Haemolytic uraemic syndrome

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Haemolytic uraemic syndrome is a form of thrombotic microangiopathy affecting predominantly the kidney and characterised by a triad of thrombocytopenia, mechanical haemolytic anaemia, and acute kidney injury. The term encompasses several disorders: shiga toxin-induced and pneumococcus-induced haemolytic uraemic syndrome, haemolytic uraemic syndrome associated with complement dysregulation or mutation of diacylglycerol kinase ε, haemolytic uraemic syndrome related to cobalamin C defect, and haemolytic uraemic syndrome secondary to a heterogeneous group of causes (infections, drugs, cancer, and systemic diseases). In the past two decades, experimental, genetic, and clinical studies have helped to decipher the pathophysiology of these various forms of haemolytic uraemic syndrome and undoubtedly improved diagnostic approaches. Moreover, a specific mechanism-based treatment has been made available for patients affected by atypical haemolytic uraemic syndrome due to complement dysregulation. Such treatment is, however, still absent for several other disease types, including shiga toxin-induced haemolytic uraemic syndrome.

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Introduction

Haemolytic uraemic syndrome is a rare but severe disease that has in the past two decades generated many studies. Experimental and genetic studies have helped to decipher the pathophysiology of various forms of haemolytic uraemic syndrome, while clinical studies have better delineated the picture and improved diagnosis. These breakthroughs have paved the way for new targeted therapies. Haemolytic uraemic syndrome is a rapidly evolving field but is one of the best examples of precision medicine—ie, tailored mechanism-based treatment—and of how translational research can improve the management of a disease. In this Seminar, we discuss the definitions and classifications, pathophysiology, genetics, clinical presentation, diagnostics, and management of haemolytic uraemic syndrome subsets.

Definitions and classifications

Haemolytic uraemic syndrome belongs to a range of thrombotic microangiopathies and arises from an initial endothelial cell injury. The term thrombotic microangiopathy refers primarily to pathological features of vascular damage. In haemolytic uraemic syndrome, these features are documented mainly in the kidney as fibrin and platelet thrombi in capillaries and arterioles, endothelial cell swelling and detachment from the glomerular basement membrane, and the appearance of so-called double contours on the glomerular basement membrane. These pathological features translate clinically into a classic triad: peripheral thrombocytopenia, mechanical haemolytic anaemia, and damage to various organs, predominantly the kidney and the brain.

The pathophysiology of haemolytic uraemic syndrome is complex because several mechanisms can lead to the same pattern of endothelial cell damage and similar clinical and biological abnormalities. Additionally, several types of haemolytic uraemic syndrome might share common mechanisms of endothelial cell damage. At least seven classifications have been previously proposed, which are continually evolving as new mechanisms are discovered (appendix pp 1–3). The 2016 classification proposed by the International Haemolytic Uraemic Syndrome group[1] will be used in this Seminar (figure 1).

The term haemolytic uraemic syndrome encompasses a heterogeneous group of disorders, including typical haemolytic uraemic syndrome due to an infection from shiga toxin-producing *Escherichia coli* (STEC), compared with atypical haemolytic uraemic syndrome during which genetic or acquired dysregulation of the complement alternative pathway is detected in 40–60% of patients.[2,3] Cobalamin C (cblC)[4,5] and diacylglycerol kinase ε (DGKE) deficiency[6] are two rare genetic forms of haemolytic uraemic syndrome. Approximately 30% of atypical haemolytic uraemic syndrome arises from unknown mechanisms. Haemolytic uraemic syndrome can occur as a complication of, or be precipitated by, various diseases, conditions, and treatments, including malignant hypertension, autoimmune diseases, cancers, use of medications or abuse of recreational drugs, haemopoietic stem-cell or solid organ transplantation, or infections. Whether secondary haemolytic uraemic syndrome should be included within the range of atypical haemolytic uraemic syndrome is debatable, which has important implications for diagnosis and treatment.

Search strategy and selection criteria

We searched PubMed between Jan 1, 1989, and March 1, 2016, with the terms "hemolytic uremic syndrome", "thrombotic microangiopathy", "shigatoxin", "pneumococcus", "cobalamin C defect", "complement", and "eculizumab" in combination with the terms "pathophysiology", "diagnosis", "causes", and "treatment". We restricted our search to English and French publications. We selected reports from the past 5 years but did not exclude important and highly cited older publications. We searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles are also cited to provide more detail.

See **Online** for appendix

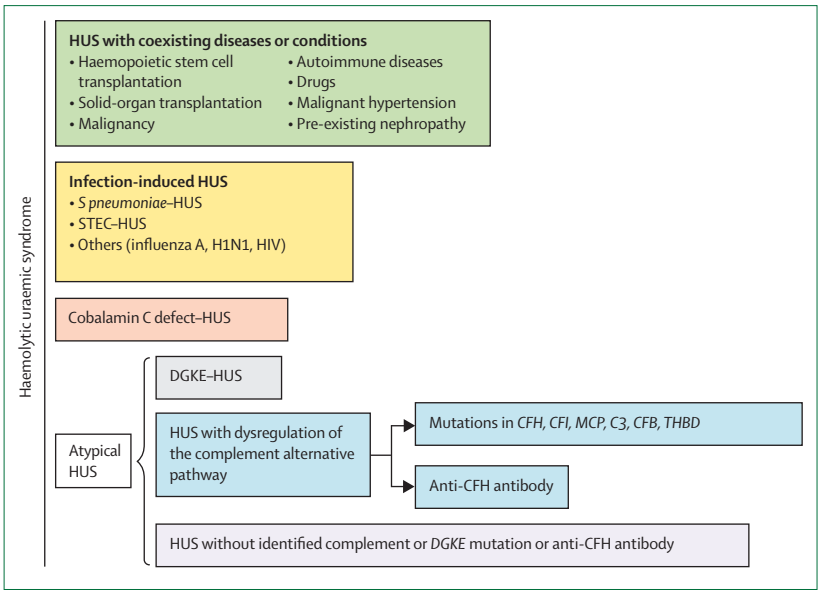


Figure 1: **Classification of various forms of haemolytic uraemic syndrome**
2016 International Haemolytic Uraemic Syndrome group classification. Adapted from Loirat and colleagues,[1] by permission of SpringerNature. HUS=haemolytic uraemic syndrome. STEC=shiga toxin-producing *Escherichia coli*. DGKE=diacylglycerol kinase ε. CFH=complement factor H. CFI=complement factor I. MCP=membrane-cofactor protein. C3=component 3. CFB=complement factor B. THBD=thrombomodulin.

The relevance of the term atypical haemolytic uraemic syndrome itself can be questioned, and new terminologies that use complement–haemolytic uraemic syndrome, *DGKE* mutation–haemolytic uraemic syndrome, and cblC defect–haemolytic uraemic syndrome might be more appropriate because they refer to the pathogenic mechanisms and, therefore, indicate targets for optimal treatment.

Incidence and epidemiology

In children with haemolytic uraemic syndrome, the proportion with STEC–haemolytic uraemic syndrome is 85–90%, atypical haemolytic uraemic syndrome is 5–10%, and *S pneumoniae*–haemolytic uraemic syndrome is about 5%. By contrast, the respective frequency of haemolytic uraemic syndrome secondary to coexisting diseases or conditions, or infections; and atypical haemolytic uraemic syndrome is not precisely documented in adults.

Although the frequency of invasive pneumococcal disease substantially decreased in children after the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7),[7] the annual incidence of *S pneumoniae*–haemolytic uraemic syndrome (0·06 cases per 100 000 children <18 years) did not decrease.[8] This finding was related to the replacement of the pre-PCV7 serotypes by non-PCV7 serotypes, especially the 19A serotype in children with *S pneumoniae*–haemolytic uraemic syndrome.[9] Whether PCV13 vaccine, including the 19A serotype, allows for a decreased incidence of *S pneumoniae*–haemolytic uraemic syndrome is not known.

STEC–haemolytic uraemic syndrome is mostly a disease of children younger than 3–5 years (annual incidence in Europe and North America of 0·6–0·8 cases per 100 000 children <15–18 years,[10–12] and 1·9–2·9 cases per 100 000 children <3–5 years[10,11]), possibly because anti-STEC antibodies develop later in life.[13] Importantly, incidence of STEC–haemolytic uraemic syndrome in Latin America remains ten times higher than in other continents (eg, 10–17 cases per 100 000 children <5 years in Argentina).[14] 5–10% of patients with sporadic STEC–gastroenteritis develop haemolytic uraemic syndrome, a frequency that can reach 20% or more during outbreaks.[15,16] Although *E coli* O157 was predominantly isolated in patients with STEC–gastroenteritis or haemolytic uraemic syndrome until 2010, non-O157 STECs (mostly O26, O111, O121, O145, O91, O103, O104, and O80) altogether are now as frequent as O157 in Europe and North America,[11,17,18] whereas O157 remains the predominant strain (>70%) in Latin America.[14]

Estimation of the annual incidence of atypical haemolytic uraemic syndrome has been revisited according to current definition of the disease (haemolytic uraemic syndrome without coexisting disease or condition, or specific infection) to be 0·23–0·42 cases per million population (0·10–0·11 in children <16–17 years per million population; appendix pp 26, 27).[2,19,20]

Pathophysiology

The common feature to all forms of haemolytic uraemic syndrome is the presence of endothelial cell lesions in the microvasculature of the kidney and, less frequently, of other organs. The trigger of endothelial cell lesions might be extrinsic and transient, such as *Streptococcus pneumoniae* or STEC infections, drugs, or cancer. In these settings, the thrombotic microangiopathy process usually abates once the trigger has been removed or controlled, with no risk of relapse. Conversely, the driving force of endothelial cell damage might be endogenous and sustained, such as inherited or acquired dysregulation of the complement alternative pathway in atypical haemolytic uraemic syndrome, permanent endothelial cell activation due to the loss of DGKE in *DGKE* mutation–haemolytic uraemic syndrome, or a deficient cobalamin metabolism (mutations in methylmalonic aciduria [cobalamin deficiency type C with homocystinuria]) in cblC defect–haemolytic uraemic syndrome. In these situations, haemolytic uraemic syndrome relapses are frequent and the outcome is poor in untreated patients.

The current understanding of the pathophysiology of various forms of primary and secondary haemolytic uraemic syndrome is shown in figure 2 and table 1. The mechanisms underlying some forms of haemolytic uraemic syndrome are unknown or only partially understood—ie, atypical haemolytic uraemic syndrome with no documented complement or *DGKE* gene variants. The implication of complement activation as a second-hit mechanism that amplifies endothelial cell

damage is suggested in STEC-induced and *S pneumoniae*-induced haemolytic uraemic syndrome (figure 2) and in several secondary forms of haemolytic uraemic syndrome (table 1). All forms of haemolytic uraemic syndrome share a final common procoagulant and proinflammatory phenotype of activated endothelial cells (figure 2). In addition to endothelial cell damage, some forms of haemolytic uraemic syndrome involve podocyte injury—eg, anti-vascular endothelial growth factor drug-associated haemolytic uraemic syndrome,[36] DGKE–haemolytic uraemic syndrome,[79,80] and STEC–haemolytic uraemic syndrome.[81]

Genetics

Haemolytic uraemic syndrome can be a familial monogenic recessive disease caused by pathogenic variants in a single gene (cblC-defect–haemolytic uraemic syndrome[4] and DGKE–haemolytic uraemic syndrome[6]). Complement–haemolytic uraemic syndrome is frequently sporadic (85% of families[2]) despite presence of pathogenic variants in complement genes in the patient and one of their healthy parents. These findings suggest that the genetic background predisposes the patient to the disease rather than directly causing the disease. The reasons underlying the incomplete penetrance of complement–haemolytic uraemic syndrome are unclear. It has been suggested that combined pathogenic variants (found in 3% of patients[82]) or associated at-risk haplotypes (in complement factor H [*CFH*], membrane cofactor protein [*MCP* or *CD46*], and CFH-related protein 1 [*CFHR1*]) increase the risk of disease occurrence.[2,3] Genetic screening of large cohorts of patients with atypical haemolytic uraemic syndrome revealed pathogenic variants in *CFH*, *MCP*, complement factor I (*CFI*), component 3 (*C3*), complement factor B (*CFB*) genes, or hybrid genes caused by non-allelic homologous recombination between *CFH* and *CFHR1* or *CFHR3* in 27–59% of adults and 19–52% of children (appendix pp 6, 7).

