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Diagnosis and Classification of Hemolytic Uremic Syndrome: The Hungarian Experience

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ABSTRACT

Background. Hemolytic uremic syndrome (HUS) is a rare disease with various etiologies, making the identification of the specific forms and appropriate treatment difficult. Therefore, clinical and laboratory data from these patients need to be analyzed in national and international registries. Herein we have described 47 Hungarian HUS patients with detailed laboratory and clinical data obtained between 2008 and 2010.

Methods. Blood samples and clinical data of 47 patients with HUS diagnosed according to characteristic clinical signs were submitted for diagnostic evaluation, including complement protein and genetic analysis, measurement of ADAMTS13 activity and antibody analysis against O157LPS and factor H.

Results. There were 8 patients with typical diarrhea-positive HUS; 13 with atypical HUS (aHUS) and 26 with secondary HUS/thrombotic thrombocytopenic purpura group characterized by signs of complement consumption and decreased ADAMTS13 activity. Thus, decreased total alternative pathway activity is a promising diagnostic parameter with good sensitivity for aHUS.

Conclusions. These observations highlight the requirement for multiple diagnostic tests together with clinical data to identify the specific cause of HUS. Because the long-term prognosis of aHUS, eg, graft survival after renal transplantation, may vary according to the molecular etiology, it is important for all affected patients to undergo a detailed molecular diagnosis of the disease. There is a clear clinical need for the development and application of novel assay in this field to allow more rapid efficient diagnosis of patients who undergo a first episode of HUS.

The triad of microangiopathic hemolytic anaemia, thrombocytopenia, and acute renal failure are characteristic features of hemolytic uremic syndrome (HUS).[1,2] Various forms of HUS can be classified as those with advanced etiology or secondary to underlying disease.[3] Recent progress in the understanding of the HUS pathogenesis has helped to identify various forms of the disease with advanced etiology, namely, diarrhea-positive (D+HUS) most frequently caused by verocytotoxin-producing bacteria,[4] atypical HUS (aHUS) linked to failure of the complement alternative pathway (AP) regulation,[5] and *Pneumococcus* HUS (P-HUS) linked to neuraminidase-producing invasive *Pneumococcus* infections.[6] Many underlying disease have also been described to produce secondary forms of HUS, including, among others, systemic autoimmune diseases such as systemic lupus erythematosus and scleroderma, various types of cancers and their treatments, bone marrow transplantation, graft-versus-host disease, infections leading to sepsis, and disseminated intravascular coagulation. The purpose of the present study was to summarize the basic clinical data and laboratory findings among a series of 47 HUS cases seen in the past 3 years in 1 laboratory offering diagnostic services for Hungary.

PATIENTS AND METHODS

All 47 patients suffered an acute or a previous HUS episode defined as microangiopathic hemolytic anemia, ie, decreased he-

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Table 1. Characteristics of the Hungarian HUS Cohort at the Time of Presentation for First Complement Analysis

Parameter (reference range)	D+HUS (n = 8)	aHUS (n = 13)	Secondary HUS/TTP (n = 26)
Clinical status at the time of complement investigation, acute episode/in remission	5/3	3/10	21/5
Female/male	4/4	12/1	17/9
ADAMTS13 activity, (67%–151%) >70%/10%–70%/<10%/nd	2/4/0/2	2/7/0/4	2/23/0/1
Renal sequelae on follow-up, none/CRI/ESRD	8/0/0	4/1/8	9/15/2
Death, yes/no	0/8	3/10	13/13
Alternative pathway activity (Wieslab-ALT: 70%–110%), >70/<70/nd	4/1/3	3/10/0	5/13/8
Complement C3 (0.7–1.8 g/L), >0.7/<0.7/nd	8/0/0	6/7/0	15/7/4
Complement factor H (127–447 mg/L), >127/<127/nd	8/0/0	10/3/0	24/0/2
Complement factor I (70–130%), >70/<70/nd	8/0/0	9/4/0	18/6/2
Complement factor B (70–130%), >70/<70/nd	8/0/0	9/4/0	18/6/2
Anti-FH IgG (neg/pos/nd)	8/0/0	11/2/0	24/0/2
Anti–*E. coli* O157 LPS IgM (neg/pos/nd)	5/3/0	13/0/0	0/0/26
Anti–*E. coli* O157 LPS IgG (neg/pos/nd)	8/0/0	13/0/0	0/0/26
Comment	All 8 cases diarrhea positive (5 of them bloody)	Mutation positive 5/13 cases, *CFHR1–3* deletion 2/13 cases; Type of mutation: *CFHR*1215Q 2 cases, T956M 1 case, *CFH* R1210C with *CFH* T1216del 1 cases, *CFI* c.772G/A 1 case	Underlying disease: sepsis 7/26, systemic autoimmune disease 6/26, Cancer or related treatment 4/26, BMT, GVHD 4/26, other 5/26

