargy and irritability to seizures, coma or stroke), pancreatic inefficiency, or hepatomegaly (Ibama et al., 2019).

Patients with aHUS may present during any stage of life. Episodes may be triggered by pregnancy, infections or transplants. A preceding infection may manifest with diarrhoea, similarly to patients with STEC-associated HUS, although onset of aHUS is generally less sudden. This similarity presents a clinical challenge; therefore, patient history plays a key role in differential diagnosis, as the disease course is characterized by recurring episodes throughout the patient's life, especially post triggering events mentioned earlier. Acute episodes generally lead to end-stage renal failure, and in some cases already occur at presentation. Extra-renal manifestations include digital gangrene, cerebral/peripheral vessel stenosis, CNS involvement, and in some cases pancreatic and pulmonary complications (Karpman et al., 2016; Afshar-Kharghan, 2016).

Laboratory Investigation of HUS

The laboratory diagnosis of haemolytic uremic syndrome (HUS) involves haematological, biochemical and microbiological assays. These include FBC (thrombocytopenia, haemoglobin <8g/dL) and clotting assays (urgent blood film if TMA suspected – schistocyte count >1%, increased reticulocyte counts), haemolysis screen (high bilirubin, high LDH, low haptoglobins, negative Coombs test), renal function (elevated serum creatinine and urea, hyperkalaemia, hyponatraemia, metabolic acidosis) and urinalysis (microscopic haematuria, proteinuria and casts - glomerular injury), liver function (elevated liver enzymes), and ADAMTS13 activity (checked if TTP suspected) (Firth, 2019; Ibama et al., 2019).

Various microbiological assays are employed to differentiate dHUS, including faeces analysis (culture on sorbitol MacConkey Agar – where colourless non-haemolytic colonies are subsequently confirmed with E. coli O157:H7 antiserum, PCR for *stx*/*eae* genes, ELISA for free Stx), serology assay (ELISA for EHEC virulence factors, i.e., Stx, serotype-specific lipopolysaccharides, adhesins), blood culture (bacteraemia usually absent) and urine culture (urinary tract infections rare). Renal biopsies are rarely performed during the acute phase of disease, due to risk of bleeding associated with the thrombocytopenia (Ibama et al., 2019; Karpman et al., 2016). Therefore, these are reserved in the case of diagnostic uncertainty in context of STEC-HUS. Patients commonly display superimposed acute tubular damage and nonspecific features of TMA, including glomerular capillary thrombosis, endothelial swelling, congested glomeruli and capillary wall necrosis (luminal narrowing and thrombosis). Cortical infarcts are characteristic of severe and fatal disease (Joseph et al., 2020). Patients presenting with abnormal neurology should undergo CT or MRI scan of the head (Firth, 2019).

Patients with aHUS generally present with moderate to severe thrombocytopenia (<50 x10^9/L), however, around 15% of cases have normal platelet counts. Renal failure is severe and presents with elevated creatinine, haematuria, proteinuria, oedema, and hypertension. Some aHUS cases associated with MCP mutations have been reported to mimic TTP, with normal kidney function and mainly neurologic findings. Triggering events of aHUS are also frequently seen in other TMAs (e.g.,

pregnancy), which further complicates diagnosis (Afshar-Kharghan, 2016). Simple laboratory tests for the quick diagnosis of defective alternative complement regulation do not exist, hence diagnosis initially requires exclusion of TTP (ADAMTS13 analysis). Clinical history and presentation are also useful, such as significant renal failure, which favours a diagnosis of aHUS over TTP (Tsai, 2014). Several more indicative assay results (e.g., complement assays) are usually not available immediately, as most of these tests are performed in reference laboratories. ADAMTS13 activity, complement functional assays, and CH50, C3, C4, FI, FH and anti-FH antibody quantitation (ELISA) may take days to complete, while genetic studies on complement genes may take weeks to months.

As early treatment correlates with better prognosis, clinicians are forced to begin therapy based on general laboratory results and clinical indicators. The course of therapy can be modified based on response and the results of more specific investigations, when these results become available. Genetic studies are used to confirm aHUS diagnosis and guide long-term management, i.e., the length of anticomplement therapy or decisions regarding kidney transplantation (Afshar-Kharghan, 2016).