An interactive database dedicated to atypical haemolytic uraemic syndrome summarises the pathogenic variants that specifically impair the protection of endothelial cells from complement damage.[83] Pathogenic changes identified in patients with atypical haemolytic uraemic syndrome are non-sense and missense variants, small and large deletions, splice site changes, and complex rearrangements. However, several new rare missense variants have been identified,[84] thus it is crucial to assess the effect of a given variant on the regulation of the complement alternative pathway. In practice, pathogenic *CFH*, *CFI*, and *MCP* missense variants generally lead to impaired protein synthesis or protein function. Whereas, pathogenic *C3* and *CFB* missense variants are usually gain-of-function mutations. The challenge for genetic testing in atypical haemolytic uraemic syndrome is to show the pathogenic relevance of all novel or rare variants, defined in this Seminar as variants with a minor allele frequency of less than 1% (appendix p 9).[85]

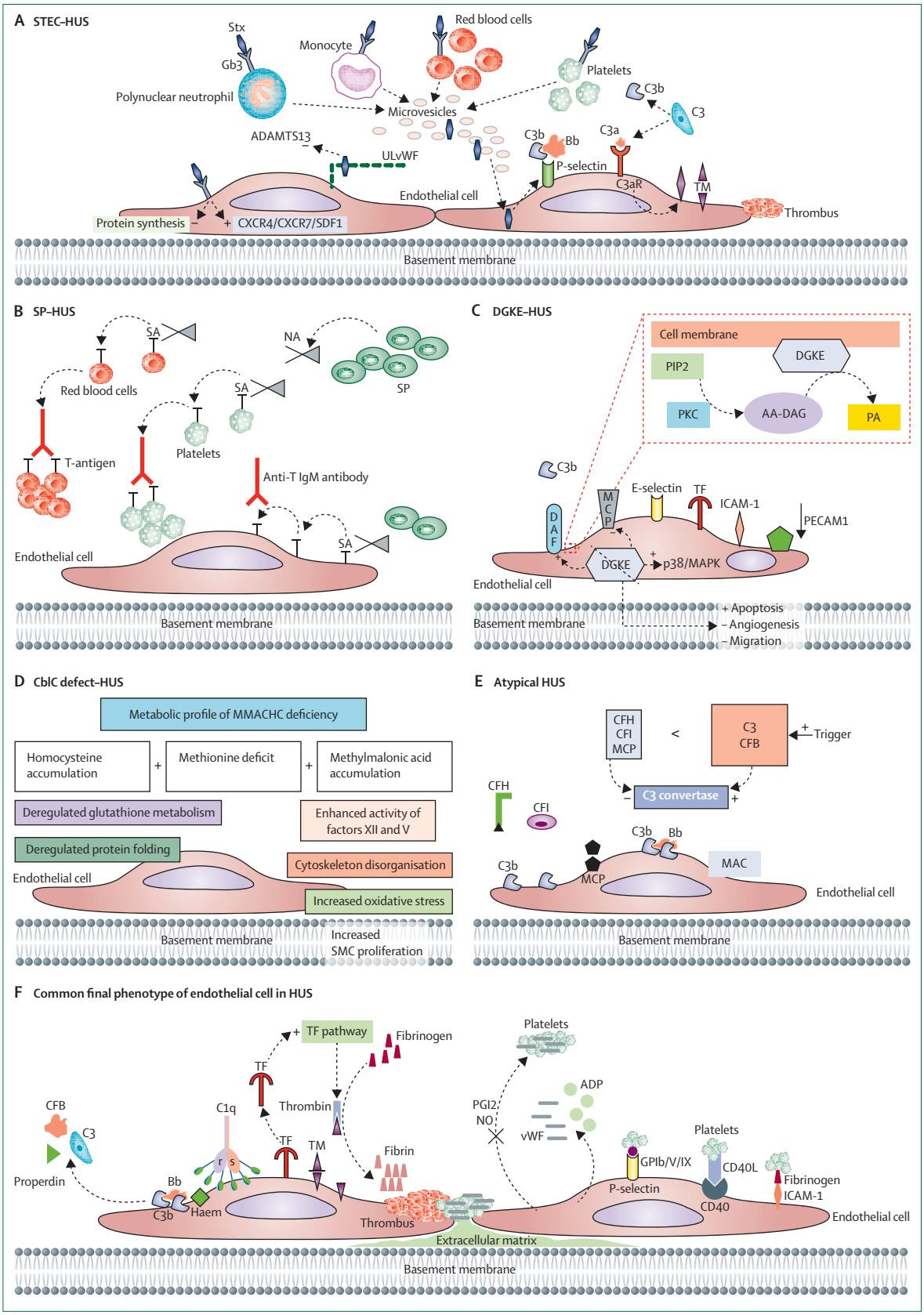
Variants in genes coding for proteins involved in the coagulation pathway, such as thrombomodulin[86] or plasminogen,[87] have been reported in patients with atypical haemolytic uraemic syndrome, but further studies are needed to confirm the role of this pathway in haemolytic uraemic syndrome. Finally, a few studies have identified novel or rare variants in complement genes in secondary forms of haemolytic uraemic syndrome—eg, rare pathogenic variants in pregnancy-associated haemolytic uraemic syndrome (86% of cases),[72] and rare variants, mostly without functional studies done as yet, in de-novo haemolytic uraemic syndrome after kidney transplantation (20%)[50] and haemopoietic stem-cell transplantation (65%).[46]

Clinical presentation

The clinical symptoms of haemolytic uraemic syndrome are non-specific and include fatigue, pallor, shortness of breath, reduced urine output, and oedema. STEC–haemolytic uraemic syndrome frequently follows prodromic bloody diarrhoea[88–90] (appendix pp 4, 5) and by contrast has seldom been reported after STEC urinary tract infection.[91] *S pneumoniae*–haemolytic uraemic syndrome occurs in individuals with severe *S pneumoniae* sepsis, associated usually with pleural or pulmonary infection, and in 30% of cases, with meningitis (appendix pp 4, 5).[9,23] In the context of atypical haemolytic uraemic syndrome, onset of the disease might follow intercurrent events (including viral gastroenteritis, influenza, vaccination, or childbirth), referred to as trigger events, in roughly half of children and a third of adults. Age of onset varies between patients and across causes of haemolytic uraemic syndrome. Although post-infectious haemolytic uraemic syndrome predominates in children younger than 3 years, the onset of complement–haemolytic uraemic syndrome occurs almost as frequently in children as in adults.[2,3] By contrast, all patients with DGKE–haemolytic uraemic syndrome have onset before 12–13 months of age (appendix pp 4, 5).[2,6]

The classic triad combining thrombocytopenia, haemolytic mechanical anaemia, and acute kidney injury remains the typical hallmarks of the disease. However, thrombocytopenia is usually mild in atypical haemolytic uraemic syndrome[92] and is absent at presentation in 15–20% of patients.[2,3] Renal presentation of atypical haemolytic uraemic syndrome is variable across patients and might include nephrotic range proteinuria resulting from glomerular basement membrane damage,[93] sometimes associated with C3 deposits in mixed atypical haemolytic uraemic syndrome and C3 glomerulopathy forms.[70] Renal failure requires prompt initiation of dialysis in more than 50% of cases regardless of the cause, and in more than 75% of adults with atypical haemolytic uraemic syndrome (appendix pp 4, 5). Additionally, some patients with atypical haemolytic uraemic syndrome develop inaugural accelerated and malignant hypertension, raising the complex issue of

For the **newly identified rare missense variants** see http://exac.broadinstitute.org/



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whether malignant hypertension complicates haemolytic uraemic syndrome or the other way around.[94] In the scenario of malignant hypertension complicating haemolytic uraemic syndrome, the control of hypertension is expected to lead to rapid resolution of thrombocytopenia and haemolysis.

Systemic presentation of haemolytic uraemic syndrome varies greatly between patients, depending on the organs affected by the thrombotic microangiopathy process. CNS involvement can be as high as 20% in paediatric STEC–haemolytic uraemic syndrome[88,95] and was reported in 50% of adults during the 2011 German *E coli* O104 outbreak.[96] Similarly, the percentage of patients with atypical haemolytic uraemic syndrome and extra-renal manifestations in retrospective studies ranges from 8% to 25% in adults and 16% to 29% in children (appendix pp 4, 5). These extra-renal manifestations predominantly include neurological symptoms[2,3,97] and pancreatointestinal involvement,[2] and less frequently gangrene of the fingers or toes,[98,99] ulcerative–necrotic skin lesions,[100] or myocardial infarction or ischaemic cardiomyopathy.[101–104]

Diagnostic investigations and differential diagnosis

A practical diagnostic approach of patients suspected of haemolytic uraemic syndrome is detailed in figure 3.[105–107] Several working groups have proposed diagnostic algorithms.[1,19,108,109] This approach relies on stepwise procedures, which aim to confirm or rule out distinct causative forms of haemolytic uraemic syndrome on the basis of direct tests. However, diagnosis of atypical haemolytic uraemic syndrome can be made by default when other causes have been eliminated with a reasonable clinical probability. The diagnostic algorithm is more straightforward in children than in adults because there is less confounding with coexisting diseases related to haemolytic uraemic syndrome;[1] additional investigations, guided by anamnesis and clinical examination, are usually needed in adults (figure 3).[19,109]

The main differential diagnosis of atypical haemolytic uraemic syndrome in children is STEC–haemolytic uraemic syndrome, whereas the two main differential diagnoses in adults are ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats-13) deficiency–thrombotic thrombocytopenic purpura and secondary haemolytic uraemic syndrome. *S pneumoniae*–haemolytic uraemic syndrome is suspected on the basis of clinical presentation.[23] cblC defect–haemolytic uraemic syndrome has long been considered as a disease of infants (<1 year) with severe forms of cblC defects.[4,5] Some reports[110–112] suggest that cblC defects can be detected in older children or adults with eculizumab-resistant atypical haemolytic uraemic syndrome (panel 1). This finding prompted experts to recommend plasma homocysteine and urine or plasma methylmalonic acid assessments as part of all investigations for STEC-negative atypical haemolytic uraemic syndrome.[113] In patients in whom stepwise investigations have led to the diagnosis of atypical haemolytic uraemic syndrome by elimination, complement and *DGKE* investigations are done (figure 3).

