Review

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Haemolytic uraemic syndrome

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Haemolytic uraemic syndrome (HUS) is defined by the simultaneous occurrence of nonimmune haemolytic anaemia, thrombocytopenia and acute renal failure. This leads to the pathological lesion termed thrombotic microangiopathy, which mainly affects the kidney, as well as other organs. HUS is associated with endothelial cell injury and platelet activation, although the underlying cause may differ. Most cases of HUS are associated with gastrointestinal infection with Shiga toxin-producing enterohaemorrhagic *Escherichia coli* (EHEC) strains. Atypical HUS (aHUS) is associated with complement dysregulation due to mutations or autoantibodies. In this review, we will describe the causes of HUS. In addition, we will review the clinical, pathological, haematological and biochemical features, epidemiology and pathogenetic mechanisms as well as the biochemical, microbiological, immunological and genetic investigations leading to diagnosis. Understanding the underlying mechanisms of the different subtypes of HUS enables tailoring of appropriate treatment and management. To date, there is no specific treatment for EHEC-associated HUS but patients benefit from supportive care, whereas patients with aHUS are effectively treated with anti-C5 antibody to prevent recurrences, both before and after renal transplantation.

Keywords: complement, enterohaemorrhagic *Escherichia coli*, haemolytic uraemic syndrome, microvesicles, Shiga toxin.

Introduction

Haemolytic uraemic syndrome (HUS) is characterized by the simultaneous development of nonimmune haemolytic anaemia, thrombocytopenia and acute renal failure. The main causes of HUS are Shiga toxin-producing *Escherichia coli* (STEC) also known as enterohaemorrhagic *E. coli* (EHEC), in which patients usually present with a gastrointestinal prodrome, and complement-mediated disease [atypical HUS (aHUS)] associated with mutations in genes encoding complement factors or autoantibodies. Less common causes are other infections, other genetic causes (i.e. not affecting the complement system), malignancies, drugs, transplantation, pregnancy or malignant hypertension. The clinical presentation and renal pathology may be similar, regardless of the primary cause. Patient investigation should therefore be geared towards defining the aetiology, as treatment strategies may differ based on the underlying disease pathogenesis.

In this review, we will define the clinical and laboratory features of HUS, as well as disease epidemiology and pathology, and describe aspects of the disease pathogenesis. We will provide a clinical investigation protocol, based on the known aetiologies of HUS, designed to achieve an appropriate diagnosis and thus suitable treatment. The prognosis of HUS, in terms of patient morbidity and mortality, is largely based on the underlying cause and the provision of appropriate treatment. Studies in recent years have generated new insights into the pathogenesis of the various forms of HUS, which will be highlighted here, as these scientific advances provide the background for novel therapies.

Classification and clinical features of HUS

Haemolytic uraemic syndrome is classified as post-infectious, complement-mediated, which may be hereditary and/or autoimmune, or associated with other co-existing conditions such as pregnancy, human immunodeficiency virus (HIV) infection, transplantation (bone marrow and solid organ), malignancy, autoimmune diseases, drugs, malignant hypertension as well as other more unusual associations, some of which are hereditary (Table 1). There is also some degree of overlap between aetiologies; for example, pregnancy-

Table 1 *Classification of haemolytic uraemic syndrome (HUS) based on aetiology*

Aetiology		Cause and features	Comment	Reference
Specific infectious agent	Shiga toxin-producing bacteria	Enterohaemorrhagic *Escherichia coli* (EHEC)	Most prevalent serotypes: O157, O26, O104, O111, O103, O145, O121, O45	[230]
		Shigella dysenteriae type 1		[231]
		Citrobacter freundii		[232]
	Streptococcus pneumoniae		Neuraminidase producing	[193]
	Influenza A	H1N1	Neuraminidase producing (possible explanation)	[192]
	Enteroviruses	Coxsackie A and B, Echo	Unclear association	[233]
	HIV			[234]
	Pseudomonas aeruginosa		Neuraminidase producing (possible explanation)	[235]
Complement dysregulation	Genetic	Mutations in genes encoding for factor H, factor I, MCP, C3, factor B, clusterin, thrombomodulin	Resulting in dysregulated complement activation via the alternative pathway	[55, 154]
		Rearrangements or deletions in genes encoding complement factor H-related proteins	Associated with antibodies to factor H	[156]
		Factor H *CFH-H3* and MCP *ggaac* risk haplotypes		[156, 161]
	Acquired	Anti-factor H antibodies	Associated with genetic rearrangements or deletions in factor H-related proteins	[169]
		Monoclonal gammopathy		[236]
Mutations in diacylglycerol kinase-ε (*DGKE*)		Loss-of-function recessive mutations		[32]
Autoimmune	Systemic lupus erythematosus			[237]
	Anti-phospholipid syndrome			[238]
	Scleroderma			[239]
Pregnancy related	HELLP syndrome		May be associated with complement dysregulation	[240]
	Postpartum			[240]
Transplantation	Solid organ			[241]
	Bone marrow			[242]
	CMV viraemia			[243]

Table 1 (*Continued*)

Aetiology		Cause and features	Comment	Reference
Malignancy	Cancer chemotherapy	Mitomycin, cisplatin, bleomycin		[244]
	Ionizing radiation			[245]
Drugs	Quinine			[244]
	Calcineurin inhibitors		Also in combination with everolimus	[244]
	Oral contraceptives			[244]
	Antiplatelet agents	Clopidogrel, ticlopidine		[244]
	VEGF inhibitors			[246]
Malignant hypertension				[247]
Cobalamin metabolism		Cobalamin type C MMACHC mutations, methylmalonic aciduria and homocystinuria		[248]
Denys–Drash syndrome		*WT1* mutations		[249]
Unknown		May be familial		[36]

MCP, membrane cofactor protein; HELLP, haemolysis, elevated liver enzymes, low platelets; VEGF, vascular endothelial growth factor; CMV, cytomegalovirus; MMACHC, methylmalonic aciduria and homocystinuria, cblC type; WT, Wilm's tumour.

associated HUS and post-transplant HUS may be associated with complement mutations [1, 2]. Patients with HUS, regardless of aetiology, present with pallor, signs and symptoms of kidney failure, possible jaundice and/or bleeding and purpura.

EHEC-associated HUS

The most common cause of HUS is gastrointestinal infection with EHEC. EHEC infection was first associated with haemorrhagic colitis during an outbreak in the USA in 1982 [3] and at approximately the same time was associated with HUS in sporadic cases [4]. EHEC-associated HUS may occur in larger or smaller outbreaks [5–10] or in sporadic cases, and typically presents as haemolytic anaemia, thrombocytopenia and acute renal failure developing after gastroenteritis, within 2–12 days after the debut of diarrhoea, which may manifest as haemorrhagic colitis with bloody diarrhoea. Approximately 15% of cases of EHEC-associated gastroenteritis will develop HUS [11], although the gastroenteritis itself may be very severe and cause morbidity (rectal prolapse, colonic gangrene or perforation) and even mortality [12]. The use of antibiotics and antimotility agents during the gastrointestinal phase of infection may increase the risk of developing HUS [13, 14]. Furthermore, young children (<5 years) and the elderly are more prone to develop HUS [11, 15] although HUS developed mostly in middle-aged women during the more recent large German outbreak of EHEC in 2011 [16].

