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ORIGINAL ARTICLE

HUS with mutations in *CFH* and STEC infection treated with eculizumab in a 4-year-old girl

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Abstract

Background Hemolytic uremic syndrome secondary to Shiga-toxin-producing *Escherichia coli* infection (STEC-HUS) generally shows a favorable outcome. Few cases develop extra-renal complications, since neurological involvement is an important cause of morbidity and mortality. The role of complement in STEC-HUS has been recently highlighted, and the use of eculizumab in severe cases has been communicated. HUS results from environmental and genetic factors, but the simultaneous occurrence of STEC and complement mutations remains undetermined.

Methods A pediatric case with severe STEC-HUS carrying *CFH* mutations, with favorable response to eculizumab is analyzed.

Results STEC-HUS was diagnosed in a 4-year-old girl with classic HUS, including low C3. Peritoneal dialysis was started due to hypertension, oligoanuria, and pleural effusion. She evolved with generalized tonic–clonic seizures and required mechanical ventilation. MRI reported multiple supra- and infratentorial ischemic lesions with laminar/striatal cortical necrosis and leukoencephalopathy. After two eculizumab doses, a significative stabilization in diuresis, blood pressure, creatinine, and C3 was achieved. At the third week, episodes of massive digestive bleeding and a life-threatening condition required a colectomy thus preserving the ileocecal valve. Due to atypical evolution, a genetic study was considered, identifying two heterozygous variants (CFH S1191L/V1197A).

Conclusion STEC-HUS in patients with a genetic predisposition has been previously reported, but the low frequency of occurrence makes it a rare disease. As in the present case, patients with atypical course might benefit from genetic analysis to evaluate early eculizumab initiation and to better understand its phenotype.

Keywords Hemolytic uremic syndrome · Factor H mutation · Thrombotic microangiopathy · *Escherichia coli*

Introduction

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy with endothelial injury that results in thrombosis of capillaries and arterioles presenting with the classic triad of hemolytic anemia, thrombocytopenia, and impaired organ function with the kidney as a main affected organ [1–4]. Most cases occur in children and represent one of the main causes of acute kidney injury with a mortality of 1–5% and long-term sequelae in almost 30% of survivors [5, 6].

A digestive infection by enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 is the most frequent etiology, which in 5–10% of cases develops HUS generally 2 weeks after the diarrhea episode. This entity is known as HUS secondary to Shiga-toxin-producing *Escherichia coli* (STEC-HUS) and causes 85% of cases [7–10], while the remaining 15% is secondary to a genetic alteration of the alternate pathway (AP) of complement and corresponds to atypical HUS (aHUS) [11, 12].

The primary mechanism of damage in STEC-HUS is endothelial damage caused by Shiga toxin (Stx); however, there is growing evidence that there is also an early secondary activation of complement, as a second hit, that

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contributes to endothelial damage [13, 14]. Different studies have shown that in children with STEC-HUS, there is increased complement consumption with low plasma C3 levels. Orth et al. showed in an in vitro model with normal human serum that preincubation with high titers of Stx2 induces direct activation of the complement AP and decreased activity of cell surface-bound factor H [15]. Kellnerová et al. demonstrated in kidney and gut cell lines that Stx2a bound specifically to C3b and C5, suggesting its potential involvement in complement regulation during STEC-HUS infection [16].

Some clinical studies have associated complement activation with a particular clinical course, although the number of patients is limited [17–19]. Balestracci et al. associated STEC-HUS with low C3 levels and a complicated course, with a higher incidence of need for dialysis and extrarenal complications with central nervous system involvement, severe intestinal damage, acute pancreatitis, diabetes, and multiple organ failure [20, 21]. However, given the predominant role of the complement system in fighting infections, it remains currently unresolved whether complement depletion arises primarily from endothelial damage caused by Stx, or is primarily responsible for the pathogenesis of the disease.

The use of eculizumab, a humanized monoclonal IgG2/4 antibody that binds to C5 preventing the conversion of C5a to C5b and the formation of the membrane attack complex (MAC), has been of high impact in the treatment of atypical HUS cases, and its off-label use in STEC-HUS with neurological involvement and severe clinical evolution has been communicated [22–25].