To date, no direct diagnostic test for atypical haemolytic uraemic syndrome exists. Available biomarkers are not completely reliable. For example, normal complement concentrations do not rule out complement–haemolytic uraemic syndrome because low concentrations of circulating C3 have low sensitivity (about 30% of patients with atypical haemolytic uraemic syndrome[2]), whereas high concentrations of circulating

Figure 2: **Pathophysiology of various forms of haemolytic uraemic syndrome**
(A) Stx enters the endothelial cell via Gb3-dependent and Gb3-independent pathways, and exerts its cytotoxic effect via protein synthesis inhibition and enhancement of the CXCR4/CXCR7/SDF1 pathway.[21] Stx also induces the translocation of P-selectin to the endothelial cell surface, favouring the assembly of alternative C3 convertase, the release of C3a, and TM shedding.[22] (B) SP–HUS is a prototypic NA-induced HUS.[23,24] The NA produced by SP cleaves SA of glycoproteins on red blood cells, platelets, and the cell surface of glomerular endothelial cells, exposing the cryptic Thomsen–Friedenreich antigen (T antigen). It is assumed that the reaction of T antigen with anti-T IgM antibodies normally present in plasma results in TMA. (C) DGKE is an intracellular lipid kinase that phosphorylates preferentially AA-DAG to PA and thus terminates DAG signalling. The loss of DGKE in endothelial cells enhances p38/MAPK pathway activation and ultimately leads to a prothrombotic and proinflammatory phenotype of the endothelial cell. In-vitro loss of DGKE does not seem to alter C3 deposition on endothelial cells.[25] (D) CblC deficiency with MMACHC is the most common congenital disorder of cobalamin metabolism (autosomal recessive; estimated incidence of one in 100 000 livebirths),[26] and results in an accumulation of homocysteine and methylmalonic acid and in decreased synthesis of methionine. Pathogenesis of organ damage in cblC deficiency remains partially understood. Endothelial cell damage can result from various consequences of homocysteine accumulation and methionine deficit, including deregulated glutathione and energy metabolism, and—of particular interest for HUS—enhanced platelet aggregation and coagulation,[27] and increased proliferation of vascular SMC and intima thickening.[28,29] (E) In atypical HUS, the loss of the inhibitory effect of CFH, CFI, or MCP (from inactivating mutations or anti-CFH antibodies) results in the loss of endothelial cell protection from CAP-induced damage. Similarly, gain-of-function mutations in the genes coding for C3 and CFB,[30,31] the two main components of the alternative C3 convertase, are associated with excessive activation of CAP, resulting in the endothelial cell acquiring a procoagulant and proinflammatory phenotype that triggers thrombosis.[32] (F) Distinct initial pathogenic mechanisms of HUS lead to a common final proinflammatory and prothrombotic phenotype of endothelial cells resulting from increased secretion of vWF multimers and ADP, decreased release of NO and PGI2, the upregulation at the endothelial cell surface of various adhesion molecules, expression and secretion of TF, alterations in the glycocalyx, and the shedding of TM. A detailed figure legend and references are provided in the appendix (pp 18–23). HUS=haemolytic uraemic syndrome. Stx=shiga toxin. Gb3=globotriaosylceramide 3. TM=thrombomodulin. SP=*Streptococcus pneumoniae*. NA=neuraminidase. SA=sialic acid. TMA=thrombotic microangiopathy. DGKE=diacylglycerol kinase ε. AA-DAG=arachidonic acid-containing diacylglycerol. PA=phosphatidic acid. TF=tissue factor. cblC=cobalamin C. MMACHC=methylmalonic aciduria. SMC=smooth muscle cell. CAP=complement alternative pathway. CFH=complement factor H. CFI=complement factor I. MCP=membrane-cofactor protein. MAC=membrane-attack complex. C3=component 3. CFB=complement factor B. vWF=von Willebrand factor. ADP=adenosine diphosphate. NO=nitric oxide. PGI2=prostacyclin.

	Mechanism of TMA
Malignancy	
Prostate, gastric, breast, lung, lymphoma, and others[33]	Intravascular tumoural emboli with coagulation activation and vessel wall proliferation.[34] Precise frequency of mutations of complement genes unknown. Anecdotal cases of eculizumab efficacy (possible bias in reporting).[35]
Drugs	
Anti-VEGF drugs	Direct dose-dependent endothelial cell toxicity. Anti-VEGF-induced TMA involves podocyte injury.[36]
Ciclosporin, tacrolimus, everolimus, gemcitabine, and mitomycin	Precise frequency of mutations of complement genes unknown. Anecdotal cases of eculizumab efficacy (possible bias in reporting).[37,38]
IFNα/β, cocaine, quinine,* oxaliplatine, and others[39]	Quinine-dependent antibodies against endothelial cell, platelets, and leukocytes.[40] Potential oxaliplatin-dependent antibodies against platelets.[40]
HSCT	Severe multivisceral TMA affecting almost invariably the kidney[41] but also the CNS, lung, and gastrointestinal tract,[42] and is associated with a high mortality (>80%).[43,44] Multifactorial endothelial cell damage: chemotherapy, total body irradiation, immunosuppressive drugs, graft *vs* host disease, and infections. Anti-CFH antibodies identified in three patients.[45] Increased frequency of variants in complement (*C3*, *C5*, *CD46*, *CD55*, and *CFD*) and *ADAMTS13* genes in patients with HSCT–TMA compared with patients without TMA after HSCT (clinical relevance of these variants to be fully assessed).[46] Increased soluble C5b-9[45] and positive C4d renal arteriolar staining[47] in patients with HSCT–TMA. In two retrospective cases series[48,49] of HSCT–TMA (30 patients in all), eculizumab use was associated with a haematological response in 56% of patients and a mortality rate of 40% (compared with 0–35% of haematological response and 78–96% mortality rate in historical controls treated mainly with PE[43,44]).
Solid organ transplantation	
Renal (de novo), lung, heart, and intestinal	Multifactorial: CNI and mTOR inhibitor toxicity, human leucocyte antigen mismatch, and infections. TMA might complicate acute humoral rejection of the renal graft. In one series, seven (29%) of 24 patients with de-novo TMA after renal transplantation had rare variants (initially reported as mutations) in *CFH* and *CFI* genes.[50] Anecdotal cases of eculizumab efficacy (possible bias in reporting).[51–53]
Infections†	
HIV	Putative direct HIV toxicity to endothelial cell. Incidence has decreased with highly active antiretroviral therapy.[54] Might be coincidental with CMV or HHV8 co-infection.[55,56]
H1N1 influenza	Unmasking of the cryptic Thomsen–Friedenreich antigen on red blood cells and endothelial cells has been suggested. Pneumococcal infection or HUS might be superimposed to H1N1 influenza infection. H1N1 influenza can trigger HUS in patients with complement or *DGKE* mutations.
CMV, HHV6, parvovirus B19, malaria, or others	Potential direct viral endothelial cell toxicity. Mainly in immunocompromised patients.[57]

(Table 1 continues on next page)

C5a and soluble C5b-9 might have insufficient specificity.[114–116] Similarly, diagnosis of atypical haemolytic uraemic syndrome should not be based on the detection of complement gene variants, which are identified in only 40–60% of patients or less (appendix pp 6, 7).[2,3] Genetic investigations might take up to several weeks and should not delay treatment of atypical haemolytic uraemic syndrome. Studies have tackled the challenge to shift atypical haemolytic uraemic syndrome from a differential diagnosis to a direct diagnosis, using complement biomarkers. Sophisticated assays have yielded promising results[115,117] but need to be validated in prospective studies.

Management and outcome

Supportive therapy (appendix p 10) is the cornerstone of haemolytic uraemic syndrome treatment and has largely contributed to the decrease in mortality following development of any form of haemolytic uraemic syndrome.

S pneumoniae–haemolytic uraemic syndrome

Early recognition and prompt initiation of antibiotics (mainly amoxicillin or third generation cephalosporin in case of meningitis) with supportive intensive care largely accounts for the improvement of *S pneumoniae*–haemolytic uraemic syndrome outcomes in the past two decades (appendix pp 4, 5). Because plasma contains anti-Thomsen–Friedenreich antibodies, which might enhance agglutination of Thomsen–Friedenreich-anti-Thomsen–Friedenreich and worsen haemolytic uraemic syndrome course, plasma and unwashed red blood cells or platelets are traditionally avoided, as long as agglutination tests are positive.[23]

STEC–haemolytic uraemic syndrome

Outcome and predictive factors

Central to management of STEC–haemolytic uraemic syndrome is early assessment of haemolytic uraemic syndrome severity, which correlates with the risk of sequelae (appendix pp 4, 5). Overall, early death rates have been reduced to 1·4–2·9% in children since the early 2000s;[89,118] whereas people aged 60 years or older still have the highest risk of death.[15] In the same period, the proportion of children who progressed to end-stage renal disease was 1·4%, or had renal (chronic kidney disease stage 1 or 2, proteinuria, or hypertension) or neurological sequels 5 years after STEC–haemolytic uraemic syndrome was 30% and 4%, respectively.[88] Risk factors for poor short-term and long-term outcomes include mainly increased leucocyte count, haemoconcentration, and dialysis need and duration (panel 2).[15,88–90,118,119]

Management

Correct management of circulatory volume is of utmost importance during the early course of STEC–haemolytic uraemic syndrome. Early fluid infusion reduces the rate of CNS involvement, need for dialysis, in-hospital stays, and long-term renal and extra-renal sequelae.[120] Therefore, volume depletion should be restricted to patients with anuria and life-threatening fluid overload.

Antibiotics have long been contraindicated in patients with STEC gastroenteritis, despite the paucity of supportive evidence from meta-analyses[121,122] or large series.[88,89,123] Antibiotics might increase the risk of haemolytic uraemic syndrome because antibiotic-induced injury to the bacterial membrane might favour the release of shiga toxin, antibiotics might give STEC a selective advantage if these organisms are not as readily eliminated from the bowel as the normal intestinal flora, and some antibiotics (such as fluoroquinolones,

particularly ciprofloxacin) are potent inducers of shiga toxin gene expression. In view of these reasons, discrepant results might occur from the class of antibiotics used: data from two prospective studies in children with O157 STEC–diarrhoea showed an increased risk of haemolytic uraemic syndrome either in the children treated with any kind of antibiotics[124] or only in those given bactericidal antibiotics.[125] This highly topical issue was re-examined after the outbreak in Germany, when O104 shedding was shown to be shortened in patients with haemolytic uraemic syndrome or in long-term carriers of O104 who were treated with azithromycin (a non-bactericidal antibiotic).[126–128] Conversely, no occurrence of haemolytic uraemic syndrome was reported in O104 carriers treated with azithromycin.[126] Azithromycin reduces the release of shiga toxin from STEC in vitro and STEC-induced mortality in animal models.[128] The current view about azithromycin has shifted towards a more balanced benefit to risk ratio, prompting an ongoing prospective study (NCT02336516).

Randomised trials did not show benefit from the use of anti-thrombotic and antifibrinolytic or shiga toxin binding agents, or plasma infusions (appendix p 11). The benefit conveyed by plasma exchanges over supportive therapy alone remains far from certain.[95,123,129,130] Notably, a few patients with severe neurological complications, which were unresponsive to plasma exchanges and eculizumab, during the 2011 German outbreak showed a prompt recovery upon immunoadsorption.[131–133] These promising results in patients with life-threatening conditions warrant confirmation in properly designed prospective studies. Innovative approaches that target shiga toxin synthesis, as well as entry and toxic effect in endothelial cells[134] might lead to new specific treatments for STEC–haemolytic uraemic syndrome.

A report of three children with O157 STEC–haemolytic uraemic syndrome that showed a prompt neurological recovery from eculizumab therapy has raised tremendous hopes.[135] However, on the one hand, two large series from the 2011 German outbreak have since shown similar outcomes in patients treated with or without eculizumab, including those with neurological impairment.[123,130] On the other hand, subsequent smaller series have reported rapid improvements with eculizumab for life-threatening heart or brain manifestations, or both, in severe forms of STEC–haemolytic uraemic syndrome.[136–138] Notably, these reports were retrospective and non-controlled, and an ongoing randomised controlled trial (NCT02205541) should clarify the benefit of eculizumab in STEC–haemolytic uraemic syndrome.

Treatment remains supportive in most cases (figure 3). However, for those with life-threatening complications, short-term eculizumab therapy and immunoadsorption sessions in unresponsive cases might be a suitable treatment strategy.