Patients typically present with acute pallor and symptoms of renal failure (oedema, nausea and emesis, oliguria and/or high blood pressure). In addition to renal failure, extra-renal manifestations may occur including cardiac, neurological, respiratory and pancreatic involvement [17–21] as well as elevated liver function tests. Neurological symptoms may vary from mild jerks to severe coma or stroke in approximately 30% of cases and are associated with a worse outcome. Other factors related to a worse outcome are leukocytosis [22, 23] and low platelet counts [22, 24]. EHEC-associated HUS usually does not recur.

aHUS

aHUS may be sporadic or familial and is associated with an underlying dysregulation of the alternative

pathway of complement. The complement abnormality may be a mutation, genetic rearrangement or deletion in a gene encoding a complement factor, or the presence of a homozygous complement gene haplotype or of an autoantibody to complement regulator factor H. The complement abnormality itself is not sufficient for development of disease as unaffected family members of patients with aHUS may carry the same genetic aberration. Patients may present during childhood or adulthood, and episodes may be triggered by infections, transplants or pregnancy [1, 25]. Recurrences triggered by infections are not associated with one specific pathogen. The preceding infection may manifest with diarrhoea, and thus, some patients may present in a similar manner to patients with EHEC-associated HUS; this represents a clinical challenge, although the onset of aHUS is generally less abrupt than that of EHEC-associated HUS. The course of disease is characterized by recurring episodes of acute disease ultimately leading to end-stage renal failure, although terminal renal failure may already occur at presentation. The disease may recur after transplantation.

Extra-renal manifestations may also occur and are, in part, secondary to vascular injury induced by complement activation. These include digital gangrene, cerebral or peripheral vessel stenosis, ophthalmological and neurological involvement as well as pulmonary and pancreatic complications [26–29].

Streptococcus pneumoniae-associated HUS

Haemolytic uraemic syndrome occurring during pneumococcal infection manifests simultaneously with pneumonia and in more severe cases sepsis, meningitis, hepatocellular injury and/or peritonitis [30]. Patients may be severely ill, exhibiting multi-organ involvement and possibly disseminated intravascular coagulation (DIC) [31].

Diacylglycerol kinase-ε (DGKE)-associated HUS

A rare but distinctive subtype of HUS is associated with mutations in the *DGKE* gene. Patients usually present with HUS as infants with hypertension, haematuria and proteinuria eventually leading to renal failure [32, 33].

Epidemiology

EHEC-associated HUS occurs primarily in children younger than 5 years of age and in the elderly [34, 35]. After an incubation period of 4–7 days, EHEC-infected patients develop diarrhoea [36] and approximately 15% of cases develop HUS [11] within an additional 2–10 days. Patients may be infected by intake of contaminated food including raw, processed or undercooked meat, vegetables, unpasteurized juice or milk products, cross-contamination of food products and utensils, intake of contaminated water, even from swimming pools [5, 37–42], person-to-person transmission [43, 44] or contact with animals bearing the strain [45]. Transmission occurs more often in summer [46], requires a very low number of bacterial organisms [47] and occurs in outbreaks or sporadically. Very large outbreaks have occurred in Japan [48] and in Germany [16], but smaller outbreaks have been reported in numerous countries [5–10]. In countries in which intake of raw meat is higher, EHEC infection is endemic and HUS rates are thus higher, such as in Argentina [49]. The incidence in Argentina has been reported to be as high as 12.2 cases per 100 000 children younger than 5 years of age [50]. It is difficult to assess the annual incidence of EHEC-associated HUS, but overall rates corresponding to two per 100 000 for all age groups have been reported and up to six per 100 000 in children younger than 5 years of age [51].

Many strains of *E. coli* have been associated with clinical disease including sorbitol non-fermenting and fermenting *E. coli* O157 as well as *E. coli* O26, O103, O111 and O145. *E. coli* O104:H4 was the specific strain isolated during the large German outbreak in 2011. This is a hybrid strain bearing characteristics of both EHEC strains (producing Shiga toxin) and enteroaggregative *E. coli* (EAEC) strains (with regard to the pattern of intestinal colonization) [52].

aHUS is an ultra-rare disease with an estimated incidence that is most probably between 0.5 and 2 per million [53, 54]. Onset may occur at any age but is more frequent in childhood [55] particularly before the age of 2 years [56]. Onset before 6 months of age is highly indicative of aHUS as EHEC-associated HUS is uncommon in this age group. The onset is usually triggered by a febrile infection in the respiratory or gastrointestinal tract. Patients who do not develop end-stage renal failure during the first episode tend to relapse, and the disease may affect several members of the same family [57].

Laboratory diagnosis of HUS

HUS is defined as the simultaneous occurrence of haemolysis, thrombocytopenia and acute renal failure. The initial laboratory investigation required to make a diagnosis of HUS should include haematological, biochemical and microbiological assays for the detection of haemolytic anaemia, thrombocytopenia, renal failure and EHEC infection (see Table 2). Biochemical abnormalities may be related to intestinal and renal losses of proteins and electrolytes, as well as extra-renal affection of the liver and pancreas. Urinalysis will reveal glomerular injury with casts, haematuria and proteinuria. Blood cultures are usually negative, except for cases of invasive *S. pneumoniae*-associated HUS. The clinical investigation for determining the underlying cause of HUS is described below.

Pathology

The pathological lesion observed in the kidneys of patients with HUS is termed thrombotic microangiopathy (TMA). Patients seldom undergo renal biopsies during the acute phase of disease due to

Table 2 *Laboratory diagnosis of haemolytic uraemic syndrome (HUS)*

Analysis	Feature	Assay
Haematological	Haemolysis	Lactic dehydrogenase
		Reticulocyte count
		Haptoglobin
		Unconjugated bilirubin
		Blood smear (red blood cell fragmentation)
		Direct antiglobulin test (also known as Coombs test)
	Thrombocytopenia	Platelet count
	Leukocytosis, neutrophilia[a]	Neutrophil count
	Normal coagulation	Coagulation screen[b]
Biochemical	Renal failure	Elevated serum creatinine and urea
		Hyperkalaemia
		Acidosis
	Gastrointestinal losses	Hyponatraemia[a]
		Hypoalbuminaemia[a,c]
	Pancreatic effects	Hyperglycaemia[a]
	Hepatic effects	Elevated LFTs[a]
Microbiological	Gastrointestinal infection	Faeces: culture, or PCR for EHEC genes (*stx*, *eae*), or ELISA for free Shiga toxin
		Serology: ELISA for EHEC virulence factors (serotype-specific lipopolysaccharide, Shiga toxin or adhesins) [250, 251]
	Bacteraemia	Blood culture, spinal fluid[d]
	Urinary tract infection	Urine culture[e]
	Streptococcus pneumoniae infection	T antigen lectin binding assay
Urinalysis	Haematuria	Dip stick, microscopy, chemistry
	Proteinuria	
	Glomerular injury	

EHEC, enterohaemorrhagic *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; LFT, liver function test; T antigen, Thomsen–Friedenreich antigen.
[a]Associated with EHEC-associated HUS. [b]To rule out consumption of coagulation factors, although fibrin split products may be elevated. [c]Low levels of serum proteins may be due to intestinal and urinary losses. [d]Usually negative in all forms of HUS except for invasive *S. pneumoniae* infection. [e]EHEC is usually detected in faeces but may also be isolated from urine [252].

ongoing thrombocytopenia. Our knowledge of renal pathology is therefore obtained either from post-mortem specimens or from biopsies carried out in more severe cases, or in those patients in whom the diagnosis is unclear. TMA is characterized by specific lesions in glomeruli including micro-thrombi and microaneurysms in glomerular capillaries. Fragmented red blood cells may be visible in the lumina. The capillary endothelial cells are swollen and detached from the basement membrane. Ultramorphological examination reveals subendothelial lucent flocculent material [58]. Mesangial expansion as well as mesangiolysis is observed by light microscopy [58]. Similar lesions are seen in arterioles and arteries of the renal cortex consisting of thrombi and endothelial detachment. The extensive vascular injury with occluded vessels leads to reduced glomerular filtration and ischaemic damage resulting in renal cortical necrosis in the most severe cases. Thus, the entire nephron is affected and tubular damage, particularly in EHEC-associated HUS, is a prominent feature [59].

Chronic renal changes, particularly associated with aHUS, include the appearance of double contours of capillary walls with mesangial interposition and the formation of new basement membrane (the latter visible by electron microscopy) [60]. Myo-intimal concentric proliferation presenting an 'onion-skin' appearance in arterioles and arteries is usually associated with severe hypertension [58].

