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ABSTRACT

Haemolytic uremic syndrome (HUS) is the most common cause of acute kidney injury in children. HUS was first described in 1955. Since then major advances have been achieved in understanding pathogenesis and improving patient management and outcomes. Major milestones in these 60 years are: linking diarrhoea associated HUS (D+HUS) to Shiga like toxins producing organisms, differentiating HUS into D+HUS and diarrhoea–ve (D-HUS) (this classification was modified later), genetic diagnosis of atypical HUS (a-HUS), plasmapheresis, Eculizumab (considered as the most exciting development in HUS history) use in a-HUS management and finally renal transplantation of HUS patients with end stage renal disease (ESRD).

HISTORY OF HAEMOLYTIC UREMIC SYNDROME

Diarrhoea associated (D+HUS)/Typical HUS

In 1955, Gasser et al first described five children with haemolytic anaemia, thrombocytopenia, and renal failure. This combination of clinical presentations was referred to as HUS[6]. This was followed by reports of similar cases including individual cases, large series and epidemics from different parts of the world[7–13].

Before 1955, cases with acute renal failure following gastroenteritis were explained as either: prerenal failure (severe dehydration), acute tubular necrosis (ATN), sepsis, renal vein thrombosis or disseminated intravascular coagulopathy. Such cases were treated by antibiotics and haemodynamic support only.

Haemolytic uremic syndrome was first described in 1955[6]. Since then major advances have been achieved in understanding aetiology, pathogenesis, diagnosis and optimum management of such cases. Major milestones in these 60 years are: linking diarrhoea associated HUS (D+HUS) to Shiga like toxins producing organisms, differentiating HUS into D+HUS and diarrhoea–ve (D-HUS) (this classification was modified later), genetic diagnosis of atypical HUS (a-HUS), plasmapheresis, Eculizumab (considered as the most exciting development in HUS history) use in a-HUS management and finally renal transplantation of HUS patients with end stage renal disease (ESRD).

Despite these advances a lot is still required; a-HUS diagnosis and management in developing countries is still mostly impossible, very high cost of Eculizumab is limiting its widespread use and still no consensus on maintenance plasmapheresis therapy or long-term Eculizumab treatment.
reported in Wales. A number of possible environmental factors were examined including food, water, and other toxins but no definite cause was detected, however a response to an infection was suspected to be underlying mechanism\cite{10}.

In 1985 Karmali et al.\cite{11} showed that patients with HUS preceded by diarrhoea contained E. coli strains in their stools which produced a toxin that caused irreversible damage to cultured vero cells (kidney cells from the African green monkey). Another working group demonstrated that the verocytotoxin produced by strains of Enterhemorrhagic E. coli (EHEC) associated with HUS is related to Stx of Shigella dysenteriae type 1\cite{12}. Following these descriptions, it is now known that E-coli-producing Stx and other factors, are the main causes of paediatric D-HUS\cite{13}.

**Hereditary/familial/Diarrhoea –ve/atypical HUS**

Hagge et al.\cite{14} (1967) reported HUS in 2 siblings, one of them had repeated attacks, developed ESRD and died at age 8 years while the other recovered completely after one attack. This was followed by many reports of HUS in families and cases from non-endemic areas\cite{15-20} which forced researchers to think that there is different group of HUS patients (other than D-HUS) with possible genetic background.

Complement factor H (CFH) mutations were the first identified genetic association with a-HUS in 1973\cite{21}, while an acquired dysfunction of CFH due to anti-CFH antibodies was described in 2005 for the first time\cite{22}. Membrane co-factor protein (MPC) mutation in a-HUS was first reported in 2003\cite{23}. More than 40 different mutations in MCP have been identified so far in patients with a-HUS\cite{24-27}. Up to 12% of a-HUS patients have different combinations of 2 or more mutations of CFH, CFI, MCP, C3, (Complement factor B) CFB or (Thrombomodulin)THBD\cite{28,29}. Despite all these discoveries still 30% of a-HUS cases are unexplained\cite{30} suggesting that pathogenic pathways and genetic susceptibility loci are still unexplored. Clinical presentation of the different subgroups of HUS and thrombotic thrombocytopenic purpura (TTP) and investigations to confirm diagnosis are summarized in (Table 1)\cite{31}.

