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A Pediatric Neurologic Assessment Score may drive the Eculizumab-based treatment of Escherichia Coli-related Hemolitic Uremic Syndrome with neurological involvement

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Abstract

Thrombotic Microangiopathy (TMA) is a clinical syndrome encompassing a large group of rare but severe disorders including Thrombotic Thrombocytopenic Purpura (TTP) and both typical and atypical forms of Hemolytic Uremic Syndrome (HUS). The key role of the Complement System iswell known in TTP and atypical HUS, but recent reports describe its involvement in the pathogenesis of HUS secondary to gastrointestinal infections due to Shiga toxin-producing Escherichia coli (STEC).

TMA mainly affects the kidney, but extra-renal complications are frequently described. The involvement of the central nervous system (CNS) represents often a life-threatening condition and it can result in serious long-term disability in HUS-patients who overcome the acute phase of illness.

In the present study we retrospectively analyzed a pediatric cohort of a single tertiary pediatric hospital in Southern Italy, in which this complication occurred in 12/54 children (22% of cases), of whom 5 with severe neurological involvement had been successfully treated with Eculizumab. The great clinical variability of brain injury in our cohort has led us to retrospectively build a "neurological score" useful to assess the clinical severity of neurologic involvement. Subjects with higher neurologic score due to the most severe CNS involvement ~~having a high neurologic score were~~ resulted in the group of patients early treated with Eculizumab, obtaining a good clinical response (4 out 5 patients). In conclusion the early treatment with Eculizumab in children with severe neurological involvement during STEC-HUS was associated with complete regression of both Acute Kidney Injury (AKI) and neurological lesions observed at Magnetic Resonance Imaging (MRI). A "neurological score" may be an useful tool to drive the early treatment of CNS complications in STEC-HUS with Eculizumab, although future perspective controlled studies are urgently needed to validate this therapeutic approach.

Key-words

Hemolytic uremic syndrome – Neurological involvement – Eculizumab – Magnetic resonance imaging

Introduction

The triad of hemolytic anemia with erythrocyte fragmentation, thrombocytopenia, and organ damage represents the clinical expression of Thrombotic Micro-Angiopathy (TMA), a clinical syndrome encompassing the Thrombotic Thrombocytopenic Purpura (TTP) and both typical and atypical forms of Hemolytic Uremic Syndrome (HUS)[1].

Unlike the TPP and the atypical HUS (aHUS), in the typical HUS secondary to gastrointestinal infections due to Shiga toxin-producing Escherichia coli (STEC-HUS) the absence of specific therapy enforces the clinicians to quickly establish the best supportive care [2] with the aim to reduce STEC-HUS related mortality and morbidity. The achievement of an organizational model is crucial to allow early detection of hemorrhagic gastroenteritis potentially due to STEC [3,4].

The best supportive care for STEC-HUS includes both the renal replacement therapy (RRT), when needed, and the blood transfusions, which are used in 80% of STEC-HUS patients with severe anemia. Infusion of platelets is not indicated due to the higher risk of *de novo* thrombosis, with the exception of severe hemorrhages or before a surgical procedure [5].

A debated issue for a long time is the use of antibiotics [6]: several authors [7,8] suggest that most antibiotics (except fosfomycin),might induce massive bacterial lysis with release of large amounts of Shiga Toxins and severe worsening HUS [9].

Plasma exchange (PEX) and plasma infusion (PI) have been recognized as best treatment for severe HUS for several years [10-13]. Nowadays, recent reports do not confirm the efficacy of these therapies in STEC-HUS [14,15]. Nevertheless, PEX and PI are actually the best "rescue therapy" for neurological HUS [16]. Neurological injury occurs in 20-25% of STEC-HUS patients, can be sudden and severe and is the most frequent cause of acute mortality in this group of patients [17,18]. This complication is caused by body fluids and electrolytes disorders secondary to AKI (electrolytic alterations, uremia, arterial hypertension) and by thrombotic microangiopathy of the cerebral microcirculation [19].

With the establishment of a pathogenic role for the complement system in the onset of STEC-HUS [20] the use of “off label” treatment of the severe forms of STEC-HUS with Eculizumab (even in

presence of neurological involvement [NI]) has been added to therapeutic regimens [21]. Eculizumab is an anti-C5-convertase monoclonal antibody, licensed in Italy for the therapy of atypical forms of HUS (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH).

