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Hemolytic Uremic Syndrome: Epidemiological and Clinical Features of a Pediatric Population in Tuscany

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Key Words
Acute renal failure · Diarrhea · Hemolytic uremic syndrome · Shiga toxin-producing *Escherichia coli*

Abstract
We retrospectively analyzed etiological, pathological and clinical features of the patients with hemolytic uremic syndrome (HUS) observed in the Pediatric Nephrology Unit at AOU Meyer of Florence. From January 1997 to December 2008, 22 cases were identified, with an annual incidence of 0.05 cases per 100,000 inhabitants, and 0.34 cases per 100,000 children <15 years old. 60% of the patients were D+ and 40% were D–, with an age distribution from 12 days to 13 years. Twenty patients (90%) had oligoanuria, lasting 6.4 ± 4 days for D+ patients versus 11.8 ± 4 days for D– patients. The development of chronic kidney disease positively correlates with the initial blood pressure value, the length of oligoanuria, and hospitalization. Microbiological investigations showed an association of D+HUS with different strains of Shiga toxin-producing *Escherichia coli* in 54% of the cases. D–HUS was associated with complement factor H deficiency in one patient. In the other cases, the triggering factors were pertussis, urinary tract infections and upper airway infections. While clinical and prognostic features correspond with literature data, in Tuscany the annual incidence is lower, and the percentage of D–HUS patients is higher than that observed in other studies.

Introduction

Hemolytic uremic syndrome (HUS) is characterized by nonimmune microangiopathic hemolytic anemia, thrombocytopenia and renal impairment, and it is the most common cause of acute renal failure (ARF) in children under 4 years of age [1–3].

In the majority of cases (90%), HUS follows an episode of gastroenteritis, often with bloody diarrhea (D+HUS), triggered by Shiga toxin-producing *Escherichia coli* (STEC) [4–6]. Approximately 5–10% of people with diarrhea due to STEC develop HUS [1]. The remaining 10% of cases are classified as atypical because they are not associated with a prodrome of diarrhea (D–HUS) and can be triggered by other bacterial and viral infections (neuroaminidase-producing *Streptococcus pneumoniae*, human immunodeficiency virus and others) [1, 7, 8] or can be caused by mutations in genes encoding complement factor H (CFH), complement factor I (CFI), complement factor B, membrane cofactor protein (MCP), complement C3 and thrombomodulin [9–13].

Metabolic diseases such as inborn errors of vitamin B_{12} intracellular metabolism are also associated with neonatal or late-onset HUS [14], and HUS has also been connected with autoimmune diseases such as LES and antiphospholipid antibodies syndrome [1]. HUS associated with malignancy, transplantation or drugs such as calcineurin inhibitors has rarely been reported [1].

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The annual incidence of D+HUS varies in the different countries, and meanwhile in western Europe it is 1.5–4 per 100,000 children aged less than 5 years; in Argentina it is in the order of 21.7 per 100,000 children <5 years old [15–18]. In a recent study, performed in Switzerland, the annual incidence of HUS was calculated to be 1.42 per 100,000 children ≤16 years old [19]. In France, from 1996 to 2006, the average annual incidence of HUS was 0.71 cases per 100,000 children <15 years old and 1.87 cases per 100,000 children <5 years old [17]. In Australia, the annual incidence of D+HUS is 0.64 per 100,000 children <15 years old and 1.35 per 100,000 children <5 years old [20]; in the US, the annual incidence of HUS is approximately 2.2 cases per 100,000 inhabitants with an incidence up to 6.1 cases/100,000 children <5 years old, although for the same group of patients an estimate as low as 1.08/100,000 has been provided [21–23]. In patients with diarrhea-associated HUS, the overall mortality rate is 3–5%; death or end-stage renal failure occurs in about 12%, and approximately 25% of survivors demonstrate long-term renal sequelae [1, 6]. Such percentages reach 30–60% in patients with D–HUS [10].

We performed a retrospective study to analyze epidemiological, etiological, pathological and clinical features of the patients with HUS observed in the Pediatric Nephrology Unit at 3rd level Meyer Hospital of Florence in Tuscany (Italy). All patients in whom HUS was diagnosed in Tuscany were referred to this specialized centre, except for mild cases, which were treated in local hospitals.