### CLASSIFICATION

Haemolytic uremic syndrome has been divided into D+HUS and D-HUS, with D+HUS resulting from verocytotoxin-producing E. coli and Shigella dysenteriae type 1, while a-HUS resulting from all other causes. Because diarrhoea is not a reliable characteristic feature, currently HUS is divided into ‘typical’ for that due to infective causes and a-HUS for all other causes (Table 2). Typical HUS is more common, with verocytotoxin-producing organisms being the commonest aetiology, however the incidence of Pneumococcal infection as a cause of HUS is higher in some countries\cite{32}.

### PATHOGENESIS OF HUS

**Diarrheal prodrome is not always bloody**

Haemolytic uremic syndrome typically follows an enteric infection with a Shiga toxin-producing E. coli, usually serotype O157:H7

| Table 1 Clinical presentation of the different subgroups of haemolytic uremic syndrome and thrombotic thrombocytopenic purpura and investigations required for diagnosis\cite{5} |
|------------------------------------------|------------------|--------------------------------------------------|
| **Age at onset and clinical presentation** | **Probable diagnosis** | **Investigations to confirm diagnosis** |
| Neonatal period                          |                  |                                                  |
| Severe jaundice                          | Congenital TTP   | ADAMTS 13 deficiency (< 10%) without anti-ADAMTS |
|                                        | (Upshaw-Schulman syndrome) | 13 antibodies                                    |
|                                        |                  | Mutation in ADAMTS13 (autosomal recessive)       |
|                                    |                  |                                                  |
| Consanguineous family and/or similar symptoms or neonatal death in siblings | Methyl-malic aciduria-associated HUS | Hyperhomocysteinemia, hypomethioninemia, methylmalonic aciduria |
|                                    |                  | Mutation in MMACHC (autosomal recessive)         |
| Failure to thrive, feeding difficulties, hypotonia ± developmental delay | HUS due to Streptococcus pneumoniae | False positive Coombs test                        |
|                                        |                  | Positive cultures (blood, CSF) or PCR             |
| Consanguineous family                  |                  | Positive T-activation test (exposure of the Thomsen-Friedenreich antigen on red blood cells) supports the diagnosis |
| < 2 years                              |                  |                                                  |
| Fever                                  | STEC-HUS          | Stool or rectal swab: culture for STEC (Mac Conkey for 0157:H7); PCR for Stx |
| Invasive S.pneumoniae infection (proven or suspected); pneumonia, meningitis, sepsicaemia, especially if empyema or subdural collection | (Shigella dysenteriae-HUS in endemic regions) | Serum: anti-LPS antibodies against the most common serotypes in the local country |
| > 6 months-5 years                    |                  |                                                  |
| Diarrhea ± melena during the last 2 weeks |                  |                                                  |
| Endemic region of STEC or Shigella dysenteriae infection |                  |                                                  |
| Adolescents and adults                 | Immune TTP       | ADAMTS 13 deficiency (< 10%) with anti-ADAMTS13 antibodies |
| Fever                                  |                  |                                                  |
| Central nervous system manifestations |                  |                                                  |
| No or mild renal involvement           |                  |                                                  |
| Autoimmune context (SLE, APLS, thyroiditis) |                  |                                                  |
| ‘From birth to adolescence and adult age’ |                  |                                                  |
| No prodromic diarrhea or prodromic diarrhea but any of the following: |                  |                                                  |
| 1. age < 6 months or > 5 years         |                  |                                                  |
| 2. insidious onset                     |                  |                                                  |
| 3. relapse of HUS                      |                  |                                                  |
| 4. suspicion of previous HUS           |                  |                                                  |
| 5. previous unexplained HUS            |                  |                                                  |
| 6. post-transplant HUS                 |                  |                                                  |
| 7. pregnancy (post-partum) HUS         |                  |                                                  |
| 8. non synchronous familial HUS\cite{33} |                  |                                                  |
| Adjuvant therapy                        |                  |                                                  |
| HUS: hemolytic uremic syndrome; TTP: thrombotic thrombocytopenic purpura; ADAMTS13: A Desintegrin And Metalloproteinase with a Thrombospondin type 1 motif, member 13; MMACHC: methylmalonic aciduria and homocystinuria; CSF: cerebro-spinal fluid; PCR: polymerase chain reaction; STEC: Shiga-toxin producing Escherichia coli; Stx: Shiga-like toxin; LPS: lipopolysaccharides

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(Figure 1). The infection is almost always non-bloody diarrhoea at first. In 80% of patients, the diarrhoea becomes bloody within one and five days after the onset of diarrhoea\[34,35\]. However, some patients may be infected with E. coli O157:H7 and develop HUS, and without having diarrhoea at all\[36\]. So the presence of non-bloody diarrhoea is not against diagnosis of D-HUS.