In the present work we propose a Pediatric Neurologic Assessment Score for HUS patients (PNAS-HUS) and analyze the efficacy and the safety of Eculizumab treatment in a pediatric cohort of STEC-HUS patients with severe NI.

Subjects and Methods

Patients

Between January 2006 and September 2016, 55 children (24 males, 31 females) (mean age 32 months, min 7-max 155) were admitted to the Pediatric Nephrology and Dialysis Unit of the Pediatric Hospital "Giovanni XXIII" in Bari with a diagnosis of STEC-HUS.

The main clinical and laboratory parameters for the diagnosis of HUS (hemoglobin, erythrocytes count, platelet count, serum creatinine, azotemia, LDH, aptoglobin, total direct and indirect bilirubin) were collected and recorded in 54/55 children; an 18 month old boy died on the first day of hospitalization for severe gastrointestinal hemorrhage and was excluded from further analysis.

In 12 cases (22.2%), clinical manifestations of CNS involvement were recorded. The mean age of children with NI (6 males and 6 females) was 25 months, ranging between 7 and 53 months. Five out of 12 showed severe NI and were treated with Eculizumab, after their parents signed informed consent for "off-label" use of this drug.

We carried out a retrospective study based on registry data and no ethics approval was required. Nevertheless, all the children's parents signed a written informed consent to collect their clinical data at time of hospital access.

Methods

The diagnosis of typical HUS was suspected in the presence of clinical signs of active thrombotic micro-angiopathy (low platelets count, hemolysis, and kidney damage). Diagnosis was confirmed by specific diagnostic assays: a) Stx free fecal examination detection by the Vero cells assay; b) Isolation of Shiga toxin-producing Escherichia coli (STEC) with serological typing and PCR detection of the genes coding for virulence factors *vtx1, vtx2,eae*; c) specific anti-lipopolysaccharide serum antibodies (LPS) against the major STEC serotypes mainly related to typical HUS (O26, O157, O103, O111 and O145) [22].

Among patients with STEC-HUS, neurological involvement (NI) was diagnosed in presence of one or more of the following clinical signs at the onset or during the acute phase of the disease:

alteration of consciousness, confusional state, disorders of communication skills, strabismus/eye fixing, nystagmus, amaurosis, seizures, hyporeactivity, disorders of muscle tone (hypo/hypertone) and neurovegetative system (heart rate, hypo~~/hyper~~tension, respiratory rate alterations).

NI was analyzed with clinical neurological evaluation, electrophysiological investigations (EEGs) and, when needed for severe neurologic sign, with neuro-imaging techniques (Computer Tomography or Magnetic Resonance of the brain).

Based on neurologic clinical signs recorded during hospitalization and on number and type of instrumental investigations, a Pediatric Neurologic Assessment Score for HUS patients (PNAS-HUS) was retrospectively developed. In detail, a variable score from 0 (absence of the symptom) to 3 (maximum symptom expression) was assigned to each of the clinical signs considered. Values 1 and 2 were attributed respectively to mild and mild-to-severe symptoms or duration. Due to great clinical variability of brain injury, the more serious episode for each clinical sign was considered for the score assignment.

A further score of 1 for each neuro-radiological examination (MRI or CT scan of the brain) and 0.5 for each EEG were added to the obtained value, regardless of pathological findings severity (for details see Supplementary Table 1~~Materials~~). The final score was assessed by two independent observers based on available clinical data with a negligible inter-observer variation.

One or more EEG tests, based on the clinical course, were performed in all 12 children at our Pediatric Hospital using 24 channels electroencephalograph (EEG 1200, Nikon Kohden, Tokyo, Japan) equipped with photo stimulator with Xenon lamp. All the tests were performed with 24 channels (12 for brain electrical activity) and the recorded data were analyzed with sampling frequencies up to 10000 Hz.

The CT scan of the brain was performed at in Radio-diagnostic Unit of our Hospital in 8/12 children with a multi-slice General Electric "Lightspeed" Computerized Tomograph, 64-layer high resolution (GE Healthcare, Little Chalfont, UK).

The MRI of the brain was performed in 11/12 patients at the Neuro-Radiology Unit of our Hospital with a General Electrics "SIGNAMR 1, 5 ES Excite" (GE Healthcare) and was completed by

spectroscopy analysis. All the patients received anesthesia for the procedure because of critical conditions and of risk of movement.