The intestinal lesion seen during EHEC-associated HUS consists of erosions and ulcerations, leading to transmural perforation in severe cases, oedema, hyperaemia, inflammatory infiltrates and haemorrhage, fibrin exudates, vascular thrombosis, mucosal or mural necrosis and pseudomembrane formation [61, 62].

Current understanding of the pathophysiology of HUS

EHEC-associated HUS

Shiga toxin-producing EHEC strains colonize the intestine after ingestion. Bacteria initially colonize the terminal ileum [63] followed by specific attachment to colonic enterocytes generating a so-called attaching and effacing lesion [64]. Colonization is facilitated by an interaction with the intestinal microflora, in a process termed quorum sensing, enabling bacterial communication between strains via genetically encoded mediators [65, 66]. The same mechanism also enables communication with host-derived hormones, such as catecholamines, thus promoting adhesion and virulence and the release of Shiga toxin in the intestine [67].

There is no bacteraemia during EHEC infection as the strain is non-invasive [68]. Thus, toxin released into the intestine must translocate via enterocytes, or between the cells, to gain access to the circulation and thus reach its target organs (mainly the kidneys and brain). The presence of toxin in the kidneys of patients and in *in vivo* models [69–71] suggests that the toxin is transferred from the intestine to the kidneys. The manner by which the toxin is taken up from the intestine *in vivo* is, as yet, unknown but may include binding of the toxin's pentameric B subunit to its receptor, globotriaosylceramide (Gb3 or CD77), on intestinal epithelial cells or Paneth cells [72, 73] and holotoxin uptake, or paracellularly, in a process enhanced by counter-migration of neutrophils towards the intestinal lumen [74]. Alternatively, the toxin may be taken up by macropinocytosis [75] or within bacterial outer membrane vesicles [76]. Intracellularly, Shiga toxin induces cell death by binding of its enzymatically active A subunit to ribosomal RNA and inhibition of protein synthesis [77]. The toxin induces intestinal cell apoptosis [62] and profound intestinal inflammation [78], which may further promote bacterial colonization and toxin release by means of quorum sensing [66]. The immune response to pathogen-associated molecular patterns (PAMPs) primed in the intestine is also essential for elimination of the organism [79, 80].

An antibody response is generated upon intestinal colonization by EHEC. Patients develop antibodies to the serotype-specific lipopolysaccharide (LPS), Shiga toxin and intestinal adhesins. It is unclear whether these antibodies are protective, but the lower incidence of EHEC infections in countries endemic for enteropathogenic *E. coli* (EPEC) infections (intestinal strains that express certain adhesins that are homologous to those expressed by EHEC) would suggest a degree of antibody-mediated protection [81], a finding confirmed by *in vivo* studies [82].

During haemorrhagic colitis, Shiga toxin, which has translocated across the intestinal mucosal barrier, will gain access to the circulation. This may be achieved by binding to and injury of intestinal endothelial cells [83]. Free toxin in the bloodstream is minimal [84, 85], but the toxin binds to neutrophils, monocytes, platelets and red

blood cells [86–91] demonstrated *in vivo* on platelets and leukocytes, and thus circulates in the bloodstream. Elevated neutrophil counts are associated with a worse prognosis [22, 23] possibly due to the enhanced ability to transfer toxin as well as the destructive properties associated with proteases released by activated neutrophils. Shiga toxin bound to blood cells may be taken up by the cells [89] although most blood cells are resistant to the cytotoxic effects of the toxin. Cells that lack protein synthesis, such as platelets and red blood cells, would not be negatively affected by the toxin, but even leucocytes appear to be resistant to the cytotoxic effects. On the contrary, platelets and leucocytes are activated by the toxin [92].

Toxin may be released from blood cells within microvesicles [71]. These microvesicles, originating from host blood cells and bearing contents of the parent cell, plus Shiga toxin, evade the host immune response and are taken up by kidney glomerular and peritubular capillary endothelial cells. Within the renal cells, the toxin is released, and the enzymatically active A subunit is transported in a retrograde manner to ribosomal RNA [71]. Thus, blood cell-derived microvesicles appear to be important for the transfer of toxin from blood cells to the target organ cell (Fig. 1). The toxin thereby reaches the glomerular endothelial cell and the tubular epithelial cell (microvesicles pass through the tubular basement membrane) in which it has been shown to induce cell death [59, 71, 93].

Blood cell-derived microvesicles originating from platelets, monocytes, neutrophils and red blood cells were shown to be elevated in acute blood samples from patients with EHEC-associated HUS [86, 92, 94, 95], decreasing after recovery to normal values. Microvesicles expose phosphatidylserine as well as tissue factor [92], both of which promote thrombosis.

The main manifestations of HUS are acute haemolytic anaemia, thrombocytopenia and renal failure. These features can be explained by the effects of Shiga toxin, in conjunction with other bacterial virulence factors, and the host response, as shown schematically in Fig. 2 (for review see [78]).

Haemolysis

Red blood cell fragmentation is a major feature of the haemolytic process during HUS. Schistocytes are seen on blood smears, sometimes in the form of helmet cells. The fragmentation has been attributed to mechanical breakdown of red blood cells in capillaries partially occluded by microthrombi [96]. Alternatively, red blood cells may fragment due to oxidative damage, as alterations in glutathione metabolism were found in one study in patients with HUS [97].

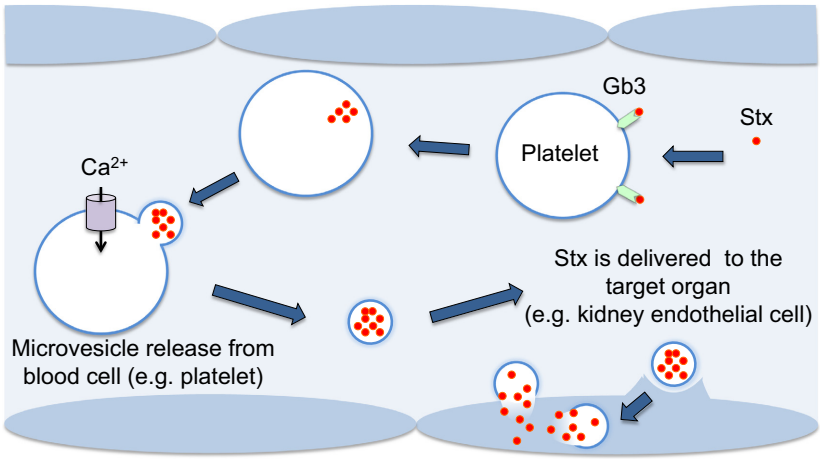

Fig. 1 *Schematic representation of Shiga toxin transfer from the circulation to the kidney. Suggested sequence of events during Shiga toxin transfer presented within a blood vessel. Once within the bloodstream, Shiga toxin binds to its receptor on blood cells, for example globotriaosylceramide (Gb3) on platelets. The toxin is internalized and the activated blood cell releases microvesicles containing the toxin. The blood cell-derived microvesicles circulate and thus reach the target organ where they are taken up by endothelial cells. In the kidney, this has been shown to occur within glomerular and peritubular capillary endothelial cells. Toxin is released from microvesicles within the cells. Stx, Shiga toxin.*