TREATMENT

TTP treatment should be started as soon as the provisional diagnosis has been made. Standard therapeutic plasma exchange (TPE) guidelines advise daily 1-1.5 times patient's plasma volume exchanges. These are continued until platelet count recovers (>150 x10^9/L) for a period of 2 consecutive days, haemolysis has ceased, and no additional organ dysfunction occurs. TPE is the treatment of choice for both iTTP and cTTP, and its use has drastically reduced mortality rates from >90% (untreated) to 5-16% (Kalpatthi and Kiss, 2020). Its use also correlates with higher survival rates compared with simple high-dose plasma infusion because it delivers greater quantities of functional ADAMTS13, without causing circulatory overload, and removes anti-ADAMTS13 antibodies. It may also remove ULVWF multimers and inflammatory cytokines, although this has yet to be proven (Kremer Hovinga et al., 2017). Regular plasma infusions are effective in preventing acute episodes in cTTP; however, no official guidelines indicate when they should be implemented.

Other available treatments include corticosteroids (immunosuppression in iTTP), Rituximab (anti-CD20 monoclonal antibody for patients with suboptimal response to TPE), N-Acetylcysteine (reduces the size of VWF multimers *in vitro* and inhibits platelet aggregation), splenectomy (last resort for patients who relapse after TPE/ Rituximab/ N-Acetylcysteine), and immunomodulatory drugs for suppression of anti-ADAMTS13 production (Cyclosporine A; last resort treatment in cases of suboptimal response to previous therapies mentioned).

Increased mortality and treatment refractoriness are generally associated with older age, increased plasma cardiac troponin (damaged myocardium), and very high LDH levels at diagnosis. The average survival rate from an initial episode is 80-90% (Kremer Hovinga et al., 2017; Rottenstreich et al., 2015). The definition of a full response to treatment includes platelets >150 x10^9/L for 2 consecutive days, with normal/normalising LDH, and clinical recovery. A full recovery is defined as a lasting response, for a minimum of 30 days, after TPE discontinuation (Joly et al., 2017).

Recent advances in TTP therapy include Caplacizumab (humanized anti-VWF antibody fragment), which inhibits the interaction between vWF multimers and platelets, reducing microvascular thrombosis. A recent study by Scully et al. (2019), has shown Caplacizumab can reduce mortality and refractoriness to 0% (compared with 4% in the placebo group), however, an 8% disease recurrence rate was observed once dosing had ceased. More bleeding was also observed in the test group (mainly epistaxis and from gums). These limitations, plus its high cost, may restrict its practical use. However, future studies on Caplacizumab combination with Rituximab and TPE may achieve lower mortality, faster recovery and reduced relapse rates, which could reduce overall health care costs (Kalpatthi and Kiss, 2020). Recombinant ADAMTS13 (BAX930) for cTTP has also shown promising results in

effectively restoring vWF-cleaving activity. Further results of ongoing trials are expected in 2023 (ClinicalTrials.gov, 2020; Kremer Hovinga et al., 2017).

HUS treatment should be started as soon as a diagnosis is suspected. Supportive care is the first line of action in any HUS subtype, but treatment is also directed towards the specific cause of the disease (dHUS or aHUS). Supportive care involves adequate nutrition and hydration, correction of acidosis and electrolyte imbalance, renal replacement therapy (RRT), as well as controlling hypertension and seizures (Karpman et al., 2016). Electrolyte and fluid imbalance (dehydration), caused by vomiting, diarrhoea and decreased fluid intake, is corrected by administering the appropriate electrolyte orally, intravenously or by tube. Hypertension (fluid overload) is treated with diuretics (oral or IV), to prevent hypertensive encephalopathy and congestive heart failure. If replacement of fluids is ineffective in correcting the imbalances, or if cardiac/pulmonary functions are already affected by severe fluid overload, the patient is switched to RRT (peritoneal dialysis or haemodialysis). Dialysis is also used to treat metabolic acidosis. Severe anaemia (Hb <7g/dL) is treated with red cell transfusion, while platelet transfusions are given only to patients with life-threatening bleeding (platelets <10 x10^9/L) or requiring surgery (Ibama et al., 2019; Karpman et al., 2016).