The aim of this report is to present the case of a patient with STEC-HUS confirmed by PCR for EHEC with a severe evolution due to neurological and intestinal complications, in whom eculizumab was used with a favorable clinical response, and in whom two mutations toward the C-terminus in *CFH* were subsequently confirmed.

Clinical case

A 4-year-old previously healthy female patient was admitted in poor general condition, with dysentery and severe dehydration of 4 days of evolution. On admission, she was somnolent and hypoactive, presenting 2 episodes of abundant diarrhea with fresh blood. In the previous history, she had consumed roasted meat 6 days prior to the onset of symptoms.

Abdominal ultrasound showed signs of severe colonic inflammation and increased echogenicity of kidney parenchyma, and initial lab tests showed thrombocytopenia and low C3 serum levels (Table 1). During the first week, she required a red blood cell and 2 platelet transfusions. She evolved to oligoanuria, with volume overload and hypertension. A peritoneal catheter was installed to start peritoneal dialysis. A positive stool culture and FilmArray for enterohemorrhagic *Escherichia coli* O157 were confirmed. A chest X-ray showed bilateral pleural effusion requiring left pleural drainage, and noninvasive ventilatory support was started. During the second week of evolution, a progressive compromise of consciousness and generalized tonic–clonic seizures were observed, requiring medical management and connection to mechanical ventilation. A cerebral tomography (CT) was reported without abnormalities at that time.

In the context of HUS, anuria, low C3 levels, and neurological compromise, a diagnosis of aHUS was made. ADAMTS 13 activity was between normal values, and management with plasmapheresis was proposed, which could not be performed due to hemodynamic instability. It was decided to use off-label eculizumab therapy, first dose of 500 mg/m^2, showing 4 days after its use a significant improvement in kidney function, creatinine decreased from 4.08 to 1.07 mg/dl, and complement C3 levels increased from 61 to 102 mg/dl. A second dose of eculizumab 870 mg/m^2 was administered 1 week after the first dose (Table 1, Fig. 1).

A brain CT scan was repeated, showing multiple occipitals, frontal, and right temporal cerebral infarcts (Fig. 2).

Table 1 Laboratory tests during hospital stay

Admission day 0	Day 1	Day 1 admission Day 3	Day 3 admission Day 10	Day 10 admission Day 18	Day 18 admission Day 30	Day 30 admission Day 78
Lab test (reference range)						
Hemoglobin (11.5–13.5 g/dl)	12	7.8	10.2	9.9	10.3	10.1
Platelet count (150–450 mil/µl)	43	42	81	185	149	506
LDH (196–373 U/L)	4726	3456	1324	1016	321	201
Creatinine (0.2–0.7 mg/dl)	1.07	3.51	3.77	2.64	0.54	0.41
C3 (99–214 mg/dl)	–	55	61	102	86	168
C4 (11–51 mg/dl)	–	18	28	30	26	69
			Eculizumab day 14		Eculizumab day 21	

The electroencephalogram (EEG) showed slow and disorganized activity. A magnetic resonance imaging (MRI) reported multiple supra and infratentorial ischemic lesions with involvement of borderline areas associated with extensive laminar and striatal cortical necrosis and leukoencephalopathy. Daily EEG showed no changes in electroencephalogram pattern, presenting a new seizure episode one week after the first episode.

At the third week of hospitalization, in the context of kidney failure and hematologic compromise, 4 episodes of massive digestive bleeding were observed, with significant hemodynamic deterioration. The patient required RBC transfusions and maintenance on mechanical ventilation. Abdominal CT showed parietal inflammatory thickening in the stomach, hepatic angle of the colon, descending colon, and rectum. After 1 month of evolution, due to severe and repeated life-threatening colonic bleeding, a colectomy with resection of the ascending, transverse, and descending colon was performed. Two weeks later, a significant improvement of consciousness, neurological status, diuresis, and hemodynamic condition was observed, with normalization of biochemical parameters. The patient was discharged on the 101st day of evolution.

