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ORIGINAL ARTICLE

C3 levels and acute outcomes in Shiga toxin–related hemolytic uremic syndrome

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Abstract

Background The correlation between complement activation and severity of hemolytic uremic syndrome related to Shiga toxin–producing *Escherichia coli* (STEC-HUS) has been examined in few studies, with conflicting results. We investigated whether C3 levels on admission are associated with worse acute outcomes.

Methods Demographic, clinical, and laboratory variables were compared between dialyzed and non-dialyzed patients and between those with or without extrarenal complications. Univariate and multivariate analyses were performed; odds ratio (OR) and 95% confidence interval (95%CI) were calculated. C3 concentrations were correlated with dialysis length (Spearman test) and ROC curves with area under the curves (AUC) were calculated to identify C3 concentrations able to discriminate patients with dialysis requirements and complicated course.

Results Among 49 children, 33 had normal and 16 had decreased C3 concentrations. Higher hemoglobin, lactic dehydrogenase, urea and creatinine and lower albumin, sodium, and C3 and C4 concentrations at admission were associated with dialysis requirement; only creatinine remained significant ($p = 0.03$, OR 2.1, 95%CI 1.34–2.7) by multivariate analysis. Patients with a complicated course presented higher leukocyte count, hemoglobin and lactic dehydrogenase and lower albumin, sodium, and C3 and C4. In the multivariate analysis, leukocyte count ($p = 0.02$, OR 2.6, 95%CI 1.4–4.3) and C3 concentration ($p = 0.039$, OR 1.7, 95%CI 1.1–2.73) were independently associated with a complicated disease. C3 levels correlated with dialysis length ($r = -0.42$, $p = 0.002$); nevertheless, they were unable to discriminate dialysis requirement (AUC = 0.25, 95%CI 0.11–0.38) and extrarenal complications (AUC = 0.24, 95%CI 0.11–0.4).

Conclusions Our study suggests that decreased C3 levels at admission are associated with a more complicated STEC-HUS episode.

Keywords Hemolytic uremic syndrome · *Escherichia coli* · Shiga toxin · Complement activation · C3

Introduction

Hemolytic uremic syndrome related to Shiga toxin–producing *Escherichia coli* (STEC-HUS) is characterized by the triad of microangiophatic hemolytic anemia, thrombocytopenia, and renal injury [1]. In children, it represents one of the most common etiologies of acute kidney injury; death occurs in 1–5% of patients and around 30% of the survivors demonstrate long-term renal sequelae [1–3].

The primary pathogenetic mechanism of the disease is endothelial damage caused by Shiga toxin (Stx) [4]; however, there is increasing evidence for complement activation as a contributing factor for organ damage [5]. Several decades ago, low plasma C3 concentrations and augmented degradation complement products were noticed in children likely to have had STEC-HUS [6–9]. In 2009, Orth et al. [10] have shown in vitro that high doses of Stx2, far above the concentrations measured in patients [11], induce direct activation of complement alternative pathway (AP), and bind factor H decreasing its activity on the cell surface. In addition, Morigi et al. demonstrated experimentally that Stx triggers complement-dependent microvascular thrombosis [12]. Complement activation was also inferred by the presence of circulating

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microvesicles derived from platelets, monocytes, and red blood cells bearing C3 and C9 in STEC-HUS patients [13, 14]. More recently, C5b-9 deposits were found in the human kidney during the disease and additional studies revealed that Stx induces complement-mediated injury in glomerular endothelial cell and podocyte [15–17].

Despite this substantial amount of findings, only a few clinical studies have correlated complement system activation with clinical course. Furthermore, they presented conflicting results and most of them consisted of small series, or even included patients without microbiological diagnosis [18–24]. Since 2010, we have incorporated serum C3 determination into the initial laboratory profile in patients with STEC-HUS; thus, we aimed to further explore the association between C3 concentrations on admission and acute outcomes in a large cohort of patients with proven STEC infection.

