CONSENSUS CONFERENCE

# Hemolytic uremic syndrome in a developing country: Consensus guidelines

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## Abstract

**Background** Hemolytic uremic syndrome (HUS) is a leading cause of acute kidney injury in children. Although international guidelines emphasize comprehensive evaluation and treatment with eculizumab, access to diagnostic and therapeutic facilities is limited in most developing countries. The burden of Shiga toxin-associated HUS in India is unclear; school-going children show high prevalence of anti-factor H (FH) antibodies. The aim of the consensus meeting was to formulate guidelines for the diagnosis and management of HUS in children, specific to the needs of the country.

**Methods** Four workgroups performed literature review and graded research studies addressing (i) investigations, biopsy, genetics, and differential diagnosis; (ii) Shiga toxin, pneumococcal, and infection-associated HUS; (iii) atypical HUS; and (iv) complement blockade. Consensus statements developed by the workgroups were discussed during a consensus meeting in March 2017.

**Results** An algorithm for classification and evaluation was developed. The management of Shiga toxin-associated HUS is supportive; prompt plasma exchanges (PEX) is the chief therapy in patients with atypical HUS. Experts recommend that patients with anti-FH-associated HUS be managed with a combination of PEX and immunosuppressive medications. Indications for eculizumab include incomplete remission with plasma therapy, life-threatening features, complications of PEX or vascular access, inherited defects in complement regulation, and recurrence of HUS in allografts. Priorities for capacity building in regional and national laboratories are highlighted.

**Conclusions** Limited diagnostic capabilities and lack of access to eculizumab prevent the implementation of international guidelines for HUS in most developing countries. We propose practice guidelines for India, which will perhaps be applicable to other developing countries.

**Keywords** Anti-factor H antibodies · Complement · Plasma exchange · Thrombotic microangiopathy

Hemolytic uremic syndrome (HUS) is an important cause of severe acute kidney injury (AKI) in childhood. A significant proportion of patients require renal replacement therapy and about one third shows features of chronic kidney disease.

Across the world, the majority of HUS follows gastrointestinal infection with Shiga toxin-producing organisms, chiefly *Escherichia coli*. HUS might be associated with systemic illnesses (secondary HUS), and with disorders of complement

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regulation (atypical HUS, aHUS; Table 1). Management of the disease is supportive, with attention to AKI and therapy of underlying disorders. The availability of eculizumab, a complement inhibitor, has had major impact on treatment of aHUS in the developed world.

There are differences in the epidemiology and management of HUS in India compared to developed countries. Improving standards of hygiene and healthcare have led to decline in incidence of Shigella-associated HUS, the chief form of Shiga toxin-associated disease in the region [1]. There is limited information on the epidemiology of enterohemorrhagic *E. coli*, with reports not suggesting a role in etiology of diarrheal or dysenteric illnesses [2–4]. The capacity for diagnosis of Shiga toxin-associated HUS and genetic screening is limited, posing hurdles in etiological classification. Unlike European cohorts, aHUS associated with factor H (FH) antibodies is more common, accounting for ~ 50% of pediatric patients [5]. Finally, in absence of access to eculizumab, plasma therapy forms the basis for managing aHUS in India and most developing countries, including patients with anti-FH-associated disease.

**Table 1** Classification of hemolytic uremic syndrome (HUS)

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**Shiga toxin-associated HUS**
Shiga toxin-producing *Escherichia coli*, *Shigella dysenteriae* type 1, *Citrobacter*, *Campylobacter*

**Pneumococcal HUS**
Invasive infection with neuraminidase-producing *Streptococcus pneumoniae*

**Infection-associated HUS**
Triggered by influenza A, human immunodeficiency virus, cytomegalovirus, Epstein-Barr virus, parvovirus B19, Coxsackie virus, echovirus, varicella virus, hepatitis A, B, and C, *Salmonella typhi*, *Bartonella*, leptospira, malaria, dengue, and rickettsia

**Secondary HUS**
Systemic lupus erythematosus; antiphospholipid antibody syndrome
Hematopoietic stem cell or solid organ transplant
Malignancy
Malignant hypertension
Drugs: quinine, mitomycin, ticlopidine, clopidogrel, calcineurin inhibitors, sirolimus, oral contraceptives, bevacizumab

**Defective cobalamin metabolism**
Homozygous or compound heterozygous mutation in *MMACHC*

**Atypical HUS**
Homozygous or heterozygous mutations in *CFH*,[a] *CFI*, *CFB*, *C3*, *CD46*, *THBD*, or *DGKE*
Autoantibodies to factor H
Unexplained

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*C3* complement C3, *CD46* membrane cofactor protein, *CFB* factor B, *CFH* complement factor H, *CFI* factor I, *DGKE* diacylglycerol kinase-ε, *MMACHC* methylmalonic aciduria and homocystinuria type C, *THBD* thrombomodulin

[a] Pregnancy is a frequent trigger for HUS, usually in patients with *CFH* mutations

International guidelines on management of HUS [6–13] that emphasize comprehensive evaluation and specific therapy with eculizumab for patients with aHUS might not be suitable in resource constrained settings. Given these challenges, the Indian Society of Pediatric Nephrology has proposed guidelines for evaluation and management of HUS in Indian children. These guidelines are likely to be suitable for developing countries with less than optimal availability and access to diagnostic and therapeutic facilities.

## Consensus development

Workgroups were constituted to address key issues in management: (i) investigations, biopsy, genetics, and differential diagnosis; (ii) Shiga toxin, pneumococcal, and other infection-associated HUS; (iii) atypical HUS; and (iv) complement blockade. Prior to the meeting on 31 March and 1 April 2017 in New Delhi, each workgroup searched databases for research articles, identified the state of knowledge and formulated questions though emails. During the meeting, the workgroups developed consensus statements through alternating breakout and plenary sessions. Research studies were rated from A to D using standard criteria [14].

A. Systematic review, well-designed randomized controlled trials, or diagnostic studies without significant limitations
B. Randomized controlled trials or diagnostic studies with methodological limitations; consistent evidence from observational studies
C. Small cohorts or case control studies; case series
D. Expert opinion; case reports

Each consensus statement was assigned one of two levels of recommendation, based on assessment of relative benefit versus harm, and relevance in context of available facilities.

Level 1. Recommendation applicable to most subjects, based on consistent information confirming benefit over harm or vice versa
Level 2. Suggestion or option based on equivocal or insufficient evidence, with unclear balance of benefit over harm

The final manuscript was circulated to participants for approval.

## Guideline 1: Diagnosis of HUS

Rapid diagnosis and prompt management of HUS is essential to limit irreversible renal damage. Patients with suspected

HUS require urgent evaluation by a specialist to initiate appropriate care.

1.1  We recommend making a diagnosis of HUS in presence of the following [**1B**]:

   (i)  Microangiopathic hemolytic anemia (MAHA), defined by anemia (hemoglobin < 10 g/dl, hematocrit < 30%), and fragmented red cells on peripheral smear (schistocytes ≥ 2%) with either elevated lactate dehydrogenase (LDH > 450 IU/l) or undetectable haptoglobin
   (ii)  Thrombocytopenia (platelet count < 150,000/μl)
   (iii)  AKI, defined as increase in serum creatinine by 50% over baseline level [15]

1.2  We recommend evaluation and exclusion of disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP), when clinically indicated (see below). [**1B**]

1.3  We suggest exclusion of common infections that mimic or trigger HUS, e.g., malaria, leptospirosis, and dengue. [**2C**]

## Rationale

The diagnosis of HUS is based on presence of MAHA, thrombocytopenia, and AKI [6, 16]. The threshold for each of these parameters is not well defined. Patients may occasionally have a subacute presentation with AKI, systemic hypertension, and renal histological features of TMA, without thrombocytopenia and microangiopathic hemolysis [13, 17–19]. The diagnostic sensitivity of HUS is increased if platelet counts are screened at multiple time points [11]. Differentiating DIC from HUS is necessary, especially in the setting of sepsis or malignancy (Fig. 1). DIC is characterized by prolonged prothrombin time or activated partial thromboplastin time, low fibrinogen, elevated D-dimer and soluble fibrin monomers [20]; haptoglobin levels are normal (30–100 mg/dl) [11].