Due to severe and atypical evolution of STEC-HUS, a genetic study of the complement was performed. Sanger sequencing was used to study the coding exons of complement factor H (*CFH*) and factor I (*CFI*), since their analyses were locally available and these genes are known to accumulate an important fraction of variants in cases of aHUS. Two heterozygous close positions were identified in the last portion of *CFH* (c.3572C > T, c.3590 T > C) that have been described in cis configuration in other patients. At the protein level, the combined predicted missense variants, p.S1191L and p.V1197A, have been described in cases of severe aHUS and functional studies have demonstrated an altered capacity to control complement activation on the cell surface [26, 27].

Discussion

STEC-HUS in patients with a genetic predisposition for complement-mediated HUS has been previously reported, but the low frequency of occurrence makes it a rare constellation. In this report, we present the clinical case of a 4-year-old female patient admitted for a STEC-HUS, in which two *CFH* mutations were later confirmed, in the context of a complicated evolution characterized by a low C3, hematological and kidney compromise that requires peritoneal dialysis, a severe neurological involvement, and massive hemorrhagic colitis requiring colectomy. Genetic evaluation in STEC-HUS has been previously described.

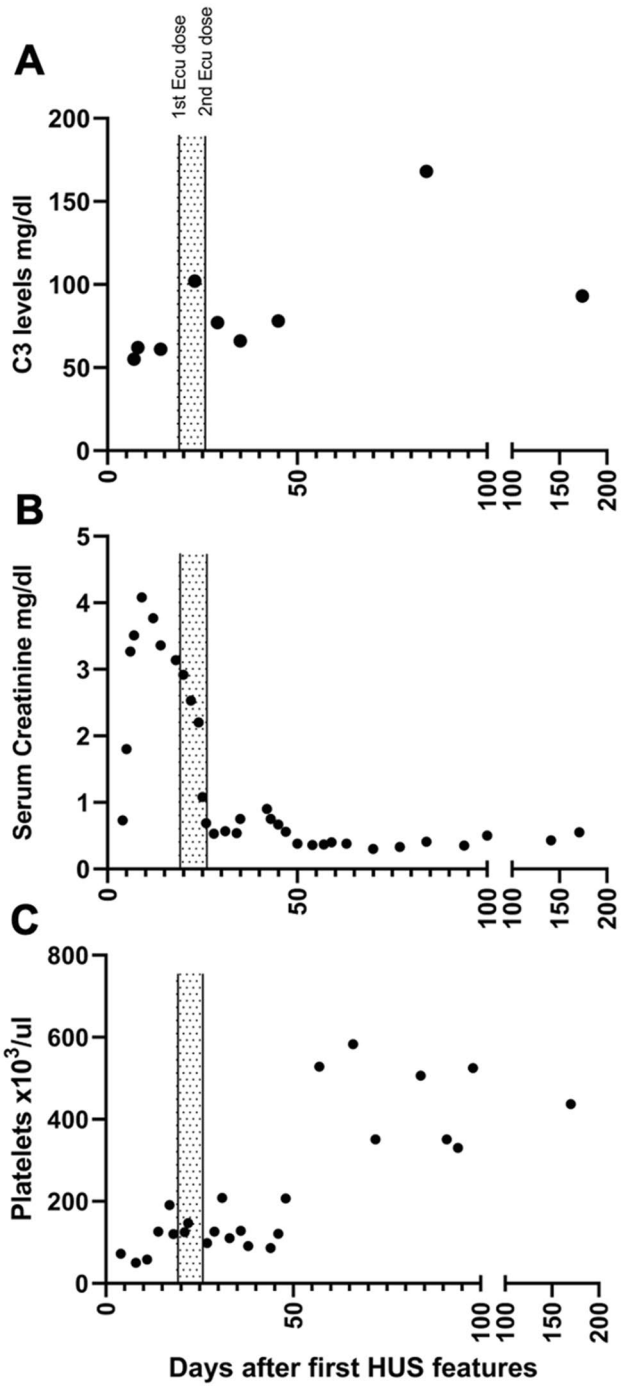

Fig. 1 Day after first HUS features

Fremeaux-Bacchi et al. assessed variants for 6 susceptibility complement genes linked to aHUS in children, 75 Stx-positive and 33 Stx-negative, with a clinical diagnosis of post-diarrheal HUS [28]. They found pathogenic variants with minor allele frequency (< 0.1%) in 12/75 (16%) Stx-positive patients, 4 of them with pathogenic variants. From a clinical