Material and methods

In this retrospective single-center study, we included patients treated at the Hospital General de Niños Pedro de Elizalde between 2010 and 2017 who met the following criteria: (1) diagnosis of HUS with confirmed STEC infection; (2) age under 18 years old; and (3) C3 levels tested at admission. Exclusion criteria were (1) recurrent or family history of hereditary HUS; (2) HUS associated with systemic diseases such as organ transplantation, systemic lupus erythematosus, pneumococcal infection, or HIV infection; and (3) pre-existing renal disease.

We recorded duration of prodromal phase, demographic characteristics (age, gender, body weight and height), and microbiological test results. Blood laboratory determinations performed at admission and analyzed included white blood cell count (WBC), hemoglobin, platelets count, urea, creatinine, C3 and C4 levels, lactic dehydrogenase (LDH), albumin, and sodium. The C3 and the C4 concentrations were measured by nephelometry (IMMAGE Beckman Coulter, Brea, CA, USA) and the normal reference values ranged within 90–180 mg/dl and 10–40 mg/dl, respectively. To assess the severity of the disease, the following data on care needs was collected: blood product administration, the need for and duration of dialysis, treatment with antihypertensive drugs and intensive care unit admission. Extrarenal complications evaluated were severe bowel or central nervous system (CNS) injury, multiple organ failure, cardiac involvement, pancreatic compromise (pancreatitis and/or diabetes mellitus), and/or death. Additionally, clinical notes were examined at 6 months after the acute phase in order to evaluate long-term renal outcomes. All patients were managed by the same nephrology team according to standard recommendations [1].

Definitions

HUS diagnosis was based on the triad composed by thrombocytopenia (< 150,000/mm3), microangiopathic hemolytic anemia (schistocytes in blood smear), and serum creatinine concentration greater than the upper limit of normal for age (at admission or during the course of the disease) with proteinuria and/or hematuria [1, 25]. STEC infection was evidenced by at least one of these three laboratory criteria: screening by polymerase chain reaction/isolation of STEC, detection of free Stx in stools and, in the last years, by the detection of anti-lipopolysaccharide antibodies O157, O145, and O121 [26]. Indications for dialysis were anuria lasting > 24 h, refractory electrolyte abnormalities, and hypervolemia [27]. Antihypertensive drugs were indicated if the child developed high blood pressure according to the Task Force standards [28]. Major CNS involvement was defined as any symptoms involving seizures, focal deficits, and/or coma [29]. Severe bowel injury was referred to the presence of prolonged bloody diarrhea associated with abdominal distension, pain, tenderness, and cramps usually associated with radiologic or ultrasonographic abdominal abnormalities or pathology findings, such as bowel wall necrosis [30]. Multiple organ failure was defined as the concurrent dysfunction of two or more systems and cardiac involvement as the presence of myocarditis, pericarditis, and arrhythmia [31]. Diagnosis of acute pancreatitis required at least two of three criteria: (1) abdominal pain suggestive of or compatible with acute pancreatitis (i.e., abdominal pain of acute onset, especially in the epigastric region, usually with nausea and vomiting), (2) serum amylase and/or lipase activity at least three times greater than the upper limit of normality, and (3) imaging findings compatible with acute pancreatitis [32]. Diabetes mellitus was defined as hyperglycemia on consecutive days treated with insulin [33]. Chronic kidney disease (CKD) was defined as the presence of at least one of the following findings: estimated glomerular filtration rate < 90 mL/min/1.73 m2 (calculated by the Schwartz formula), abnormal proteinuria or microalbuminuria, or hypertension at 6 months of follow-up [34].

