Guidelines on Hemolytic Uremic Syndrome by Indian Society of Pediatric Nephrology: Key Messages

Priyanka Khandelwal and Arvind Bagga

From the Division of Pediatric Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, India. Correspondence to: Dr Arvind Bagga, Division of Pediatric Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. arvindbagga@hotmail.com

Hemolytic uremic syndrome is an important cause of acute kidney injury that requires dialysis in children. The diagnosis and management is difficult due to limited diagnostic facilities and non-availability of specific complement inhibitors. We describe salient features of the recent Indian Society of Pediatric Nephrology consensus guidelines on hemolytic uremic syndrome.

Keywords: *Factor H antibodies, Plasma exchange, Thrombotic microangiopathy*.

Hemolytic uremic syndrome (HUS) is an important cause of acute kidney injury (AKI) requiring renal replacement therapy. Rapid diagnosis and management is necessary to limit irreversible renal damage. Although Shiga toxin-associated HUS constitutes the chief form of the disease worldwide, burden of this illness in India is not clear. School-going children show high prevalence of anti-factor H (FH) antibody-associated HUS. While International guidelines emphasize comprehensive diagnostic evaluation and complement blockade with eculizumab, access to these facilities is limited in India. Given the difference in epidemiology and challenges in management, guidelines for treatment of HUS were recently published by the Indian Society of Pediatric Nephrology (ISPN) [1]. This article highlights key messages from these guidelines.

DIAGNOSIS

Importance of Demonstrating Schistocytes and Thrombocytopenia

The diagnosis of HUS requires all of the following: *(i)* microangiopathic hemolysis characterized by anemia (hemoglobin <10 g/dL), fragmented red cells on peripheral smear (schistocytes ≥2%) and either high lactate dehydrogenase >450 IU/l or undetectable haptoglobin; (*ii*) thrombocytopenia (platelets <150,000/μL), and (*iii*) AKI (rise in creatinine by 50% over baseline). Guidelines for identifying schistocytes on peripheral smear are available [2]. Rarely, HUS may have an indolent presentation with AKI and systemic hypertension without thrombocytopenia or microangiopathic hemolysis. Renal biopsy is usually not required.

Rule-out Infections

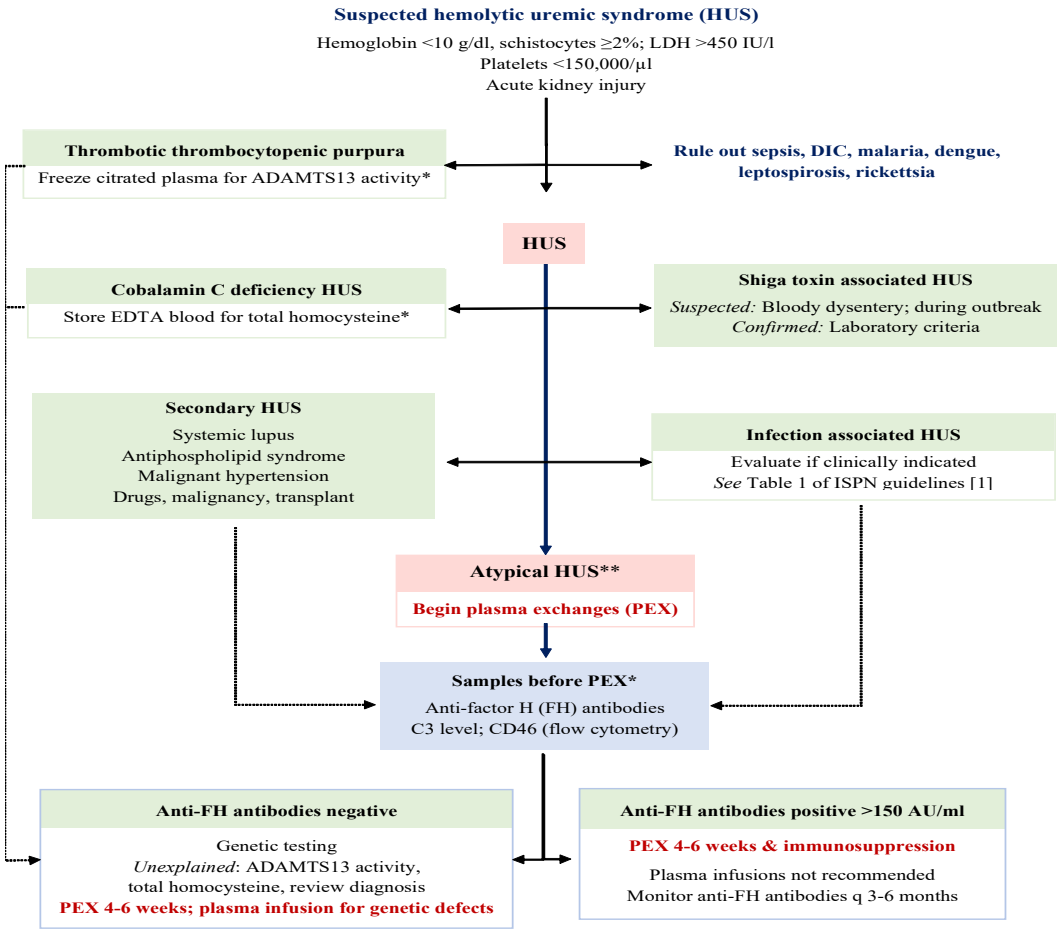
Disseminated intravascular coagulation (DIC) and thrombotic thrombocytopenic purpura (TTP) should be ruled out in patients with suspected HUS. Infections that mimic/trigger HUS, *e.g*., malaria, leptospirosis, dengue, rickettsia and H1N1 infection should be excluded, if clinically suspected.

