

Successful treatment of DEAP-HUS with eculizumab

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Abstract

Background Deficiency of complement factor H-related (CFHR) proteins and CFH autoantibody-positive hemolytic uremic syndrome (DEAP-HUS) represents a unique subgroup of complement-mediated atypical HUS (aHUS). Autoantibodies to the C-terminus of CFH block CFH surface recognition and mimic mutations found in the genetic form of (CFH-mediated) aHUS. CFH autoantibodies are found in 10–15 % of aHUS patients and occur—so far unexplained—almost exclusively in the background of *CFHR1* or *CFHR3/CFHR1* deletions.

Methods As a well-defined role for eculizumab in the treatment of complement-mediated aHUS is becoming established, its role in DEAP-HUS is less conspicuous, where a B-cell-depleting and immunosuppressive treatment strategy is being proposed in the literature.

Results We here show eculizumab to be safe and effective in maintaining a disease-free state, without recurrence, in a previously plasma-therapy-dependent DEAP-HUS patient, and in another patient in whom, although showing a good clinical response to plasma therapy, the therapy was hampered by allergic reactions to fresh frozen plasma and contend there is a rationale for the use of eculizumab in concert with an immunosuppressive strategy in the treatment of DEAP-HUS. Considering the high rate of early relapse, the possible coexistence and contribution of both known and unknown complement-gene mutations, the probable pathogenic role of CFHR1 as a complement alternative pathway (CAP) regulator, the experimental nature of measuring and using anti-CFH autoantibodies to guide management, and until the positive reports of immunosuppression in addition to plasma therapy are confirmed in prospective studies, we feel that a complement-directed therapy should not be neglected in DEAP-HUS. Serial CFH autoantibody titer testing may become a valuable tool to monitor treatment response, and weaning patients off eculizumab may become an option once CFH autoantibody levels are depleted.

Conclusions A prospective study of eculizumab treatment in a larger cohort of DEAP-HUS patients is required to validate the applicability of our positive experience.

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Introduction

Hemolytic uremic syndrome (HUS) describes the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. In 80 % of cases, HUS is initiated by Shiga toxin (Stx)-producing *Escherichia coli* (STEC) [1]. HUS in association with pregnancy, drugs such as calcineurin

inhibitors, defective cobalamin metabolism, and genetic defects of complement regulation make up the remainder of cases, collectively termed atypical HUS (aHUS) [2].

STEC-HUS was traditionally not recognized as complement-mediated disease, although there is emerging evidence that complement plays a role in its pathology [3, 4]. In aHUS, however, 40–60 % of cases are linked to mutations in components of the complement alternative pathway (CAP) [5, 6], where the disease-linked regulators are the plasma proteins complement factors H (CFH) and I (CFI), and the cell-surface-associated membrane cofactor protein (MCP/CD46) and thrombomodulin (THBD/CD141). These proteins interfere with the assembly, activity, and stability of the CAP C3 convertase complex (C3bBbP) both in plasma and on cell surfaces. Gain of function mutations in the C3 convertase components C3 and complement factor B (*CFB*) and loss-of-function mutations in regulators (above; primarily *CFH*) have been linked to CAP hyperactivity and aHUS [5]. A substantial subgroup of patients with complement-mediated aHUS (10–15 % of cases) [7–9] have also been found to have autoantibodies to CFH. More than 90 % of these cases are associated with homozygous deletions within the complement factor H-related (*CFHR*) gene cluster on chromosome 1q32, resulting in the loss of genes coding for CFH-related proteins 3 (*CFHR3*) and 1 (*CFHR1*). This condition has a typical age of onset ranging from 4 to 17 years and is termed deficiency of CFHR proteins and CFH autoantibody-positive HUS (DEAP-HUS) [8].

Approximately 50 % of DEAP-HUS cases present with a diarrheal prodrome similar to STEC-HUS [10], and the disease has a similar clinical course to non-*CFH* mutation associated aHUS in terms of chronic kidney disease (CKD), progression to end-stage kidney disease (ESKD), and death (occurring in about 40 %, 30 %, and 10 % of patients, respectively). There is a high rate of disease relapse (58 %) due to accumulation of autoantibodies [10], and with each recurrence, the risk of CKD and ESKD increases [11]. In the largest series of patients with DEAP-HUS (44 patients), almost 27 % had ESKD and almost 40 % had evidence of CKD [10].

Specific clinical practice guidelines for DEAP-HUS are lacking; strategies have been adopted from genetically determined forms of aHUS or other autoimmune diseases [12, 13]. The available literature supports therapeutic plasma exchange (TPE) initially, as in the other forms of aHUS, but with the addition of an antibody-suppressing or depleting treatment, such as azathioprine, mycophenolate mofetil (MMF), cyclophosphamide, or rituximab [10, 14, 15]. Expanding the scope of treatment options for DEAP-HUS, we report here the safe and successful use of the complement C5 blocker eculizumab (Soliris®) in two DEAP-HUS patients. One patient was in a more acute phase of the disease and responding to plasma exchange

but developed increasingly severe allergic reactions to fresh frozen plasma (FFP); the other was dependent on biweekly plasma infusions as maintenance therapy. We also discuss current treatment strategies for DEAP-HUS and highlight the distinctive characteristics of this aHUS subgroup that support the use of a complement-directed therapy, at least initially.