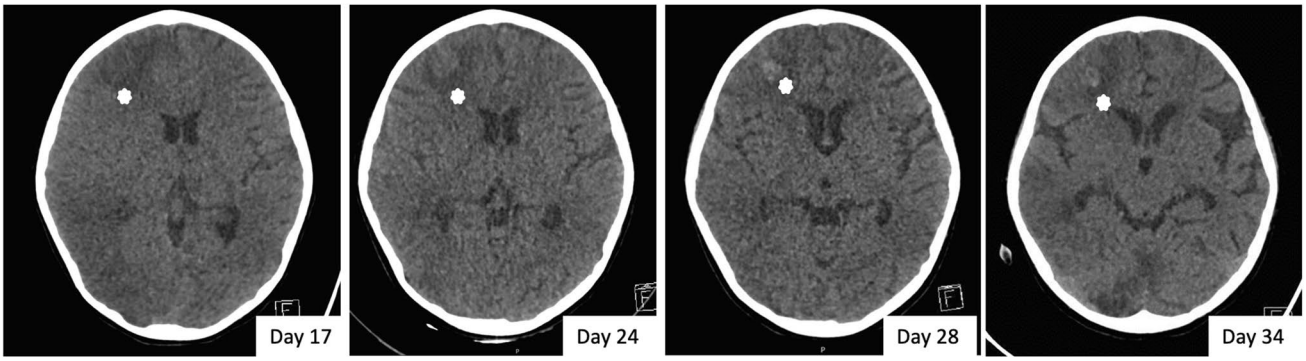

Fig. 2 Brain CT scan

point of view, a significant difference in complement pathogenic variants was not found in those patients with/without a complicated clinical course, central nervous system involvement, or kidney replacement therapy requirement.

In our patient, off-label eculizumab was administered at the second week of hospitalization due to the severe neurological involvement and the suspected complement activation in a STEC-HUS case. The neurological impairment observed in some cases of STEC-HUS has been largely known to be a life-threatening condition, leading to severe long-term neurological sequelae and patient death. In the last few years, it has been observed that an early intervention with eculizumab could make a difference in the unfavorable outcome of these patients, blocking the activated AP of complement in the classic HUS form, as suggested by multiple studies [15, 17–21, 29].

The early use of eculizumab has shown to improve neurological outcome in 11 children with STEC-HUS and CNS involvement who presented with seizures and coma [23]. Only 2/10 surviving patients showed neurological impairment at the time of discharge, and 8/10 showed no further seizures after the first dose of eculizumab. Platelet counts normalized at a mean of 4 days after eculizumab dose. A Letter to the Editor published in the *NEJM* confirmed the remarkable effect of C5 blockade by this antibody in 3 children with Shiga toxin-producing *E. coli* and severe involvement of the central nervous system [22]. The dramatic neurological response within 24 h of the first eculizumab dose was followed by the normalization of markers of disease activity, platelet count, and LDH levels. As opposed to our patient, no mutation could be demonstrated later for the genes which encode complement regulatory proteins, including anti-CFH antibodies.