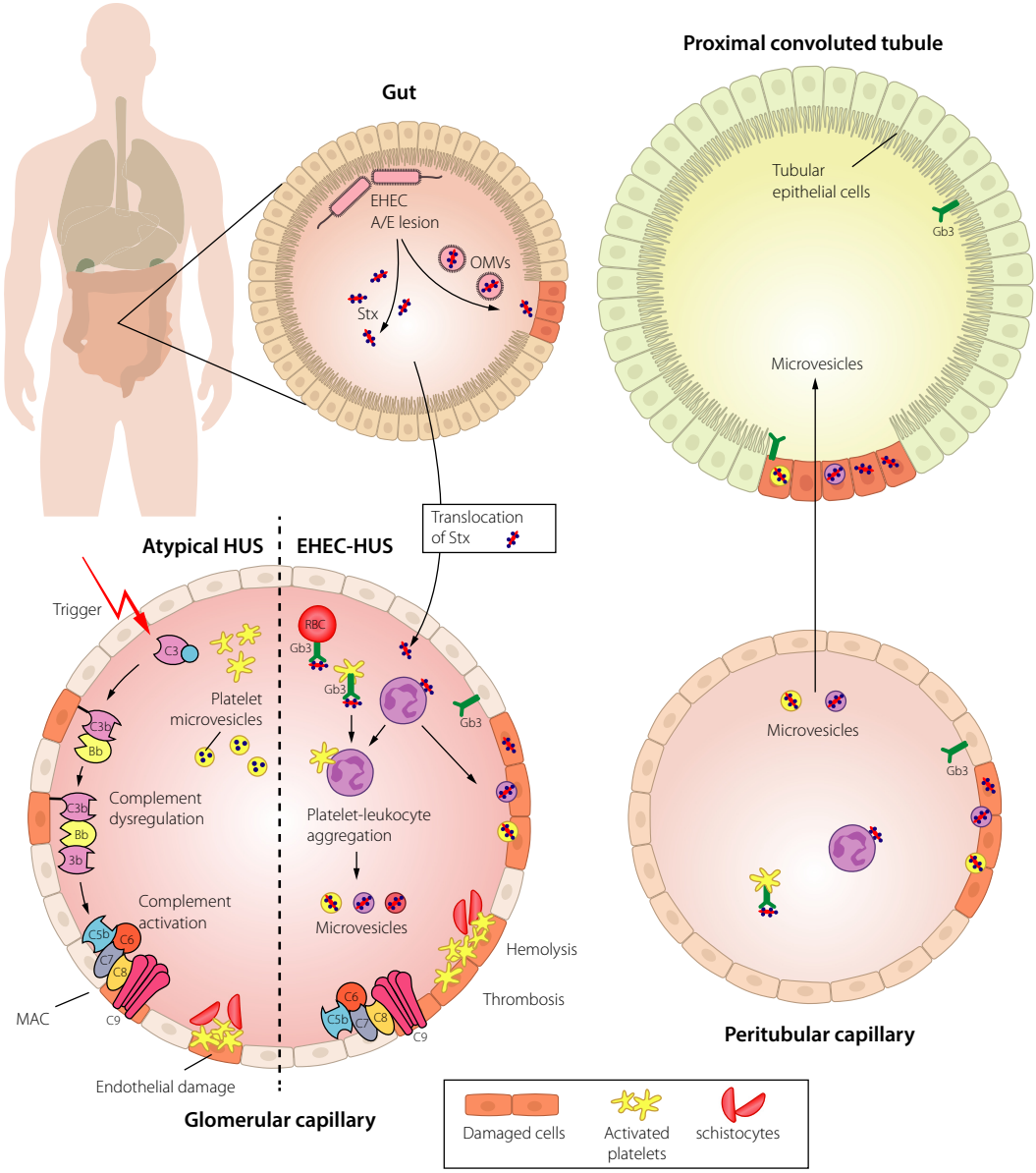

Fig. 2 *Pathophysiology of enterohaemorrhagic* Escherichia coli *(EHEC)-associated haemolytic uraemic syndrome (HUS) and atypical HUS (aHUS). EHEC-associated HUS is presented in all panels except the lower left panel, which shows the proposed pathophysiology of aHUS. EHEC colonizes the gut, mainly the colon, forming intimate attaching and effacing lesions and releasing Shiga toxin. After injury to the intestinal epithelium and endothelium, the toxin gains access to the circulation and binds to blood cells on which it circulates. Binding to platelets and leucocytes activates these cells. Toxin released in the circulation or within microvesicles undergoes endocytosis in glomerular and peritubular capillary endothelial cells damaging these cells. The combination of activated platelets and damaged endothelium induces thrombosis. Red blood cells are fragmented mechanically on microthrombi in combination with complement-induced haemolysis. Microvesicles transfer toxin between cells, as well as via the basement membrane to the tubular epithelium, thus affecting the entire nephron. In aHUS, uninhibited complement activation on the host endothelium and platelets induces cell injury and a prothrombotic state with fragmentation of red blood cells. Complement is deposited on the cells, and platelet-derived microvesicles are released into the circulation. A/E lesion, attaching and effacing lesion; Stx, Shiga toxin; OMV, outer membrane vesicle; RBC, red blood cell.*

Shiga toxin binds to red blood cells via the Gb3 receptor known as the Pk antigen (an antigen within the P1PK blood group system) and present on most red blood cells [87]. Our group recently showed that Shiga toxin induced haemolysis and that this process involved complement deposition on red blood cells [86]. This is of interest because complement activation on red blood cells is known to induce haemolysis [98]. Patients with EHEC-associated HUS were shown to have complement activation on their red blood cells as well as circulating red blood cell-derived microvesicles coated with C3 and C9 [86]. Thus, as deposition of complement on red blood cells occurs during EHEC-associated HUS, it seems plausible that its presence contributes to the haemolytic process. The role of complement in EHEC-associated HUS will be reviewed below.

Shiga toxin was recently shown to modulate erythroid maturation *in vitro* [99]. Patients with EHEC-associated HUS exhibit reticulocytosis during the acute phase of disease, i.e. bone marrow erthryoid maturation does not seem to be affected, and thus, this finding may not have clinical bearing.

Thrombocytopenia

Low platelet counts in HUS are the result of platelet activation and deposition of aggregates in microthrombi along the damaged vascular wall. Platelet activation occurs due to exposure of the subendothelium secondary to toxin-induced endothelial cell damage whereby platelets interact with fibrinogen, collagen and von Willebrand factor to form aggregates [100]. In addition, platelets are activated directly by Shiga toxin and LPS [89, 92, 101] and by cytokines released by activated monocytes or endothelial cells [102, 103]. Platelets derived from patients with HUS show evidence of activation as they are degranulated [104], and have reduced intracellular β-thromboglobulin levels and an impaired response to aggregation [105]. O157 LPS and Shiga toxin can activate platelets [89, 106], and platelet-derived microvesicles are released *in vitro* by stimulation with these bacterial virulence factors [92, 107], and *in vivo* in patients, reflecting the degree of platelet activation.

Platelets have a role in the inflammatory process by interacting and forming complexes with leucocytes [92] and by releasing proinflammatory cytokines [108, 109]. Platelets play a most important role in the formation of microangiopathic lesions during HUS, and low platelet counts are correlated with the degree of renal dysfunction [22, 24].

The prothrombotic process

The thrombotic events that occur during EHEC-associated HUS are secondary to endothelial cell injury, enhanced platelet activation on the subendothelium, thrombin generation, tissue factor release, elevated levels of microvesicles in the circulation and decreased fibrinolysis. Coagulation abnormalities occur during the gastrointestinal phase of infection, preceding the development of HUS. Children who later developed HUS exhibited elevated plasma concentrations of prothrombin fragment 1 + 2, tissue plasminogen activator (t-PA) antigen, t-PA–plasminogen activator inhibitor type 1 (PAI-1) complex and D-dimer [110]. Prothrombotic markers were elevated during HUS and fibrinolysis was inhibited. Likewise, tissue factor levels were shown to be high [111] and platelet–leucocyte complexes that expressed tissue factor were elevated in patient samples [92]. Microvesicles released from monocytes and platelets expressed tissue factor as well as phosphatidylserine [92], both of which contribute to thrombosis. These findings could be reproduced *in vitro* by stimulation of endothelial cells with Shiga toxin [112], and whole blood with Shiga toxin and O157 LPS, suggesting that the toxin together with LPS induces the prothrombotic state via damage to the endothelium, activation of platelets and release of tissue factor and microvesicles [92].