The mechanisms by which hemorrhagic colitis and HUS occurs are not fully clear. The bacteria adhere to the mucosa of the colon causing bloody diarrhea then shiga like toxins reach the blood stream and attach to the endothelium of the small arterioles of the kidneys and other organs. The endothelial cells express a toxin-specific receptor that enables the contact between toxin and cells leading to endothelial damage which causes platelet aggregation , activation and fibrin deposition\[37\].

**Role of Podocyte dysfunction in HUS**

Abnormal complement signalling as the only cause of a-HUS has been recently challenged as mutations in diacylglycerol kinase ε (DGKE) (which encodes a protein, diacylglycerol kinase ε) have been reported in children with a-HUS which is not a component of the complement system. Patients with DGKE mutations developed proteinuria which highlighted podocyte dysfunction as a complication of this form of a-HUS\[38\]. Complement activation in a-HUS patients with genetic or autoimmune complement abnormalities, might also result in podocyte dysfunction and vice versa podocyte dysfunction that leads to nephrotic-range proteinuria might also predispose patients to the development of thrombotic microangiopathy (TMA)\[39\].

In 2013, Lemaire and colleagues identified compound heterozygous and homozygous mutations in DGKE as the cause of a recessive form of a-HUS. Affected patients had a specific clinical phenotype with disease onset in the first year of life, several relapses before age of 5 years and all progressed to CKD before adulthood, long after the last relapse of a-HUS had occurred. Three of 12 patients developed nephrotic syndrome (NS) due to glomerular filtration barrier dysfunction. Compound heterozygous or homozygous mutations of DGKE were found in 27% of those patients with no anti-CFH autoantibodies or mutations in known complement a-HUS-associated genes detected\[37\]. A Spanish cohort of 83 a-HUS children with onset before age of 2 years in 2014 described homozygous or compound heterozygous DGKE mutations in four patients (5%); three of these patients had recurrence of disease, however, also carried homozygous mutations in THBD or C3\[39\].

A two-way relationship between podocyte dysfunction and a-HUS pathogenesis is suggested. On one side, nephrotic-range proteinuria may occur in childhood forms of a-HUS associated with complement dysregulation and also in patients with DGKE genetic. Activation of Diaacylglycerol - Protein kinase C (DAG–PKC) signalling is a common pathogenetic mechanism in a-HUS with both complement dysregulation and DGKE deficiency, leading to podocyte dysfunction. Conversely, podocyte dysfunction leading to nephrotic range proteinuria predisposes HUS cases to TMA by inducing prothrombotic abnormalities and endothelial dysfunction\[40\].

**Complement regulator factor H autoantibodies**

Complement regulator factor H (CFH) autoantibodies (CFH-AAs) formation is a common cause of a-HUS; these patients have autoantibodies against CFH domains 19-20 (CFH19-20) which are nearly identical to CFH related protein-1 domains 4-5 (CFHR1 4-5). It is not known why nearly all the patients with autoimmune a-HUS lack CFHR1. CFH-AAs bind to a common site on the loop R1182-L1189 of CFH next to the buried two residues different in CFH19-20 and CFHR14-5. The crystal structure of CFHR1 4-5 also showed that the conformation of the autoantigenic loop is different on CFH and CFHR1. These data provided the basis for the suggested novel model to explain how CFHR1 deficiency is linked to CFH-AA formation\[41\], the autoantigenic epitope of CFH and its homologous site in CFHR1 are structurally different which provides an explanation for formation of autoantibodies caused by deficiency of CFHR1 in autoimmune a-HUS\[41\].

**CLINICAL PRESENTATION OF HUS**

**Extra renal manifestations of D+HUS and Diabetes risk**

Whereas the kidney and gastrointestinal tract(GIT) are the most commonly affected organs in HUS, central nervous system (CNS), pancreatic, myocardial and skeletal, involvement is also reported\[42-44\]. GIT involvement with severe colitis may lead to transmural necrosis with perforation and/or colonic stricture later on\[43,44\]. CNS involvement in D+HUS is common and manifest as irritability and seizures, and in more severe cases, paresis, coma, and cerebral oedema. Skeletal muscle involvement manifested as rhabdomyolysis occurs in rare cases, and myocardial involvement is very rare as well\[45,46\].