Online scoring criteria for diagnosis of DIC are available [21]: www.thecalculator.co/health/Disseminated-Intravascular-Coagulation-(DIC)-Score-Calculator-1048.html. Patients with Shigella-associated HUS may occasionally show features of DIC [1].

TTP is a rare disorder in children, with inherited and acquired TTP reported in 2.4% and 4.6% patients, respectively of a French cohort of patients with thrombotic microangiopathy (TMA) [22]. Confirmation is feasible, but assays for ADAMTS13 (*A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13*) activity are available at limited centers and have a long turnaround time.

Due to its rarity, a recent meeting of the KDIGO did not advise routine evaluation for ADAMTS13 activity in children with HUS [23]. TTP should be suspected in patients with severe, persistent thrombocytopenia (< 30,000/μl) [13, 24] and mild or no AKI [25, 26]. Severe AKI is, however, reported in 10–12% patients with idiopathic TTP [22, 25]. Congenital TTP (Upshaw Schulman syndrome) has varied phenotype, and may present in neonates with MAHA and jaundice, or in children with unexplained thrombocytopenia. It is characterized by ADAMTS13 activity < 5%, absent antibodies to ADAMTS13, and a homozygous or compound heterozygous mutation in *ADAMTS13* [27].

Due to limited availability of the ADAMTS13 assay, we *suggest storing blood samples (collected in 3.2% sodium citrate) at − 20° to − 70 °C in patients with suspected HUS.* The possibility of TTP should be revisited if the etiology of TMA is unclear. The diagnosis is confirmed by demonstrating ADAMTS13 activity < 10% on fluorescence resonance energy transfer (FRET)-based assays and/or the presence of anti-ADAMTS13 antibodies.

HUS may at times mimic or occur in association with infections such as malaria [28], dengue [29], and leptospirosis [30]. These disorders might result in anemia, thrombocytopenia, and AKI that is not due to renal microangiopathy.

### Guideline 2: Shiga toxin-associated HUS

Shiga toxin-associated gut infection is one of the chief causes of HUS and confirmed by stool examination.

2.1  We recommend that diagnosis of Shiga toxin-associated HUS, either due to Shiga toxin-producing *Escherichia coli* (STEC) or *Shigella dysenteriae*, be based on the following criteria. [**1A**]

**Confirmed case** HUS associated with infection with Shiga toxin-producing organisms confirmed by positive stool culture and either of the following:

(i)  Detection of virulence genes on fecal extracts or cultures (*stx1*, *stx2*, and *eae* by PCR)
(ii)  Free fecal Shiga toxin (detected on tissue culture or immunoassay)
(iii)  Serum IgM antibodies to serogroup specific lipopolysaccharide (by ELISA, passive hemagglutination assay) (Table 2)

**Suspected case** HUS occurring within 2–3 weeks of bloody diarrhea and/or occurring during a known outbreak of STEC-HUS in patients above 6 months of age

**Suspected hemolytic uremic syndrome (HUS)**

Hemoglobin <10 g/dl, schistocytes ≥2%; LDH >450 IU/l (or haptoglobin <30 mg/dl)
Platelets <150,000/μl; Acute kidney injury*

Rule out sepsis, DIC, malaria,
dengue, leptospirosis

Consider probable or confirmed **TTP** (*see* text)

**HUS**

**Shiga toxin associated HUS**
Suspected: Bloody dysentery; during outbreak
Confirmed: Laboratory criteria (*see* text)

**Pneumococcal HUS**
Clinical features; culture or antigen positive

HUS with other infections (*see* Table 1)

**Cobalamin C deficiency HUS**
Elevated total homocysteine
*MMACHC* gene mutation

**Secondary HUS**

Systemic lupus, antiphospholipid syndrome
Drug induced HUS; Malignancy
Hematopoietic stem cell, solid organ transplant
Malignant hypertension; Postpartum HUS

**Atypical HUS** (aHUS)**

**Evaluation** (*see* Table 2)
C3, CD46 flow cytometry
Anti-factor H antibodies
Genetic screening

**Complement mediated aHUS**

**Unexplained thrombotic microangiopathy**
Review for TTP, cobalamin C deficiency, other conditions
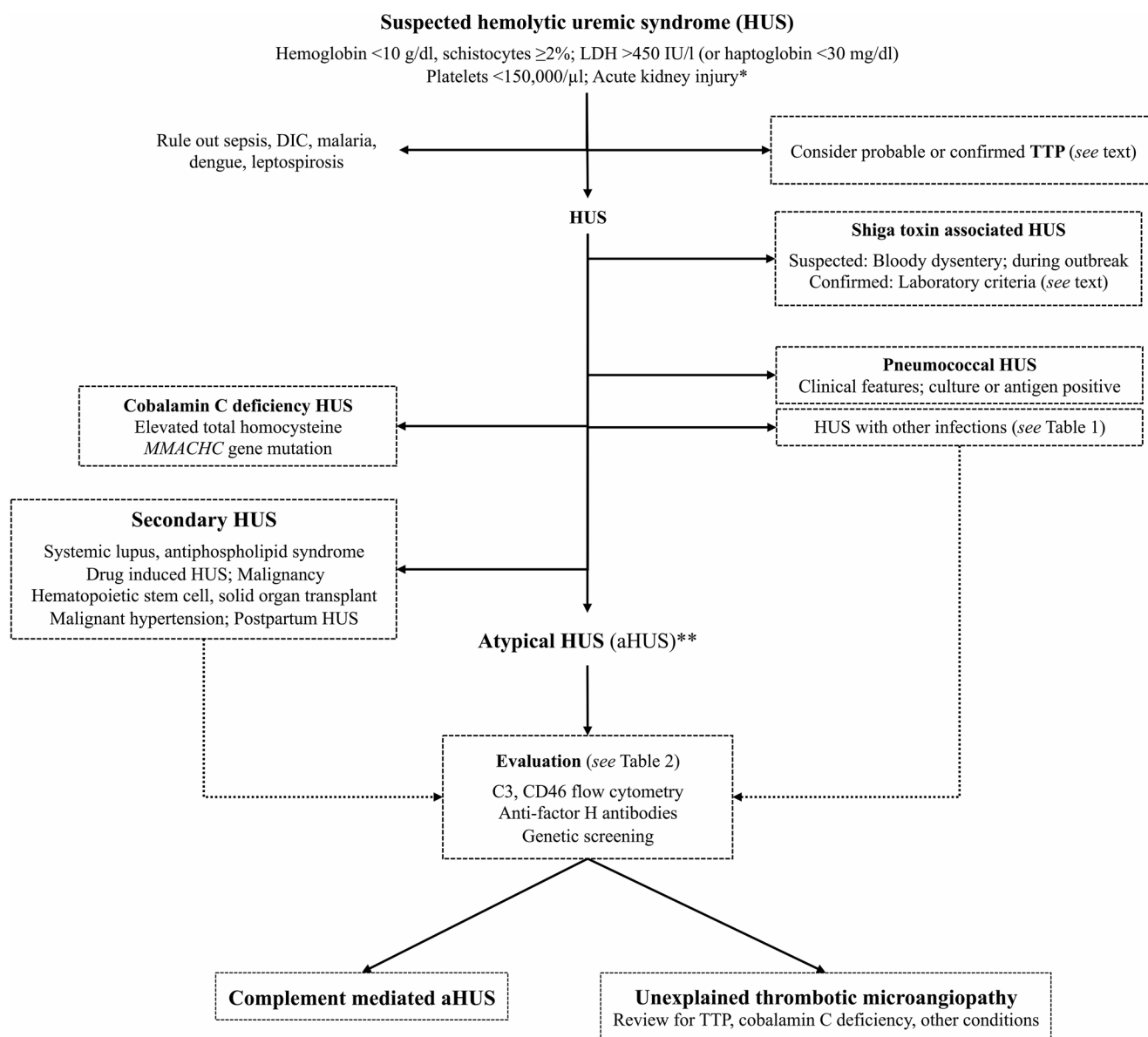
**Fig. 1** Evaluation of patients with hemolytic uremic syndrome (HUS). Patients with secondary and infection triggered HUS should also be screened for abnormalities of the alternate complement pathway. CD46 membrane cofactor protein; DIC, disseminated intravascular coagulation; LDH, lactate dehydrogenase; TTP, thrombotic thrombocytopenic purpura. *Consider renal biopsy if triad not present and/or unexplained acute kidney injury. **Also consider atypical HUS if positive non-synchronous family history, recurrent disease, or insidious disease