Treatment with Eculizumab

After counseling and informed consent procedures, Eculizumab was administered in five children, all treated after 2011. They received two doses of 300 mg of Eculizumab at day 1 and day 8 together with transient antibiotic coverage and meningococcal vaccination.

Statistical analysis

The statistical analysis was performed with SPSS Statistics Software (SPSS 17.0 Inc., Evanstone, USA). Normality of variable distribution was tested using Kolmogorov-Smirnov test. Comparison of variables between the different groups was obtained with Student's *t*-test and Mann-Whitney *U*-test owing to normal or non-parametric distribution. Frequencies were compared among groups by X^2-test.

Pediatric Neurologic Assessment Score for HUS patients (PNAS-HUS), obtained as described before, was converted in percentile rank and patients affected by neurologic STEC-HUS were divided in two groups, according to a total score above or below ~~with a total score above~~ the 50th percentile ~~were considered eligible for treatment with Eculizumab~~.

A *p*-value of ≤0.05 was considered statistically significant. Results are expressed in the text as mean and as [minimum-maximum] range, unless otherwise stated.

Results

Between January 2006 and September 2016, 55 children affected by STEC-HUS were treated at our Hospital. Frequency analysis showed an average of about 5 cases per years (Figure 1). Noteworthy, an epidemic cluster was recorded in 2013, with a total of 20 cases, 17 of which were treated in our Unit[22].

Based on clinical and laboratory data, the diagnosis of STEC-HUS was confirmed by the positivity of at least one of the three specific test. Shiga Toxin (Stx) free fecal examination, performed at laboratory of Hygiene and Public Health Unit of University of Bari, was positive in 42 specimens (77.8%).

Isolation of Stx-producing Escherichia coli (STEC) was obtained only in 32 stool specimens from 32 patients (59.3%), which were sent to laboratory of National Reference Laboratory for E.Coli in Rome. All the STEC isolated from HUS cases belonged to the “top-five” STEC serogroups (O157, O26, O111, O145, O103). The STEC serotype most commonly associated with the diagnosis of HUS in our cohort was O26 (53%), and was also present in community-wide outbreak in august-september 2013 [22]. Others serotypes as O145 (18%), O111 (15%), O153 and O107 (both 7%), were less frequently involved. Noteworthy, no differences in main baseline clinical and laboratory data were observed in sporadic STEC-HUS patients as compared with epidemic cases treated during community-wide outbreak in august-september 2013 [22].

Among the patients enrolled in the study, regarding renal function, twenty-nine of our patients (53.7%) required RRT, while supportive medical therapy was successfully administered in the remaining 25 patients (46.3%).

After recovery from HUS, all the patients were analyzed in a follow-up ranging from 1 to 60 months. Among the entire study group, 37 patients (68.5%) gained complete clinical remission without apparent renal sequelae to date, while in thirteen patients (24.2%) and in three patients (5.5%) were non-nephrotic proteinuria and glomerular micro-hematuria respectively. Only one STEC-HUS patient (1.8%) developed chronic renal failure requiring a dialysis-transplant program.

The involvement of the central nervous system (CNS) was present in 12 children (22.2%). These patients showed higher levels of serum creatinine (3.5 [3.0-5.4] vs. 1.3 [0.6-1.8] mg/dL, $p<0.001$), serum urea (176.8 [22.8-225.8] vs. 99.9 [58.2-175.4] mg/dL, $p<0.002$), leucocytes count (17.8 [13.6-21.3] vs. 11.0 [8.2-12.6] $x10^3$/µL,$p<0.001$) and C-reactive protein [19.7 [6.7-30.0] vs. 1.1 [0.5-3.5] mg/dL, $p<0.002$], while serum levels of sodium were lower (129 [range 124-132] vs. 134 [range 130-140] mEq/L, $p<0.005$), as compared to STEC-HUS patients without CNS involvement. No other parameters showed statistically significant differences [Table 1].

The spectrum of neurological symptoms was extremely variable in severity and type. The neurological signs and symptoms recorded in the 12 STEC-HUS patients with CNS involvement are detailed in Table 2, as well as the serotype of E. Coli responsible of STEC-HUS.