The positive and rapid clinical and biochemical response to the blockade of the alternative complement pathway by this antibody strongly supports the hypothesis that Stx in fact activates the complement cascade, as shown three decades ago, when 68 children with STEC-HUS were evaluated, showing that a subgroup had low serum levels of C3, which, when associated with leukocytosis represented a poor prognosis [30]. In a more recent communication, in 17 STEC-HUS children aged 1.2–14 years, measurement of the serum levels of two complement pathway products, Bb and soluble membrane attack complex (sC5b-9), showed a significative increase in serum levels of both AP components when compared with healthy controls the day of enrollment [31]. One month after recovery, a normalization of these fragments were found, validating that STEC-HUS activates the AP of the complement. However, retrospectively, it can be argued that the diagnosis of STEC-HUS was made only under clinical and laboratory criteria, without culture or molecular identification of the pathogen, leaving open the possibility that some patients could have corresponded to atypical HUS. Our findings in our patient confirm that in STEC-HUS with severe neurological involvement, one must consider the associated complement compromise and, as we found, a concomitant mutation in the complement cascade. Ferraris et al. [32] communicated 18 STEC-HUS with confirmation of Stx in stool cultures by PCR, thus avoiding the inclusion of atypical cases in the sample of patients. They measured plasma levels of complement activated factors Bb and the fluid phase SC5b-9 of the MAC complex, both showing significantly increased levels at the time of admission, with a rapid decline during the first week of evolution. The authors concluded that the results confirmed the activation of the alternative pathway of complement during the acute phase of STEC- HUS. Current evidence from case reports shows that patients with mutations in complement genes associated with aHUS can manifest with STEC-HUS. A case by case approach is required to evaluate the treatment with complement blocking agents.

The role of Stx in the activation of the AP of the complement has also been the subject of studies in patients with STEC-HUS. Stx is composed of one enzymatically active A subunit, non-covalently associated with a pentameric B subunit, representing the virulence factors of EHEC,

which after absorption in the gastrointestinal tract reach the organs expressing the binding molecules, globotriaosylceramide receptors Gb3 and Gb4. After entering the cell via endosomes, the toxin is transported to the ribosomes, inhibiting the synthesis of protein and leading to endothelial cell death. Both Stx express many subtypes, Stx1 (stx1a, stx1c, stx1d) and Stx2 (stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, stx2g), and the risk of developing STEC-HUS has been previously linked to the stx2 family. In a recent study in Argentina, the Stx genotypes were evaluated in 280 patients who fulfilled the inclusion criteria of HUS [33]. They found 2 genotypes as the more frequently pathogenic factors, the *stx2a/2c* genotype in 63.9% of the patients and *stx2a* in 33.6% of the patients, although no relationship to the severity of the disease could be confirmed. In this setting, the role of a purified sample of Stx was explored in the activation of complement, finding that Stx2 activated complement in vitro predominantly via the alternative pathway [15]. They also evaluated the role of Stx2 over the regulator factors of the C3 activation, FH and FI, showing that, although Stx2 does not cleave those factors, purified Stx2 was able to bind FH in a concentration-dependent manner without affecting its function as a cofactor for FI in the cleavage of C3b. To date, data show that the complement AP is activated in D + HUS patients.

In our patient, C3 serum levels normalized 4 days after the administration of the first eculizumab dose, suggesting that the alternative complement pathway had been activated by the Stx. The off-label use of this drug in STEC-HUS has been communicated frequently in the last few years, due to its powerful inhibitory effect on the terminal complement pathway by binding to C5, preventing the generation of C5a and C5b, the first protein of the MAC. A recent report by the Pediatric Nephrology Research Consortium Study evaluating the use of eculizumab in 152 patients, mean age 9.1 ± 6.8 years, stated that 12% of the target population corresponded to STEC-HUS, most of them due to neurological impairment [34]. A higher proportion of eculizumab off-label use was reported by the French National Hospitalization database, where almost 80% of the indications corresponded to acquired hemolytic anemias, which include approved conditions like paroxysmal nocturnal hemoglobinuria, but also non-approved disease such as typical HUS [35].