2.2   We suggest screening for STEC infection in all *suspected* cases of STEC-HUS on stool samples collected at admission. [**2A**]

## Rationale

Guidelines from the Center for Disease Control recommend stool culture and testing for Shiga toxin to detect serotypes of STEC [31]. Infection with STEC is the chief leading cause of HUS in developed countries [18], but fecal isolation is difficult if patients present after the first 7–10 days of illness and following antibiotic administration [32]. Direct screening of fecal extracts or cultures for Shiga toxin is possible using molecular biology, biochemical techniques, or Vero cell cultures [13, 31]. PCR analysis on primary fecal cultures is the most sensitive and specific investigation for screening for the toxin-producing organisms [33]. Isolation of the STEC strain and its characterization by serotype, phage type, *eae* genes, and subtypes of vt1/vt2 should be done if possible [31]. The presence of serum IgM antibodies against specific *E. coli* lipopolysaccharide is important evidence for recent STEC infection [34].

While circulation of *S. dysenteriae* is extremely low in recent years in India [35], there is limited data regarding the epidemiology of STEC. In a study from north India, STEC

**Table 2** Evaluation of patients with hemolytic uremic syndrome (HUS)

Diagnosis

  Complete blood counts; peripheral smear for schistocytes; reticulocyte count[a]

  Lactate dehydrogenase, haptoglobin[a]

  Direct Coombs test[b]

  Blood: creatinine, electrolytes, transaminases, bilirubin, complement C3[a]

  Urinalysis

  Rapid test for malaria, dengue; IgM antibodies for dengue, leptospirosis (if suspected)

  Coagulation profile[a] (suspected systemic sepsis)

  Ultrasound abdomen

  If clinical features present: Echocardiogram, neuroimaging, amylase, troponin T

Determining cause of HUS

  Essential

    Investigate for infection associated or secondary HUS, if clinically suspected (see Table 1; below)

    Anti-factor H antibodies[a]; antinuclear antibodies

    CD46 expression on neutrophils (flow cytometry)[a]

    Store blood for ADAMTS13 activity[a,c]; total homocysteine[a,c]

  Selected patients

    Suspected Shiga toxin-associated HUS: Stool sample for culture (sorbitol MacConkey agar, selective media); PCR for *stx*1, *stx*2, intimin (*eae*), and enterohemolysin (*ehxA*) genes; ELISA for free Shiga toxin; IgM antibodies to specific lipopolysaccharide(s)

    Suspected pneumococcal HUS: Culture, PCR or ELISA; peanut lectin agglutination assay

    Sequencing genes: *CFH, CFI, CFB, C3, CD46, DGKE; THBD, MMACHC*

    Multiplex ligation-dependent probe amplification: Copy number variations in *CFHR1*–5 and *CFH*

*ADAMTS13* A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; *C3* complement C3; *CD46* membrane cofactor protein; *CFB* complement factor B; *CFH* factor H; *CFHR* CFH related; *CFI* factor I; *DGKE* diacylglycerol kinase-ε; *ELISA* enzyme-linked immunosorbent assay; *MMACHC* methylmalonic aciduria and homocystinuria type C; *PCR* polymerase chain reaction; *stx* shiga toxin; *THBD* thrombomodulin

[a] Blood samples should be drawn before plasma exchanges or infusion in appropriate tubes according to the recommendations of the local laboratory

[b] May be positive with pneumococcal infection, systemic lupus tested prior administration of blood products

[c] Fresh citrated blood (ADAMTS13) and EDTA plasma (homocysteine) samples to be frozen at −20 to −70 °C within 2-h of collection

was isolated in 1.7% stool samples of acute non-bloody diarrhea and 1.6% samples of bloody diarrhea [2]. The authors found serogroup diversity of non-O157 STEC; O157 was not isolated, signifying either its absence or presence in low numbers [2]. Non-O157:H7 STEC was detected by PCR for *stx* and *eae* in 23% of 198 patients with acute diarrhea in southern India; only three isolates were identified as STEC by serotyping [3]. In the absence of epidemiological data of STEC-HUS in India, we suggest confirming the diagnosis by detection of fecal Shiga toxin as well as by culture. Since

excretion of STEC is short-lasting (median 10–14 days; 92% detection rate within 6 days) [36, 37], delayed referral and prior antimicrobial use reduce the likelihood of detection. In such patients, presence of IgM antibodies against specific lipopolysaccharide enables the diagnosis [34]. Demonstration of elevated serum IgG antibodies against Shiga toxin, by ELISA, Western blot, or tissue culture cytotoxicity neutralization assay is chiefly an epidemiologic tool.

Five to 10% patients with sporadic STEC infection and ~20% patients during outbreaks develop HUS. Risk factors for occurrence of HUS are: young age (< 5 years), > 3 days of diarrhea, blood in stools, and high leukocyte count (> 15,000/μl) [38, 39]. While international guidelines state that patients with HUS should be screened for STEC infection, access to microbiological diagnosis is difficult in developing countries. In view of insufficient information on epidemiology, lack of surveillance of food borne pathogens, and limited facilities for microbiological confirmation, the diagnosis of STEC-HUS is usually clinical. The present guidelines suggest suspecting STEC-HUS in patients with prior history of bloody diarrhea, or if occurring during an outbreak of STEC infection. However, it is emphasized that ~ 30–40% cases of STEC-HUS may not have dysentery; the condition is also reported in patients without diarrhea and infants below 6 months of age [13].