Renal failure

The pathogenetic mechanisms leading to acute renal failure during EHEC-associated HUS are associated with prothrombotic vascular injury, as outlined above, triggering the formation of occluding microthrombi in glomeruli, as well as acute toxin-induced tubular injury [59, 113]. The toxin itself reaches the kidney [69–71] affecting glomerular (endothelial cells, podocytes and mesangium) and tubular cells [59, 114–116]. In addition, there is activation and influx of neutrophils, corresponding to the severity of renal failure [117, 118], and of platelets within microthrombi [109]. Thus, multiple cell types may release potent inflammatory mediators and enzymes. Furthermore, cytokines, chemokines, soluble adhesion molecules, growth factors, cytokine receptors and acute-phase response proteins are elevated in EHEC-associated HUS patients [78, 119–130] and may contribute to

the progression of renal damage particularly as elevated cytokine levels have been demonstrated in the urine of patients with HUS [121]. The chemokine receptor CXCR4/CXCR7/stromal cell-derived factor 1 pathway is also activated *in vivo*, and *in vitro* by Shiga toxin, thus also contributing to renal damage [131]. Finally, activation of the complement system [132] may induce chemotaxis and cytolysis and further contribute to the tissue injury as described below.

Injury to the central nervous system

Central nervous system (CNS) affection carries a worse prognosis for full recovery and is observed in 30–60% of patients [18, 133]. The pathogenetic mechanisms involved are similar to those described in the kidney with toxin binding to neurons and endothelial cells in the CNS [134], damage to the blood–brain barrier and the induction of multiple inflammatory mediators (for review, see [78]).

Complement activation during EHEC-associated HUS

There is evidence for complement activation during EHEC-associated HUS, primarily via the alternative pathway. Patients have been found to have low plasma levels of C3 [135, 136] and elevated levels of complement degradation products such as factors Bb, C3a and soluble C5b-9 [94, 137, 138]. Levels of factors Bb and C5b-9 correlated with the presence of oliguria [137]. Circulating platelet- and monocyte-derived microvesicles coated with C3 and C9 as well as C3 deposits on platelet–monocyte aggregates were observed in paediatric HUS patients during the acute phase of disease [94]. Likewise, C3 deposits were observed on red blood cells, and red blood cell-derived microvesicles were coated with both C3 and C9 [86]. C5b-9 deposits were also found in the human kidney during EHEC-associated HUS [139]. Thus, the extensive endothelial injury and blood cell activation during EHEC-associated HUS lead to secondary complement activation.

In vitro studies have shown that Shiga toxin incubated with normal whole blood induced the formation of leucocyte–platelet aggregates and the release of platelet- and monocyte-derived microvesicles coated with C3 and C9 deposits [94]. Similarly, red blood cell-derived microvesicles coated with C9 were demonstrated, in a process dependent on activation of the alternative pathway [86].

In vivo models using EHEC infection or Shiga toxin and LPS have also confirmed the importance of the alternative pathway for complement activation in the kidneys. These studies demonstrated C5b-9 deposition in glomeruli when mice were infected with EHEC [139], an effect inhibited by anti-C5 antibody. Similarly, complement deposits were observed on podocytes, associated with their dysfunction, after mice were injected with Shiga toxin and LPS; this effect was inhibited by a C3a receptor antagonist [132]. Glomerular fibrinogen deposition was decreased in EHEC-infected mice treated with anti-C5 as well as C6-deficient mice [139] and reduced in Shiga toxin/LPS-injected mice treated with the C3a receptor antagonist [140], in which platelet aggregates also decreased. This aspect is of importance as circulating C3a and C5b-9 may activate platelets [141, 142] and soluble C5b-9 enhances expression of tissue factor on the endothelium [143].

The mannan-binding lectin (MBL) pathway of complement activation is triggered by binding to bacterial surface components. Although MBL deficiency may predispose to infection, it does not seem to predispose to EHEC-associated HUS [144].

Overactivation of the complement system on host renal and blood cells may have an injurious effect. Shiga toxin and other EHEC virulence factors such as LPS are capable of activating complement, mainly via the alternative pathway. Complement activation most probably contributes to toxin-induced cell injury and prothrombotic reactions in concert with other harmful effects induced by the bacteria and the host response.

Atypical HUS

Atypical HUS is primarily mediated by dysfunctional complement regulation resulting in complement activation on host cells via the alternative pathway [57]. Complement deposition occurs in an uninhibited manner on the endothelium and on platelets [94, 145, 146]. A majority of patients with aHUS have heterozygous mutations in complement components, either loss-of-function mutations in regulators such as factor H [147], factor I [148], membrane cofactor protein (MCP/CD46) [149] or thrombomodulin [150] or gain-of-function mutations in C3 [151, 152] or factor B [153]. One pedigree has been described with a heterozygous mutation in clusterin, which affects regulation of the terminal complement pathway [154]. In

addition, patients may have hybrid genes between factor H and factor H-related proteins [155], rearrangements or homozygous deletions in factor H-related proteins (mostly factor H-related proteins 1 and 3), which are often associated with antibodies to factor H [156–158]. These deletions are also prevalent in unaffected individuals in the general population, but the presence of anti-factor H antibodies predisposes the individual to aHUS.

Certain polymorphisms in the factor H, MCP or factor H-related protein 1 genes have been ascribed a risk profile [157, 159, 160], and the constellation of certain haplotypes in the factor H [156] or the MCP [161] genes is associated with enhanced risk of developing aHUS. The presence of risk haplotypes in both the factor H and the MCP genes increases the penetrance of disease amongst mutation carriers [162].

Most, but not all, studied mutation phenotypes lead to activation of complement *in vitro*. A disease-associated complement mutation or antibodies to factor H are found in about 70% of patients [57]. A small percentage of patients (3–5%) may have mutations in more than one complement gene [162]. aHUS occurring in more than one family member is associated with 20–30% of cases, and this is due to variable penetrance, except for rare cases with homozygous mutations, in which the disease penetrance is high [57]. The complement gene products reported to be associated with aHUS are depicted in Fig. 3, and their known functions are summarized in Table 3. The mechanism by which cell injury occurs on the endothelium and platelets is shown in Fig. 2.

Factor H

Factor H mutations account for approximately 30% of aHUS complement mutations. Factor H is the main regulator of the alterative pathway functioning both in the fluid phase and on cell surfaces. It is composed of 20 short consensus repeats and the gene is composed of 23 exons. The N-terminus of the protein is associated with cofactor activity for factor I and decay of the C3 convertase, whereas the host recognition properties are localized at the C-terminus (Table 3) [163]. Many factor H mutations have been described and the majority of the aHUS-associated mutations are localized at the C-terminus [156]. aHUS-associated mutations are listed in a database available online (http://www.fh-hus.org/). Patients with factor H mutations do not necessarily have low factor H or C3 levels, although rare cases of homozygous mutations in factor H usually do [146, 164]. Studies

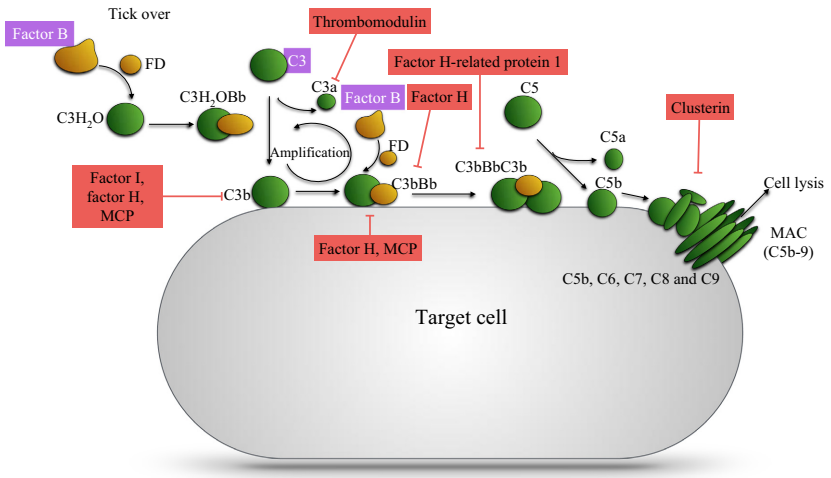

Fig. 3 *Complement activation via the alternative pathway on cells and mutations in atypical haemolytic uraemic syndrome (aHUS). The figure shows alternative pathway activation, from the low-grade 'tick-over' binding of $C3H_2O$ to factor B in the presence of factor D (FD) to formation of the definitive C3 convertase (C3bBb). The C3 convertase continuously cleaves C3 via the amplification loop, when uninhibited, and proceeds to form the C5 convertase (C3bBbC3b) by binding more C3b. C5 convertase cleaves C5 and thus contributes to formation of the membrane attack complex (MAC or C5b-9). Complement regulators that are mutated or deleted in aHUS are shown in red; complement proteins contributing to the formation of the C3 convertase and mutated in aHUS are shown in purple. A preliminary version of this figure appeared in the Ph.D. thesis of I. Arvidsson.*