Mechanism of TMA	
(Continued from previous page)	
Autoimmune diseases	
SLE	Endothelial cell injury mediated by immune complexes.[58] Might be related to intravascular immunoglobulin thrombi[59] and coexist with proliferative lupus nephritis or APS, or both. Anecdotal reports of the efficacy of eculizumab (possible bias in reporting).[60,61]
APS	Two-hit hypothesis.[62] First hit: binding of anti-b2GPI antibodies to their target on endothelial cells leads to the upregulation of adhesion molecules and TF, and the displacement of the anticoagulant annexin A5. Second hit: inflammatory trigger (eg, infection or surgery) and activation of the complement cascade leading to thrombosis.[63] C5a increases the release of TF by neutrophils.[64] Suggested role of b2GPI as a complement regulator (binding to C3b).[65] Few cases of eculizumab efficacy in catastrophic APS (possible bias in reporting).[66–68]
Systemic sclerosis	Vessel wall intimal proliferation and lumen obstruction. Might be precipitated by steroids.
Polymyositis or dermatomyositis, Still's disease, or other	Few cases of HUS reported.[69] Dermatomyositis is a complement-mediated disorder.
Malignant hypertension	Differential diagnosis with atypical HUS might prove difficult because atypical HUS might present with malignant hypertension, and any form of malignant hypertension might lead to TMA.
Coexisting nephropathies	HUS might complicate the course of IgA nephropathy, C3 glomerulopathy, or other membranoproliferative glomerulonephritis,[70] ANCA, or anti-GBM vasculitis. Possible link between complement activation and TMA in ANCA vasculitis.[71]
Pregnancy–HUS	In a retrospective study,[72] among 21 patients with pregnancy–HUS (79% in post-partum), 52% reached end-stage renal disease within 6 months of onset *vs* 57% in women with non-pregnancy-related atypical HUS, and 86% had mutations of complement genes *vs* 74% in female patients with non-pregnancy-related atypical HUS. Patients with pregnancy–HUS might have disease relapse outside pregnancy.[73] Few cases of eculizumab efficacy in pregnancy–HUS or post-partum–HUS.[73,74]
Differential diagnosis of pregnancy–HUS	
HELLP syndrome, pre-eclampsia, or eclampsia	Endothelial cell injury results from an imbalance between antiangiogenic (soluble Flt1 and endoglin) and angiogenic factors (placental growth factor).[75] Initial cause of the disease is unknown but probably multifactorial. Variants of complement genes reported in 10–12% of patients with HELLP syndrome,[76] in 18% of patients with SLE-associated or APS-associated pre-eclampsia, and in 8% of patients with non-immune pre-eclampsia.[77]
Post-partum haemorrhage	High risk of renal cortical necrosis in the setting of gravid renal endothelium.[78] Current data not supportive of a definitive role of complement.

The main causes of secondary HUS and their underlying proven or supposed mechanisms. TMA=thrombotic microangiopathy. VEGF=vascular endothelial growth factor. mTOR=mammalian target of rapamycin. IFN=interferon. HSCT=haemopoietic stem cell transplantation. CFH=complement factor H. ADAMTS13=a disintegrin and metalloprotease with thrombospondin type 1 repeats-13. PE=plasma exchange. CNI=calcineurin inhibitors. CFI=complement factor I. CMV=cytomegalovirus. HHV8=human herpesvirus-8. SLE=systemic lupus erythematosus. APS=antiphospholipid syndrome. TF=tissue factor. HUS=haemolytic uraemic syndrome. ANCA=antineutrophil cytoplasm antibodies. GBM=glomerular basement membrane. HELLP=haemolysis, elevated liver enzymes, low platelet count. *Includes quinine containing beverages. †STEC–HUS and SP–HUS are discussed separately.

Table 1: **HUS associated with coexisting diseases or conditions, and pregnancy–HUS and its differential diagnosis**

Atypical haemolytic uraemic syndrome

Outcome and predictive factors

Before eculizumab was available for treatment of haemolytic uraemic syndrome, the death rate (reported in two European cohorts)[2,3] was higher in children than in adults (8–14% *vs* 2–4%) at 3–5 years' follow-up. Conversely, the rate of end-stage renal disease was higher in adults than in children (table 2 and appendix pp 4, 5).[2,3] In adults, outcomes were similarly poor irrespective of whether a patient had a complement variant or not. *CFH*–haemolytic

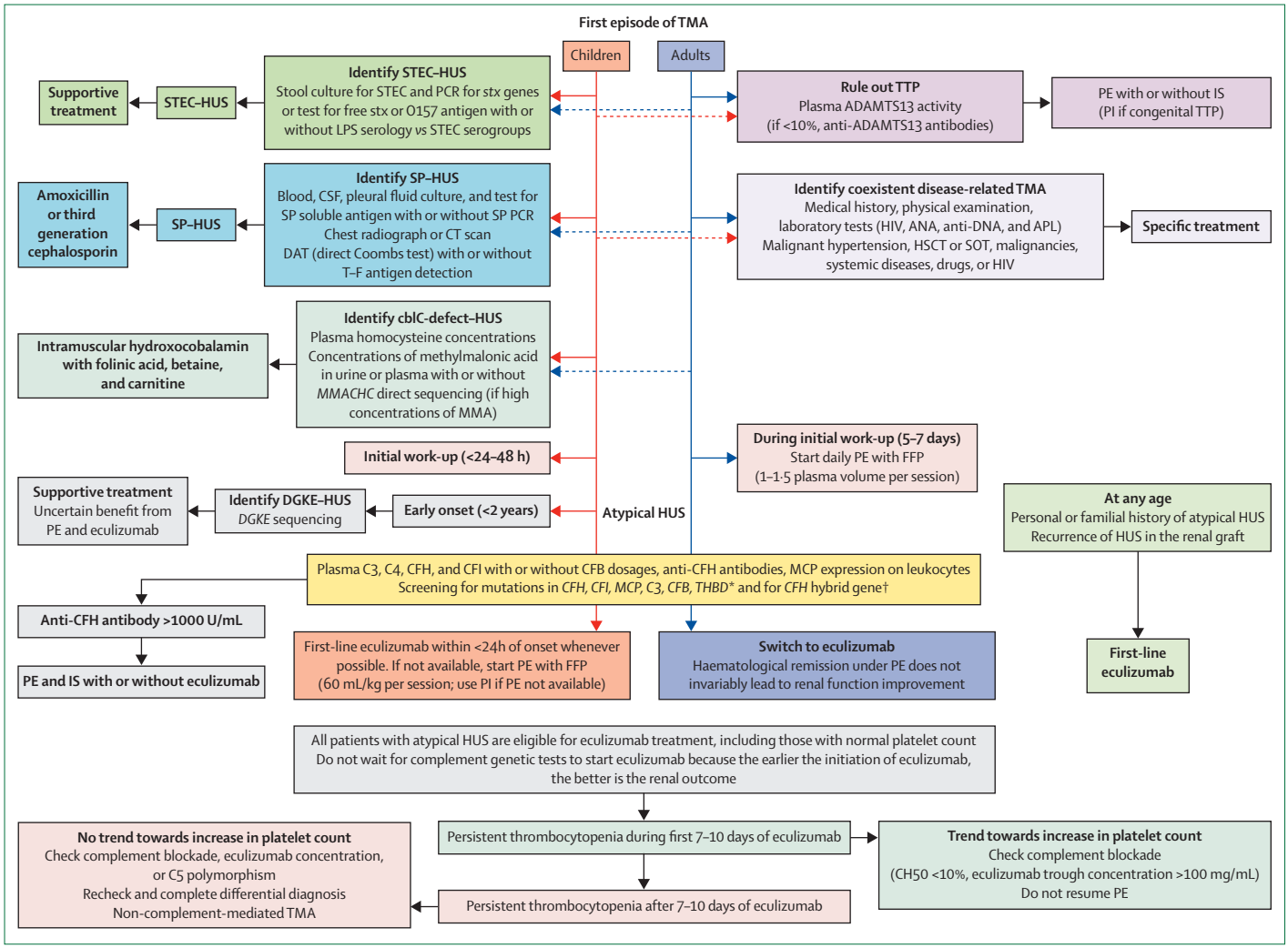

Figure 3: **Practical diagnostic approach and treatment options for HUS according to age at onset**
Dashed arrows point to a clinical situation less frequent than those pointed with solid arrows. HUS=haemolytic uraemic syndrome. TMA=thrombotic microangiopathy. STEC=shiga toxin-producing *Escherichia coli*. Stx=shiga toxin. LPS=lipopolysaccharide. SP=*Streptococcus pneumoniae*. CSF=cerebrospinal fluid. DAT=direct anti-globulin test. T–F=Thomsen–Friedenreich. cblC=cobalamin C. MMA=methylmalonic aciduria. MMACHC=methylmalonic aciduria (cbl deficiency) cblC type. DGKE=diacylglycerol kinase ε. PE=plasma exchange. TTP=thrombotic thrombocytopenic purpura. IS=immunosuppressive treatment. ADAMTS13=a disintegrin and metalloprotease with thrombospondin type 1 repeats-13. PI=plasma infusion. ANA=antinuclear antibodies. APL=antiphospholipid antibodies. HSCT=haemopoietic stem cell transplantation. SOT=solid organ transplantation. FFP=fresh frozen plasma. MCP=membrane cofactor protein. CH50=total complement activity 50. CFH=complement factor H. CFI=complement factor I. MCP=membrane-cofactor protein. C3=component 3. C4=component 4. THBD=thrombomodulin. *Variants can be screened either by Sanger or new generation sequencing methods. †Complex gene rearrangements are sought through multiplex ligation-dependent probe amplification. See the appendix (pp 23–26) for a detailed figure legend.

uraemic syndrome had the most severe outcomes for children and adults.[2,3] Only children with *MCP* variants retained relatively favourable outcomes despite frequent relapses (25% end-stage renal disease at median follow-up of 17·8 years).[2] Although a similar percentage (approximately 30%) of adults and children had relapses during the first year, relapse was more common in children in subsequent years (approximately 50% *vs* 20%) because of the high frequency of relapses in children with *MCP* variants.[2] Patients with DGKE–haemolytic uraemic syndrome usually progress to severe chronic kidney disease and end-stage renal disease by the age of 20–25 years (appendix p 12). The use of immunosuppressive drugs has substantially improved the renal survival and decreased the risk of relapse in patients with anti-CFH antibodies (appendix p 13).

Management

There are no prospective randomised controlled trials assessing the safety and efficacy of any therapeutics for atypical haemolytic uraemic syndrome, primarily because of the rarity of the disease. Industry-sponsored trials with eculizumab, the first licensed complement blocker, in atypical haemolytic uraemic syndrome were prospective but not controlled.[139–144] Therefore, the assessment of eculizumab efficacy relies on a

Panel 1: Main clinical characteristics in patients with early-onset[4] and late-onset[5,110–112] cobalamin C defect-related haemolytic uraemic syndrome

Clinical presentation

- Early-onset forms (<1 year): feeding issues (vomiting or poor sucking), failure to thrive, neurological symptoms (hypotonia, lethargy, developmental delay, seizures, microcephaly, or hydrocephalus), and visual impairment (pigmentary retinopathy or nystagmus)
- Late-onset forms: (≥1 year) pulmonary hypertension and neuropsychiatric symptoms (cognitive impairment, ataxia, seizures, or myelopathy)

Epidemiology

- Around 37 cases of haemolytic uraemic syndrome reported so far (17 with an onset >1 year)
- Haemolytic uraemic syndrome in 5% of cases with onset ≤1 year; 25% of cases with onset >1 year

Natural history

- Both early-onset and late-onset haemolytic uraemic syndrome present with severe hypertension (sometimes misdiagnosed as malignant hypertension), proteinuria, with or without nephrotic syndrome, haematuria, progressing chronic kidney disease, with or without acute kidney injury, mechanical haemolytic anaemia, macrocytosis, thrombocytopenia, with or without leucocytopenia
- Early-onset forms: progression to multivisceral failure, end-stage renal disease, cardiomyopathy, neurological deterioration, and eventually death (roughly 100% if untreated)
- Late-onset haemolytic uraemic syndrome forms frequently associated with pulmonary hypertension (40%)

Treatment

- Intramuscular hydroxocobalamin (doses titrated to target plasma homocysteine concentrations <40–60 µmol/L); normalisation of total homocysteine concentration (<15 µmol/L) rarely achieved, except in some patients with late onset
- Supplements in folinic acid, betaine, and carnitine
- Protein restriction not recommended

Outcomes upon early-initiated hydroxycobalamin treatment

- Early-onset forms: treatment prevents death, allows renal recovery, but does not protect from neurocognitive and vision deterioration
- Late-onset forms: treatment allows renal recovery, prevents haemolytic uraemic syndrome relapses, and improves pulmonary hypertension and neuropsychiatric symptoms

comparison between historical controls from the pre-eculizumab era and patients treated with eculizumab, who were enrolled in either prospective or retrospective studies.