### Pneumococcal and other infection-associated HUS

HUS may also be associated with specific infections (Table 1). A diagnosis of *probable* pneumococcal HUS is made in patients, usually younger than 2 years, with sepsis, pneumonia or meningitis, and positive direct Coombs' test without features of DIC [40, 41]. The diagnosis is *confirmed* by either: (i) *S. pneumoniae* isolated by bacterial culture, or detection of pneumococcal antigen by PCR or ELISA in appropriate body fluids or, (ii) positive peanut lectin (*Arachis hypogaea*) agglutination assay [40, 41]. Therapy is with parenteral cephalosporins; vancomycin is used if there is high prevalence of multidrug resistance [42]. Some authors suggest avoiding plasma infusions or plasma exchanges (PEX) and using washed red cells and platelets, if necessary.

If indicated, serology for human immunodeficiency virus; polymerase chain reaction (PCR) for influenza virus (H1N1), cytomegalovirus, and Epstein-Barr virus; peripheral smear and quantitative buffy coat for malaria parasite; NS-1 antigen or dengue-IgM antibody; and investigations for leptospirosis and rickettsia are performed.

## Guideline 3: Cobalamin deficiency-associated HUS

3.1  We suggest screening for cobalamin C (cblC) deficiency by estimating total homocysteine levels. [**2C**]

**Probable cblC deficiency-related HUS** HUS and elevated plasma total homocysteine level (> 50–100 μM/l by chromatography, immunoassay) with normal levels of vitamin B$_{12}$ and folate.

**Confirmed cblC deficiency-related HUS** Homozygous or compound heterozygous mutation in the *MMACHC* gene in a patient with probable cblC deficiency.

3.2   We recommend that patients with probable or confirmed cblC deficiency should receive prompt therapy with parenteral hydroxycobalamin, oral betaine and folic acid. [**1B**]

### Rationale

Cobalamin deficiency accounts for a small (~ 6–8%) proportion of patients with HUS. The condition may present at all ages, ranging from neonates to adults, and does not respond to PEX [18]. Features include failure to thrive, feeding difficulties, seizures, abnormal muscle tone, visual impairment, and developmental delay. Megaloblastic anemia is present in 10–25%. One-third patients do not have extrarenal features, although neurological (44%) or cardiopulmonary (39%) disease and pulmonary hypertension (17%) are frequent [43].

Elevated blood levels of total homocysteine are characteristic. *Samples may be stored and processed later, if the assay is not immediately available.* Plasma samples should be centrifuged within 30 min of collection and stored at − 20° to − 70 °C (Fig. 1). Free homocysteine levels are not required; vitamin B$_{12}$ and folate deficiency also result in elevated total homocysteine [44]. Plasma and urine methylmalonic acid levels are high in cblC deficiency, but may be normal in cblD (*MMADHC*) deficiency [44].

The mortality in patients with untreated disease is high [18, 43]; early treatment is therefore recommended [44]. Patients respond to therapy with parenteral hydroxycobalamin (1 mg daily) to lower homocysteine levels < 40–60 μM/L; cyanocobalamin is not effective. Betaine (250 mg/kg/day in 3 divided doses) helps methylating homocysteine to methionine. Folate (5–30 mg divided in 2–3 doses per week) is a useful adjunct [44]. Specific therapy allows renal recovery and prevents relapses.

### Guideline 4: Diagnosis of atypical HUS (aHUS)

4.1   We suggest the diagnosis of aHUS where suspected STEC and pneumococcal HUS, TTP, and secondary HUS are excluded based on clinical and laboratory features. [**2D**]

4.2   We also suggest a diagnosis of aHUS in patients with (i) negative results for infection by Shiga toxin-producing organism, (ii) positive non-synchronous family history, or (iii) recurrent disease. [**2C**]

### Rationale

Many algorithms are proposed for evaluation of patients with TMA [7, 8, 11, 13]. In resource-limited settings, it is important to follow a pragmatic approach without compromising the principles of management. We endorse the etiology-based classification of HUS proposed by international guidelines, emphasizing the importance to differentiate between infection-associated illness, cblC deficiency, secondary HUS, and aHUS (Table 1, Fig. 1) [19, 45, 46]. Since TTP and cblC deficiency are relatively infrequent causes, units lacking facility to screen for these disorders might store samples for subsequent analysis. Confirmation of STEC-HUS is difficult due to limited diagnostic facilities, delayed analysis, and prior use of antibiotics. Plasma specimens may be stored for later serological investigations.

### Guideline 5: Evaluation of atypical HUS (Table 2, Fig. 1)

5.1   We recommend the following evaluation in patients with aHUS: (i) Complement C3; (ii) test for anti-factor H (FH) antibodies; (iii) flow cytometry for expression of membrane cofactor protein (MCP, CD46) on neutrophils; and (iv) sequencing of *CFH, CFI, CFB, C3, CD46, THBD,* and *DGKE.* Blood specimens for C3 and anti-FH antibodies should be drawn prior to instituting therapy. [**1B**]

5.2   We recommend estimating anti-FH antibodies using enzyme-linked immunosorbent assay (ELISA). A positive threshold for the assay should be determined based on data in the local population. [**1C**]

5.3   We suggest that patients with anti-FH antibody-associated HUS do not routinely require screening for genetic variants. Genetic screening is advised with (i) early age of onset, (ii) relapsing course, (iii) family history of HUS, (iv) illness that is refractory to therapy with PEX, and (v) prior to renal transplantation. [**2C**]

### Rationale

Atypical HUS is characterized by dysregulation of the alternative complement pathway, resulting in endothelial damage and microvascular thrombosis [47]. About 40% patients have a pathogenic variant in genes encoding regulatory proteins or

factors of the alternative complement pathway (factor H, factor I, CD46, factor B, C3, and thrombomodulin) or rearrangements of genes encoding factor H (FH)-related proteins [48]. In addition to inherited defects, antibodies to FH (anti-FH), the chief regulatory protein of the alternative pathway, is an acquired cause of aHUS comprising a distinct subgroup of patients. Recent reports suggest that mutations in genes outside the complement pathway might be associated with aHUS phenotype. Recessive mutations in *DGKE*, which encodes diacylglycerol kinase-ε and is expressed in endothelial cells, platelets, and podocytes were identified in 2–3% patients with aHUS, presenting in the first 2 years of life [49]. Others report the association of aHUS with mutations in genes encoding plasminogen, inverted formin-2 and vitronectin [49–51]. The distinction between disease due to complement and non-complement genes is important for therapeutic purposes.

Evaluation of patients with aHUS is done in a step-wise manner, adapted to availability of resources (Table 2). Blood levels of C3 are reduced in 30–40% patients but are often normal. A low C3 level is not specific for aHUS, nor does it correlate with severity of disease. Flow cytometry for surface expression of CD46 is a useful screening test; labs are advised to simultaneously run specimens from healthy controls. Reduced expression that persists following hematological remission helps identify patients with possible *CD46* mutation. Because these patients are unlikely to benefit from PEX, a precise diagnosis is important [52, 53].

Immune assays for FH, FI, and FB are expensive and require to be performed at specialized laboratories. Their normal levels do not rule out the possibility of normally expressed, but functionally impaired protein [9]. In our experience, measurement of blood levels of FH and FI has not been useful in screening children with aHUS. Estimation of levels is useful if a variant is found in the corresponding gene; if deficient, these levels may serve as a marker of efficacy of supplemental therapy.