In STEC-HUS (dHUS), supportive therapy is the cornerstone of treatment. Early IV fluid administration has shown to reduce CNS-complications and mortality, however, it must be balanced against the risk of fluid overload (hypertension) once AKI is established. Fluid overload is generally managed with diuretics, calcium receptor blockers or angiotensin-converting enzyme inhibitors. Haemodialysis is preferred in adult patients, while children are frequently put on acute peritoneal dialysis. Packed red blood cell transfusions are preferred. Plasma exchange therapy has been theorized to remove the circulating toxins or complement factors, but there is little evidence of its effectiveness and randomised trials are currently pending (Joseph et al., 2020). Renal transplantation may be necessary if renal function cannot be restored after the acute phase of disease (Karpman et al., 2016). Other possible treatments currently undergoing trials include complement blockade therapy, Shiga toxin competitive inhibitors and anti-Stx monoclonal antibodies. STEC-HUS has relatively low lethality in paediatrics (<3%), but mortality can rise to 15-33% in adult and fragile populations, with long-term sequelae affecting a third of patients (Joseph et al., 2020).

In aHUS, plasma infusion (20-30 mL/kg body weight) or plasma exchange (1.5-2 times patient's plasma volume) are the standard treatments. They are effective at improving hematologic parameters and preventing relapse post kidney transplant. However, despite plasma therapy, many patients progress to ESRD (end-stage renal disease) or death because complement-mediated organ damage continues, as plasma exchange, in most cases, does not stop complement overactivation (Afshar-Kharghan, 2016). Even those who initially respond favourably can become dependent on, or resistant to, this treatment (Baines and Brodsky, 2017). Long-term follow-up is indicated, as 20-25% of patients develop some degree of CKD (Ylinen et al., 2020). Therapeutic response to plasma therapy mainly depends on complement mutations; therefore, genetic studies are useful in long-term management of the patient. However, specific mutations are identified in only about 50% of cases, hence prognosis is mainly dependent on presence of ESRD, extra-renal involvement, frequency of recurrence after kidney transplant, and the time delay between symptom onset and commencement of treatment. Therefore, anticomplement reagents (Eculizumab) should be considered as first-line therapy in relapsing patients, or in siblings of a patient with confirmed aHUS diagnosis (Afshar-Kharghan, 2016).

Eculizumab is a humanised monoclonal IgG antibody against complement C5. It effectively inhibits the terminal complement pathway and MAC formation, by preventing the conversion of C5 to C5a and C5b. Eculizumab is effective and safe for the treatment of aHUS in both adults and children (Firth, 2019; Walsh and Johnson, 2018). It significantly improves thrombocytopenia and renal function, leading to discontinuation of dialysis in some cases, especially if implemented early. The optimal treatment duration has not been defined; however, it may be safe to discontinue once the trigger of the acute episode has resolved. Some risk of relapse/recurrence still remains, with increased risk of Neisserial infections (require vaccination) (Baines and Brodsky, 2017). Treatment efficacy should be monitored by haematological (platelet counts) and biochemical (LDH, haptoglobin, creatinine) markers

of disease activity, levels of complement activation (CH50) and complement deposition on cells (Karpman et al., 2016). Immunosuppressive therapy might additionally be required if anti-FH antibody is detected. The length of therapy is determined based on anti-FH antibody titre monitoring. Kidney transplantation is considered a last resort for patients with ESRD. It requires rigorous risk assessment and preparation (vaccinations, prophylactic eculizumab). Patients with high risk of reoccurrence (FH, C3 and FB mutations) require life-long prophylactic Eculizumab therapy. Patients with low titres of anti-FH can discontinue the prophylaxis after a 12-month relapse-free period (Afshar-Kharghan, 2016).

CONCLUSION

Correct diagnosis and immediate treatment are crucial for positive patient prognosis. The similarities in clinical presentation of iTTP and STEC-HUS emphasise the importance of specific diagnostic assays (ADAMTS13 quantitation and PCR) in the differential diagnosis. Treatment of TTP and HUS should be reviewed as soon as more effective methods become validated and approved for use. Further studies are required on Caplacizumab, which has already shown promising results by potentially reducing mortality and refractoriness to 0%. Caplacizumab may also prove to be more effective if combined with Rituximab and TPE, potentially achieving faster recovery with reduced mortality and relapse rates. Novel STEC-HUS treatments currently undergoing trials (complement blockade therapy, Shiga toxin competitive inhibitors and anti-Stx monoclonal antibodies) may prove to be more effective and simple treatment options where available. Both TTP and HUS mortality rates remain significantly high (5-16% and 15-33% respectively) thus further innovation in therapeutics and development of staff educational programs, to further improve patient prognosis is required.

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