have shown that mutant variants of factor H are incapable of protecting endothelial cells [145] and platelets [165] from complement activation via the alternative pathway, thus explaining the endothelial cell injury and platelet activation occurring in aHUS cases with these mutations. Serum complement deposition on endothelial cells may be used as an assay to monitor disease activity [166]. Furthermore, mutant factor H enables complement activation to occur on platelets and the release of tissue factor- and phosphatidylserine-expressing platelet microvesicles contributing to the prothrombotic process [165].

The role of factor H in aHUS was demonstrated *in vivo* in a mouse model lacking the C-terminus five short consensus repeats of factor H (FHΔ16-20 mice) that developed spontaneous HUS [167]. Mice that were, in addition, C5-deficient were protected from this phenotype, demonstrating the importance of the terminal complement cascade for the development of renal lesions in thrombotic microangiopathy [168].

Anti-factor H antibodies

In addition to factor H mutations that neutralize the host cell recognition properties of the protein, autoantibodies may have a similar effect [169]. The antibodies are mostly, but not only, directed to the C-terminal and can affect cell surface protection as well as the interaction between factor H and C3 [170, 171]. Factor H antibodies account for approximately 5–10% of aHUS cases. The level of antibodies is related to disease activity and may affect C3 levels as well [172]. Anti-factor H antibodies are associated with rearrangements or deletions in factor H-related proteins. Factor H-related protein 1 may have a regulatory function in the terminal complement cascade [173], and thus, its deletion or the presence of hybrid genes may promote formation of the membrane attack complex (MAC). Furthermore, hybrid genes may affect the regulatory function of factor H [174] and thus promote complement activation.

Factor I

Factor I mutations account for <10% of aHUS-associated mutations. Factor I is encoded by a gene consisting of 13 exons [175]. It is a serine protease active in the fluid phase that, within the alternative pathway, cleaves C3b to its inactive form iC3b in the presence of the cofactors factor H, complement receptor 1, MCP or von Willebrand factor [176]. Similarly, within the classical pathway, it cleaves C4b in the presence of C4-binding protein, complement receptor 1 or MCP [177]. Most aHUS-associated mutations are located within the serine protease domain. Mutations may affect protein secretion or enzymatic function [178], but not all mutations have been shown to affect protease activity [179]. Factor I mutations in conjunction with additional aHUS-associated mutations may affect the patient phenotype [180].

MCP/CD46

Membrane cofactor protein is a membrane-bound protein with an intracellular anchor, a transmembrane domain and four extracellular short consensus repeats [181]. It functions as a cofactor for factor I-mediated cleavage and inactivation of C3b and C4b. The *MCP* gene is composed of 14 exons, and heterozygous mutations, mostly localized in the region encoding the extracellular domain, are the cause of up to 15% of aHUS cases [149]. Mutations affect expression of the extracellular domain, thereby binding to C3b and cofactor activity [182]. Decreased MCP expression on the cell surface can be detected by flow cytometry of leucocytes [183].

C3

Mutations in C3 account for up to 10% of aHUS-associated mutations. The gene is composed of 41 exons, and mutations may be localized throughout the gene [151]. C3 levels are usually low. Mutations affect binding to factor H, thus reducing its regulatory capacity, or enhance binding to factor B resulting in a hyperfunctional C3 convertase and complement deposition on endothelial cells and platelets [151, 152, 184].

Factor B

The factor B gene is composed of 18 exons. Factor B is cleaved *in vivo* into factors Ba and Bb, the latter binding to C3b to form the C3 convertase. Similar to C3 mutations, mutations in factor B may result in a hyperfunctional C3 convertase [185] or a C3 convertase resistant to decay by factor H [186]. However, not all mutations have been shown to cause protein dysfunction *in vitro* [27, 186]. Only a limited number of mutations in factor B have been demonstrated in patients with aHUS to date and these account for up to 4% of aHUS cases [187].

Thrombomodulin

Thrombomodulin mutations are rare in aHUS, observed in approximately 3% of cases. The thrombomodulin gene is intron-depleted [188].

Table 3 *Complement proteins associated with atypical haemolytic uraemic syndrome (aHUS) and their function*

Complement protein	Pathway	Soluble or membrane bound	Complement factor or regulator	Function
Factor H	Alternative	Soluble	Regulator	• Cofactor for factor I in C3b cleavage • Accelerates decay of the C3 convertase • Host cell recognition
Factor H-related protein 1	Terminal	Soluble	Regulator	• Inhibits the C5 convertase
Factor I	Alternative and classical	Soluble	Regulator	• Cleaves C3b to iC3b (inactive form) in the presence of cofactors: factor H, C4-binding protein, MCP, complement receptor 1 or von Willebrand factor
MCP (CD46)	Alternative	Membrane bound	Regulator	• Cofactor for factor I-mediated C3b cleavage
Thrombomodulin	All	Membrane bound	Regulator	• Enhanced factor I-mediated C3b cleavage with cofactor factor H • Generates TAFI, which inactivates C3a and C5a
Clusterin	Terminal	Soluble	Regulator	• Inhibits MAC formation
C3	Alternative and classical	Soluble	Factor	• C3 cleavage to C3a and C3b has anaphylactic, chemotactic and antimicrobial properties • C3b forms the C3 convertase with factor B and further binds to form the C5 convertase • C3b and its inactive form, iC3b, are opsonins
Factor B	Alternative	Soluble	Factor	• Binds to C3 and is cleaved by factor D to form the C3 convertase C3bBb

MCP, membrane cofactor protein; TAFI, thrombin-activatable fibrinolysis inhibitor; MAC, membrane attack complex. Modified with permission from [253].

Thrombomodulin is a transmembrane glycoprotein expressed on vascular endothelial cells. It serves as a cofactor for thrombin-mediated activation of thrombin-activatable fibrinolysis inhibitor (TAFI) to TAFIa. TAFIa inactivates C3a and C5a. In addition, thrombomodulin binds to C3b and factor H and enhances factor I-mediated inactivation of C3b in the presence of factor H. Mutations were shown to enhance complement activation by diminishing these functions [150].

Clusterin

A heterozygous clusterin mutation has been described in one family in which siblings were affected by aHUS and poststreptococcal glomerulonephritis [154]. The prevalence of mutations in the clusterin gene is hard to assess, as it is not assayed regularly. Clusterin regulates the formation of the terminal complement cascade MAC. The mutant variant could not prevent assembly of the MAC on platelets and red blood cells, thus promoting platelet activation and haemolysis.

Mutations, gene rearrangements and auto-antibodies are all predisposing factors for the development of aHUS. However, family members of patients with aHUS may carry the same mutation without being affected. It is thus assumed that a 'second hit' is necessary to trigger aHUS such as additional complement mutations or risk-associated haplotypes, infection or pregnancy [57] (Fig. 2). Once haemolysis is induced, heme is released and may further activate the complement system in the fluid phase and on cell surfaces particularly in the setting of mutated complement proteins [189].

An important aspect of aHUS is that disease recurrences occur in the presence of viable renal tissue. Thus, haematological recurrences associated with haemolysis and thrombocytopenia occur only in patients with residual renal function and cease to recur once terminal renal failure occurs. This may indicate that an interaction between components of renal tissue and the complement system could

activate disease activity. Patients may, however, have ongoing complement activation in the vasculature even in the absence of renal tissue [27].