Anti-FH antibody-associated illness constitutes ~50% of pediatric patients with aHUS in India, chiefly affecting children between the age of 5–15 years [5]. Most patients have high levels of antibodies (1000 to 20,000 AU/ml) [54]. Given the therapeutic implications, it is necessary to screen for anti-FH antibodies in all patients with aHUS. Methods for ELISA differ in approach to coating, blocking, incubation, and color development, with comparable results [55]. Sample-specific subtraction is done to rule out false positives and a reference arbitrary unit (AU/ml) scale, based on positive anti-FH serum is used for reporting. Using this method, a positive threshold of 150 AU/ml has been established for Indian controls [5]. There are efforts to establish an international standard that would enable comparison of data across centers. The current protocols are preferred over commercial ELISA kits, that apart from being expensive, may show false positive results and underestimate antibody titers, limiting their role during follow up; a positive threshold for these kits is also not defined [55].

Next-generation sequencing (NGS) allows rapid and simultaneous sequencing of large gene panels. Screening for the following genes using a NGS panel: *CFH*, *CFI*, *CFB*, *C3*, *CD46*, *THBD*, *CFHR1–5*, and *DGKE* and for rearrangements of *CFH-CFHR5* by multiplex ligation-dependent probe amplification (MLPA) is recommended in patients with suspected aHUS to guide regarding prognosis, risk of relapses, transplantation, and genetic counseling. Homozygosity for risk haplotypes of *CFH* and *MCP* has been shown to increase disease penetrance and severity [56]. Variants identified on NGS should be validated by Sanger sequencing. Expert opinion is necessary for ascribing pathogenicity. A database of gene variants is available at: http://www.complement-db.org/home.php.

Anti-FH-associated aHUS is associated with an 84 kb homozygous deletion of the *CFHR1* gene, present in ~5–10% healthy individuals across the world [54, 57]. MLPA or end-point PCR is useful in confirming the deletion, but not required in clinical practice [58]. Detection of deletion helps confirm antibody mediated disease, in patients with (i) low antibody titers, especially if received PEX before screening; (ii) onset of illness < 3 years of age; and (iii) atypical presentation.

The need for additional genetic screening in patients with anti-FH-associated aHUS is debated. Of 296 patients with anti-FH positive illness screened, 5% show change in complement regulatory genes, mostly polymorphisms or variants of unknown significance [59 unpublished data]. Thus the likelihood of detecting pathogenic mutations in complement genes in patients with anti-FH-associated aHUS is low. Evaluation for additional defects is suggested in patients with a refractory, relapsing or atypical illness and before renal transplant.

## Guideline 6: Indications for renal biopsy

6.1 We recommend that renal biopsy be considered in patients with (i) unclear diagnosis of HUS, (ii) unsatisfactory clinical response, to determine extent of renal damage and help in prognosis, and (iii) distinguish between causes of allograft dysfunction, including recurrent HUS. [**1B**]

### Rationale

The diagnosis of HUS is clinical. A renal biopsy is not required to demonstrate TMA and adds little to the etiology or prognostic information at onset. Histological diagnosis is required in patients with acute or subacute onset of unexplained renal dysfunction, proteinuria and/or hypertension, who might not show hemolysis or thrombocytopenia. A renal biopsy is important for confirming the diagnosis and establishing reliable renal prognosis in secondary HUS, particularly in context

of lupus nephritis, antiphospholipid syndrome, associated malignancies and following hematopoietic stem cell, or solid organ transplant. Biopsy of the allograft may provide the first clue of TMA, especially in patients with aHUS undergoing renal transplantation. Adequate precautions are necessary before the biopsy, with attention to thrombocytopenia, coagulation parameters, and hypertension. The biopsy should be interpreted by a renal pathologist; histology findings of TMA have recently been reviewed [23].

## Guideline 7: Therapy of Shiga toxin-associated HUS

7.1   We recommend maintaining hydration by early use of isotonic fluids in patients with dysentery, starting from onset of bloody diarrhea to the day of onset of HUS, and monitoring for fluid overload in patients with renal failure. [**1B**]

7.2   We recommend therapy with appropriate antibiotics for bloody diarrhea. [**1A**]

7.3   While we do not suggest the use of PEX in patients with Shiga toxin-associated HUS, therapy may be considered for patients with severe neurological or cardiac involvement. [**2D**]

7.4   We do not recommend the use of plasma infusions, heparin, urokinase, dipyridamole, antimotility agents, glucocorticoids, and Shiga toxin binders. [**1B**]

### Rationale

Patients with HUS due to proven or presumed STEC infection show an association between dehydration and adverse outcomes [60]. A review of cohort studies in children with presumed or proven STEC infection showed that high hematocrit (> 23%) at presentation and lack of fluid administration before onset of HUS was associated with higher risk of oliguric AKI, need for renal replacement therapy, and neurological complications [60–65]. Ringer lactate or dextrose saline should be infused during bloody diarrhea and including the day of diagnosis of HUS to prevent dehydration. Since administration of intravenous fluids is not always feasible, the use of oral rehydration solution to maintain euvolemia is an alternative, but which has not been examined. *Since fluid overload is an important predictor of mortality in AKI* [15]*, we recommend judicious monitoring of fluid balance subsequently* [7].

Patients with shigellosis require prompt antimicrobial treatment in order to reduce mortality, fecal shedding and complications [66]. While there is limited circulation of *S. dysenteriae* in India [35], bacteriological confirmation is often not feasible. The WHO recommends ciprofloxacin as first-line therapy for shigellosis [66]: ceftriaxone,

azithromycin, and cefixime are alternate agents [67]. The role of antibiotics in STEC gastroenteritis is debated, since these might induce expression of Shiga toxin [68] and increase the risk of HUS [69]. Recent reviews and cohort studies do not show an increased risk of HUS with use of antibiotics [39, 68, 70–74]. Since clinical distinction between shigellosis and STEC infection may be difficult, we recommend that patients with bloody diarrhea receive treatment with oral ciprofloxacin, azithromycin or cefixime for 5 days.

The American Society of Apheresis does not recommend PEX for patients with Shiga toxin HUS [75]. Two multicenter randomized trials did not show benefit of plasma infusions over supportive care [76, 77]. Data in adults [72, 78] and children [79] from the recent German outbreak did not show benefit of PEX in reducing the need for dialysis and respiratory or neurological complications. While PEX reduced mortality in adults (31% versus 83% with or without PEX, respectively) [80], it did not improve outcomes of patients with severe neurological involvement [81]. Based on weak evidence, a recent systematic review of observational data suggests a possible improvement in outcomes of patients older than 60 years of age and children with severe systemic involvement administered PEX within 24–48 h of onset [82].

Since there is evidence of activation of the complement pathway during acute STEC infection [83], complement blockade may have a role in treating patients with severe disease. While there was no proven benefit with use of eculizumab, a humanized monoclonal antibody against C5 that blocks the terminal complement pathway, in the German epidemic [72, 78], anecdotal reports suggest that patients with severe neurological and cardiac features might benefit from such therapy [84, 85]. A retrospective study on 28 patients treated with eculizumab for severe STEC-HUS showed neurological improvement in 67.9%, the majority improving after the first dose of the medication [86]. A review reported no benefit with plasma infusions, heparin, urokinase, dipyridamole, antimotility agents, steroids, and Shiga toxin binders [87].

## Guideline 8: Managing atypical HUS without anti-FH antibodies

Inherited defects of the alternative pathway are the chief cause of aHUS in Europe and North America. The standard of care for patients is complement blockade with eculizumab, a humanized anti-C5 antibody [88]. *However, eculizumab is expensive and unlikely to be available soon in India and other developing countries.* Thus, PEX or plasma infusions remain the chief option for patients with aHUS, especially those with genetic defects in the complement pathway.