Patients and Methods

We collected the medical records of 22 patients with a diagnosis of HUS between January 1997 and December 2008. All the patients satisfied the diagnostic criteria for HUS: ARF (increased serum creatinine and/or BUN above the limit for age), acute Coombs-negative hemolytic anemia (Hb <11 g/dl) with microscopic evidence of fragmented erythrocytes, and thrombocytopenia (platelet count <150,000/mm^3).

Epidemiological data collected included annual incidence, seasonal distribution of the cases, sex and age of the patients and duration of hospitalization. Clinical data included information about prodromal phase and development of the disease, laboratory findings and treatment. Biochemical parameters were measured for all patients by automatic biochemistry analyzer. Microbiological investigations were performed both by local laboratories and by the Department of Food Safety and Veterinary Public Health of Istituto Superiore di Sanità in Rome, Italy. Genetic analyses (screening for CFH, MCP and CFI mutations), when considered suitable, were performed by the laboratory of Istituto Mario Negri, in Bergamo, Italy. STEC association was determined using one of the following criteria: positive stool cultures, evidence of Shiga toxin in stools and/or positive serum analysis for LPS antibodies.

We performed statistical t test for normally distributed variables: age of patients, WBC, RBC, platelets, LDH, bilirubin, hemoglobin, creatinine, BUN, systolic (SBP) and diastolic (DBP) blood pressure, duration of oligoanuria and hospitalization.

Results

Among the 22 patients with a diagnosis of HUS, 14 were female (64%) and 8 male (36%). All the children were in prepubertal age, between 12 days and 13 years of age, with an average age of 44 ± 39 months. 60% of the patients were D+, and the average age of these patients was 35 ± 15 months (range 12–54 months). The remaining 40% were D–, and the average age of these patients was 53 ± 58 months (range 12 days to 13 years).

Because of the extreme heterogeneity of the age of the D– group, the average age in the survey of children with D–HUS was not significantly higher than in the survey of children with D+HUS (p = 0.3017).

D+ and D– patients were hospitalized for 18.5 ± 5 and 38 ± 14 days, respectively (p = 0.0002).

The annual average of the cases was 1.7 per year, but with a great variability: in 1998, there was a peak of 4 cases, with no cases in 1999, 2001, 2002 and 2006. The monthly distribution of D+HUS peaked during the summer, with 80% of cases occurring from June to September. There was no difference in seasonal distribution in D–HUS cases. The annual incidence was 0.34 cases per 100,000 children <15 years old and 0.05 cases per 100,000 inhabitants.

Clinical characteristics, neurological complications and treatment applied are resumed in table 1.

During hospitalization, 20 patients (90%) had oligoanuria (diuresis <250 ml/m^2/24 h – <1 ml/kg/h). 77% of the patients presented persistent oligoanuria for more than 5 days, and its duration was significantly longer (p = 0.008) in D– patients (11.8 ± 4 days), compared with D+ patients (6.4 ± 4 days). During the acute phase of the disease, arterial hypertension with SBP and or DBP higher than the 95th percentile occurred in 46% (6/13) of D+ patients and 66% (6/9) of D– patients. The average SBP was 112 ± 13 (D+) and 136 ± 29 mm Hg (D–; p = 0.0266), and the average DBP was 69 ± 12 (D+) and 82 ± 12 mm Hg (D–; p = 0.0417). Neurological symptoms, such as drowsiness and seizures, were found in 4 (18%) patients: 2 D+ and 2 D– patients. 44% of D– patients required pediatric intensive care unit services versus 23% of D+ patients. Among

Table 1. Characteristics of patients with HUS (median ± SD)