Pancreatic involvement is uncommon in HUS. Autopsy studies reported thrombosis of vessels supplying the islets of Langerhans with preservation of the exocrine pancreas\[47,48\]. Little is known about the incidence and management of diabetes mellitus (DM) during D+HUS. A systematic review and metanalysis was conducted in 2005 to detect incidence of DM in D+HUS, severity of HUS attacks associated with DM and long term prognosis of such patients. The incidence of DM (hyperglycaemia requiring insulin) during acute D+HUS in children below 16 years of age was 0-15%, with pooled incidence estimated at 3.2% (95% CI 1.3-5.1). The development of DM was associated with severe disease, marked by CNS symptoms, the need for acute dialysis, and mortality. Children who developed DM during D+HUS and survived, one-third had permanent DM requiring insulin 6 months to 15 years after the acute phase, whereas two-thirds were reported to recover\[49\]. No evidence supports that individuals infected with E. coli O157:H7 who develop gastroenteritis without HUS are at increased risk of DM\[40\].

So frequent monitoring of blood glucose during acute D+HUS is a must, especially for patients on peritoneal dialysis (PD). Early aggressive treatment of hyperglycaemia will prevent ketoacidosis and improve acute outcomes, as in other critically ill patients\[51,52\]. Continued loss of islet cells after recovery of acute attack of HUS may be due to ongoing inflammation and fibrosis, or decreased islet reserve which manifests clinically in those predisposed to type 2
DM. So, even cases that were not identified to have hyperglycaemia during the acute phase of D+ HUS may be at long-term risk of DM. Thus, consideration should be given to long-term screening of D+ HUS survivors for DM; the optimal timing of screening is unclear[29].

LABORATORY TESTS FOR HUS
Diagnosis of HUS depends on careful history taking (Family history, history of diarrhoea and other infections), exclusion of D+HUS (screening for Shiga-toxin producing E-coli (STEC)) first followed by ADAMTS13 level assessment to exclude TTP and anti-CFH antibodies followed by full complement pathway assessment including genetic studies (Figure 2).

Lactate Dehydrogenase levels in HUS
Lactate dehydrogenase (LDH) level is elevated in HUS and TTP cases. It is not diagnostic but used mainly in follow up. Total serum LDH rises mainly due to the release of red blood cell LDH due to intravascular haemolysis[30] as in other haemolytic anaemias[34,35]. This widely accepted belief has not been supported by LDH isoenzyme analysis[36]. A large proportion of the increase in total serum LDH in patients with TTP is the result of systemic microvascular compromise, rather than erythrocyte lysis. Widespread tissue ischemia caused by occlusion of microvasculature by platelet thrombi has been well described in autopsy studies of patients with fulminant forms of HUS and TTP[37].

IMAGING OF HUS WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT
The central nervous system (CNS) is involved in 20–50% of HUS cases[43,47,57]. A toxin-mediated vasculopathy involving the small intracerebral vessels similar to the kidneys is the probable mechanism[56,59]. Severe fluid and electrolyte disturbances and hypertension may be responsible for encephalopathy in HUS patients[43,47]. In many cases, Computed tomography (CT) has been the initial diagnostic imaging study of choice because of its greater availability, However magnetic resonant imaging (MRI) is nowadays the modality of choice for the evaluation of most non-traumatic CNS disease in children[46].

Basal ganglia lesions was reported as the most frequent with HUS[43–46]. Other findings, including territorial infarction or diffuse white-matter changes similar to posterior leukoencephalopathy, have been described, reflect complications rather than specific changes of the disease[56,57,58]. Involvement of the basal ganglia is not specific for HUS and is seen in different conditions as severe hypoxia, intoxication and infectious diseases. This supports the theory of a direct or receptor-mediated verotoxin-induced injury as a mechanism of CNS involvement in HUS patients[59].