8.1   In the absence of eculizumab, we recommend prompt initiation of PEX in patients with aHUS. For initial

therapy, we recommend that PEX be preferred to plasma infusions. [**1C**]

8.2 We suggest that PEX be administered daily until hematological remission and then tapered over 4–6 weeks (Table 3). [**2D**]

8.3 Patients on plasma therapy should be monitored for plasma or filter reactions, complications of catheter insertion, infection or thrombosis, and blood-borne infections. [**1C**]

8.4 We recommend efforts to enable therapy with eculizumab in the following: (i) lack of remission despite 7–10 days of PEX, (ii) life-threatening features (seizures, cardiac dysfunction), (iii) complications due to PEX or vascular access, and (iv) inherited defect in complement regulation. [**1C**]

## Rationale

Prior to approval and use of eculizumab, the 2009 European guidelines recommended prompt therapy of aHUS with PEX within 24-h of presentation [9]. PEX may be performed by

**Table 3** Protocol for plasma exchanges or infusion

| |
|---|
| Method: Membrane filtration or centrifugation |
| Replacement fluid: Fresh frozen plasma[a] |
| Anticoagulation: Heparin or citrate |
| Medications |
|   Intravenous premedication: Pheniramine and hydrocortisone |
|   Maintain ionized calcium > 1mMol/l; potassium 3.5–5 mEq/l |
|   Prime circuit if extracorporeal volume > 10% of blood volume |
| Schedule |
|   Daily with 1.5 times plasma volume (60–75 ml/kg) per session for 5 days or until hematological remission, whichever is later |
|   Alternate days at 40 ml/kg for 2 weeks; twice a week for 2–3 weeks |
|   Anti-FH positive HUS |
|     Discontinue plasma exchanges after 4–6 weeks |
|   Anti-FH negative HUS |
|     Pending genetic screening: Switch to plasma infusions; fresh frozen plasma 10–20 ml/kg once every 7–10 days |
|     *CD46*, *DGKE* variants, no significant genetic variation: Discontinue exchanges or infusions |
|     *CFH*, *CFI*, *C3*, *CFB*, and *THBD* variants: Long-term plasma infusions; strongly consider eculizumab |
| Definition of response |
|   Hematological remission: Platelets > 100,000/μl, schistocytes < 2%, LDH less than upper limit of normal on 2 consecutive days |
|   Relapse: Recurrence of anemia with schistocytes ≥ 2%, elevated LDH, and/or thrombocytopenia (platelets < 150,000/μl) with or without AKI, following remission for > 2 weeks |
|   Refractory illness: Platelets < 100,000/μl and/or persistent hemolysis (fall in hemoglobin; persisting ≥ 2% schistocytes) despite 7–10 daily adequate volume plasma exchanges |

*AKI* acute kidney injury, *FH* factor H, *LDH* lactate dehydrogenase

[a] Alternative is Octaplas®, a solvent/detergent treated pooled human plasma that is not available in most developing countries

membrane filtration or by centrifugation based methods [89] (Table 3). PEX is preferred to plasma infusions for initial therapy, since large volumes of plasma can be infused even in patients with oliguria. PEX also enables removal of dysfunctional proteins and antibodies, which might be present in a proportion of children with aHUS.

An audit on the safety of PEX from centers in Europe and North America showed procedure-related complications and hypersensitivity to plasma in one-third patients [90]. While there were risks of securing vascular access in infants, a multi-center study confirmed the safety of the procedure [91]. An audit of 2024 PEX sessions ($n = 109$ patients) in New Delhi showed adverse events in $\sim 10\%$, including chills, abdominal pain, hypotension, urticaria, seizures, and hypocalcemia (unpublished data). Plasma hypersensitivity was rare, and rate of catheter-related infections was 1.45/1000 catheter-days. Parents should be counseled about the risk of blood-borne infections with repeated therapies. While use of Octaplas, sterile pooled human plasma treated by solvent detergent process, reduces the risk of hypersensitivity reactions and blood-borne infections [92], the product is not available in most developing countries.

**Eculizumab** The outcome of aHUS from large cohorts managed with plasma therapy alone is unsatisfactory, with 30–50% patients progressing to end-stage renal disease at 1–5 years follow up [19, 93]. Complement blockade with eculizumab has emerged as specific therapy for aHUS with inherited defects in complement regulation. The evidence for its benefit is derived from prospective single arm industry sponsored trials in adults and children [88, 94–96]. In a trial on 17 adults refractory to plasma therapy, hematological remission was achieved by 1–2 weeks and sustained in 88% [88]; renal functions improved with 12% patients on chronic dialysis after 2 years therapy [95]. Another trial on 20 plasma-dependent adults with chronic kidney disease showed hematological remission in 90% [88] and increase in glomerular filtration rate over the next 2 years [95]. Two subsequent trials underscored the medium term efficacy of eculizumab as first-line treatment of aHUS in children [96] and adults [94]. Progression to end-stage renal disease or death occurred in fewer patients (6–15%), compared to those managed with plasma therapy [19, 93].

Despite lack of availability of eculizumab in developing countries, all efforts must be made to make the medication accessible for patients with aHUS who fail to respond adequately to PEX. Standard guidelines should be followed while instituting therapy with eculizumab including monitoring for complement blockade, dose interval, and duration of therapy (Supplementary Table 1). Hematological remission is expected at median of 7–8 days; patients lacking response by 10–14 days of therapy should be reviewed for: (i) inadequate dose, suggested by trough level < 50 μg/ml and CH50 > 20%; (ii) losses (nephrotic range proteinuria, or PEX); (iii)

resistance due to ongoing infection, inflammation or surgery; and (iv) C5 variant with impaired binding to eculizumab [97].

Eculizumab is not indicated for therapy of non-complement disorders, e.g., *DGKE* or *MMACHC* mutations [98]. Information on efficacy of eculizumab for extrarenal manifestations is limited. Therapy was useful in a patient with digital gangrene and another with skin necrosis and intestinal perforation [99–101]. The medication has variable efficacy in managing neurological or myocardial manifestations [102–104]. The duration of therapy is not clear. Discontinuation of treatment results in relapses in 60–72% patients with *CFH* mutations, 43% with *C3* defects, 37–50% with *MCP* mutations and ~ 10% with no identified defect [97].

## Guideline 9: Managing anti-FH antibody-associated HUS (Fig. 2)

9.1  We recommend a combination of prompt PEX (with fresh frozen plasma as replacement fluid) and immuno-suppressive therapy for patients with anti-FH antibodies (Fig. 2). [**1B**]

9.2  We do not recommend use of immunosuppressive med-ications without confirming the presence of anti-FH an-tibodies. [**1D**]

9.3  We suggest daily PEX until hematological remission and then taper over 3–5 weeks. We do not recommend plas-ma infusions as a substitute for PEX. [**2D**]

9.4  Since high anti-FH levels might predict a relapse, we recommend monitoring antibody titers frequently during the first 12–24 months. [**1C**]

9.5  We suggest therapy with eculizumab in the following: (*i*) lack of remission despite 7–10 PEX; (ii) life-threatening features (seizures, cardiac dysfunction); (iii) complica-tions due to PEX or vascular access; and (iv) inherited defect in complement regulation. [**2C**]

### Rationale

The aim of therapy for patients with anti-FH-associated HUS is reduction of antibody titers. The American Society for Apheresis assigns level I category to anti-FH-associated HUS, implying that PEX is a primary therapeutic intervention [75]. Since antibodies are chiefly IgG, 5–7 PEX achieve 80% reduc-tion in antibody titers [105]. *Prompt initiation and continued PEX for at least 3–5 weeks, is advised.* Blockade of the com-plement pathway by eculizumab is effective in inducing hema-tological remission, but has limited impact on antibody titers.