Statistical analysis

Continuous variables were non-normally distributed (Shapiro–Wilk test) and therefore, they were expressed as median values (range) while categorical data was presented as absolute figures and percentages. We have analyzed the association between C3 concentrations at admission and disease severity, considering two main outcomes: (1) the need for dialysis and (2) the presence of extrarenal complications, including death. Univariate analysis was performed in order to identify factors associated with each outcome of interest,

using the Wilcoxon test for continuous variables and by chi-square test or Fisher exact test for categorical data. Then, a multivariate logistic regression analysis was performed with those variables previously identified as significant and odds ratio (OR) and 95% confidence intervals (95%CI) were calculated. In addition, the C3 levels were correlated with dialysis length with the Spearman test. Finally we explored by receiver operator curve (ROC) analysis and area under the curve (AUC) determination, with their respective 95%CI, if there was a C3 concentration able to discriminate patients who needed dialysis and who developed a complicated disease form. A p value < 0.05 (two tailed) was considered statistically significant. Data were analyzed using Statistix ver. 7 (IBM version; Analytical Software, Tallahassee, FL) and Medcalc ver. 9.3.2.0 (MedCalc Software, Mariakerke, Belgium).

Results

During the study period, 79 patients were admitted with postdiarrheal HUS, all of them having C3 dosage at admission. None had family or recurrent HUS history or specific causes associated with the disease development. In 49 patients, the STEC infection was evidenced; thus, they constituted the study sample (Fig. 1). The demographic, laboratory, and clinical features of the acute illness of this subgroup were comparable with those with no proven STEC infection and also to the full cohort (data not shown). Two patients were diagnosed with STEC-HUS based on the presence of diarrhea followed by renal injury, microangiopathic hemolytic anemia, and evidence of STEC infection, despite the absence of thrombocytopenia criterion. The clinical courses of these two patients have been extensively described; of note, one of them needed dialysis and their biopsy findings showed signs of thrombotic microangiopathy [35].

Median age of patients was 2.16 years and 24 (49%) were females. Time from first symptoms to STEC-HUS diagnosis was 6 days (1–21); bloody diarrhea was found in 73.4% of cases. By stool culture and/or anti-lipopolysaccharide antibodies, serogroup O157:H7 accounted for 21 cases and O145 and O121 for 3 each. Stx type 2 was positive in 23 children while type 1 was identified in only 1 case.

Patients were grouped according to whether they needed dialysis ($n = 28$) or not ($n = 21$). In univariate analysis, demographics characteristics were comparable; in contrast, hemoglobin, urea, creatinine, LDH, albumin, sodium, and C3 and C4 concentrations were significantly different between both groups. In multivariate analysis, only serum creatinine concentration remained significant (Table 1).

Regarding the treatment received, patients with hypocomplementemia required more acute care needs including red blood cell transfusions, intensive care unit admission, and dialysis requirements (Table 2). They also needed longer periods of dialysis; however, despite having observed a good correlation between the C3 levels and the dialysis length ($r = -0.42$, $p = 0.002$) (Fig. 2), it was not possible to identify an optimal cutoff concentration of C3 (AUC = 0.25, 95%CI 0.11–0.38) able to predict the need for dialysis.

Patients with ($n = 16$) or without ($n = 33$) extrarenal complications were also similar in demographics characteristics. Conversely, the former presented a significantly higher WBC count and hemoglobin value at the time of diagnosis and lower albumin, sodium, and C3 and C4 concentrations. By multivariate analysis, WBC count ($p = 0.02$, OR 2.6, 95%CI 1.4–4.3) and C3 concentrations ($p = 0.039$, OR 1.7, 95%CI 1.1–2.73) were independently associated with a worse STEC-HUS