Panel 2: Predictors of short-term and long-term outcome of patients with *STEC*–haemolytic uraemic syndrome

In-hospital death

Older than 60 years[15,118]

In children:[89]

- White blood cell count >25 400 cells per mL
- Haemoconcentration (haematocrit [Ht] ≥20%)
- Recent respiratory tract infection

Three greater risk profiles:

- White blood cell count >41 900 cells per mL (n=44, nine deaths, 20·5% probability of death, OR 45)
- White blood cell count 25 400–41 900 cells per mL and Ht >19·6% (n=86, eight deaths, 9·3% probability of death, OR 18)
- White blood cell count ≤25 400 cells per mL and recent respiratory tract infection (n=37, one death, 2·7% probability of death, OR 4·9)

Sequelae in the long term

In children:[88]

- White blood cell count >20 000 cells per mL
- Use of plasma exchange during the acute phase
- Dialysis duration
- Hypertension at the acute phase
- Haemoconcentration (haemoglobin >5·6 mmol/L)[119]

STEC=shiga toxin-producing *Escherichia Coli*. OR=odds ratio. For a detailed explanation of panel 2 see the appendix (pp 28,29).

There is no strong evidence for plasma therapy efficacy in atypical haemolytic uraemic syndrome. Data from a 2015 study suggest that plasma therapy does not decrease serum concentrations of complement alternative pathway activation markers during acute atypical haemolytic uraemic syndrome.[145] Moreover, in two independent large cohort studies,[2,3] plasma therapy had little effect on renal survival. In an Italian study,[3] although plasma therapy induced haematological remission in 78% of children and 53% of adults with episodes of atypical haemolytic uraemic syndrome, half of the children and two-thirds of the adults progressed to end-stage renal disease or died at 3 years' follow-up. Similarly, in a French study,[2] children and adults had poor renal outcomes after the first episode of atypical haemolytic uraemic syndrome, whether they had high-dose plasma therapy (>5 plasma exchanges or plasma infusions >10 mL/kg per day for >5 days) or not. Moreover, the improved long-term renal outcomes observed in children with *MCP* variants cannot not be attributed to plasma efficacy because MCP is a membrane-anchored protein. Plasma exchange is associated with high morbidity in children (31%), mainly due to central catheter complications.[20]

Eculizumab, a monoclonal anti-C5 antibody that blocks the entry of C5 into C5 convertase,[109] has revolutionised the treatment of atypical haemolytic uraemic syndrome. Recommended doses and treatment regimens for

	Children			Adults				
	Pre-eculizumab era		Eculizumab	Pre-eculizumab era		Eculizumab		
	French cohort[2] (n=89)	Italian cohort[3] (n=149)	Trial 3[139,140] (n=22)	French Cohort[2] (n=89)	Italian cohort[3] (n=149)	Trial 1[141,142] (n=17)	Trial 2[141,142] (n=20)	Trial 4[143,144] (n=41)
First episode	16%	..	..	46%	..	..	..	..
6-month follow-up	..	..	9%	..	..	6%	10%	15%
1-year follow-up	29%	..	9%	56%	..	6%	10%	15%
2-year follow-up	..	..	..	..	..	12%	10%	..
3-year follow-up	..	48%	..	..	67%	..	..	..
5-year follow-up	36%	..	..	64%	..	..	..	..

For a detailed table legend see the appendix (pp 27,28). HUS=haemolytic uraemic syndrome.

Table 2: **Percentage of patients with atypical HUS who progressed to end-stage renal disease or who died in four prospective trials of eculizumab compared with the Italian and French registries of the pre-eculizumab era**

eculizumab, according to patient weight, along with recommendations for monitoring complement blockade are shown in the appendix (p 14). Eculizumab tolerability is generally good. The main risk of complement blockade is meningococcal meningitis, which occurred in two of 100 patients included in prospective trials.[139,141–144] Specific meningococcal prophylaxis is therefore mandatory in patients receiving eculizumab.[1] Phase 4 studies are required to fully assess the increased risk of other bacterial or viral infections, particularly in patients who have had a transplant.[146]

Eculizumab for treating atypical haemolytic uraemic syndrome has been tested in four prospective trials (three in adults[141–144] and one in children[139,140]) and one retrospective series[93] (table 2). In plasma-responsive or plasma-dependent adult patients with chronic kidney disease, eculizumab maintained haematological normalisation in 90% of patients despite plasma therapy cessation, and was associated with a small but significant increase in estimated glomerular filtration rate (eGFR; 6–8 mL/min) at 1-year and 2-year follow-up.[141,142] This finding suggests that cryptic active thrombotic microangiopathy was ongoing in some patients who had plasma therapy despite haematological remission. Presently, long-term plasma therapy is infrequently the preferred first-line treatment, and is mostly used when access to eculizumab is limited (figure 3).

In the other prospective studies, treatment with eculizumab was initiated shortly after plasma therapy was proved inefficacious (ie, haematological remission was not achieved) or as first-line treatment. Haematological normalisation was achieved and maintained in 82% of children at 1-year follow-up,[139,140] and in 88–90% of adults at 1-year[143,144] and 2-year follow-up.[142] The increase in eGFR was more pronounced in children (64 mL/min)[139,140] than in adults (30–35 mL/min).[141–144] This finding is in accords with the overall better renal prognosis of atypical haemolytic uraemic syndrome in children than in adults.[2] However, early initiation of eculizumab as first-line therapy accounts for better outcomes in more than half of children. Indeed, the time between onset of atypical haemolytic uraemic syndrome episode and treatment initiation inversely correlates with the increase in eGFR.[141,142,147] None of the children and only one (1%) of the 78 adults died within the 1–2 years of study follow-up with use of eculizumab. End-stage renal disease or death at 1-year or 2-year follow-up occurred in 6–15% in adults and 9% in children, which is far lower than occurred in the pre-eculizumab era (table 2). A retrospective study showed a similar significant benefit from eculizumab treatment.[93] Several case reports[99,148–151] of patients with plasma exchange and plasma infusion-resistant atypical haemolytic uraemic syndrome show the capability of eculizumab to relieve neurological, cardiac, and peripheral ischaemic complications.[1] On the basis of these studies, which support the superiority of eculizumab over plasma therapy in atypical haemolytic uraemic syndrome, all patients with atypical haemolytic uraemic syndrome are eligible for treatment with eculizumab, if it is available (figure 3).

Important information can also be yielded from these studies that help to address pending questions about long-term management of eculizumab treatment (appendix p 15). Increasing the time between eculizumab infusions would reduce the treatment burden and cost but requires close monitoring of the complement blockade by CH50 (complement haemolytic 50) and eculizumab trough concentration, if available. Some patients (mostly paediatric), receiving eculizumab every 3–4 weeks can still show a potent drug effect as reflected by sustained undetectable CH50 activity.[152] The most crucial question is, however, the duration of therapy. If renal function fails to recover, in the absence of other signs of active disease, a 3–6 month treatment is nonetheless recommended before considering eculizumab discontinuation, because late renal recovery is possible.[1,104] In patients who had stable haematological and renal remission following treatment with eculizumab, the issue of whether or not to discontinue eculizumab therapy after a few months is debated. One

argument is that the unpredictable relapse rate, the increased risk of meningococcal infection by roughly 5000 times,[153] and the high cost of the drug supports discontinuation. However, the risk of relapse with ensuing acute kidney injury and potentially irreversible chronic kidney disease favours long-term treatment. Ongoing prospective studies (NCT02574403) will provide valuable information in this debate.

Preliminary data from retrospective studies suggest that pathogenic variants in complement genes might be the most important predictors of relapse of atypical haemolytic uraemic syndrome after treatment discontinuation (appendix p 8). Patients with *CFH* variants seem to have the highest risk (five [50%] of ten patients) whereas those without complement variants seem to have a very low risk (one [5%] of 18 patients). Regardless of the genetic background, eculizumab discontinuation requires a close monitoring of the biological features of thrombotic microangiopathy using urinary dipsticks (twice weekly, increased to daily in case of infection) and blood tests (once weekly initially). Education of the patient and prompt resuming of eculizumab for early-diagnosed relapses should reduce the risk of irreversible chronic kidney disease. Progressive tapering of eculizumab doses before its discontinuation is not supported by any data.

Two subtypes of atypical haemolytic uraemic syndrome have specific considerations. Firstly, no clear benefit—especially in terms of proteinuria reduction—from plasma exchanges and plasma infusions or eculizumab has been reported in children with DGKE–haemolytic uraemic syndrome (appendix p 12). Secondly, plasma exchange associated with corticosteroids and immunosuppressors are the established treatment for anti-CFH antibody–haemolytic uraemic syndrome. But some case reports show that eculizumab is efficient to induce remission and rescue life-threatening complications in this subtype (appendix p 13),[154–157] suggesting that further studies are necessary to define the role of eculizumab in these settings.

Secondary haemolytic uraemic syndrome

Haemolytic uraemic syndrome associated with coexisting diseases or conditions comprise a heterogeneous group, of a variety of types of endothelial cell damage (table 1). The identification of complement alternative pathway dysregulation as a major driver of atypical haemolytic uraemic syndrome has prompted some investigators to reconsider the pathogenesis of secondary haemolytic uraemic syndrome, leading for instance to the reclassification of pregnancy–haemolytic uraemic syndrome. Similarly, the implication of complement alternative pathway dysregulation has been suggested in several secondary haemolytic uraemic syndrome (table 1).

The treatment of secondary haemolytic uraemic syndrome relies on treatment and withdrawal of the triggering condition whenever feasible. Plasma therapy is often empirically used despite the lack of established benefit. A paucity of case reports suggest that complement blockade might be a potential second-line treatment in some secondary haemolytic uraemic syndrome that is resistant to conventional management (table 1). However, the reporting bias inherent to these cases constitutes a strong limitation. Screening for variants of complement genes in large cohorts of secondary haemolytic uraemic syndrome and studies assessing complement blockade in this setting are urgently needed.

Haemolytic uraemic syndrome in pregnancy

Pregnancy can trigger different types of thrombotic microangiopathies, including ADAMTS13-deficiency–thrombotic thrombocytopenic purpura (mostly during the second and third trimesters), haemolytic uraemic syndrome (mostly in peripartum or post-partum), and HELLP (haemolysis, elevated liver enzymes, and low platelet count) syndrome, a thrombotic microangiopathy affecting the liver and inconstantly the kidney (table 1). Features of thrombotic microangiopathy might also be encountered in severe pre-eclampsia or eclampsia and post-partum haemorrhage. A retrospective study showed that pregnancy–haemolytic uraemic syndrome shares features with atypical haemolytic uraemic syndrome, including severity at diagnosis and during follow-up, frequency of variants of complement genes, and a similar pattern of relapse.[72] Thus, most investigators assume that pregnancy-related atypical haemolytic uraemic syndrome is an atypical haemolytic uraemic syndrome precipitated by pregnancy. Large international studies are needed to achieve consensus across the field. Diagnosis of haemolytic uraemic syndrome might be difficult when thrombotic microangiopathy develops during a complicated peripartum course. Clinical history, measurement of ADAMTS13 activity, the presence of marked liver enzymes elevation (HELLP syndrome), or rapid (48–72 h) regression of thrombotic microangiopathy features after delivery (usual in HELLP syndrome and pre-eclampsia or eclampsia) might help clinicians to distinguish atypical haemolytic uraemic syndrome from other thrombotic microangiopathy disorders.