DIC is characterized by prolonged prothrombin time or activated partial thromboplastin time, low fibrinogen, elevated D-dimer and soluble fibrin monomers. TTP is rare in childhood; persistent thrombocytopenia (<30,000/μL) and mild/no AKI is suggestive. Blood samples should be stored and later processed for ADAMTS13 activity, if etiology of microangiopathic anemia is unclear.

Evaluation

ISPN guidelines endorse the etiology-based classification of HUS (***Fig.*1**) [3]. Epidemiology of HUS in India differs from that in developed countries. Worldwide, the chief cause of HUS is gastrointestinal infection with Shiga toxin producing *E. coli* (STEC-HUS), which is seen in 80% patients. In India, infection with *S. dysenteriae* has declined significantly and prevalence of patients with STEC-HUS is also low. STEC-HUS is suspected if occurring within 2 weeks of bloody diarrhea – infection is diagnosed by stool culture and demonstration of virulence genes, fecal Shiga toxin or IgM antibodies to serogroup specific lipopoly-saccharide.

Cobalamin deficiency accounts for ~6-8% patients with HUS. Feeding difficulties, seizures, abnormal



*ADAMTS13, A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CD46, membrane co-factor protein; DIC, disseminated intravascular coagulation; LDH, lactate dehydrogenase; *See Box I for evaluating patients with HUS, including storing and processing of samples; **Also consider atypical HUS if positive non-synchronous family history or recurrent disease.*
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Fig. 1 *Approach to patients with hemolytic uremic syndrome (HUS).*

muscle tone, developmental delay and megaloblastic anemia are common; one-third lack extra-renal features. High blood levels of total homocysteine (>100 µM/L) followed by genetic screening is confirmatory [4]. Samples may be stored and processed later (***Box* I**). Specific therapy includes parenteral hydroxycobalamin, oral betaine and folate [4]. Secondary causes of HUS include systemic lupus, malignant hypertension and antiphospholipid antibody syndrome.

In a significant proportion of patients, HUS is associated with uncontrolled activation of the alternate complement pathway, termed atypical HUS (aHUS). A diagnosis of aHUS is made when infection, cobalamin-associated and secondary forms of HUS are excluded (***Fig.* 1**). These patients need detailed evaluation to determine the underlying cause. However, access to microbiological and complement assays is limited in India. Given the implications of accurate diagnosis, physicians taking care of these patients must be aware regarding appropriate screening. Units lacking facilities for assays must store samples for later analyses.

Screen for anti-FH Antibodies

Unlike European cohorts, anti-FH antibody-associated illness accounts for ~50% pediatric atypical HUS (aHUS) in India, chiefly affecting children aged between 5 and 15 years. Given therapeutic implications of this diagnosis, experts recommend prompt screening for anti-FH antibodies prior to instituting plasma exchange (PEX) therapy. ELISA test is available at multiple centers, with

BOX I Evaluation of Patients With Hemolytic Uremic Syndrome

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
- Anti-factor H antibodies[a,d]; antinuclear antibodies
- CD46 expression on neutrophils (flow cytometry)[a,d]
- Store blood for ADAMTS13 activity[a,c,e]; total homocysteine[a,c]

Selected patients

- Suspected Shiga toxin associated HUS: Stool culture; PCR for *stx*1, *stx2* genes[f]
- Suspected pneumococcal HUS: Culture, PCR, ELISA; peanut lectin agglutination assay
- Gene sequencing: *CFH*, *CFI*, *CFB*, *C3*, *CD46*, *DGKE, THBD, MMACHC*
- Multiplex ligation-dependent probe amplification: Copy number variations *CFHR1*-5