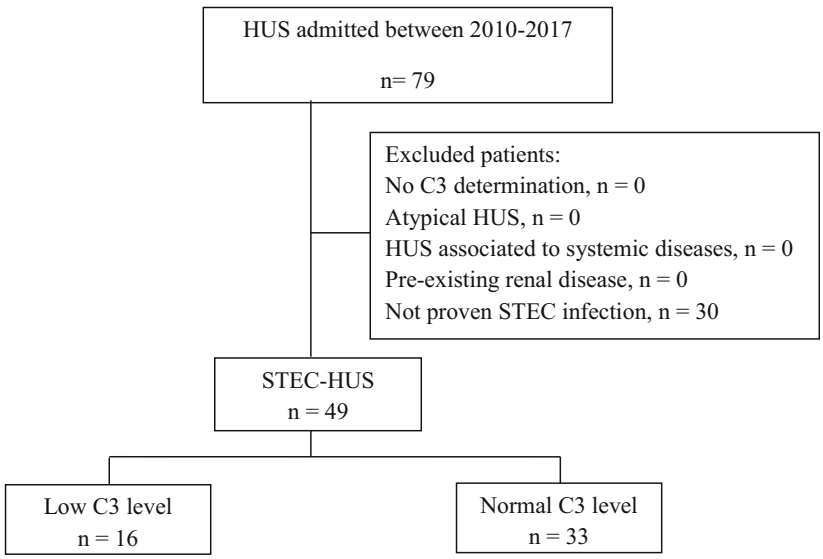

Fig. 1 Flow of patient selection

HUS hemolytic uremic syndrome, *STEC* Shiga-toxin-producing *Escherichia coli*

Table 1 Variables associated with dialysis at disease onset in children with Shiga toxin–related hemolytic uremic syndrome. Univariate and multivariate analysis

Variable	Univariate analysis			Multivariate analysis	
	Dialyzed ($n = 28$)	Non-dialyzed ($n = 21$)	p value	OR (95%CI)	p value
Demographics					
Age (years)	2.12 (0.75–11)	2.25 (0.9–14.5)	0.51	NA	NA
Gender (female/male), n	12/16	12/9	0.32	NA	NA
Weight (k)	12 (8.8–32)	13 (7.1–58)	0.83	NA	NA
Height (cm)	85 (73–140)	87 (70–168)	0.93	NA	NA
Initial laboratory parameters					
WBC (mm^3)	22,250 (8700–55,000)	18,000 (7100–62,700)	0.17	NA	NA
Hemoglobin (g/dL)	9.15 (3.2–16.5)	7.7 (3.7–11.7)	0.04	0.97 (0.85–1.32)	0.7
Platelets (mm^3)	82,500 (21,000–570,000)	53,000 (23,000–143,000)	0.11	NA	NA
Urea (mg/dL)	127 (72–510)	81 (19–278)	0.003	0.93 (0.8–1.07)	0.15
Creatinine (mg/dL)	2.94 (1.29–15)	0.8 (0.28–1.75)	0.00001	2.1 (1.34–2.7)	0.03
LDH (IU/L)	4533 (338–13,889)	2680 (486–14,444)	0.03	1 (0.99–1.01)	0.99
Albumin (mg/dL)	2.31 (1.4–3.83)	3.2 (2.51–4)	0.001	0.5 (0.25–1.19)	0.92
Sodium (mEq/L)	131 (124–145)	138 (122–151)	0.002	1.3 (0.84–2.3)	0.12
C3 (mg/dL)	87 (29–193)	117 (70.4–164)	0.003	0.84 (0.31–2.27)	0.84
C4 (mg/dL) *	19.8 (6–33)	23 (11.3–38)	0.03	1.3 (0.6–3.1)	0.97

Data are given as the median (range) or as frequency of distribution (n). * Data available in 45 patients

OR odds ratio, *CI* confidence interval, *NA* not assessed, *WBC* white blood cell count, *LDH* lactate dehydrogenase

episode (Table 3). In detail, those with decreased C3 concentrations were more likely to have extrarenal complications, including multiple organ failure and severe CNS and intestinal injury. Patients with cardiac ($n = 1$; C3 76 mg/dl), pancreatic (n = 1, C3 68 mg/dl), or who died ($n = 2$, C3 81 mg/dl and 29 mg/dl each) also had reduced C3. However, we did not find a difference between groups which could be related to the sample size, given the rare frequency of these complications. Moreover, they did indeed show CKD more frequently at 6 months of follow-up (Table 2). We have additionally explored the ability of C3 levels to determine a complicated form of the disease, but the AUC was very low (AUC = 0.24, 95%CI 0.11–0.4). Finally, 4 out of 45 children whose C4 levels were measured showed reduced values (all of them had low C3 concentrations also); they had severe extrarenal complications (3 required intensive care and 1 of them died).