As for atypical haemolytic uraemic syndrome, eculizumab has been found remarkably efficient to control pregnancy–haemolytic uraemic syndrome.[74,158,159] Data mainly from cohorts of patients with paroxysmal nocturnal haemoglobinuria suggest that the use of eculizumab appears safe during pregnancy.[160] Transplacental passage of the drug was documented in 35% of women—however, at concentrations below the therapeutic range—and the drug was not detected in the milk of breastfeeding mothers. No eculizumab-related side-effects were reported in newborn babies and infants. Nevertheless, up to half of pregnant patients might require an increase in the dose or the frequency of eculizumab infusions, or both, to maintain an optimal complement blockade.[160]

Renal transplantation and haemolytic uraemic syndrome

The different forms of haemolytic uraemic syndrome whose pathogenic mechanism primarily involves damage to the endothelial cells by environmental factors have a low rate of recurrence after kidney transplantation. By contrast, atypical haemolytic uraemic syndrome related to complement alternative pathway dysregulation, involving circulating factors, is associated with a high rate of recurrence after kidney transplantation (appendix p 16). Importantly, in the pre-eculizumab era, atypical haemolytic uraemic syndrome recurrence was strongly and independently associated with kidney graft failure in patients with atypical haemolytic uraemic syndrome (risk ratio 4·86, 95% CI 1·30–13·81; p=0·001), because of the poor efficacy of plasma exchange in the treatment of overt recurrence.[161] However, the past 5 years have seen a shift toward safer and more successful outcomes, based on targeted and individualised strategies (appendix p 16).

The risk of recurrence in atypical haemolytic uraemic syndrome is dependent on the genetic background. Patients with isolated variants in membrane-anchored (eg, MCP) or intracellular (eg, DGKE) proteins have a low risk of post-transplant recurrence, because the kidney allograft expresses a functional protein, whereas patients with variants in circulating factors have a risk of recurrence that ranges from 50% to nearly 100%.[3,161] Consequently, the risk of post-transplant recurrence differs in carriers of *MCP* variants from 7·6% to 30%, depending on whether or not the *MCP* variant is with other variants involving circulating factors.[82] With respect to DGKE, none of the three reported variants of *DGKE* carriers transplanted so far have had a recurrence.[6]

The greatest risk of post-transplant recurrence (>90%) is associated with *CFH*, *C3*, and *CFB* variants, whereas patients with neither pathogenic variant nor homozygous at-risk *CFH* haplotype (polymorphism) have a much lower post-transplant recurrence rate (about 20%), and patients with *CFI* pathogenic variant or at-risk *CFH* haplotype have an intermediate risk (about 50%).[161] Hence, tailored therapeutic strategies have been proposed, based on complement and genetic investigations, to prevent recurrence of atypical haemolytic uraemic syndrome. Combined liver and kidney transplantation with perioperative plasma or eculizumab has also been done in a few patients with mostly *CFH* variants (appendix p 16). With respect to overt atypical haemolytic uraemic syndrome recurrence, long-term eculizumab therapy has emerged as the new gold standard first-line treatment. The earlier treatment is initiated after the onset of the recurrence, the better the recovery of graft function.

Conclusion

Much has been achieved in the field of haemolytic uraemic syndrome in the past 10 years (appendix p 17). A mechanistic approach of haemolytic uraemic syndrome has been developed, an innovative efficacious treatment—the anti-C5 antibody eculizumab—has been made available for atypical haemolytic uraemic syndrome, and new mechanisms of pathogenesis have been identified. Several aspects remain unclear. Reliable biomarkers for the diagnosis and monitoring of haemolytic uraemic syndrome are missing. The optimal duration of complement blockade in patients with atypical haemolytic uraemic syndrome, and the place of this treatment in STEC–haemolytic uraemic syndrome and secondary haemolytic uraemic syndrome need further assessment. A specific treatment of STEC–haemolytic uraemic syndrome is urgently needed. Ongoing studies will undoubtedly provide clues or definite answers to these questions.

Contributors

All authors contributed equally to the preparation of this Seminar.

Declaration of interests

FF, JZ, VF-B, and CL served on advisory boards or in teaching courses, or both, for Alexion Pharmaceuticals. VF-B serves as coordinator and FF serves as member of the scientific advisory board of Alexion M11-001 atypical Haemolytic Uraemic Syndrome international registry, and CL serves as coordinator for France for this registry. JZ's research is supported by the Emmanuel Boussard Foundation.

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References

1 Loirat C, Fakhouri F, Ariceta G, et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. *Pediatr Nephrol* 2016; **31:** 15–39.

2 Fremeaux-Bacchi V, Fakhouri F, Garnier A, et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin J Am Soc Nephrol* 2013; **8:** 554–62.

3 Noris M, Caprioli J, Bresin E, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 2010; **5:** 1844–59.

4 Fischer S, Huemer M, Baumgartner M, et al. Clinical presentation and outcome in a series of 88 patients with the cblC defect. *J Inherit Metab Dis* 2014; **37:** 831–40.

5 Huemer M, Scholl-Burgi S, Hadaya K, et al. Three new cases of late-onset cblC defect and review of the literature illustrating when to consider inborn errors of metabolism beyond infancy. *Orphanet J Rare Dis* 2014; **9:** 161.

6 Lemaire M, Fremeaux-Bacchi V, Schaefer F, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet* 2013; **45:** 531–36.

7 Pai R, Moore MR, Pilishvili T, Gertz RE, Whitney CG, Beall B. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis* 2005; **192:** 1988–95.

8 Veesenmeyer AF, Edmonson MB. Trends in US hospital stays for *Streptococcus pneumoniae*-associated hemolytic uremic syndrome. *Pediatr Infect Dis J* 2013; **32:** 731–35.

9 Banerjee R, Hersh AL, Newland J, et al. *Streptococcus pneumoniae*-associated hemolytic uremic syndrome among children in North America. *Pediatr Infect Dis J* 2011; **30:** 736–39.

10 Ong KL, Apostal M, Comstock N, et al. Strategies for surveillance of pediatric hemolytic uremic syndrome: Foodborne Diseases Active Surveillance Network (FoodNet), 2000–2007. *Clin Infect Dis* 2012; **54** (suppl 5): S424–31.

11 Bruyand M, Mariani-Kurkdjian P, Gouali M, Van Cauteren D, de Valk H et le réseau des néphrologues pédiatres. Surveillance du syndrome hémolytique et urémique post-diarrhéique chez l'enfant de moins de 15 ans en France en 2014. Institut de veille sanitaire. 2014. http://invs.santepubliquefrance.fr/content/download/114287/401538/version/2/file/Bilan_SHU_2014.pdf (accessed Oct 30, 2015).

12 Ardissino G, Salardi S, Colombo E, et al. Epidemiology of haemolytic uremic syndrome in children. Data from the North Italian HUS network. *Eur J Pediatr* 2016; **175:** 465–73.

13 Guirro M, Piazza RM, de Souza RL, Guth BE. Humoral immune response to Shiga Toxin 2 (Stx2) among Brazilian urban children with hemolytic uremic syndrome and healthy controls. *BMC Infect Dis* 2014; **14:** 320.

14 Rivas M, Chinen I, Miliwebsky E, Masana M. Risk factors for Shiga toxin-producing *Escherichia coli*-associated human diseases. *Microbiol Spectr* 2014; **2**.

15 Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 2011; **365:** 1771–80.

16 Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med* 2011; **365:** 1763–70.

17 Gould LH, Mody RK, Ong KL, et al. Increased recognition of non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog Dis* 2013; **10:** 453–60.

18 European Centre for Disease Prevention and Control. Annual epidemiological report 2014—food- and waterborne diseases and zoonoses. Shiga toxin/verocytotoxin-producing *Escherichia coli* infection. 2014. http://ecdc.europa.eu/en/publications/Publications/food-waterborne-diseases-annual-epidemiological-report-2014.pdf (accessed Oct 30, 2015).

19 Sheerin NS, Kavanagh D, Goodship TH, Johnson S. A national specialized service in England for atypical haemolytic uraemic syndrome-the first year's experience. *QJM* 2016; **109:** 27–33.

20 Johnson S, Stojanovic J, Ariceta G, et al. An audit analysis of a guideline for the investigation and initial therapy of diarrhea negative (atypical) hemolytic uremic syndrome. *Pediatr Nephrol* 2014; **29:** 1967–78.

21 Petruzziello-Pellegrini TN, Yuen DA, Page AV, et al. The CXCR4/CXCR7/SDF-1 pathway contributes to the pathogenesis of Shiga toxin-associated hemolytic uremic syndrome in humans and mice. *J Clin Invest* 2012; **122:** 759–76.

22 Morigi M, Galbusera M, Gastoldi S, et al. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. *J Immunol* 2011; **187:** 172–80.

23 Spinale JM, Ruebner RL, Kaplan BS, Copelovitch L. Update on *Streptococcus pneumoniae* associated hemolytic uremic syndrome. *Curr Opin Pediatr* 2013; **25:** 203–08.

24 Loirat C, Saland J, Bitzan M. Management of hemolytic uremic syndrome. *Presse Med* 2012; **41:** e115–35.

25 Bruneau S, Neel M, Roumenina LT, et al. Loss of DGKε induces endothelial cell activation and death independently of complement activation. *Blood* 2015; **125:** 1038–46.

26 Martinelli D, Deodato F, Dionisi-Vici C. Cobalamin C defect: natural history, pathophysiology, and treatment. *J Inherit Metab Dis* 2011; **34:** 127–35.

27 Undas A, Brozek J, Szczeklik A. Homocysteine and thrombosis: from basic science to clinical evidence. *Thromb Haemost* 2005; **94:** 907–15.

28 Geraghty MT, Perlman EJ, Martin LS, et al. Cobalamin C defect associated with hemolytic-uremic syndrome. *J Pediatr* 1992; **120:** 934–37.

29 Tsai JC, Perrella MA, Yoshizumi M, et al. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. *Proc Natl Acad Sci USA* 1994; **91:** 6369–73.

30 Fremeaux-Bacchi V, Miller EC, Liszewski MK, et al. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. *Blood* 2008; **112:** 4948–52.

31 Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J, et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci USA* 2007; **104:** 240–45.

32 Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361:** 1676–87.

33 Lechner K, Obermeier HL. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. *Medicine* 2012; **91:** 195–205.

34 Morton JM, George JN. Microangiopathic hemolytic anemia and thrombocytopenia in patients with cancer. *J Oncol Pract* 2016; **12:** 523–30.

35 Favre GA, Touzot M, Fremeaux-Bacchi V, et al. Malignancy and thrombotic microangiopathy or atypical haemolytic and uraemic syndrome? *Br J Haematol* 2014; **166:** 802–05.

36 Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 2008; **358:** 1129–36.

37 Al Ustwani O, Lohr J, Dy G, et al. Eculizumab therapy for gemcitabine induced hemolytic uremic syndrome: case series and concise review. *J Gastrointest Oncol* 2014; **5:** e30–33.