Characteristics	All patients	D+	D–	p value
Sample size	22 (100)	13 (60)	9 (40)	
Females	14 (63)	8 (61)	6 (66)	0.8058
Age, months				
Median ± SD	44 ± 39	35 ± 15	53 ± 58	0.3017
Range	0.4 – 156	12 – 54	0.4 – 156	
Length of hospital stay, days				
Median ± SD	26 ± 14	18 ± 5	38 ± 14	0.0002
Range	9 – 60	9 – 30	21 – 60	
Clinical features at the time of hospitalization				
Diarrhea	13 (60)	13 (100)	0	
Bloody diarrhea	9	9 (70)	0	0.0012
Abdominal pain	13 (60)	13 (100)	0	
Pallor	18 (81)	9 (70)	9 (100)	0.0658
Vomiting	10 (45)	6 (50)	4 (44)	0.9369
Fever	6 (27)	4 (33)	2 (22)	0.6581
Oligoanuria	20 (90)	11 (84)	9 (100)	0.0468
Length of oligoanuria days	8 ± 5	6.4 ± 4	11.8 ± 4	0.008
Hypertension	12 (54)	6 (46)	6 (66)	0.3421
SBP, mm Hg	120 ± 25	112 ± 13	136 ± 29	0.0266
DBP, mm Hg	74 ± 13	69 ± 12	82 ± 12	0.0417
Seizures	4 (18)	2 (15)	2 (22)	0.6827
Intensive care unit	7 (30)	3 (23)	4 (44)	0.2901
Treatment				
Dialysis	17 (77)	9 (70)	8 (88)	0.2794
Blood transfusion	22 (100)	13 (100)	9 (100)	
Platelet infusion	2 (9)	1	1	0.7839
FFP	5 (23)	0	5 (55)	0.0022
Plasma exchange	2 (9)	0	2 (22)	0.0746

Figures in parentheses indicate percentages.

Table 2. STEC strain detected in D+ patients

STEC strain	Cases among D+HUS
O157	3 (23%)
O145	2 (15%)
O103	1 (8%)
O26	1 (8%)
O111	0
Microbiologic and serologic test negative	3 (23%)
Other	3 (23%)

the patients with D+HUS, one developed acute pancreatitis. Among the patients with D–HUS, 2 showed unilateral renal agenesis with vesicoureteral reflux grade II, and in both cases the onset of HUS was a urinary tract infection due to no-STEC. Finally, in one case D–HUS was associated with *Bordetella pertussis* infection.

Dialytic treatment was required by 70% of D+ patients and 88% of D– patients. In all the 16 dialyzed patients, the duration of oligoanuria was longer than 5 days; 15 were treated with hemodialysis, one with peritoneal dialysis. Blood transfusions were required for all patients; only 2 patients received platelet transfusions.

Fresh frozen plasma (FFP) was administered to 5 children with recurrent D–HUS. In 2 cases, FFP was associated with plasma exchange. The children with no recurrences of D–HUS received only symptomatic therapy.

Among the 5 children with D–HUS recurrence, 3 developed end-stage renal disease and hypertension during the acute phase in spite of the treatment with FFP. Among these, one received a successful kidney transplant, one died during a combined kidney-liver transplant, one required chronic dialysis. Finally, one patient developed chronic kidney disease (CKD) stage 3, and the renal function remained normal only in one child with of D–HUS recurrence.

So far, no D+ patients and no children without recurrences of D– have either suffered CKD or developed long-term hypertension.

The results of microbiological investigations showed a D+HUS associated with STEC in 54% of cases. Stool cultures for O157 strain were found positive in 2 patients (15%). In 5 children (38%), the infection with different strains of *E. coli*, i.e. O145 (2), O103 (1), O26 (1), O157 (1), was demonstrated using serum analysis for antibodies to LPS (table 2).

In 3 D+HUS patients (23%), despite prodromal diarrhea, no microbiological or serologic evidence of STEC infections was found. In three D+ cases, stool culture was negative but we cannot exclude STEC infection because they date back to a time when serum analysis for Shiga toxin antibodies was not performed. All D–HUS patients were negative for any STEC infection criteria. Nobody showed a reduction in the activity of von Willebrand factor-cleaving protease (VWF-cp) or ADAMTS 13, and no patient was found to have inhibitor antibodies against the VWF-cp. CFH serum level was within the normal range (350–750 mg/l) in all children with D–HUS; a heterozygosis 'non-sense' mutation in the CFH gene [(3587 G>T) in the SCR19–20 region], was found in a patient suffering of relapsing severe HUS. Three heterozygosis polymorphisms in the CFH gene, associated with a high risk of

developing HUS (275 C>T, 2089 A>G, 2881 G>T), were found in one patient [24]. None of our patients had mutations in the MCP gene. In one case, HUS was associated with a defect in the activity of the complex II of the mitochondrial respiratory chain (succinate coenzyme Q1 reductase) and rhabdomyolysis [25, 26].