We endorse guidelines that emphasize combined therapy with PEX and immunosuppressive agents for patients with anti-FH antibodies (Fig. 2) [13, 18]. Induction therapy with

oral corticosteroids and IV cyclophosphamide is preferred ini-tially. Immunosuppression inhibits further production of anti-bodies, especially following PEX, with improved short and medium term outcomes [54]. Outcomes were similar for pa-tients managed by rituximab (*n* = 14) and intravenous cyclo-phosphamide (*n* = 31) [106].

Antibody titers are monitored closely between days 7–28 and then every 3–6 months. Elevated titer (> 1500 AU/ml) during the first 12–24 months is associated with an increased risk of relapse [54]. Relapses follow minor infectious illnesses in the initial 2 years. Therapy with azathioprine or mycophe-nolate mofetil and tapering doses of prednisolone is useful in significant reduction of this risk [5].

Many centers in the developed world initiate empiric thera-py with eculizumab, pending anti-FH antibody results. Once high titer of anti-FH antibodies is detected, there is controversy whether eculizumab therapy be continued, or replaced by PEX and immunosuppression. Treatment with eculizumab does not affect generation of antibodies and immunosuppression might be required. The use of eculizumab has been reported in 18 patients with anti-FH-associated disease, either after failing PEX or as first-line therapy [97]. Since relapses were uncom-mon, the efficacy of concomitant immunosuppression in reduc-ing antibody titers and the risk of relapse could not be deter-mined. Prospective studies are required to determine the com-parative efficacy of eculizumab to PEX and immunosuppres-sion in these patients. *Until then, we recommend the use of PEX with immunosuppression for management of these patients.* Therapy with eculizumab should be considered in patients who are refractory to PEX, show life-threatening features, or have concomitant defect(s) in complement regulation [54].

## Supportive care and monitoring

Standard recommendations should be followed for AKI with attention to fluid and electrolytes, avoiding radiocontrast and nephrotoxic agents, and timely institution of dialysis [15]. The choice of dialytic modality is based on feasibility and physi-cian expertise. We suggest avoiding platelet transfusions un-less count is < 10,000/μl, or to enable vascular catheter inser-tion. Blood transfusion is recommended for patients with he-moglobin < 6 g/dl or hemodynamic instability. Standard mea-sures are instituted to retard CKD progression, especially con-trol of hypertension and proteinuria [107]. Complications such as malignant hypertension, seizures, bowel infarction, stenosis or perforation, pancreatitis, and cardiomyopathy re-quire appropriate management. All patients require follow up for at least 5 years for hypertension, proteinuria, and estimated GFR. Patients and their families should be counseled regard-ing the risk and recognition of relapses, and complications of the disease.
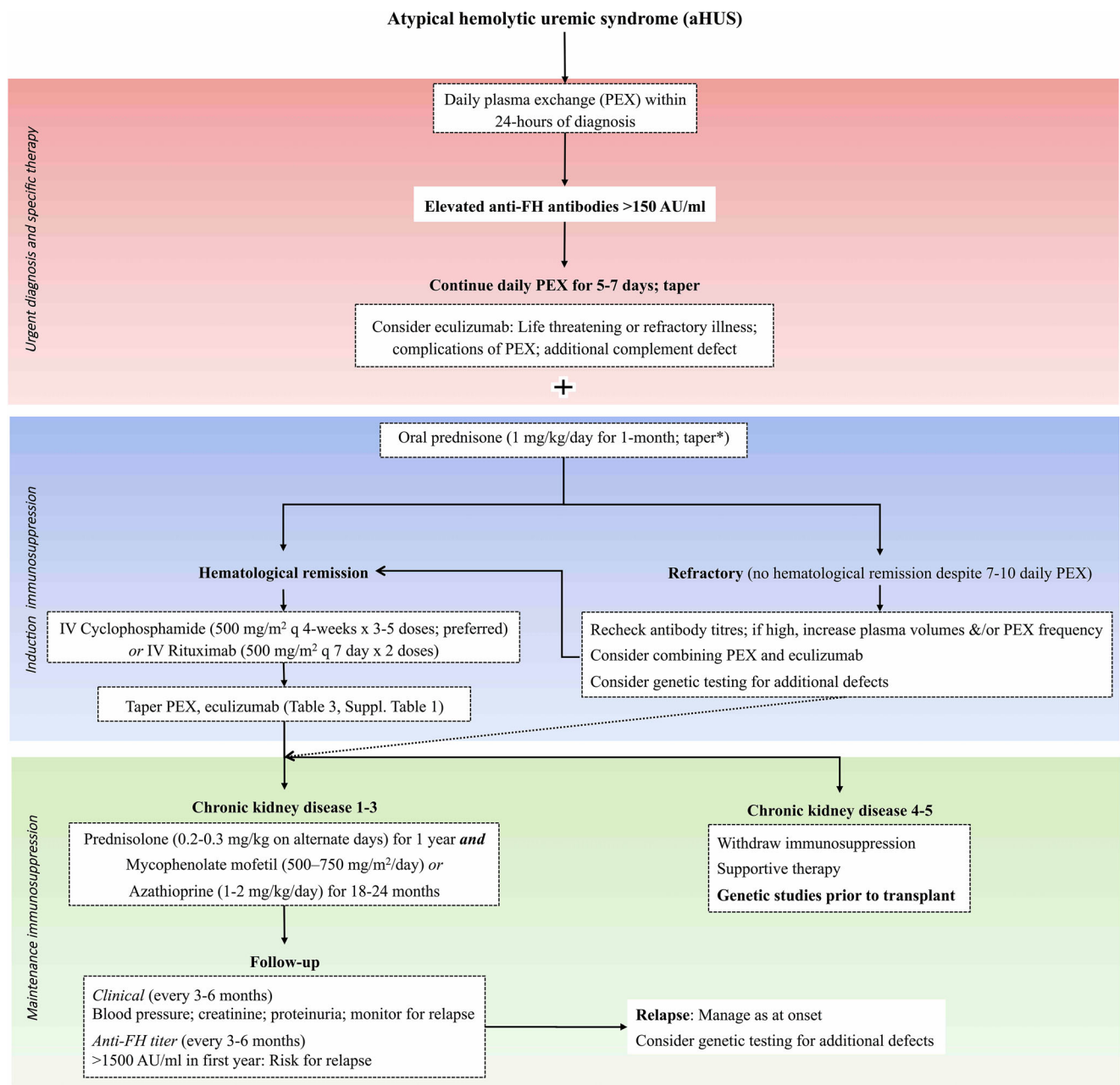
**Fig. 2** Management of patients with anti-factor H (FH)-associated atypical hemolytic uremic syndrome (aHUS). Initial therapy with plasma exchanges (PEX) is continued until hematological remission, followed by tapering over next 4–6 weeks (Table 3). Modification of therapy may be required in specific instances. *Prednisolone is given at a dose of 1 mg/kg/day for 4 weeks, then on alternate days for next 4 weeks, followed by taper every 2 weeks to 0.2–0.3 mg/kg/alternate days for 10–12 months. AU, arbitrary units; IV, intravenous; PEX, plasma exchange

## Transplantation

Patients with aHUS show variable risk of recurrent disease in the allograft. Donors and recipients need careful evaluation and peri-transplant management (Supplementary Table 2). Screening of recipients for mutations in relevant genes and for anti-FH antibodies is recommended. While the risk of recurrence is high in patients with dysregulation of the alternate pathway, those with STEC-HUS and abnormalities in membrane anchored (CD46) and intracellular (DGKE)

proteins have low risk [56, 98, 108]. Families should be counseled about the risk of recurrence, based on presence and type of mutations and titer of anti-FH antibodies.