All the signs and symptoms that compose a Pediatric Neurologic Assessment Score for HUS patients (PNAS-HUS) were assessed retrospectively in 12 STEC-HUS patients with CNS involvement. A median score of 9 arbitrary unit allow us to divide all the enrolled patients respectively to lower score group (<9) or to higher score group (>9), depending on severity of neurological signs and symptoms (see Supplemetary Figure 1). The group with lower neurologic score encompassed seven patients with mild neurological manifestations, while five patients with severe NI, requiring treatment in Pediatric Intensive Care Unit, were included in the group with higher neurologic score.

As shown in Table 2, alterations of consciousness were reported in all the patients, although of variable severity, while recurrent seizures affected 58% of children. Ocular alterations were variably reported (stabismus or eye fixing in 10 cases, nistagmus in 3 case, visus abnormalities in 2 cases), while muscle tone evaluation showed alterations in 5 cases. Language disorders were observed only in 4 case, while incidence of neurovegetative disorders (heart rate alterations, hypotension, respiratory rate alterations) accounted for 25% of cases (3 children).

The children with higher neurologic score (n=5)~~, all observed after 2011,~~ were treated with Eculizumab due to severity of CNS involvement. In three of them, PEX was the first line therapy without clinical results, while the last two were treated at the beginning with Eculizumab. This therapeutic approach resulted in 80% of successful clinical response (4/5) with both regression

neurological lesions and normalization of laboratory findings (Hemoglobin, platelets count, LDH, serum creatinine, C3 levels) suggestive of active TMA (Figure 2-3-4).

CNS examination with MRI in most cases of neurologic HUS showed local acute lesions at the basal ganglia and at white matter, mainly in semioval centers, as shown in diffusion weighted images (Figure 5A). The hypoxic lesions of basal ganglia and necrotic-haemorrhagic features of putamen and body of the caudate nucleus were combined with large ischemic vascular lesions of brain cortex (Figure 5A). After Eculizumab treatment, a regression of these lesions and the disappearance of neurologic signs and symptoms were observed (Figure 5B) except the first case. In this child, despite Eculizumab treatment, the local acute lesions at the basal ganglia evolved at second NMR in necrotic-haemorrhagic lesion of putamen.

In the seven case with mild neurologic involvement, the supportive care resulted in complete clinical remission with normalization of main laboratory parameters.

Discussion

The most severe and frequent extra-renal manifestation of HUS is neurological involvement (NI), whose incidence is not well established, despite it represents the main HUS-related cause of death and morbidity. The incidence of STEC-HUS related NI in children is variable, ranging from 3% (French Registry of Epidemiological Surveillance of the HUS) to 69% (Germany 2011) [24-25], the latter probably related to the strong virulence of the STEC serotype (O104) responsible for the epidemic episode. The spectrum of neurologic signs may be also wide, from headache and confusion to language, visual and behavioral disorders to convulsions and coma [25]. The variable clinical criteria to define NI during STEC-HUS may be a further confounder for diagnosis.

A recent report describes a NI incidence of 17% in a single center case study ~~of 46 patients observed along a follow-up of 10 years~~; 8/46 patients presented signs of NI as convulsions, hemiparesis or consciousness disorders [17]. This incidence rate appears closer to the data reported in our cohort study in which NI encompassing a wide spectrum of neurological symptoms involved 12 out 54 children with an incidence of 22.2%.

The etiopathogenetic mechanism for NI onset seems to be due to TMA related hypoxic-ischemic phenomena in association with uremia, electrolyte abnormalities and high blood pressure [19]. Although this typical secondary lesion of thrombotic microangiopathy was not observed in the human cerebral endothelium [26,27], in experimental models (i.e. rabbit) ~~it has been shown that~~ Stx directly induced brain ischemic lesions due to arterial necrosis and endothelial damage [23,28]. In addition, evidence of increased levels of TNF1, TNF-R1 and TMP-1 seemed to play a crucial role in localized inflammatory phenomena in NI~~related to onset of NI in STEC-HUS~~. [29]. Some authors suggested that both TNF-alpha and interleukin-1 (IL-1), either separately and in combination, strengthened the Stx1-mediated inhibition of protein synthesis in endothelial cells of the cerebral microcirculation and also induced the expression of enzymes involved in the synthesis of GB3, the receptor of Stx [30]. Higher levels of Caspase-3, a pro-apoptotic enzyme able to induce DNA fragmentation, were induced after incubation of endothelial cells with TNF-alpha and before exposure to Stx [31]. Taken together, these data underline the crucial role of pro-

inflammatory cytokines in the onset of Caspase-3-related apoptotic phenomena in the brain endothelium during STEC-HUS. Moreover detection of leukocytosis or high plasma levels of C reactive protein (CRP) have been related to severe neurologic damage during STEC-HUS [24]. In our cohort both leukocyte count and CRP were significantly higher at hospital admission in patients affected by STEC-HUS with neurologic involvement, as compared with the other group.