nd = not done; BMT = bone-marrow transplantation; GVHD = graft-versus-host disease; CRI = chronic renal insufficiency; ESRD = end-stage renal disease; CFH = complement factor H; CFI = complement factor I; LPS = lipopolysaccharide; ADAMTS13 = a-disintegrin and metalloprotease with thrombospondin motifs 13.

moglobin according to age and gender with increased lactate-dehydrogenase activity, with fragmentocytes or schistocytes in the peripheral blood smear and thrombocytopenia (<150 g/L). Impaired renal function was present in all of the patients who were subsequently diagnosed as D+HUS or aHUS, whereas those with secondary HUS/TTP showed variable severities of renal or neurologic dysfunction.

The following determinations were performed during the diagnostic evaluation of the patients (Table 1): complement determinations, including C3, factor I, and factor B (radial immunodiffusion); factor H (sandwich ELISA); total activity of the alternative pathway (Wieslab AP ELISA Kit) and anti–factor H IgG autoantibody measurements (direct ELISA using recombinant factor H obtained from Calbiochem as antigen); ADAMTS13 activity using FRET-VWF73 fluorigenic substrate and functional determination of blocking anti-ADAMTS13 autoantibodies by mixing studies; and anti-*E. coli* O157 LPS IgM and IgG antibodies by direct ELISA. If aHUS was suspected, we performed a bidirectional DNA sequencing of PCR products generated from selected exons of *CFH, CFI, CD46* (MCP), *CFB,* and *THBD* (thrombomodulin) genes. Details on primer sequences and PCR conditions are available on request. In anti–factor H autoantibody–positive cases, copy number of the CFHR1–3 genes was analyzed by multiplex ligation-dependent probe amplification (MLPA). For statistical analysis we used GraphPad Prism.

RESULTS

Altogether, blood samples from 47 patients with clinical signs of HUS had been received in the past 3 years. Laboratory determinations were performed according to the clinical signs and availability of appropriate biologic materials. Clinical and laboratory data are presented in Table 1.

The final diagnosis of D+HUS was established when the patient had bloody diarrhea before the onset of HUS and/or the serologic determination of anti–*E. coli* O157 LPS IgM antibodies was positive for patients with an acute first episode of HUS. Eight patients were identified as D+HUS; 6 were children (4 ± 2.5 years old) and 2 were adults (42 and 46 years old). The clinical prognosis was good for this group of patients, without renal sequelae or death. Mean serum creatinine levels at first presentation of the disease was 445 ± 256 μmol/L. Interestingly, though low levels of complement proteins or activity were not observed in these patients, ADAMTS13 activity was moderately decreased in one-half of them. The most characteristic laboratory finding was positivity for IgM anti–O157 LPS antibodies (3/5 acute cases).