The hospital stay and the duration of oligoanuria, SBP, DBP, and the development of CKD were significantly higher in the D– group ($p < 0.05$). With regard to other analyzed parameters, there were no significant differences between the D+ and D– groups (table 3). The duration of oligoanuria and the values of SBP and DBP are positively correlated with the development of chronic renal disease ($p < 0.05$).

Table 3. Laboratory findings at admission

Parameters	D+	D–	p value
White cell count, $\times 10^3/mm^3$	16 ± 6.6	27.2 ± 26.9	0.1816
Red cell count, $\times 10^3/mm^3$	3,121 ± 990	2,652 ± 584	0.5569
Platelet count, $\times 10^3/mm^3$	66.1 ± 50.8	55.8 ± 39.4	0.3572
Hemoglobin, g/dl	8.6 ± 2.3	7.1 ± 1.5	0.5159
LDH, U/l	4,626 ± 2,934	4,975 ± 2,364	0.9405
Serum creatinine, mg/dl	3.6 ± 3.1	2.3 ± 2.1	0.4516
Bilirubin, mg/dl	1.8 ± 0.8	3.6 ± 1.8	0.0675

Discussion

Despite its low incidence, HUS it is the primary cause of ARF in children between the ages of 1 and 4 years, and is the second most common cause in children aged under 1 or over 4 years. Moreover, it is the seventh cause of CKD in children over 5 years [1]. In Italy, the national annual incidence from 1988 to 2004 was 0.28 cases per 100,000 children <15 years old [27], results lower than in other European countries [15–17, 19]. Because risk factors for HUS in western European countries show similar frequency and characteristics, the lower incidence of HUS observed in Italy might be explained by the difficulty in epidemiologic detection of HUS. As far as D+HUS is concerned, Italy has no mandatory notification system for either cases of infections caused by STEC O157 or cases of HUS; monitoring is therefore achieved via voluntary organizations or initiatives, for the most part by The HUS National Monitoring System for Pediatric Patients, active since 1988. This organization has registered a total of 439 cases of pediatric HUS between 1988 and 2004 [27]. The absence of mandatory notification of HUS and/or *E. coli* infection holds up epidemiological studies, especially in Italy, where the regional organization of health facilities represents an additional obstacle. In fact, the practice of notification may differ from a geographic area to another. In our study, the annual incidence of HUS in Tuscany is 0.05 cases per 100,000 inhabitants and 0.34 cases per 100,000 children <15 years old. The incidence of HUS in Tuscany is higher than the national annual incidence but lower than in other countries and, in some years, no cases have been observed, probably due to the rarity of the disease and to environmental factors difficult to decipher. Considering cattle as a major reservoir of STEC pathogenic for humans and comparing the HUS incidence in Tuscany to other European countries, the difference can be related to the low presence of cattle breeding in Tuscany [28]. Besides, we have to consider that many D+HUS cases were probably treated in local hospitals and not in our Nephrology Unit, because they were mild forms, and thus we did not gain knowledge of them. In 1998, in Tuscany, the pediatric units of all hospitals established a network that allows cultural exchanges and easier diagnosis. This network makes it also possible to treat some mild renal diseases in hospitals without pediatric nephrology facilities. It is, however, possible that many mild cases are undiagnosed, because oligoanuria and renal failure are explained as due to post-diarrhea dehydration. Furthermore, rehydration performed in a peripheral hospital, might prevent severe HUS, as recent data suggest [29]. These considerations also help explain the higher percentage of D–HUS patients in our series compared to the series in other studies. In fact, in the 12-year span of our survey, the percentage of D+ patients was 60%, versus 40% for D– patients. On the other hand, in other studies, the proportions are very different, ranging between 90 and 97% for D+HUS and 3 and 10% for D–HUS [1, 15–17]. STEC infection can manifest without diarrhea, leaving HUS to be classified as D–, even though it is actually an undetected D+ form. In fact, one of the largest multicenter studies demonstrated that 50% of D–HUS cases showed evidence of STEC infection from microbiological and/or serological investigations [16, 30]. However, even if in the 3 oldest cases of our series, serological investigations for STEC infection were not available and the assignment to the D–HUS group is uncertain, the percentage of D–HUS remains 25%. In the US and Europe, *E. coli* O157 is the principal cause of D+HUS, both for sporadic cases and epidemics [4, 15–17, 30]. In other parts of the world, for example in Australia, infections from non-O157 strains have been more frequently

reported, especially O111 [20]. The cause of these variations in the distribution of the serotypes in various areas and countries has not yet been clarified. In our survey, only 23% of patients with diarrhea resulted positive for the O157 strain, whereas 30% resulted positive for non-O157 STEC strains, which further confirms the territorial variability of the diffusion of STEC.