Live-related donation should be avoided in patients at high risk of disease recurrence [109]. Such patients require either combined liver kidney transplantation or therapy with eculizumab (peri- and post-transplant) [109], at an appropriate center. Patients with high titer of anti-FH antibodies and no additional defects are managed by PEX prior to and following transplantation, and use of rituximab. Live donors should be

**Table 4** Comparison of present and international guidelines on management of hemolytic uremic syndrome (HUS) in children

| Guideline | Current guidelines | International consensus [13] |
|---|---|---|
| Diagnosis of HUS | Microangiopathic hemolytic anemia, thrombocytopenia and AKI | Similar |
| | Disseminated intravascular coagulation and thrombotic thrombocytopenic purpura (TTP) excluded, *when clinically indicated* | Exclude TTP in patients with suspected atypical HUS (aHUS) |
| | Exclude infections that mimic HUS, e.g., malaria, leptospirosis and dengue | Conditions mimicking HUS not discussed |
| | Influenza and HIV infection included in infection associated HUS | Similar |
| Shiga toxin associated HUS | *Confirmed* based on clinical and laboratory criteria | STEC infection diagnosed on stool culture and detecting Shiga toxin by fecal PCR or immune assay |
| | *Suspected* in patients with HUS occurring within 2-3 weeks of bloody diarrhea, and/or during an outbreak of STEC-HUS in patients above 6 months of age | No criteria for *suspected* STEC-HUS |
| Cobalamin C deficiency | Screen all patients with aHUS using total homocysteine levels | Screen all patients; plasma homocysteine and methionine; plasma and urine methylmalonic acid |
| | Therapy with parenteral hydroxycobalamin, oral betaine and folic acid administered in probable or confirmed cases | Similar therapy advised |
| Diagnosis of aHUS | Exclude STEC-HUS, secondary HUS; store samples for TTP, cobalamin deficiency | Diagnose when TTP, STEC-HUS, cobalamin C deficiency, infection associated and secondary HUS are ruled out |
| | Consider aHUS: Negative for Shiga toxin; non-synchronous family history; recurrent disease | |
| Evaluation of aHUS | Evaluate for complement abnormalities, anti-FH antibodies | Similar |
| | Recommend genetic screening, except patients with anti-FH associated aHUS[a] | Recommend genetic testing in all, including with anti-FH antibodies |
| Renal biopsy | Recommended for unclear diagnosis, unsatisfactory clinical response, or allograft dysfunction | Similar |
| Therapy of STEC-HUS | Hydration recommended in dysentery; monitor for fluid overload | Japanese [32] and Australian [12] guidelines recommend supportive care, adequate fluids, and consideration of PEX in patients with STEC-encephalopathy |
| | Recommend 5-days therapy with ciprofloxacin, azithromycin or cefixime for bloody diarrhea | |
| | Consider therapy with PEX if severe neurological or cardiac involvement | |
| Atypical HUS without anti-FH antibodies | Prompt PEX recommended; PEX preferred to plasma infusions | Eculizumab is preferred; patients commencing therapy with PEX should be switched to eculizumab once diagnosis of aHUS is made |
| | Daily PEX until hematological remission; then taper over 4-6 weeks | |
| | Consider eculizumab if: Refractory to 7-10 PEX; life threatening features (seizures, cardiac dysfunction); complications of PEX or vascular access; inherited defect in complement regulation | Specific indications for eculizumab use (following 5-daily PEX): Platelets $<150 \times 10^9$/l; LDH > upper normal; <25% decrease in serum creatinine; extrarenal features |
| Anti-FH associated HUS | Recommend therapy with PEX and immunosuppressive agents | Combined PEX (or eculizumab) and immunosuppression |
| | Daily PEX until hematological remission; taper in 4-6 weeks, plasma infusions not recommended | Stop PEX/eculizumab when anti-FH titer <1000 AU/ml |
| | Consider eculizumab if: Refractory to 7-10 PEX; life threatening features (seizures, cardiac dysfunction); complications of PEX or vascular access; additional inherited defect in complement regulation | |
| | Monitor antibody titers during first 12-24 months to predict relapse | Recommend regular monitoring of anti-FH levels |

*AKI* Acute kidney injury, *ELISA* enzyme linked immunosorbent assay, *FH* factor H, *HIV* human immunodeficiency virus, *HUS* hemolytic uremic syndrome, *IV* intravenous, *PCR* polymerase chain reaction, *PEX* plasma exchange, *STEC* Shiga toxin producing *Escherichia coli*, *TTP* thrombotic thrombocytopenic purpura

[a] Genetic screening in patients with anti-FH associated HUS: Early age of onset; relapsing course; family history of HUS; prior to transplantation

tested by MLPA for copy number variations in *CFHR1/3*. Since homozygous *CFHR1* deletion is a risk factor for anti-FH-associated aHUS, it is unclear whether healthy live-related donors with a homozygous deletion should be accepted as donors.

Patients should be monitored for recurrence following transplantation [54, 110]. If recurrence of HUS is confirmed, all efforts should be made to procure and institute therapy with eculizumab.

## Conclusions and perspectives

Guidelines from developed countries recommend comprehensive diagnostic assessment of patients with HUS, especially those with atypical disease. Compared to PEX, while terminal complement blockade with eculizumab has significantly improved outcomes in patients with aHUS, patients with anti-FH antibody-associated aHUS are satisfactorily managed with PEX and immunosuppression. Given limited diagnostic capabilities and lack of access to eculizumab, the international guidelines are not likely to be implemented in most developing countries in the near future. Table 4 summarizes practice guidelines for patients with HUS in India and other developing countries and compares them to international recommendations for the disease. We believe that it is desirable to invest resources in our country for setting up facilities for diagnosis and therapy (Table 5). Changing epidemiology of the disease and availability of novel agents that block the complement cascade might necessitate revision of these guidelines.

**Table 5** Priorities for capacity building in regional and national laboratories in India

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Microbiology
 Stool culture on sorbitol MacConkey agar or selective media
 Real-time polymerase chain reaction (PCR) for *stx1* and *stx2*[a]
 ELISA for free Shiga toxin or O157 antigen
 ELISA for antibodies to *Escherichia coli* lipopolysaccharide(s),
  antibodies to Shiga toxin

Immunology and biochemistry
 Flow cytometry for CD46 expression[b]
 ELISA for anti-factor H antibodies[b]
 Assay for ADAMTS13 activity; anti-ADAMTS13 antibody
 ELISA for complement factors H (FH), FI, and FB
 Genetics[c]
 Next-generation sequencing for *CFH, CFI, CFB, C3, CD46, THBD,
  DGKE*
 Sanger validation of changes on next-generation sequencing
 *CFHR1* deletion: Multiplex ligation-dependent probe amplification or
  end-point PCR

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[a] Performed at Postgraduate Institute of Medical Education and Research, Chandigarh

[b] All India Institute of Medical Sciences, New Delhi, other centers

[c] Research centers; private laboratories

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