The diagnosis of aHUS was established when there were >1 episodes of HUS or complement abnormalities either in the acute stage of the disease, or in remission. There were 8 children and 5 adults, with an average age at disease onset of 11 ± 9 years. Interestingly, there was a female predominance (92%) among our aHUS series, with a fairly bad clinical prognosis; 70% chronic renal insufficiency or end-stage renal disease and 3/13 died, and the mean serum creatinine levels at first presentation of the disease was 172 ± 30 μmol/L. The most characteristic laboratory finding among our aHUS series (77% of all patients) was a decreased total activity of the AP, including all patients in

the acute stage and 70% of those in remission. Factor I, factor B, and C3 levels were also decreased in most patients, indicating pathogenic overactivation of the AP. Mutations of complement regulatory proteins were identified in 5 patients (38%); the position of the mutations is detailed in Table 1. Two cases among the 13 aHUS patients were identified as anti–factor H autoantibody–positive patients (15%): One patient carried homozygous deletion of *CFHR1* and *CFHR3* genes, and the other carried homozygous deletion of *CFHR1* but heterozygous deletion of *CFHR3*. Seven healthy mutation-positive family members were identified by family studies. None of the aHUS patients were positive for anti–O157 LPS antibodies, but 78% of those investigated for ADAMTS13 activity showed moderately decreased levels of the protease.

HUS (TTP) as a complication of an underlying disease or of its treatment (Table 1) was observed in 26 patients, 4 of whom were children with the age at disease onset of 38 ± 23 years. All of the patients had hemolytic anemia with fragmentocytes and low platelet counts, but renal sequelae were present in only 65% of them. The mean serum creatinine levels at first disease presentation was 230 ± 102 μmol/L, and neurological signs in 40%. Sixty-five percent of patients were female, and the overall mortality was 50%. All but 2 patients showing secondary HUS/TTP had moderately decreased ADAMTS13 activity (typically 10%–50%); 72% of those investigated for total AP activity showed decreased levels, but low levels of factors I and B were observed in only 25%, indicating lack of pathogenic overactivation of AP.

Two infants (9 and 12 months old) with invasive pulmonary pneumococcal infection and sepsis were identified as P-HUS (not presented in Table 1). The disease was verified in both cases by positive cultures and detection of plasma neuraminidase activity by 5-methylumbelliferyl neuraminic acid. Both patients died due to complications of sepsis; ADAMTS13 activity and AP activity was decreased in both cases.

DISCUSSION

In this report, we have described a series of 47 patients with HUS referred to a single research laboratory for diagnostic evaluation over 3 years. Because the laboratory specializes in complement determinations, there was a potential positive selection for aHUS cases and a relative underrepresentation of typical D+HUS. During the diagnostic evaluation of the patients with a first acute episode of HUS, tests were performed in 3 steps. First, during the first 48 hours, the presence of (auto)antibody-positive forms of thrombotic microangiopathies were investigated among anti–factor H, anti–O157 LPS IgM, and anti-ADAMTS13 autoantibody determinations. Next, functional assessment of AP and antigenic determinations of complement proteins determined the presence of complement consumption and pathologic overactivation. Finally, if aHUS was the most probable form of the disease, we performed genetic analysis of complement and thrombomodulin genes. This algorithm, together with clinical data,[7] helped us to quickly identify autoantibody-positive patients requiring early intensified immunosuppressive treatment with plasmapheresis. Furthermore, the functional measurement of AP by the Wieslab ELISA showed promising sensitivity to early identify patients with complement consumption.

For subjects having mutations in complement regulatory genes, a combined liver-kidney transplantation might be a therapeutic option.[8] Further studies are required to determine the exact role of eculizumab, a C5-blocking monoclonal antibody, in the treatment arsenal for aHUS patients.[2] However, there is a clear clinical need for the development and application of novel assays in this field to allow more rapid efficient diagnosis for patients with a first episode of HUS. There is currently no available functional test to assess the AP C3 convertase, because the hemolysis- and ELISA-based tests depend on the activation of the terminal pathway. Furthermore, it would be important to identify the most reliable complement activation markers with high specificity for aHUS.

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