Survivor patients normalized the C3 and C4 levels within 1 month after disease onset, except for a girl with persistent low C3 levels for 3 months. In this particular case, thrombotic thrombocytopenic purpura was excluded with a normal ADAMTS 13 activity (104%, normal range: 40–130%), and screening for mutations in the genes encoding complement regulatory proteins (CFH, CFI, CFB, C3, MCP, CFHR 1–5, DGKE, and THBD) and testing for anti-CFH antibodies were negative.

Discussion

Despite the increasing understanding of the role of complement in STEC-HUS physiopathology, clinical data on whether complement activation can influence the course of illness are still scarce. Moreover, a closer analysis of major pediatric studies linking complement activation and disease evolution shows conflicting results (Table 4) [18–24]. While some of them reported a relationship between elevated activity of complement AP and poor prognosis, other groups either failed in demonstrating such association, showed mixed results (i.e., complement activation predicted dialysis but not CNS injury), or just observed trends towards more severe disease but without reaching statistically significant differences [18–24]. In the last cases, the small samples analyzed may be responsible for those inconclusive results. It should also be pointed out that in most of the abovementioned studies, biological confirmation of STEC infection was not required to enter in the investigation.

The main result of our study is that decreased C3 concentrations were significantly associated with dialysis requirement and extrarenal complications. Remarkably, hypocomplementemia was an independent determinant of a worse STEC-HUS episode. This finding supports that the clinical outcome depends not only of the direct effects of Stx

Table 2 Care needs and complications during the acute phase of 49 children with Shiga toxin–related hemolytic uremic syndrome according to C3 levels

	All ($n = 49$)	Normal C3 levels ($n = 33$)	Low C3 levels ($n = 16$)	p
Care needs during the acute phase				
RBC transfusions	2 (0–10)	1 (0–6)	3 (0–10)	0.01
Platelets transfusions, *n* (%)	4 (8.1)	3 (9)	1 (6.2)	1.00
Need for dialysis, *n (%)*	28 (57.1)	13 (39.4)	15 (93.8)	0.0005
Days of dialysis	10 (2–34)	7 (2–7)	10 (4–28)	0.04
Antihypertensive medication, *n* (%)	8 (16.3)	4 (12.1)	4 (25)	0.41
ICU admission, *n* (%)	7 (14.2)	–	7 (43.7)	0.0001
Complications				
Number of patients with complications	16 (32.6)	5 (15.1)	11 (68.7)	0.0003
Major CNS involvement, *n* (%)	12 (24.5)	3 (9)	9 (56.2)	0.0007
Severe bowel injury, *n* (%)	8 (16.3)	2 (6)	6 (37.5)	0.01
Multiple organ failure, *n* (%)	4 (8.1)	–	4 (25)	0.008
Cardiac compromise, *n* (%)	1 (2)	–	1 (6.25)	0.32
Pancreatic compromise, *n* (%)	1 (2)	–	1 (6.25)	0.32
Death, *n* (%)	2 (4)	–	2 (12.5)	0.1
Renal outcomes at 6 months*				
No CKD#	26	23	3	0.0005
CKD#	13	4	9	

Data are given as the median (range) or as frequency of distribution (%)