The German and Italian studies report a larger incidence of infection from non-O157 STEC in the youngest children [16, 30]; among our patients we have not observed that, because the children with evidence of a non-O157 STEC infection are not different in age to those of the group infected with O157 STEC.

The traditional stool culture appears to be ineffective in the diagnosis, as it resulted positive in only 2 cases (17%). This is mainly due to the short time span during which it is possible to find the bacteria in the feces, and to the fact that often the stool culture is necessarily made 5–7 days after the first appearance of diarrhea. Furthermore, it is clear that research into the emerging problem of non-O157 STEC strains is essential, as our study also confirmed.

In accordance with the literature data, the average age was higher in the D– group (53.1 ± 58 months) than in the D+ group (35.1 ± 15 months), with an extreme heterogeneity of age of D– children (table 1), which ranged from 12 days to 13 years, whereas almost all D+ patients were in the age range of <4 years.

The widened age range at the appearance of D–HUS is already known [4] and is due to the presence of patients both with the onset of disease in the first months of life and in adolescent or adult patients.

Genetic factors play a considerable role in D– HUS. CFH or CFI mutations carriers often develop the disease before 3 months of age, while patients with MCP mutations develop it later in life [9]. So we might hypothesize that mutations in different genes involved in D–HUS can influence the age at onset. On the other hand, the penetrance of HUS among CFH, MCP and CFI mutations carriers appears to be between 40 and 50% and, even in patients with multiple genetic risk factors, the syndrome might not occur until middle age [9, 10]. Moreover, the newborns present an increased risk of Gram-negative sepsis and endothelial toxic damage with secondary HUS.

Even in our series, the time of hospitalization and recovery was significantly higher in D– patients (38 ± 14 days) than in D+ patients (18 ± 5 days), due to a greater overall seriousness of the disease. The average duration of hospitalization in D+HUS was similar to that observed in a similar survey [31], but in the French study, the average duration of hospitalization for D+ patients was 10 days [17]. This might be due to differences in patient management or, more likely, to a greater complexity of cases under our observation in a 3rd level pediatric hospital.

According to other epidemiological reports, most of the D+HUS cases (83%) manifest during the summer months, whereas no seasonal fluctuation was detected for the D– cases [4, 5, 16, 17]. In our study, the duration of oligoanuria and the values of SBP and DBP were positively correlated with the development of CKD ($p < 0.05$) and were considerably higher in D–HUS patients. A recent retrospective study demonstrated that the prevalence of long-term renal damage in D+HUS rises as the duration of oligoanuria increases, and is considerably less among patients who had oligoanuria for less than 5 days compared to those who had oligoanuria for more than 10 days [32, 33]. However, none of our D+ patients with oligoanuria lasting more than 5 days developed long-term renal damage.

Treatment of STEC associated with HUS consists of the management of ARF and its complications; meanwhile, D–HUS treatment should be related to etiology [34]. The German study reported that 61% of patients received dialysis treatment, 75% RBC transfusions, 16% platelet transfusions, and 9% plasmapheresis. The American study, including only D+HUS cases, reported that 55% of subjects needed dialysis, and 37% RBC transfusions [16, 21]. Among our D+HUS patients, dialysis was given to 75%, RBC transfusion to 100%, while only 2 received platelet transfusion. So, comparing our data with those of the other series, the percentage of patients requiring dialysis and RBC transfusions was higher, and this seems to support the thesis that the cases under our observation were actually characterized by more severe clinical manifestations.

In conclusion, despite the low number of our patients, our study verified important epidemiological, etiological and clinical features of HUS in Tuscany. The salient points are the high percentage of D–HUS patients, the low percentage of O157 STEC strain and, despite the severity of HUS among many patients, no death occurred during the acute phase of disease. In order to get more epidemiological, clinical, prognostic and therapeutic information, to guarantee greater reliability in terms of statistical estimation, and to correct the probable underestimation of the disease, it would be useful if HUS notification became mandatory and if a regional register of the disease was created, where the patients' clinical data and disease course could be recorded.

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