38 Faguer S, Huart A, Fremeaux-Bacchi V, Ribes D, Chauveau D. Eculizumab and drug-induced haemolytic-uraemic syndrome. *Clin Kidney J* 2013; **6:** 484–85.

39 Al-Nouri ZL, Reese JA, Terrell DR, Vesely SK, George JN. Drug-induced thrombotic microangiopathy: a systematic review of published reports. *Blood* 2015; 125: 616–18.

40 Reese JA, Bougie DW, Curtis BR, et al. Drug-induced thrombotic microangiopathy: experience of the Oklahoma registry and the BloodCenter of Wisconsin. *Am J Hematol* 2015; **90:** 406–10.

41 George JN. Hematopoietic stem cell transplantation-associated thrombotic microangiopathy: defining a disorder. *Bone Marrow Transplant* 2008; **41:** 917–18.

42 Jodele S, Laskin BL, Dandoy CE, et al. A new paradigm: diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. *Blood Rev* 2015; **29:** 191–204.

43 Daly AS, Xenocostas A, Lipton JH. Transplantation-associated thrombotic microangiopathy: twenty-two years later. *Bone Marrow Transplant* 2002; **30:** 709–15.

44 Kennedy GA, Kearey N, Bleakley S, Butler J, Mudie K, Durrant S. Transplantation-associated thrombotic microangiopathy: effect of concomitant GVHD on efficacy of therapeutic plasma exchange. *Bone Marrow Transplant* 2010; **45:** 699–704.

45 Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. *Blood* 2013; **122:** 2003–07.

46 Jodele S, Zhang K, Zou F, et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. *Blood* 2016; **127:** 989–96.

47 Laskin BL, Maisel J, Goebel J, et al. Renal arteriolar C4d deposition: a novel characteristic of hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Transplantation* 2013; **96:** 217–23.

48 de Fontbrune FS, Galambrun C, Sirvent A, et al. Use of Eculizumab in patients with allogeneic stem cell transplant-associated thrombotic microangiopathy: a study from the SFGM-TC. *Transplantation* 2015; **99:** 1953–59.

49 Jodele S, Fukuda T, Vinks A, et al. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Biol Blood Marrow Transplant* 2014; **20:** 518–25.

50 Le Quintrec M, Lionet A, Kamar N, et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. *Am J Transplant* 2008; **8:** 1694–701.

51 Safa K, Logan MS, Batal I, Gabardi S, Rennke HG, Abdi R. Eculizumab for drug-induced de novo posttransplantation thrombotic microangiopathy: a case report. *Clin Nephrol* 2015; **83:** 125–29.

52 Chandran S, Baxter-Lowe L, Olson JL, Tomlanovich SJ, Webber A. Eculizumab for the treatment of de novo thrombotic microangiopathy post simultaneous pancreas-kidney transplantation—a case report. *Transplant Proc* 2011; **43:** 2097–101.

53 Wilson CH, Brown AL, White SA, Goodship TH, Sheerin NS, Manas DM. Successful treatment of de novo posttransplant thrombotic microangiopathy with eculizumab. *Transplantation* 2011; **92:** e42–43.

54 Gervasoni C, Ridolfo AL, Vaccarezza M, et al. Thrombotic microangiopathy in patients with acquired immunodeficiency syndrome before and during the era of introduction of highly active antiretroviral therapy. *Clin Infect Dis* 2002; **35:** 1534–40.

55 George JN, Terrell DR, Vesely SK, Kremer Hovinga JA, Lammle B. Thrombotic microangiopathic syndromes associated with drugs, HIV infection, hematopoietic stem cell transplantation and cancer. *Presse Med* 2012; **41:** e177–88.

56 Rerolle JP, Canaud G, Fakhouri F, et al. Thrombotic microangiopathy and hypothermia in an HIV-positive patient: importance of cytomegalovirus infection. *Scand J Infect Dis* 2004; **36:** 234–37.

57 Belford A, Myles O, Magill A, Wang J, Myhand RC, Waselenko JK. Thrombotic microangiopathy (TMA) and stroke due to human herpesvirus-6 (HHV-6) reactivation in an adult receiving high-dose melphalan with autologous peripheral stem cell transplantation. *Am J Hematol* 2004; **76:** 156–62.

58 Chua JS, Baelde HJ, Zandbergen M, et al. Complement factor C4d is a common denominator in thrombotic microangiopathy. *J Am Soc Nephrol* 2015; **26:** 2239–47.

59 Descombes E, Droz D, Drouet L, Grunfeld JP, Lesavre P. Renal vascular lesions in lupus nephritis. *Medicine* 1997; **76:** 355–68.

60 El-Husseini A, Hannan S, Awad A, Jennings S, Cornea V, Sawaya BP. Thrombotic microangiopathy in systemic lupus erythematosus: efficacy of eculizumab. *Am J Kidney Dis* 2015; **65:** 127–30.

61 Coppo R, Peruzzi L, Amore A, et al. Dramatic effects of eculizumab in a child with diffuse proliferative lupus nephritis resistant to conventional therapy. *Pediatr Nephrol* 2015; **30:** 167–72.

62 Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med* 2013; **368:** 1033–44.

63 Girardi G, Berman J, Redecha P, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest* 2003; **112:** 1644–54.

64 Redecha P, Tilley R, Tencati M, et al. Tissue factor: a link between C5a and neutrophil activation in antiphospholipid antibody induced fetal injury. *Blood* 2007; **110:** 2423–31.

65 Gropp K, Weber N, Reuter M, et al. β(2)-glycoprotein I, the major target in antiphospholipid syndrome, is a special human complement regulator. *Blood* 2011; **118:** 2774–83.

66 Zikos TA, Sokolove J, Ahuja N, Berube C. Eculizumab induces sustained remission in a patient with refractory primary catastrophic antiphospholipid syndrome. *J Clin Rheumatol* 2015; **21:** 311–13.

67 Kronbichler A, Frank R, Kirschfink M, et al. Efficacy of eculizumab in a patient with immunoadsorption-dependent catastrophic antiphospholipid syndrome: a case report. *Medicine* 2014; **93:** e143.

68 Bakhtar O, Thajudeen B, Braunhut BL, et al. A case of thrombotic microangiopathy associated with antiphospholipid antibody syndrome successfully treated with eculizumab. *Transplantation* 2014; **98:** e17–18.

69 Couvrat-Desvergnes G, Masseau A, Benveniste O, et al. The spectrum of renal involvement in patients with inflammatory myopathies. *Medicine* 2014; **93:** 33–41.

70 Servais A, Noel LH, Roumenina LT, et al. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int* 2012; **82:** 454–64.

71 Manenti L, Vaglio A, Gnappi E, et al. Association of serum C3 concentration and histologic signs of thrombotic microangiopathy with outcomes among patients with ANCA-associated renal vasculitis. *Clin J Am Soc Nephrol* 2015; **10:** 2143–51.

72 Fakhouri F, Roumenina L, Provot F, et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol* 2010; **21:** 859–67.

73 Ardissino G, Wally Ossola M, Baffero GM, Rigotti A, Cugno M. Eculizumab for atypical hemolytic uremic syndrome in pregnancy. *Obstet Gynecol* 2013; **122:** 487–89.

74 De Sousa Amorim E, Blasco M, Quintana L, Sole M, de Cordoba SR, Campistol JM. Eculizumab in pregnancy-associated atypical hemolytic uremic syndrome: insights for optimizing management. *J Nephrol* 2015; **28:** 641–45.

75 Zeisler H, Llurba E, Chantraine F, et al. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. *N Engl J Med* 2016; **374:** 13–22.

76 Crovetto F, Borsa N, Acaia B, et al. The genetics of the alternative pathway of complement in the pathogenesis of HELLP syndrome. *J Matern Fetal Neonatal Med* 2012; **25:** 2322–25.

77 Salmon JE, Heuser C, Triebwasser M, et al. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med* 2011; **8:** e1001013.

78 Frimat M, Decambron M, Lebas C, et al. Renal cortical necrosis in postpartum hemorrhage: a case series. *Am J Kidney Dis* 2016; **68:** 50–57.

79 Zhu J, Chaki M, Lu D, et al. Loss of diacylglycerol kinase epsilon in mice causes endothelial distress and impairs glomerular Cox-2 and PGE2 production. *Am J Physiol Renal Physiol* 2016; **310:** F895–908.

80 Ozaltin F, Li B, Rauhauser A, et al. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. *J Am Soc Nephrol* 2013; **24:** 377–84.

81 Locatelli M, Buelli S, Pezzotta A, et al. Shiga toxin promotes podocyte injury in experimental hemolytic uremic syndrome via activation of the alternative pathway of complement. *J Am Soc Nephrol* 2014; **25:** 1786–98.

82 Bresin E, Rurali E, Caprioli J, et al. Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype. *J Am Soc Nephrol* 2013; **24:** 475–86.

83 Rodriguez E, Rallapalli PM, Osborne AJ, Perkins SJ. New functional and structural insights from updated mutational databases for complement factor H, Factor I, membrane cofactor protein and C3. *Biosci Rep* 2014; **34:** e00146.

84 Lek M, Karczewski KJ, Minikel EV, et al; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; **536:** 285–91.

85 Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17:** 405–24.

86 Delvaeye M, Noris M, De Vriese A, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361:** 345–57.

87 Bu F, Maga T, Meyer NC, et al. Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2014; **25:** 55–64.

88 Rosales A, Hofer J, Zimmerhackl LB, et al. Need for long-term follow-up in enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome due to late-emerging sequelae. *Clin Infect Dis* 2012; **54:** 1413–21.

89 Mody RK, Gu W, Griffin PM, et al. Postdiarrheal hemolytic uremic syndrome in United States children: clinical spectrum and predictors of in-hospital death. *J Pediatr* 2015; **166:** 1022–29.

90 Spinale JM, Ruebner RL, Copelovitch L, Kaplan BS. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. *Pediatr Nephrol* 2013; **28:** 2097–105.

91 Chiurchiu C, Firrincieli A, Santostefano M, Fusaroli M, Remuzzi G, Ruggenenti P. Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin *Escherichia coli* O157:H7 bacteremia and urinary tract infection. *Am J Kidney Dis* 2003; **41:** e4.

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

93 Fakhouri F, Delmas Y, Provot F, et al. Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am J Kidney Dis* 2014; **63:** 40–48.

94 Mathew RO, Nayer A, Asif A. The endothelium as the common denominator in malignant hypertension and thrombotic microangiopathy. *J Am Soc Hypertens* 2015; **10:** 352–59.

95 Nathanson S, Kwon T, Elmaleh M, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2010; **5:** 1218–28.

96 Magnus T, Rother J, Simova O, et al. The neurological syndrome in adults during the 2011 northern German *E. coli* serotype O104:H4 outbreak. *Brain* 2012; **135:** 1850–59.

97 Durkan AM, Kim S, Craig J, Elliott E. The long-term outcomes of atypical haemolytic uraemic syndrome: a national surveillance study. *Arch Dis Child* 2016; **101:** 387–91.

98 Malina M, Gulati A, Bagga A, Majid MA, Simkova E, Schaefer F. Peripheral gangrene in children with atypical hemolytic uremic syndrome. *Pediatrics* 2013; **131:** e331–35.

99 Rigothier C, Delmas Y, Roumenina LT, et al. Distal angiopathy and atypical hemolytic uremic syndrome: clinical and functional properties of an anti-factor H IgAλ antibody. *Am J Kidney Dis* 2015; **66:** 331–36.

100 Ardissino G, Tel F, Testa S, et al. Skin involvement in atypical hemolytic uremic syndrome. *Am J Kidney Dis* 2014; **63:** 652–55.

101 Dragon-Durey MA, Sethi SK, Bagga A, et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol* 2010; **21:** 2180–87.

102 Noris M, Remuzzi G. Cardiovascular complications in atypical haemolytic uraemic syndrome. *Nat Rev Nephrol* 2014; **10:** 174–80.