Low C3 levels < 90 mg/dl

RBC red blood cell, *ICU* intensive care unit, *CNS* central nervous system

* Data available in 39 out of 47 survivors of the acute phase of the disease

CKD (chronic kidney disease) according to Kidney Disease Improving Global Outcomes Guidelines 2012[34]

but also on secondary effects induced by an activated complement system [5, 21]. Consistently, the subset of patients with low C3 levels presented a complicated course and subsequently required more care needs. Similarly to C3 concentrations, multivariate analysis also identified leukocytosis independently associated with a poor prognosis, as already known [36, 37]. Moreover, early work by Robson et al. found an inverse significant correlation between white blood cell count and C3 levels [18]. In univariate analysis, patients with a complicated course exhibited a greater hemoglobin concentration, a surrogate marker of dehydration, a finding repeatedly associated with adverse outcomes [25, 38, 39]. Likewise, they had lower serum sodium levels, a predictor of death recently identified in a multicentric study involving 466 children [26]. The presence of hypoalbuminemia is a common finding in STEC-HUS [40]; nevertheless, in complicated patients, it may also reflect hypercatabolism. Regarding dialysis requirement, dialyzed patients presented lower C3 concentrations compared with those non-dialyzed. However, by multivariate analysis, only creatinine values were independently associated with dialysis. In addition, patients with hypocomplementemia needed dialysis for longer periods. Consistent with this observation, Ferraris and coworkers found higher levels of sC5b-9 and Bb levels in patients presenting with oligoanuira when compared with those non-oliguric [20]. In the same line, in a preliminary study, Karnisova et al. recently reported a significant correlation between the initial C3 concentration and the duration of renal replacement therapy [23], a finding corroborated in our series (Fig. 1). Interestingly, they also identified that patients with C3 < 82.5 mg/dl were more likely to need renal replacement therapy and to have extrarenal complications [23]. In contrast, as also noted by Thurman et al. [19], among our patients, no cutoff value for C3 could be defined in order to discriminate which would require dialysis or suffer complications. Finally, patients with low C3 levels were more likely to develop CKD after 6 months of follow-up; an expected finding since they required longer dialysis, which is the main predictor of renal sequelae [41].

Reduced concentrations of C3 levels were transient returning to normal during convalescent phase, as noted by other authors [19, 20]. It is interesting to stand out that hyperactivation of complement was not found in all subjects. In fact in our patients, this accounts for 32.6%. Earlier studies revealed C3 consumption in half of the patients [8, 9], contrasting with more recent reports, where the percentage was lower. Robson and Alesteil Grunow found a rate of 19% each [18,

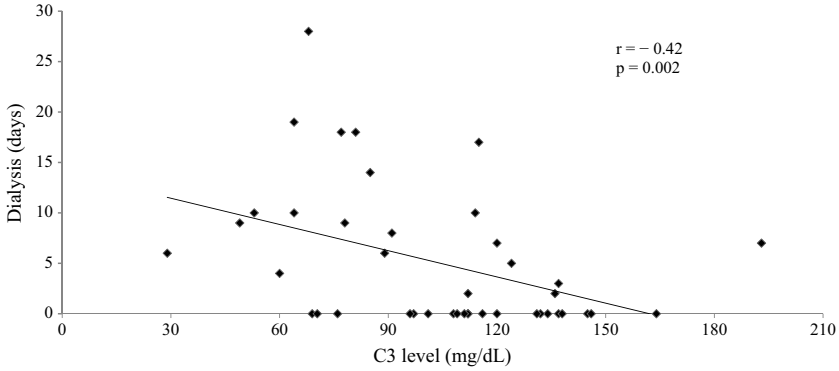

Fig. 2 Correlation between C3 levels and dialysis duration in children with Shiga toxin–related hemolytic uremic syndrome

21], while others reported a consumption of 28% [22]. In addition, Westra et al. noted decreased C3 levels in 6 (5 slightly and one markedly) out of 22 patients with STEC-HUS (26%), but the median concentration of the full cohort was not significantly different from those of healthy pediatric age-matched controls [42]. Otherwise, Ferraris et al. found elevated C3 levels as well as Frémeaux-Bacchi, the latter also comparing with healthy controls [20, 24]. Here it is worth recognizing that our study lacks data on the complement values provided by a control group. It must also be mentioned that in studies where the breakdown products were assayed, markers of AP activation were seen in a higher proportion of patients [19, 20]. Furthermore, AP activation was detected even when the C3 levels were normal [7, 21, 22].