ADAMTS13 disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CD46 membrane cofactor protein; CFHR complement factor H related; ELISA enzyme linked immunosorbent assay; PCR polymerase chain reaction; stx Shiga toxin; [a]Blood samples should be drawn before plasma exchanges or infusion; [b]Positive with pneumococcal infection, lupus; must be tested prior to administering blood products; [c]Plasma to be separated from fresh citrated blood (ADAMTS13) and EDTA blood (homocysteine) within 1-hr of collection and frozen at -20 to -70°C; [d]Division of Nephrology, Department of Pediatrics, All India Institute of Medical Sciences, New Delhi; [e]Department of Hematology, Christian Medical College, Vellore; [f]Postgraduate Institute of Medical Education and Research, Chandigarh.

turn-around of 7-14 days [5]. Commercial ELISA kits may show false positive results and underestimate antibody titers, limiting their role on follow-up; a positive threshold for these kits is also not defined [6].

Pathogenic variants in genes encoding proteins of complement and coagulation pathways: *CFH*, *CFI*, *CFB*, *C3*, *CD46*, *THBD* and *DGKE* are associated with aHUS in 30-40% cases. Patients without anti-FH antibodies require sequencing of these and other genes by next-generation sequencing (NGS) and *CFHR1-5* copy number variations by multiplex ligation-dependent probe amplification (MLPA). These studies are useful for guiding management, prognosis, risk of relapses and allograft recurrence, and allow genetic counseling. NGS allows rapid, simultaneous sequencing of multiple genes; declining costs have made studies more accessible. Patients with anti-FH antibody-associated HUS do not require genetic screening, except if: (*i*) onset before 4 years of age, (*ii*) relapsing course, (*iii*) family history of HUS, (*iv*) illness that is refractory to PEX, and (*v*) prior to kidney transplantation.

MANAGEMENT

Atypical HUS: Across the developed world, complement blockade with eculizumab, the C5 monoclonal antibody, is the standard of care for patients with aHUS. However, eculizumab is expensive and not available in India and developing countries. In absence of anti-complement therapies, intensive PEX is less than ideal, but the only alternative. For our country, timely institution of PEX (60-75 mL/kg; fresh frozen plasma as exchange fluid) is most appropriate for patients with suspected aHUS.

PEX by filtration or centrifugation method, must be done at centers with expertise [7]. PEX is administered daily until hematological remission (platelets >100,000/μL, schistocytes <2%, LDH less than upper limit of normal for 2 consecutive days) and tapered over 4-6 weeks. Maintenance therapy with plasma infusions is

advised every 10-14 days for patients with mutations in complement genes, especially *CFH* and *CFI.* There is limited benefit of PEX in patients with: (*i*) microbiologically confirmed STEC-HUS, without cardiac or neurological involvement, (*ii*) infection-associated HUS or, (*iii*) pathogenic variants in *CD46* and *DGKE.*

Anti-FH antibody associated aHUS: Since aim of therapy for patients with anti-FH associated HUS is reduction of titers, PEX are most appropriate to achieve this goal. Plasma infusions do not remove antibodies and are not a substitute for PEX. Findings from a nationwide database on 436 patients with anti-FH disease show that high antibody titers ≥8000 AU/mL at onset, delayed PEX and short duration PEX (<14 days) predict adverse outcomes [8]. Combination of PEX and immunosuppression was most useful [1,8].

Immunosuppression must not be used without confirming presence of anti-FH antibodies. Therapy is initiated with prednisolone 1 mg/kg/day for 4 weeks, then alternate-day followed by tapering over 10-12 months. Therapy includes cyclophosphamide (500 mg/m^2 intravenously once in 4-weeks) for 5 doses. About 15-30% patients relapse; high anti-FH titers (>1300 AU/mL) during remission predict early relapses [8,9]. Antibody titers should be sequentially measured, especially in the first 12-24 months of follow up. Maintenance therapy with mycophenolate mofetil or azathioprine for 18-24 months, and tapering prednisolone further reduces the risk of relapses.

CONCLUSIONS

Given limited diagnostic capabilities and lack of access to eculizumab, international guidelines on aHUS are not likely to be implemented in developing countries in the near future. The present guidelines provide a systematic and algorithmic approach to management of patients with HUS, tailored to the distinct epidemiology and available repertoire of investigations and therapy. The guidelines underscore the importance of appropriate supportive care, and need for regular and prolonged follow-up. Capacity building for diagnosis and therapy of HUS and other complement related disorders is also required.

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