Streptococcus pneumoniae-associated HUS

Streptococcus pneumoniae-associated HUS is a rare form of HUS occurring in both children and adults during invasive neuraminidase-producing pneumococcal infection. Neuraminidase activity cleaves N-acetyl neuraminic acid (sialic acid) on red blood cells, endothelial cells, renal epithelial cells and platelets and thus exposes the Thomsen–Friedenreich antigen (T antigen, Galb1-3GalNAc) [30, 190]. Exposure of the T antigen is used to diagnose this condition with a lectin assay [191]. It has been assumed that neuraminidase activity may precipitate HUS. This assumption is strengthened by the fact that influenza A infection may also precipitate HUS [192], and these influenza strains also produce neuraminidase. However, the evidence is circumstantial and no specific pneumococcal strain or neuraminidase profile has been associated with HUS [193].

Interestingly, factor H binds to sialic acid on host cells. Thus, cleavage of sialic acid by neuraminidase may reduce the capacity of factor H to protect host cells from complement deposition. Factor H also conferred resistance to invasive pneumococcal infection [194] but did not attenuate vascular leakage in a mouse model of pneumococcal sepsis [195]. Complement is activated during *S. pneumoniae*-associated HUS, and some patients may also have complement mutations [196]. In this form of HUS, the haemolysis is direct antiglobulin test (DAT) positive, whereas all other forms of HUS are DAT negative.

DGKE-associated HUS

DGKE-HUS is associated with homozygous or compound heterozygous mutations in the *DGKE* gene [32]. The mechanism by which these mutations lead to thrombotic microangiopathy is, as yet, unclear but DGKE, demonstrated in endothelium, platelets and podocytes, inactivates diacylglycerol signalling, thus preventing thrombosis. Mutated loss-of-function variants can thus promote thrombosis [32]. This form of HUS is usually not associated with complement activation; however, certain patients exhibit complement consumption as well as complement mutations [197, 198], which may predispose to disease.

Differential diagnosis

The clinical and pathological features of thrombotic microangiopathy overlap between HUS and thrombotic thrombocytopenic purpura (TTP) as well as DIC. TTP is characterized by haemolytic anaemia, thrombocytopenia, variable renal and neurological manifestations and fever and is associated with deficient or dysfunctional ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif 13), the von Willebrand factor-cleaving protease. TTP is either congenital or acquired, due to mutations in ADAMTS13 or autoantibodies, respectively [199]. Episodes of TTP can be precipitated by pregnancy or infections, as in aHUS, and as these may be gastrointestinal infections, differentiation from EHEC-HUS may be difficult to assess initially. Most TTP patients exhibit neurological symptoms.

DIC is usually associated with septicaemia and may be difficult to differentiate from *S. pneumoniae*-associated HUS. The major difference is the consumption of coagulation factors in DIC, which is not a feature of HUS.

Evans syndrome is a rare autoimmune disease manifesting as recurrent episodes of thrombocytopenia and DAT-positive haemolytic anaemia [200]. The condition does not affect the kidneys.

Paroxysmal nocturnal haemoglobinuria (PNH) is rare disease characterized by haemolytic anaemia, thrombosis, renal manifestations or renal failure due to mutations in the phosphatidylinositol glycan class A (*PIG-A*) gene, which leads to deficiency of glycosylphosphatidylinositol (GPI)-linked proteins. Certain GPI-linked proteins, such as CD55 and CD59, are associated with complement regulation [201]. Thus, patients exhibit complement activation and present a clinical phenotype similar to aHUS.

Clinical investigation of the patient with HUS

Once a diagnosis of HUS has been made, and other diagnoses have been excluded, a clinical and laboratory investigation should be carried out in order to determine the underlying cause of HUS. Disease manifestations may overlap; for example, cases of aHUS may be preceded by gastroenteritis, and thus resemble EHEC-associated HUS. For this reason, comprehensive investigation of the patient with HUS should

address the main causes of disease, as shown in Table 1. Table 4 presents laboratory assays recommended for this investigation. Patients should be assessed based on the presumptive diagnosis, and thus, if a diagnosis of EHEC-associated HUS is assumed, based, for example, on a prodrome of bloody diarrhoea and temporal relationship to an ongoing epidemic, then a comprehensive complement analysis is not required. If, however, the diagnosis is unclear, complete testing may be necessary.

Treatment

The treatment of the various subtypes of HUS is supportive but also directed towards the specific cause of disease. Supportive care includes renal replacement therapy (preferably peritoneal dialysis, or continuous haemodialysis in the unstable patient), adequate hydration and nutrition, correction of electrolyte disturbances and acidosis, and control of hypertension and seizures [202]. Fluid replacement should consist of insensible losses and urine output in order to avoid excess hydration in the patient with renal failure. Blood transfusions are usually not recommended unless haemoglobin levels drop. In children, haemoglobin levels below 60 g/L may necessitate transfusion, but in adults comorbidities may influence the level at which a blood transfusion should be given. Platelet transfusions should be avoided unless the patient has a platelet count below 10×10^9/L and is at risk due to active bleeding or requires surgery.

Management of EHEC-associated HUS

Volume expansion using isotonic fluids was shown to have a nephroprotective effect when given before the onset of HUS [203, 204] and may be administered cautiously even after the development of HUS [205] to reduce the prerenal component of acute kidney injury due to fluid loss during the gastrointestinal phase of EHEC infection. Thus, fluid administration reduces the risk of developing HUS and the need for dialysis during established HUS. Antibiotics should be avoided during the pre-HUS phase [13, 206] presumably due to their effect on bacteriophage lysogenesis and toxin release, thus increasing the risk of developing HUS. One study indicated, however, that fosfomycin may prevent the development of HUS [207]. Once HUS has developed, there is no evidence that antibiotic treatment is harmful; on the contrary, data from the large German outbreak in 2011 indicated that antibiotic treatment was associated with fewer seizures, less abdominal surgery and faster eradication of the bacterial strain from the gut [12, 208]. These observations may, however, be specific for the outbreak strain and require confirmation in other cases.

Plasma infusions or exchange have been given during EHEC-associated HUS. There is to date little evidence for its efficacy although data differ between paediatric and adult HUS cases. Children do not seem to benefit from plasma therapy [209, 210] whilst uncontrolled case studies reported some benefit in adults [211–213]. These results could not be confirmed during the large outbreak in Germany in 2011 in which many adults were treated with plasma exchange [12, 202, 214]. Moreover, as Shiga toxin does not circulate in free form, it is unclear how plasma exchange could affect the course of disease other than by the removal of toxic microvesicles as well as prothrombotic and proinflammatory factors and replenishment of coagulation and complement factors. However, by the time the patient has presented with HUS, the toxic damage to target organs has already occurred and plasma exchange may therefore not be beneficial.