We additionally noted low concentrations of C4 together with reduced C3 values, in 4 out of 45 patients in whom it was measured. Although decreased C4 levels have been occasionally documented in some patients [9, 21], its significance remains unclear given that currently there is no evidence of activation of classical and/or lectin pathways in STEC-HUS [5, 12, 20]. Alternatively, as these 4 patients were severely affected and C4 is activated in response to infection [43], it could be speculated that they had a greater infection load than others.

In recent years, mutation in the complement genes responsible for atypical HUS has also been detected in STEC-HUS patients. However, the role of these abnormalities has not been completely elucidated yet [21, 42]. Ahlenstiel-Grunow

Table 3 Variables associated with extrarenal complications at disease onset in children with Shiga toxin–related hemolytic uremic syndrome. Univariate and multivariate analysis

Variable	Univariate analysis			Multivariate analysis	
	Extrarenal complications ($n = 16$)	No extrarenal complications ($n = 33$)	p value	OR (95%CI)	p value
Demographics					
Age (years)	2.16 (0.75–14.5)	2.16 (0.75–11.3)	0.97	NA	NA
Gender (female/male), n	7/9	17/16	0.61	NA	NA
Weight (k)	12 (7.1–32)	13 (7.6–58)	0.41	NA	NA
Height (cm)	85 (71–105.1)	87 (70–168)	0.34	NA	NA
Initial laboratory parameters					
WBC (mm^3)	33,750 (14,200–62,700)	15,000 (7100–51,000)	0.00001	2.6 (1.4–4.3)	0.02
Hemoglobin (g/dL)	9.25 (3.7–16.5)	8 (3.2–11.7)	0.04	1.25 (0.8–1.97)	0.32
Platelets (mm^3)	62,700 (21,000–122,000)	71,000 (23,000–570,000)	0.44	NA	NA
Urea (mg/dL)	116 (19–478)	109 (32–510)	0.95	NA	NA
Creatinine (mg/dL)	1.93 (0.7–6.79)	1.5 (0.28–15)	0.5	NA	NA
LDH (IU/L)	4414 (1169–13,889)	3105 (338–14,444)	0.52	NA	NA
Albumin (mg/dL)	2.2 (1.4–3.98)	3.2 (1.96–4)	0.0005	0.57 (0.3–6.7)	0.65
Sodium (mEq/L)	130.5 (124–143)	137 (122–151)	0.03	0.97 (0.93–1)	0.69
C3 (mg/dL)	79.5 (29–137)	117 (60–193)	0.0003	1.7 (1.1–2.73)	0.039
C4 (mg/dL)*	16.2 (6–33)	25 (7–38)	0.003	0.9 (0.79–1.23)	0.91

Data are given as the median (range) or as frequency of distribution (n). * Data available in 45 patients

OR odds ratio, *CI* confidence interval, *NA* not assessed, *WBC* white blood cell count, *LDH* lactate dehydrogenase

Table 4 Major studies correlating complement activation in postdiarrheal hemolytic uremic syndrome with acute phase severity