Recently, Mahat et al. performed a systematic review of the literature searching for the use of eculizumab in STEC-HUS, identifying 16 related reports, 8 of them corresponding to case reports/series, 7 retrospective studies, and 1 prospective cohort study [36]. The authors remark that, although most of the studies of the use of this drug in typical HUS communicate a positive clinical response, the evidence is mostly based on nonrandomized retrospective studies and case reports. The only prospective study was performed by Gitiaux et al., who evaluated 7 children, aged 16 months to 7 years, with typical STEC HUS and neurological compromise by means of brain magnetic resonance (MRI) during a 6-month follow-up [37]. Eculizumab efficiency was verified using serum hemolytic activity (CH50). Neuropsychological tests and brain MRI were evaluated at inclusion and after 6 months of follow-up. Two out of 7 patients died due to neurological and cardiac complications, respectively. The neurological examination and MRI imaging at 6 months were normal in all surviving children, accenting the role of complement blockade with this C5 antibody in STEC HUS. In a clinical setting, eculizumab therapy has been limited by the very high cost of the drug, as in our patient where only 2 doses were available, limiting its use in low-resource areas of the world. However, not all studies have found a favorable response to eculizumab in STEC-HUS patients. In a retrospective cohort study based on the German HUS Registry, 491 adult patients with HUS were evaluated [24]. Eculizumab combined with plasma exchange was compared to plasma exchange alone and supportive therapy. This nonrandomized retrospective study did not find any difference in outcome variables (kidney, neurological recovery, and survival) between the 3 groups. A cohort of 298 adults with EHEC-HUS at 23 centers were analyzed during the 2011 German EHEC outbreak [38]. Main outcomes were death, need for dialysis, neurological involvement, gastrointestinal complications, and mechanical ventilation. A subgroup of 67 patients treated with eculizumab was compared to 65 patients selected in terms of baseline characteristics similar to the monoclonal antibody treated group. Although groups were assigned in a nonrandomized manner, which represents a potential bias, no difference in the rate of complications, platelet recovery, creatinine, hemoglobin, and lactate dehydrogenase were found between the groups.

A major issue of clinical relevance is the role of soluble complement fractions as reliable markers of complement activation in aHUS, as recently reviewed by Raina et al. in an extensive meta-analysis [39]. Noris et al. found increased levels of sC5b-9 only in 10/19 patients during the active phase of aHUS, while 23/36 patients in remission did not show a return to normal values of this factor, as well as 8 patients after receiving eculizumab [40]. By contrast, other research groups have highlighted the complexity in the significance of MAC in aHUS, describing patients in the acute phase of disease that had significantly elevated serum C5b-9 levels, returning to normal levels during the remission phase [41–43]. Karnisova et al. showed in a retrospective study of 33 STEC-HUS pediatric patients that low C3 serum levels at admission were associated with severe kidney failure and a higher risk of dialysis in STEC-HUS children [44], as the case presented here. Unlike Karnisova et al., Fremeaux-Bacchi et al. found C3 plasma levels within normal limits in HUS patients, significantly higher compared with controls,

but when comparing Stx-negative with Stx-positive patients, the latter group showed significantly lower C3 and C4 serum levels.

A recent and remarkable study by Palomo et al. evaluated the complement activation in aHUS patients by a modification of the technique described by Noris et al. [45]. The authors exposed human dermal vascular endothelial cells to sera or plasma of aHUS patients during both the acute and remission clinical stages. Plasma from the acute phase induced a marked C5b-9 deposition on endothelial cells, returning to normal levels when patients were in remission. As well as Noris et al., the authors found that soluble C3, C4, and CH50 were normal in most patients regardless of clinical status, confirming that soluble complement fractions are of limited prognostic significance in aHUS, which represents a point of caution when using these markers in clinical settings. The lack of a fully reliable diagnostic test for aHUS, and a critical approach to the use of complement inhibitors in kidney diseases have been recently analyzed by Fakhouri et al. [46].