As complement activation via the alternative pathway may occur during EHEC-associated HUS and contribute to renal damage, complement inhibition was attempted using eculizumab, a monoclonal anti-C5 antibody. An initial report in three children with neurological complications was encouraging [215], but a clear beneficial effect could not be demonstrated during the large German outbreak of *E. coli* O104:H4 in children [209] or adults [12, 214]. Complement-induced cellular injury may, however, contribute to the renal and neurological manifestations during HUS, and there is a possibility that a selection bias may have affected the results of these reports, that is that patients more severely affected by HUS were selected for treatment and that patients may have exhibited a worse outcome without treatment. This possibility was controlled for, albeit retrospectively, in one study [12] which still showed that eculizumab treatment did not affect the course of disease. Moreover, patients treated with eculizumab were simultaneously treated with antibiotics, and, as antibiotic treatment alone seemed to have a beneficial effect in patients with HUS during the

Table 4 *Clinical investigation of the patient diagnosed with haemolytic uraemic syndrome (HUS)*

	Laboratory tests				
Type of HUS	Faeces	Urine	Serum/plasma	DNA	Cell assays
EHEC-associated	PCR for EHEC virulence genes *stx*, *eae*, *uidA*[a]	PCR for EHEC virulence genes *stx*, *eae*, *uidA*[a]	Antibodies against LPS[b] or adhesins[c]		DAT negative
	Culture of the faecal strain on sorbitol MacConkey agar plates[a]	Culture of the strain on sorbitol MacConkey agar plates[a]			
	Isolation of a faecal strain	Isolation of a urinary strain			
aHUS			Protein levels of C3, C3dg, factor H, factor I, factor B, TCC	Gene mutations in factor H, factor I, MCP, C3, factor B, thrombomodulin, clusterin	MCP expression on leucocytes[d]
			Antifactor H antibodies	Genetic rearrangements or deletions in factor H-related proteins	C3 and C5b-9 deposition on endothelial cells
				Risk haplotypes in the factor H (*CFH-H3*) and/or MCP *ggaac* genes	DAT negative
Streptococcus pneumoniae-associated HUS					T antigen lectin assay DAT positive
DGKE-associated HUS				Mutations in the *DGKE* gene	DAT negative
Cobalamin disorder		Homocystinuria, methyl malonic aciduria	Homocysteinaemia, methyl malonic acidaemia	Cobalamin type C MMACHC mutations	DAT negative

EHEC, enterohaemorrhagic *Escherichia coli*; *eae*: gene encoding the adhesin intimin; DAT, direct antiglobulin test; C3dg, C3 degradation product; TCC, terminal complement cascade (i.e. soluble C5b-9); MCP, membrane cofactor protein; T antigen, Thomsen–Friedenreich antigen; DGKE, diacylglycerol kinase-ε; MMACHC, methylmalonic aciduria and homocystinuria, cblC type.
[a]For detection of *E. coli* O157:H7. [b]These antibodies are serotype-specific. [c]These antibodies are not serotype-specific [251]. [d]Assayed by flow cytometry.

E. coli O104:H4 outbreak [12], this combined therapy may confound the clinical findings. Therefore, randomized clinical trials are required to determine whether patients with severe EHEC-associated HUS benefit from complement blockade. In a mouse model of EHEC infection, an anti-C5 antibody prevented renal injury when given early on after infection but not when given 6 days after inoculation [139], suggesting that complement blockade is not effective when given late in the course of murine EHEC infection.

Novel therapies are being tested for EHEC-associated HUS. These include antibodies to Shiga toxin, Gb3 analogues, vaccines and manganese (reviewed in ref. [202]) to neutralize the toxin in the circulation, prevent its binding to its receptor or block its intracellular toxicity. Furthermore, recombinant thrombomodulin may reduce endothelial damage and was shown to be protective in mice [216]. These treatments are not yet commercially available.

In most cases, EHEC-associated HUS does not recur after the acute phase of disease. If it does, or if a family member develops HUS at a separate time-point, a diagnosis of aHUS should be considered [217]. Renal transplantation may be necessary for the EHEC-associated HUS patient who does not regain renal function after the acute phase of disease. If there is doubt regarding the initial diagnosis of EHEC-associated HUS, a diagnosis of aHUS should be ruled out (Table 4) particularly in the patient requiring a transplant.

Treatment of aHUS

Patients may require dialysis and intensive care during the acute phase of aHUS. As the disease is often associated with complement activation, plasma therapy was considered the primary treatment for many years [218, 219]. Plasma infusion or exchange would theoretically replenish and exchange mutated complement factors, if soluble (Table 3), and remove anti-factor H antibodies. Because large quantities of plasma were required, plasma exchange was the preferred modality to prevent colloid overload in the patient with decreased renal function.

Plasma exchange combined with immunosuppressive therapy (prednisolone, cyclophosphamide pulses or rituximab) during the acute phase, followed by maintenance therapy (prednisolone with either mycophenolate mofetil or azathioprine) appears to be suitable treatment for most patients with anti-factor H antibodies [172, 220]. Patients are monitored by measurement of their antibody levels, and levels >1300 AU/mL have been associated with the risk of relapse [221].

In most other patients with aHUS, that is those who do not have circulating anti-factor H antibodies, the use of plasma to treat or prevent aHUS episodes has not been as successful [222]. Plasma could induce remission in patients who nevertheless progressed to develop renal failure over time [55, 57, 179]. The same was true for patients with aHUS who had undergone renal transplantation; pre-emptive plasma therapy could not prevent renal deterioration in many cases [27, 57]. Patients with isolated MCP mutations do not generally respond to plasma treatment, as MCP is a membrane-bound protein.

Eculizumab, an orphan drug approved for the treatment of aHUS, has proved to be a most efficient therapy for these patients, blocking C5 and thus the formation of the terminal complement cascade. Its efficacy has been demonstrated in multiple case reports as well as in controlled studies with 26 weeks of observation in adults [223, 224] and children [225]. Treatment prevented haematological recurrences and renal failure. In patients with decreased renal function, improvement was noted during treatment. Furthermore, eculizumab could prevent aHUS relapses after transplantation [226]. Current consensus recommends the initiation of treatment as soon as possible, before thorough complement genetic investigation is completed. Delay in the initiation of treatment may confer a worse prognosis [57]. Treatment is associated with an increased risk of infection with encapsulated bacteria, primarily meningococci [223]. Patients should therefore be vaccinated against meningococcal infection at least 2 weeks before commencing treatment. Vaccination against other encapsulated strains (*Hemophilus influenzae* and *S. pneumoniae*) is also recommended. If treatment is given during an acute episode, patients are treated prophylactically with antibiotics to prevent meningococcal infection until vaccination is given.

Treatment efficacy should be monitored by haematological and biochemical markers of disease

activity (Table 2), levels of complement activation (CH50 or complement activity kits [227]) and complement deposition on cells [166].

Discontinuation of eculizumab treatment may be associated with an increased risk of aHUS recurrence [57]. The same may be true for increasing treatment intervals. However, individual dosage regimens can be achieved with appropriate monitoring of complement activity [227].

Management of S. pneumoniae-associated HUS

The primary aim of treatment of patients with *S. pneumoniae*-associated HUS is eradication of the bacterial strain with antibiotics. Recommendations to avoid the use of plasma or unwashed red blood cells are based on the finding that these products may contain agglutinins against the T antigen and thus worsen the disease [31]. However, anecdotal evidence suggests that some patients respond favourably to plasma therapy [228].

Management of DGKE-associated HUS

This form of HUS usually does not respond to plasma therapy [32]. However, some cases exhibiting low C3 levels have been found to respond to intensive plasma therapy [197]. Furthermore, DGKE-associated HUS does not respond to eculizumab and does not recur after transplantation [32].

Treatment of HUS associated with cobalamin dysfunction

Patients with cobalamin C disorders resulting in HUS should receive treatment for the underlying disorder with hydroxocobalamin, betaine and folinic acid [229] although in some cases plasma exchange may also be beneficial.

Prognosis

Most patients with EHEC-associated HUS make a full recovery. The presence of neurological symptoms may be an ominous sign associated with worse outcome. Likewise, high neutrophil counts [22, 23], low platelet counts and long duration of anuria have been associated with a worse prognosis [22, 24]. In aHUS, prognosis and response to treatment are largely dependent on the presence of a specific mutation [57] or autoantibodies. Since the advent of eculizumab therapy, the prognosis of these patients has improved immensely and the risk of recurrence has decreased, both in patients with native kidneys and in those with a renal graft. However, eculizumab treatment is very expensive and certain national healthcare systems have therefore not recommended reimbursement, thereby restricting its use.

Summary

Haemolytic uraemic syndrome is associated with severe endothelial damage and platelet activation, caused by a wide spectrum of toxic and/or immunological reactions, all leading to similar disease manifestations and histopathological lesions. The varying aetiologies require extensive investigation, as the success of a treatment strategy is largely dependent on obtaining a correct diagnosis and thereby choosing appropriate treatment.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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