Reference	Year	Patients with proven STEC infection	Correlation between complement findings and acute disease outcomes
Robson et al. [18]	1992	–/68	Cases with ↓ C3 ($n = 13$) tended to a higher need for and a longer duration of dialysis, hemorrhagic colitis and CNS complications.
Thurman et al. [19]	2009	–/17	↑ Bb and ↑ sC5b–9 in all patients, levels no related to disease severity
Ferraris et al. [20]	2015	18/18	↑ Bb and ↑ sC5b–9 in all patients (higher in oliguric ones). No differences between those with or without severe CNS injury. ↑C3c in oliguric and non-oliguric patients. ↑C3 and ↑C4 in non-oliguric patients. Normal C4d levels.
Ahlenstiel-Grunow et al. [21]	2016	24/25	↑ sC5b–9 (n = 16), ↑C3d ($n = 7$). In 21 cases C3 and C4 were measured: ↓ C3 (n = 4), ↓C4 ($n = 5$). ↑ sC5b–9 related with hypertension and edema. No differences between need for and duration of dialysis, CNS involvement and death.
Ağbaş et al. [22]	2018	10/32	↓ C3 ($n = 9$) tended to more extrarenal complications.
Karnisova et al. [23]	2018	23/33	↓ C3 ($n = 18$) related to a higher need for dialysis and extrarrenal complications. Initial C3 levels correlated with duration of dialysis.
Frémeaux-Bacchi et al.[24]	2019	75/108	Median levels of C3 and sC5b-9 were higher and membrane cofactor protein expression decreased compared with controls. ↑ sC5b-9 in 66% of patients with STEC hemolytic uremic syndrome; not correlated with dialysis requirements or CNS involvement or chronic kidney disease.
Present study	2019	49/49	↓ C3 ($n = 16$) related to a higher need for dialysis and extrarrenal complications. Initial C3 levels correlated with duration of dialysis. C 4 levels measured in 45 patients: ↓ C4 (n = 4); all of them had also ↓C3.

STEC Shiga toxin–producing *Escherichia coli*, *CNS* central nervous system

– Not reported

identified a mutation in 3/25 (12%) and Westra in 7/25 (28%) of children with STEC-HUS [21, 42]. In a larger study, Frémeaux-Bacchi identified a rare variant in 12 out of 75 (16%) pediatric Stx-positive patients with HUS, but only 5% of these patients carried a pathogenic variant that is known to impair the complement regulatory activity [24]. In addition, the genetic background did not significantly affect the severity of the acute phase and incident CKD during follow-up [24]. Currently, genetic screening does not seem justified for all patients with postdiarrheal HUS, but should be considered among those who progressed rapidly to end-stage kidney disease or had a relapsing episode or family story of HUS [24]. In our series, none of the patients presented recurrent or familial forms of the disease. Moreover, in a girl with late normalization of C3 level mutation, screening for atypical HUS resulted negative.

So far, treatment of the disease is mainly symptomatic. The involvement of complement AP provided the rationale for terminal complement blockade by eculizumab in this form of thrombotic microangiopathy [2]. This monoclonal antibody effectively blocks cleavage of complement factor C5 inhibiting the formation of the terminal complement complex (C5b-9) [1]. However, as data regarding its efficacy arose from uncontrolled studies with conflicting results, prospective studies are awaited to define whether it might be a therapeutic option [2, 44, 45].

Although our study represents one of the larger series of children with STEC-HUS diagnosed microbiologically that investigate the association between C3 concentrations and disease outcomes, some limitations deserve consideration. The association noted here did not imply causality. In fact, patients with decreased C3 also presented higher hemoglobin reflecting dehydration [25, 38, 39], which could be responsible for the worse disease course. Nevertheless, C3 remained associated with a severe evolution even after the multivariate analysis. Moreover, C3 consumption may indicate complement hyperactivation, which could have biologic plausibility as a contributing factor for such clinical evolution [10, 12–17]. Also, as we have measured C3 level rather than complement pathway fragments, it is possible that complement system hyperactivity has not been noticed in some patients. Furthermore, C3 dosages along the disease trajectory were not performed. However, the data presented here could be useful since the C3 determination is readily available in most laboratories facilitating its clinical use.

Conclusion

Our data suggests that children with STEC-HUS with decreased C3 concentrations at admission are more likely to need dialysis and are at increased risk of having serious extrarenal complications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics The study was performed in accordance with the ethical standards of the institutional research and ethics committee (IRB 186/18).

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