Another life-threatening complication in our patient was the severe gastrointestinal involvement. In EHEC forms of HUS, which account for 90% of cases in children, gastrointestinal symptoms usually precede hematological and kidney involvement, and although any segment of the gastrointestinal system can be affected, the transverse and ascending colon show the most frequent and severe compromise. A recent review confirmed that gastrointestinal complications are related to mortality in these patients, and surgery may be decided early to avoid a lethal complication, as was decided in the case of our patient [47]. Roessingh et al. describe 2 children who required surgical management secondary to complication of the lower GI tract [48]. The first patient was a 5-year-old child with D + HUS, requiring a left colectomy with ileostomy and colostomy, as in our patient, with a later ileocolic anastomosis, and the second patient was a 1.5-year-old infant who presented with seizures and coma on the second day of hospitalization, developing rectal prolapse at day 20 of evolution. Another 4 children developed pancreatic compromise, 3 children developed hepatic complications, and 5 children developed with both systems involved. The authors did not find predictive symptoms or signs of the gastrointestinal involvement, or a correlation with the kidney evolution of HUS; however, as in our patient, gastrointestinal involvement with hemorrhagic colitis could represent a life-threatening condition including pancolitis, colonic perforation or toxic megacolon, and surgery must be evaluated early. Atypical HUS has been linked to gastrointestinal complications in patients with FH autoantibodies in 45 patients—38 children and 7 adults—with aHUS secondary to anti-FH antibodies [49]. Severe abdominal pain, vomiting, diarrhea, hepatitis, and pancreatitis were described. Hepatitis and pancreatitis were not found in our patient, and factor H autoantibodies are not performed on a routine clinical basis, but it should be considered according to the reported experience in this group of patients. Ultimately, the genesis of the severe gastrointestinal bleeding is not clear, but it is quite conceivable that the severe bloody diarrhea resulted in gross inflammation of involved intestinal segments. In our case, surprisingly, the symptoms appeared late in the course of the disease.

Mutation screening is recommended in aHUS cases to orientate clinical management, particularly before considering the use of C5 antibody and/or kidney transplant. Although aHUS represents a rare disease, increasing data have been generated in the last decade resulting in heterozygous variants in *CFH* responsible for the predisposition to aHUS in 20–30% of the cases with a high rate of CKD stage 5 and death. Together, the combined variants S1191L and V1197L identified in the patient might be de novo changes originated by nonallelic homologous recombination; parent testing is pending to confirm their status. These combined variants are located toward the C-terminal protein segment and, although no structural impairment has been demonstrated and the protein seems to fold correctly, they have shown to be functionally deficient in complement activation, as observed for other single C-terminal mutations [50]. These combined *CFH* variants were described in 2006 in a large family with classic features of HUS, disease recurrence after kidney transplantation with allograft loss, and high mortality [51]. Later in 2012, a pediatric case was reported with aHUS at the age of 4 years and the patient developed neurological symptoms that resolved with plasma exchange sessions. The patient underwent bilateral nephrectomy due to severe hypertension and received kidney transplantation without signs of recurrence during the first 16 months, under continuous eculizumab treatment [52]. Two cases carrying combined de novo S1191L/V1197A variants presented with aHUS at the age of 11 months, reached kidney failure, and one of them received kidney transplant with favorable outcome during the following 12 months [27]. Of note, studies have shown that tapering or withdrawing eculizumab is ethically reasonable and feasible, but a 50–60% risk of relapse has been observed in patients carrying *CFH* mutations. Consequently, we cannot rule out that our patient carrying two rare pathogenic variants of *CFH* is at risk of relapse if exposure to a triggering factor occurs [53, 54].

Conclusion

In recent years, the activation of the complement AP in STEC-HUS has been exhaustively explored and there is evidence for an initial involvement of the complement

system in the development of STEC-HUS. On the other hand, genetic mutations of the complement have been well-documented in aHUS. However, the association of genetic mutations of the complement associated with STEC-HUS has only been scarcely documented. We propose that our patient corresponds to a *CFH* mutation, as a case of aHUS triggered by an STEC infection. The impact on the outcome of early use of C5 blockade in STEC-HUS with a severe course and neurological involvement has been communicated, although the current evidence comes only from observational reports, and not from RCTs, which means a note of caution must be expressed until controlled studies are available. The reported STEC-HUS cases associated with a genetic mutation allow us to recommend that complement evaluation be included in all complicated STEC-HUS cases, as well as genetic analysis if low C3 levels are confirmed.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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