Patients with severe NI~~neurologic involvement~~ showed significantly lower serum levels of sodium, as compared with the group with mild clinical phenotype (Table 2).This data may be confounder due to fluid resuscitation required by supportive care of STEC-HUS [32], although it may exasperate the brain damage thorough several mechanisms [33-36].

Due to the lack of specific therapy, correction of electrolyte disorders and hydration status are major issues in the best supportive care of patients with STEC-HUS [2]. In detail, when diuresis is preserved, hyperhydration increases capillary blood flow and reduces the risk of capillary thrombosis and consequent ischemia with better short and long term outcome [3-4]. This observation underlines the importance of early diagnosis of HUS and the achievement of an organizational model which allows an early detection of hemorrhagic gastroenteritis potentially due to STEC in pediatric patients [37]. The onset of oligo-anuria during hemorrhagic gastroenteritis imposes fluid restriction to prevent fluid overload. In our cohort 53.7% of patients required renal replacement therapy for AKI onset and fluid overload while blood transfusion was~~is~~ required in almost 80% of STEC-HUS patients~~, due to severe anemia, while platelets transfusion is generally contraindicated because of the risk of more severe thrombotic microangiopathy, with exception of severe hemorrhage or when surgical procedures are required~~ [5]. Treatment of STEC-HUS has been a matter of debate for a long time and plasma exchange (PEX) and plasma infusion (PI) have been used as gold standard therapy for this disease, as for atypical HUS [10,11]. Recently the efficacy of these therapies has been discussed in treatment of STEC-HUS, while PEX remains the preferred rescue therapy for severe forms of neurologic HUS [12,13,16].

The activation of complement system, as established in atypical HUS, has been observed also in STEC-HUS, due to toxin effects of Stx on the endothelial cells and consequent activation of alternative pathway of complement system. Moreover Stx is able to directly activate the alternative

pathway of complement system, but also to inhibit complement regulatory proteins (CFH, CD59, thrombomodulin), mimicking loss of function mutations observed in atypical HUS [35]. The knowledge of the complement system role in the onset of this form of TMA has implied a potential therapeutic role for Eculizumab, an anti-C5-convertase monoclonal antibody, for the treatment of severe forms of STEC-HUS [38,39,40,41]. This therapeutic approach is not considered as first-line therapy in STEC-HUS pediatric patients, because of milder clinical phenotype and lower mortality as compared to adults, although the HUS Italian Registry reports a raw mortality of 2.8% [43], a potentially disabling CNS involvement in 20% of cases and a worse renal outcome (End Stage Renal Disease) in 3.4% [44].

Moreover supportive neurologic care and intensive extracorporeal blood purification (plasma exchange, selective immunoabsorption) have provided limited advantages for treatment of neurologic involvement during STEC-HUS [40, 45-48].

In 2011, it was reported the first use of Eculizumab for treatment of three pediatric cases of STEC-HUS-related encephalopathy. All the patients showed serological signs of complement activation (low C3 levels and high C3d levels) and were refractory to PEX treatment. After 24h of drug administration, a good clinical and laboratory response was observed within 5 days [21]. Further studies have described the efficacy and safety of early infusion of Eculizumab in children with neurologic STEC-HUS. In detail early administration of Eculizumab after appearance of CNS involvement gained complete clinical remission and absence of neurologic sequelae, unlike what was observed with only supportive therapy [25,49,50]. However, Eculizumab treatment of STEC-HUS patients during Germany epidemic in 2011 did not reach similar efficacy, because of late administration after prolonged PEX treatment [2].