103 Hu H, Nagra A, Haq MR, Gilbert RD. Eculizumab in atypical haemolytic uraemic syndrome with severe cardiac and neurological involvement. *Pediatr Nephrol* 2014; **29:** 1103–06.

104 Emirova K, Volokhina E, Tolstova E, van den Heuvel B. Recovery of renal function after long-term dialysis and resolution of cardiomyopathy in a patient with aHUS receiving eculizumab. *BMJ Case Rep* 2016; published online Feb 15. DOI:10.1136/bcr-2015-213928.

105 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol* 2005; **129:** 93–100.

106 Mody RK, Luna-Gierke RE, Jones TF, et al. Infections in pediatric postdiarrheal hemolytic uremic syndrome: factors associated with identifying shiga toxin-producing Escherichia coli. *Arch Pediatr Adolesc Med* 2012; **166:** 902–09.

107 Dallman TJ, Byrne L, Launders N, Glen K, Grant KA, Jenkins C. The utility and public health implications of PCR and whole genome sequencing for the detection and investigation of an outbreak of Shiga toxin-producing *Escherichia coli* serogroup O26:H11. *Epidemiol Infect* 2015; **143:** 1672–80.

108 Scully M, Goodship T. How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. *Br J Haematol* 2014; **164:** 759–66.

109 Zuber J, Fakhouri F, Roumenina LT, Loirat C, Fremeaux-Bacchi V. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8:** 643–57.

110 Koenig JC, Rutsch F, Bockmeyer C, et al. Nephrotic syndrome and thrombotic microangiopathy caused by cobalamin C deficiency. *Pediatr Nephrol* 2015; **30:** 1203–06.

111 Cornec-Le Gall E, Delmas Y, De Parscau L, et al. Adult-onset eculizumab-resistant hemolytic uremic syndrome associated with cobalamin C deficiency. *Am J Kidney Dis* 2014; **63:** 119–23.

112 Grange S, Bekri S, Artaud-Macari E, et al. Adult-onset renal thrombotic microangiopathy and pulmonary arterial hypertension in cobalamin C deficiency. *Lancet* 2015; **386:** 1011–12.

113 George JN. Cobalamin C deficiency-associated thrombotic microangiopathy: uncommon or unrecognised? *Lancet* 2015; **386:** 1012.

114 Cataland SR, Holers VM, Geyer S, Yang S, Wu HM. Biomarkers of terminal complement activation confirm the diagnosis of aHUS and differentiate aHUS from TTP. *Blood* 2014; **123:** 3733–38.

115 Noris M, Galbusera M, Gastoldi S, et al. Dynamics of complement activation in aHUS and how to monitor eculizumab therapy. *Blood* 2014; **124:** 1715–26.

116 Bu F, Meyer NC, Zhang Y, et al. Soluble c5b-9 as a biomarker for complement activation in atypical hemolytic uremic syndrome. *Am J Kidney Dis* 2015; **65:** 968–69.

117 Gavriilaki E, Yuan X, Ye Z, et al. Modified Ham test for atypical hemolytic uremic syndrome. *Blood* 2015; **125:** 3637–46.

118 Gould LH, Demma L, Jones TF, et al. Hemolytic uremic syndrome and death in persons with *Escherichia coli* O157:H7 infection, foodborne diseases active surveillance network sites, 2000–2006. *Clin Infect Dis* 2009; **49:** 1480–85.

119 Ardissino G, Dacco V, Testa S, et al. Hemoconcentration: a major risk factor for neurological involvement in hemolytic uremic syndrome. *Pediatr Nephrol* 2015; **30:** 345–52.

120 Ardissino G, Tel F, Possenti I, et al. Early volume expansion and outcomes of hemolytic uremic syndrome. *Pediatrics* 2016; **137:** 1–9.

121 Safdar N, Said A, Gangnon RE, Maki DG. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. *JAMA* 2002; **288:** 996–1001.

122 Freedman SB, Xie J, Neufeld MS, Hamilton WL, Hartling L, Tarr PI. Shiga toxin-producing *Escherichia coli* infection, antibiotics, and risk of developing hemolytic uremic syndrome: a meta-analysis. *Clin Infect Dis* 2016; **62:** 1251–58.

123 Menne J, Nitschke M, Stingele R, et al. Validation of treatment strategies for enterohaemorrhagic *Escherichia coli* O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ* 2012; **345:** e4565.

124 Wong CS, Mooney JC, Brandt JR, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis* 2012; **55:** 33–41.

125 Smith KE, Wilker PR, Reiter PL, Hedican EB, Bender JB, Hedberg CW. Antibiotic treatment of *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatr Infect Dis J* 2012; **31:** 37–41.

126 Nitschke M, Sayk F, Hartel C, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative *Escherichia coli* O104:H4. *JAMA* 2012; **307:** 1046–52.

127 Vonberg RP, Hohle M, Aepfelbacher M, et al. Duration of fecal shedding of Shiga toxin-producing *Escherichia coli* O104:H4 in patients infected during the 2011 outbreak in Germany: a multicenter study. *Clin Infect Dis* 2013; **56:** 1132–40.

128 Agger M, Scheutz F, Villumsen S, Molbak K, Petersen AM. Antibiotic treatment of verocytotoxin-producing *Escherichia coli* (VTEC) infection: a systematic review and a proposal. *J Antimicrob Chemother* 2015; **70:** 2440–46.

129 Loos S, Ahlenstiel T, Kranz B, et al. An outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 hemolytic uremic syndrome in Germany: presentation and short-term outcome in children. *Clin Infect Dis* 2012; **55:** 753–59.

130 Kielstein JT, Beutel G, Fleig S, et al. Best supportive care and therapeutic plasma exchange with or without eculizumab in Shiga-toxin-producing *E. coli* O104:H4 induced haemolytic-uraemic syndrome: an analysis of the German STEC–HUS registry. *Nephrol Dial Transplant* 2012; **27:** 3807–15.

131 Greinacher A, Friesecke S, Abel P, et al. Treatment of severe neurological deficits with IgG depletion through immunoadsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: a prospective trial. *Lancet* 2011; **378:** 1166–73.

132 Combe C, Bui HN, de Precigout V, Hilbert G, Delmas Y. Immunoadsorption in patients with haemolytic uraemic syndrome. *Lancet* 2012; **379:** 517–18.

133 Immunoadsorption in patients with haemolytic uraemic syndrome—Authors' reply. *Lancet* 2012; **379:** 518–19.

134 Melton-Celsa AR, O'Brien AD. New therapeutic developments against Shiga toxin-producing *Escherichia coli*. *Microbiol Spectr* 2014; **2.**

135 Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011; **364:** 2561–63.

136 Delmas Y, Vendrely B, Clouzeau B, et al. Outbreak of *Escherichia coli* O104:H4 haemolytic uraemic syndrome in France: outcome with eculizumab. *Nephrol Dial Transplant* 2014; **29:** 565–72.

137 Saini A, Emke AR, Silva MC, Perlman SJ. Response to eculizumab in *Escherichia coli* O157: H7-induced hemolytic uremic syndrome with severe neurological manifestations. *Clin Pediatr* 2015; **54:** 387–89.

138 Pape L, Hartmann H, Bange FC, Suerbaum S, Bueltmann E, Ahlenstiel-Grunow T. Eculizumab in typical hemolytic uremic syndrome (HUS) with neurological involvement. *Medicine* 2015; **94:** e1000.

139 Greenbaum LA, Fila M, Ardissino G, et al. Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. *Kidney Int* 2016; **89:** 701–11.

140 Rees L, Greenbaum LA, Fila M, et al. A prospective study of paediatric patients with atypical hemolytic uremic syndrome treated with eculizumab: 1-year update (abstract). *Pediatr Nephrol* 2015; **30:** 1564.

141 Legendre CM, Licht C, Muus P, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **368:** 2169–81.

142 Licht C, Greenbaum LA, Muus P, et al. Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int* 2015; **87:** 1061–73.

143 Fakhouri F, Hourmant M, Campistol JM, et al. Terminal complement inhibitor eculizumab in adult patients with atypical hemolytic uremic syndrome: a single-arm, open-label trial. *Am J Kidney Dis* 2016; **68:** 84–93.

144 Fakhouri F, Hourmant M, Campistol JM, et al. Eculizumab inhibits thrombotic microangiopathy, and improves renal function in adult atypical hemolytic uremic syndrome patients: 1-year update. *J Am Soc Nephrol* 2014; **25:** 751A.

145 Cofiell R, Kukreja A, Bedard K, et al. Eculizumab reduces complement activation, inflammation, endothelial damage, thrombosis, and renal injury markers in aHUS. *Blood* 2015; **125:** 3253–62.

146 Gomez-Cibeira E, Ivanovic-Barbeito Y, Gutierrez-Martinez E, et al. Eculizumab-related progressive multifocal leukoencephalopathy. *Neurology* 2016; **86:** 399–400.

147 Walle JV, Delmas Y, Ardissino G, Wang J, Kincaid JF, Haller H. Improved renal recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of eculizumab treatment. *J Nephrol* 2016; published online March 19. DOI:10.1007/s40620-016-0288-3.

148 Ito N, Hataya H, Saida K, et al. Efficacy and safety of eculizumab in childhood atypical hemolytic uremic syndrome in Japan. *Clin Exp Nephrol* 2015; **20:** 265–72.

149 Avila A, Vizcaino B, Molina P, Gavela E, Perez-Ebri M, Pallardo L. Remission of aHUS neurological damage with eculizumab. *Clin Kidney J* 2015; **8:** 232–36.

150 Baskin E, Gulleroglu K, Kantar A, Bayrakci U, Ozkaya O. Success of eculizumab in the treatment of atypical hemolytic uremic syndrome. *Pediatr Nephrol* 2015; **30:** 783–89.

151 Greenwood GT. Case report of atypical hemolytic uremic syndrome with retinal arterial and venous occlusion treated with eculizumab. *Int Med Case Rep J* 2015; **8:** 235–39.

152 Cugno M, Gualtierotti R, Possenti I, et al. Complement functional tests for monitoring eculizumab treatment in patients with atypical hemolytic uremic syndrome. *J Thromb Haemost* 2014; **12:** 1440–08.

153 Walport MJ. Complement. First of two parts. *N Engl J Med* 2001; **344:** 1058–66.

154 Hisano M, Ashida A, Nakano E, et al. Autoimmune-type atypical hemolytic uremic syndrome treated with eculizumab as first-line therapy. *Pediatr Int* 2015; **57:** 313–17.

155 Noone D, Waters A, Pluthero FG, et al. Successful treatment of DEAP-HUS with eculizumab. *Pediatr Nephrol* 2014; **29:** 841–51.

156 Green H, Harari E, Davidovits M, et al. Atypical HUS due to factor H antibodies in an adult patient successfully treated with eculizumab. *Ren Fail* 2014; **36:** 1119–21.

157 Diamante Chiodini B, Davin JC, Corazza F, et al. Eculizumab in anti-factor h antibodies associated with atypical hemolytic uremic syndrome. *Pediatrics* 2014; **133:** e1764–68.

158 Zschiedrich S, Prager EP, Kuehn EW. Successful treatment of the postpartum atypical hemolytic uremic syndrome with eculizumab. *Ann Intern Med* 2013; **159:** 76.

159 Ardissino G, Wally Ossola M, Baffero GM, Rigotti A, Cugno M. Eculizumab for atypical hemolytic uremic syndrome in pregnancy. *Obstet Gynecol* 2012; **122:** 487–89.

160 Kelly RJ, Hochsmann B, Szer J, et al. Eculizumab in pregnant patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2015; **373:** 1032–39.

161 Le Quintrec M, Zuber J, Moulin B, et al. Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. *Am J Transplant* 2013; **13:** 663–75.