MANAGEMENT OF HUS
Antidiarrheal and Antibiotics in D+HUS
Antidiarrheal agents should be avoided in haemorrhagic colitis due EHEC, as it is thought that it lead to retention of Stx within the colon, which could enhance absorption of the toxin and confers greater risk for developing HUS[70–72]. There is a long history of the discussion of antibiotic treatment for EHEC-induced diarrhea. In vitro studies demonstrated that EHEC produces more toxins when stimulated by nonlethal concentrations of antibiotics, this issue has been under controversial discussion[18]. During the large EHEC outbreak in Japan in 1996, it was suggested that treatment with Fosfomycin on day 2 after disease onset reduced the risk of developing HUS but this suggestion has several drawbacks. Fosfomycin is rarely used for this indication at all outside of Japan. Furthermore, recent epidemiological studies conducted by the Centre for Disease Control proved that antibiotic therapy for EHEC enteritis resulted in a significantly higher risk of developing HUS[73]. This adverse outcome may reflect the effect of specific antimicrobial agents on phage induction and subsequent Stx gene expression and transcription or increased Stx release after induced bacteria lysis[70–74,76].
Some studies demonstrated a harmful effect of antibiotic therapy in haemorrhagic colitis\(^{[75-77]}\). Other studies have not demonstrated such an association. So currently, there is no consensus on the use of antibiotic therapy in children with haemorrhagic colitis or HUS; however, antibiotics are not usually prescribed in children with HUS until there are specific indications for antibiotic therapy. In conclusion, during the diarrheal phase, antibiotic treatment should be avoided, as beneficial effects regarding initiation of HUS cannot be deduced from recent studies\(^{[78,79,80]}\).

**Role of steroids in HUS**

In a-HUS as a result of CFH, CFI, C3, THBD, CFB, MCP mutations steroids are of no value, hence not indicated. Nevertheless a small subset of a-HUS patients (6-10\%) develops CFH autoantibodies. They bind to C-terminus of CFH and decrease CFH binding to C3b with lack of complement control on cells.

Plasma exchange (PE) removes anti-CFH antibodies, but this effect is transient. Immunosuppressive therapy (steroids, azathioprine, MMF) and Rituximab (anti-CD20 antibody) combined with PE allowed long term dialysis free remission in 60-70\% of patients\(^{[25,70,81]}\). In countries where rapid assessment of CFH antibodies is not readily available or delayed, it is controversial wether to start empirical steroid therapy in all a-HUS cases.

**Renal replacement therapy in D+HUS**

To date, there is no effective preventive or specific treatment for D+HUS. Symptomatic and supportive measures are the main options\(^{[16,81]}\). About two thirds of D+HUS children need dialysis therapy. General management of AKI as fluid and electrolyte balance, antihypertensives if required, and initiation of renal replacement therapy when indicated\(^{[16]}\).

In most centers, PD is the preferential choice. However, there is no priority to one or the other. Haemodialysis may be started if a-HUS is suggestive. This is true in older children and those without clear diarrhoea. In younger children, most centres prefer PD. It has been argued that PD may have a higher risk of peritonitis in patients with bloody diarrhoea. However this has not yet been reported\(^{[77]}\).

**Platelet transfusions in HUS**

Despite thrombocytopenia (30\(\times\)10\(^3\) - 60\(\times\)10\(^3\)/mm\(^3\)) or even less, purpuric eruptions are uncommon with no or minimal bleeding reported in children with HUS\(^{[82]}\). It has been theoretically postulated that transfusions of platelets should be avoided unless there is severe bleeding or an invasive procedure is to be done. A study compared 22 (30\%) cases who received platelet transfusion with 51 (70\%) who did not and observed no bleeding complications related to these procedures in either group despite the associated thrombocytopenia\(^{[83]}\).

The rationale for avoiding platelet transfusion is the possibility that infused platelets may worsen the microvascular thrombosis and aggravating course of the disease\(^{[84,85,86]}\). However, information on the effects of platelet transfusions in D+HUS patients is scarce. In 2009 a report of 22 children with D+HUS showed that seven of them (31.8\%) required platelet transfusions without reported additional morbidity or mortality\(^{[87]}\). Due to limitations of studies, platelet transfusion should be avoided or minimized as possible in patients with HUS and decision to give a platelet transfusion must include the consideration that the perceived benefits outweigh the potential complications.

**Intravenous Volume Expansion during E-coli O157:H7 infections**

HUS can be categorized as either oligoanuric (which probably signifies ATN) or non-oligoanuric. Children with oligoanuric renal failure during HUS generally require dialysis, have more complicated courses, and at increased risk for chronic sequelae than children who experience non-oligoanuric HUS\(^{[80]}\).

Volume expansion during acute E-coli O157:H7 infection may oppose the small vessel thrombi formation by improving renal perfusion, preventing glomerular tubular imbalance from hypoperfusion and ischemia, and maintaining tubular flow. Volume expansion may also mitigate the nephrotoxicities of filtered urate\(^{[85,88]}\), and haemoglobin and of Shiga toxin’s effects on renal tubular epithelial cells\(^{[89]}\) and monococytes\(^{[90]}\) that are independent of thrombotic changes. However, renal injury can still follow E. coli O157:H7 infections in well-hydrated children. The only way to prevent HUS is prevention of E-coli O157:H7 infections\(^{[90]}\).