In our cohort, Eculizumab was administered in five STEC-HUS patients with severe NI. A good clinical and serological response was gained in 80% of patients (n=4), ~~without infusion-related side effects and~~ with normalization of laboratory findings (Figure 3, 4) and complete regression of neurologic signs and lesions, as assessed by EEG test and NMR imaging (Figure5). Only one patient with higher PNAS-HUS showed persistent neurologic sequelae and evolved to end stage renal disease requiring hemodialysis. It is conceivable that lack of response in this case was due to

late administration of Eculizumab (>72h) after prolonged PEX treatment. The remaining patients were treated within 24-48h the onset of CNS signs, thus supporting the hypothesis that anti-C5-convertase inhibitor is more effective in treatment of severe forms of STEC-HUS if early administered. Of note the more severe forms of STEC-HUS with NI were observed in the last decade, probably to increased virulence of isolated STEC stains [22].

~~Interestingly~~In our study, the pediatric neurologic score, applied retrospectively in STEC-HUS patients, showed that those with severe clinical sign (PNAS-HUS >9 arbitrary units) had needed the treatment with Eculizumab, while in those with milder phenotype (PNAS-HUS <9 arbitrary units) supportive care had been resolutive.

Small number of patients, single center experience and absence of a control group (alternative treatment in severe cases of NI) are certainly limitations of the present study. However the proposed pediatric neurologic score may help to differentiate STEC-HUS patients with severe or mild neurologic involvement and, finally, to support the therapeutic option of Eculizumab. Nevertheless further studies are needed to validate the PNAS-HUS scoring system, as well as the development of a prospective registry for assessing neurologic outcomes in STEC-HUS patients.

In conclusion, the combined role of complement system activation, pro-inflammatory and pro-apoptotic cytokines and electrolyte disorders seem to play a crucial role in determining the neurologic damage during STEC-HUS. CNS involvement in this cohort of patient worsens the morbidity and mortality and require adequate supportive therapy (correction of electrolyte disorders, anti-epileptic therapy and cardio-respiratory support when needed). When characterized by severe clinical presentation, as confirmed by neurologic score, the prompt administration of Eculizumab may significantly improve both the neurologic signs and the laboratory findings.

Early treatment of severe forms of neurologic STEC-HUS with Eculizumab after adequate clinical and instrumental neurologic examination may significantly improve the clinical outcome of these form. The validation of a Pediatric Neurologic Assessment Score for STEC-HUS (PNAS-HUS), as proposed in the present work, may improve our ability to identify patients with CNS involvement at higher risk of morbidity and mortality and may drive the prompt employ of Eculizumab in this cohort of patients.

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Table 1. Main laboratory characteristics STEC-HUS patients with and without CNS involvement

	Total (n=54)	**CNS involvement (n=12)**	**No CNS involvement (n=42)**	***p***
Gender (M/F)	23/31	6/6	19/23	0.770
Age (months)	32 [7-62]	25 [7-53]	38 [13-62]	0.299
BodyWeight (kg)	11.9 [6.8-17.4]	12.0 [7.2-15.7]	11.2 [6.8-17.4]	0.791
Hospitalization (before diagnosis of STEC-HUS)	5.0 [2.5-9.0]	6 .0[4.5-8.0]	4.5 [2.5-9.0]	0.489
Blood Pressure Systolic Diastolic	 110 [100-125] 70 [60-80]	 110 [100-125] 70 [60-80]	 108 [103-115] 65 [60-70]	 0.734 0.631
SerumCreatinin (mg/dL)	**2.8 [0.6-5.4]**	**3.5 [3.0-5.4]**	**1.3 [0.6-1.8]**	***<0.001***
Serum Urea (mg/dL)	**140.4 [58.2-225.8]**	**176.8 [22.8-225.8]**	**99.9 [58.2-175.4]**	***<0.002***
Leucocyte count (x10^3/µL)	**14.9 [10.1-18.0]**	**17.8 [13.6-21.3]**	**11.0 [8.2-12.6]**	***<0.001***
C reactive protein (mg/dL)	**3.7 [0.5-30.0]**	**19.7 [6.7-30.0]**	**1.1 [0.5-3.5]**	***<0.001***
Hemoglobin (g/dL)	9.7 [8.1-10.6]	9.9 [9.6-10.6]	9.3 [8.1-10.0]	0.082
Platelets (x10^3/µL)	68.0 [30.0-81.2]	63.5 [37.0-76.7]	64.3 [27.2-86.5]	0.785
LDH (UI/mL)	3695 [1205-5170]	3780 [1660-5250]	3540 [1090-5300]	0.502
Sodium (mEq/L)	**133 [124-140]**	**129 [124-132]**	**134 [130-140]**	***<0.005***
Potassium (mEq/L)	4.5 [3.5-5.1]	4.1 [3.5-4.7]	4.5 [3.9-5.1]	0.473
Albumin (g/dL)	3.1 [2.7-3.5]	3.0 [2.7-3.5]	3.1 [3.0-3.5]	0.696
Uric acid (mg/dL)	8.6 [6.4-10.9]	8.9 [6.8-12.0]	7.7 [5.6-8.7]	0.142

Values are expressed as mean and as [minimum – maximum] range. A comparison between main clinical and laboratory parameters of patients with CNS involvement and patients without CNS involvement is shown and a p-value is reported.

Table 2. Neurologic signs and symptoms included in Pediatric Neurological Assessment Score of STEC-HUS patients with CNS involvement.

Patient	Gender	Age (months)	E. Coli	Alteration of consciousness	Epileptic seizures	Strabismus/Eye fixing	Nystagmus	Visusdisorders/Amaurosis	Disorders of muscle tone (hypo/hypertensive)	~~Dysartria~~Disorders of communication skills*	Neurovegetative system disorders**	NMR	CT Scan	EEG	*Neurological Score*	Eculizumab
1	F	18	O111	1	0	2	0	0	0	0	0	1	0	1,5	5,5	No
2	M	13	O26	2	2	1	0	0	1	0	0	0	1	2	9	No
3	M	20	-	1	2	1	1	0	0	0	0	1	1	2	9	No
4	F	25	O26	1	0	0	0	0	0	0	0	1	1	0,5	3,5	No
5	M	16	O26	1	0	2	0	0	0	0	0	1	0	1	5	No
6	F	27	O26	1	0	1	1	0	0	0	0	1	1	0,5	5,5	No
7	F	53	O111	1	0	1	0	0	1	1	0	1	1	1,5	7,5	No
8	M	33	O111	3	3	3	0	3	2	3	3	4	1	0,5	25,5	Yes
9	M	46	O26	3	3	2	0	1	2	3	3	2	1	2	22	Yes
10	F	23	O111	1	2	1	2	0	0	0	0	2	1	2,5	11,5	Yes
11	M	7	O26	2	3	0	0	0	0	0	2	2	0	4	13	Yes
12	F	17	O26	2	3	2	0	0	3	1	0	1	0	2	14	Yes

*Disorders of communication skills: regression of acquired communication skills for younger patients, dysartria for others patients

**Neurovegetative system disorders: heart rate alterations, hypo~~/hyper~~tension, respiratory rate alterations; NMR nuclear magnetic resonance

Supplementary Tabel 1. Pediatric Neurological Assessment Score of STEC-HUS patients with CNS involvement

Neurologic signs and symptoms	Score
1. Alteration of consciousness	
Pediatric GCS* 0	0
Pediatric GCS 1-2	1
Pediatric GCS 3-4	2
Pediatric GCS 5	3
2. Epileptic seizures	
No episode	0
Absence seizures	1
Focal seizures	2
Generalized seizures	3
3. Strabismus/Eye fixing	
Absence	0
Transient	1
Short term (<6h)	2
Long term (>6h)	3
4. Nystagmus	
Absence	0
Transient	1
Short term (<6h)	2
Long term (>6h)	3
5. Visus disorders/Amaurosis	
Absence	0
Transient	1
Short term (<6h)	2
Long term (>6h)	3

Neurologic signs and symptoms	Score
6. Disorders of muscle tone (hypo/hypertensive)	
Absence	0
Transient	1
Short term (<6h)	2
Long term (>6h)	3
7. ~~Dysartria~~Disorders of communication skills**	
Absence	0
Transient	1
Short term (<6h)	2
Long term (>6h)	3
8. Neurovegetative system disorders***	
Absence	0
Onset of 1 disorder	1
Onset of 2 disorders	2
Onset of 3 disorders	3
Neuro-radiological and electrophysiological examination	**Score**
Magnetic Resonance Imaging (MRI) of the Brain	
1 point for each examination	+1
CT Scan of the Brain	
1 point for each examination	+1
Electroencephalography (EEG)	
0.5 points for each examination	+0.5

GCS Glasgow Coma Scale; **Regression of acquired communication skills for younger patients, dysartria for others patients;
****Heart rate alterations, hypotension, respiratory rate alterations***