**GUIDELINES OF THE EUROPEAN PAEDIATRIC STUDY GROUP FOR HUS**

The European Paediatric Study Group for HUS in 2006 published “Classification of HUS, TTP and related disorders” (Table 3)\(^{[81]}\). While the Guidelines for the investigation and initial therapy of D-HUS were published in 2009. The guideline describes a clinical pathway for cases of HUS and is intended to offer an approach based on opinion, as evidence is lacking. It is designed to streamline the recognition of those cases of HUS that have aetiologies other that the perceived benefits outweigh the potential complications.

![Table 3](image)

**Table 3 Step 2 of the guideline: Recommended list of investigations for patients identified as having atypical HUS\(^{[81]}\).**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.i. Disorders of complement regulation</td>
<td>C3 (plasma/serum)</td>
</tr>
<tr>
<td>1.ii.</td>
<td>Factor H and factor I concentration (plasma/serum)</td>
</tr>
<tr>
<td>1.iii.</td>
<td>Factor H autoantibody</td>
</tr>
<tr>
<td>1.iv.</td>
<td>MCF (CD46) (surface expression on mononuclear leukocytes by FACS)</td>
</tr>
<tr>
<td>1.v.</td>
<td>Gene mutation analysis in factor H, factor I, MCP, factor B and C3</td>
</tr>
<tr>
<td>2.i.</td>
<td>Plasma vWF protease (ADAMTS13) activity ± inhibitor (plasma)</td>
</tr>
<tr>
<td>2.ii.</td>
<td>Measure in acute phase of illness. Significant if activity &lt;10% of normal. If low, check for autoantibody inhibitor. Repeat in remission. If persistently low activity in absence of inhibitor, inherited deficiency likely. Genetic confirmation optional at specialized genetic laboratories</td>
</tr>
<tr>
<td>2.iii.</td>
<td>Homocysteine, methylnalonic acid (plasma and urine) ± mutation analysis in MMACHC gene</td>
</tr>
<tr>
<td>2.iv.</td>
<td>Pregnancy test, liver enzymes. Always consider pregnancy in teenage girl with HUS or TTP. Investigate as in 1.i. and 1.ii. Above</td>
</tr>
<tr>
<td>2.v. Miscellaneous</td>
<td>Antinuclear antibody, lupus anticoagulant, anti-phospholipid antibodies</td>
</tr>
</tbody>
</table>

ADAMTS13 a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, vWFp von Willebrand factor cleaving protease, HIV human immunodeficiency virus, HELLP haemolysis, elevated liver enzymes, low platelet count, MCP membrane co-factor protein, FACS fluorescence activated sorter MMACHC methylmalonic aciduria and homocysteinaemia type C protein.
than EHEC (Table 4, Figures 3, 4)[90]. Since then these guidelines are a standard and being followed worldwide and led to marked improvement in understanding and management of this disease. However after introduction of Eculizumab, clinicians worldwide believe that it should be the standard of care for all a-HUS cases[92-94].

**Interventions to promote endothelial cell health**

Endothelial cell dysfunction, due to complement activation, is an intermediate stage in the pathogenesis of HUS[95] so targeting endothelial cell dysfunction is gaining importance in the management of TMA[96] with promising results in experimental settings. Inhibitors of angiotensin-converting enzyme (ACE), HMG-CoA reductase (statins), and xanthine oxidase as well as antioxidants (such as ascorbic acid) may have beneficial effects a-HUS. ACE inhibitors reduce angiotensin II-mediated oxidative stress within the vessel wall[97]. By reducing oxidative inactivation of nitric oxide, ACE inhibitors improve endothelium-dependent vasodilation[97,98].

Therefore, ACE inhibitors may be useful in a-HUS treatment by decreasing oxidative stress and increasing bioavailability of nitric oxide[99]. Statins improve endothelial cell dysfunction by nitric oxide and decreased thrombogenicity. Statins also have immunomodulatory and anti-inflammatory properties[99,100]. Allopurinol, is an inhibitor of xanthine oxidase, improve endothelial cell dysfunction[101-103]. Ascorbic acid restores endothelium-dependent vasodilation by oxidative stress[104] and increases the bioavailability of nitric oxide by scavenging reactive oxygen species[105].

Haemolytic uremic syndrome after introduction of Eculizumab

For years, the only available treatment for a-HUS was plasma exchange, outcomes were poor and up to 60% of patients with CFH mutations (the most severe form) developed ESRD shortly after onset[106]. First report of successful treatment HUS patients with Eculizumab was in 2000[107]. The drug was approved by the Food and Drug Administration (FDA) for use in a-HUS treatment in September 2011 (FDA. News release September 23, 2011).

Eculizumab (Soliris) is a humanised monoclonal IgG2/4κ antibody produced from murine myeloma cells. It is a complement inhibitor, binding to complement protein C5 with high affinity, thus inhibiting cleavage to C5a, a proinflammatory and prothrombotic and C5b, so prevent the generation of the terminal complement complex C5b-9[94]. It is now used for paroxysmal nocturnal haemoglobinuria (PNH) and was tested for rheumatoid arthritis without success[108].

A favourable outcome after use of eculizumab in three patients with severe D+HUS and CNS involvement was reported[99]. A retrospective review of the excessive uncontrolled use of eculizumab, during outbreak of D+HUS in Germany, did not demonstrate a benefit for patients who received eculizumab, compared to patients who did not receive eculizumab[109]. So no sufficient evidence is available currently to support the use of eculizumab for D+HUS patients[105,109]. With time, the use of Eculizumab in a-HUS has increased. Renal function recovery has been described even after 6 months of dialysis[110], neurological and ocular involvement (bilateral serous retinal detachment) were reported also to reverse with eculizumab[112,113].

Therefore, the presence of extrarenal symptoms is critical when deciding to maintain anti-C5 therapy, regardless of renal replacement therapy requirement. Damage to extrarenal organs can progress in patients without renal function[114,115]. This indicates subclinical activity, and that an increased platelet count is not always a reliable recovery marker, so other biomarkers of disease activity are required[107]. Preventive measures (vaccination and if needed prophylactic antibiotics) should be initiated against Neisseria meningitides prior to starting treatment with eculizumab[117]. Studies of long-term safety and efficacy of eculizumab are still few[118,119].

**Trials of discontinuation of life long maintenance Eculizumab**

Discontinuing eculizumab therapy has been described in few reports[120-125]. The main rationale for discontinuing eculizumab therapy was to protect patients from the risk of the potentially side effects as meningococcal infection[126,127] and immune-mediated drug reactions, including the theoretical risk of developing neutralizing anti-drug antibodies that ultimately would deprive the patient of a life-saving therapeutic resource. Additionally, eculizumab is among the most expensive life-long medical treatments[124].

![Figure 3](image-url) Step 1 of the guideline. Recognition of atypical HUS[92]. HUS: Haemolytic uremic syndrome, EHEC: enterohemorrhagic E.coli.

Table 4 Genetic abnormalities and clinical outcome in patients with a-HUS[92].

<table>
<thead>
<tr>
<th>Complement abnormality</th>
<th>Main effect</th>
<th>Frequency, %</th>
<th>Death or ESRD within one year of first presentation, %</th>
<th>TMA post-transplantation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor H mutations</td>
<td>Increased activity of C3 convertase (decreased inhibition)</td>
<td>20 – 30</td>
<td>50 – 60</td>
<td>75 – 90</td>
</tr>
<tr>
<td>Factor I mutations</td>
<td>Decreased C3b inactivation</td>
<td>2 – 12</td>
<td>42 – 50</td>
<td>45 – 80</td>
</tr>
<tr>
<td>C3 mutations</td>
<td>C3 convertase resistant to inhibition</td>
<td>5 – 10</td>
<td>43 – 63</td>
<td>40 – 70</td>
</tr>
<tr>
<td>Factor B mutations</td>
<td>C3 convertase stabilisation (increased activity)</td>
<td>1 – 2</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Thrombomodulin (THBD)  mutations</td>
<td>Reduced C3b inactivation</td>
<td>0 – 5</td>
<td>50</td>
<td>1/1</td>
</tr>
<tr>
<td>Membrane cofactor Protein (MCP) mutations</td>
<td>Increased activity of C3 convertase (decreased inhibition)</td>
<td>3 – 15</td>
<td>0 – 63</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Factor H antibodies</td>
<td>Inactive factor H (increased activity of C3)</td>
<td>6 – 10</td>
<td>30 – 40</td>
<td>Greater with elevated antibody levels</td>
</tr>
</tbody>
</table>

Adapted from Noris and Remuzzi[97], Campistol et al[97], Zubel et al[114] and Fremeaux-Bacchi et al[111].

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Recommendations for plasmatherapy to prevent post-kidney recovery renal sequel during long term follow up.

Long term follow up of all D+HUS patients is mandatory. Studies did not differentiate patients who apparently completely recovered after the acute illness from those who demonstrated persistent renal abnormalities regarding development of long term renal sequel. Patients with oliguria of 8 days or less, those with oliguria greater than 8 days or anuria 1-8 days and those with anuria greater than 8 days had a step-wise worsening of prognosis. Dialysis therapy required for more than 4 weeks was associated with worse prognosis and no patient achieved full renal recovery.

OUTCOME OF TRANSPLANTED HUS CHILDREN

Patient with D+HUS rarely develop ESRD so renal transplantation is rarely required. However, reviews support that, a recurrence of HUS is the absolute exception in D+ HUS patients so transplantation can be performed without increased risk for failure. Before transplantation all a-HUS cases should have a complete genetic analysis to detect known complement mutations and anti-CFH antibodies and possibly the recurrence risk (Table 4). TMA presents in the transplanted kidney in around 50% of patients who undergo transplantation, and graft failure occurs in 80% – 100% of those with TMA.

With a lack of guidelines, patients in whom a kidney transplant is considered should be evaluated on an individual basis, based on the risk of graft failure and availability of eculizumab. Different protocols to prevent recurrence of TMA including prophylactic plasmapheresis (Figure 5) or both are followed with variable results.

Combined liver and kidney transplantation in HUS

The most common mutation of complement regulatory proteins associated with a HUS is in the gene encoding CFH. Combined liver-kidney transplantation may correct this complement abnormality and prevent recurrence when the defect involves genes encoding circulating proteins that are synthesized in the liver, such as factor H or I. Good outcomes are reported when surgery is combined with intensive plasma therapy.

LONG TERM FOLLOW UP OF D+HUS

Diarrhea associated HUS generally has a good prognosis as more than 95% of children recover from the acute phase, however long-term renal sequelae have been reported in up to 25% of cases. Long term follow up of all D+HUS patients is mandatory. Studies have suggested that patients with less severe forms of HUS including those with a preserved urine output, may also develop renal sequel at follow up.

Studies did not differentiate patients who apparently completely recovered after the acute illness from those who demonstrated persistent renal abnormalities regarding development of long term renal sequel. A study suggested that a quarter of those who recovered with an absence of proteinuria (<250 mg/day) went to develop renal sequel during long term follow up. Prognostic factors associated with poor outcomes in D+HUS cases include: severity of acute illness (greater infection or host response) including elevated white blood cell count higher than 2×10^9 with neutrophilia (141), a high serum creatinine (142), CNS involvement (134,136,143) and hypertension (138,144). Compared with patients with oliguria of 8 days or less, those with oliguria greater than 8 days or anuria 1-8 days and those with anuria of greater than 8 days had a step-wise worsening of prognosis. Dialysis therapy required for more than 4 weeks was associated with worse prognosis and no patient achieved full renal recovery.

Progression of renal disease in HUS

Children with most severe forms of HUS do not recover from AKI and become dialysis dependent. A second group recovers renal function partially, with persistent proteinuria and hypertension and progress to ESRD within 2-5 years. The third group may recover normal serum creatinine and creatinine clearance but with persistent proteinuria. They are at risk of progressing to CKD and ESRD after 5-20 years following the acute attack.

Hyperfusion (hyperfiltration injury) of the remaining nephrons after acute attack of HUS is a probable mechanism of deterioration of kidney function after recovery of acute illness leading to progressive scarring and loss of renal function. Histologic changes in biopsied HUS patients during follow up (Biopsies done because of late or persistent proteinuria, hypertension, and prolonged renal failure) show focal and segmental glomerulosclerosis (FSGS) and mesangial expansion in the glomeruli. This may highlight HUS as one of the possible causes of secondary FSGS. Close follow up and prompt control of hypertension to average or low-normal values, and treatment of proteinuria persisting for 6 months after the acute period with ACE inhibitors is recommended.

ATYPICAL HUS IN DEVELOPING COUNTRIES

An adequate diagnostic work-up as an essential requirement for proper therapy, is currently impossible and unaffordable for most a-HUS patients in developing countries. International cooperation facilitating a proper diagnostic work-up in a stringent and cost-efficient manner are indispensable for diagnosis and therapy of many individuals suffering from these serious and life-threatening diseases. Nevertheless, while the costs for complement targeting drugs remain high, treatment of a-HUS patients especially in developing countries will remain a challenge.
CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


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