Patients, methods, and results

Patient 1

An 11-year-old girl with no past medical history of note travelled to Syria where she became unwell with nausea, vomiting, fever, and hematuria. There was evidence of acute kidney injury (creatinine 180 $\mu\text{mol/L}$), hyperuricemia [506 $\mu\text{mol/L}$ (normal 110–310 $\mu\text{mol/L}$)] anemia (hemoglobin 89 g/l), and thrombocytopenia ($68 \times 10^9/\text{L}$). She maintained good urine output and avoided dialysis. Initial management consisted of blood and platelet transfusions and pulsed methylprednisolone followed by prednisone and allopurinol. She underwent a bone marrow biopsy, which showed a hypocellular marrow with normal maturation. Her creatinine peaked at 660 $\mu\text{mol/L}$. At the time of discharge from the hospital, her creatinine was 447 $\mu\text{mol/L}$ and platelets were still low ($68 \times 10^9/\text{L}$). Upon return to Canada, she presented to our institution a month post discharge from the hospital in Syria and 7 weeks after initial presentation. On admission, she was cushingoid, edematous, and hypertensive (blood pressure 134/100 mmHg). Initial hematological investigations were remarkable for anemia (hemoglobin 109 g/L), reticulocytosis [$651 \times 10^9/\text{L}$ (normal 10–100 $\times 10^9/\text{L}$)], low haptoglobin [<0.07 g/L (normal 0.32–1.98)], and thrombocytopenia (platelets $48 \times 10^9/\text{L}$). There were schistocytes on blood film. This was accompanied by significant renal impairment (creatinine 403 $\mu\text{mol/L}$, urea 26.7 mmol/L), hypokalemia (potassium 2.9 mmol/L), hypocalcemia (ionized calcium 1.01 mmol/L), hyperuricemia [510 $\mu\text{mol/L}$ (normal 120–360)], and hyperphosphatemia (phosphate 3.7 mmol/L). Serum bilirubin was normal but aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were elevated (LDH 4,510 U/L). Parathyroid hormone (PTH) at presentation was also significantly raised [301 ng/L (normal 10–65)]. Urine analysis showed >3 g/L protein and large blood, there were no casts on microscopy, and a 24-h assessment had 5.54 g of protein (protein/creatinine ratio 1,183 mg/mmol). Regarding complement studies, her C3 was normal [0.79 g/L (normal 0.77–1.43)], as was her C4 [0.25 g/L (normal 0.07–0.4 g/L)]. Her a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS-13) protease activity was normal (52 %, normal range 30–120 %). A full vasculitic and microbiological screen was negative; renal biopsy showed a

thrombotic microangiopathy (TMA) with acute and chronic features, with both the glomerular capillaries and smaller-caliber interstitial vessels being affected.

Total plasma exchange (TPE) was commenced on day 12 of admission, with FFP at $1.5 \times$ plasma volume. She received a total of nine TPE sessions over the first 2 weeks, with a slow increase in platelet count and no real improvement in creatinine (Fig. 1a). She was switched to FFP infusions daily for a week, then three times weekly for 2 weeks, then twice weekly for 2 months. The steroids (60 mg/m^2) initiated at first presentation were weaned off over the course of 2 months (Fig. 1a). Autoantibodies to CFH were confirmed. Complete absence of CFHR1 and CFHR3 was found on Western blot analysis and a homozygous deletion of *CFHR3/CFHR1* on chromosome 1q32 subsequently confirmed by multiplex ligation-probe amplification (MLPA) analysis. Genetic analysis for mutations in *CFH*, *CFI*, *Factor B*, *MCP/CD46*, *CFHR5*, and *C3* genes was negative.

Two weeks after discontinuation of steroids and while on twice-weekly FFP infusions, the patient developed a recurrence, as evidenced by an increase in creatinine ($398 \mu\text{mol/L}$), and drop in platelets ($116 \times 10^9/\text{L}$), hemoglobin (85 g/L), and C3 (0.71 g/L). She began daily FFP infusions and was recommenced on high-dose prednisone, which was continued for 4 months (Fig. 1a). The FFP infusions were continued biweekly for 38 months with no further relapse. On two separate occasions, with an intercurrent illness, biochemical changes (LDH, platelets) were suggestive of a possible impending relapse; therefore, a decision in favor of chronic plasma therapy was made. Her creatinine slowly improved and was $119 \mu\text{mol/L}$ 1 year later.

Four years from initial presentation, she was entered into an open-label, multicenter, controlled clinical trial of eculizumab for adolescent patients with plasma-therapy-sensitive aHUS (C08-003 ClinicalTrials.gov numbers, NCT00844428 [adolescent]). This consisted of weekly eculizumab for 4 weeks (induction phase) and thereafter biweekly eculizumab (maintenance phase) for 2.5 years. Plasma therapy was successfully discontinued (Fig. 1b). CFH autoantibodies remained positive on a low level, which was in keeping with ongoing signs for complement activation [e.g., soluble terminal complement complex (SC5b-9)], but there was no further evidence of disease activity (Table 1) [16]. Creatinine remained stable at $100 \mu\text{mol/L}$, with an estimated glomerular filtration rate (eGFR) of $80 \text{ ml/min/1.73 m}^2$. A plan to attempt an antibody-depleting or immunosuppressive strategy with a careful wean of eculizumab is now underway.

Patient 2

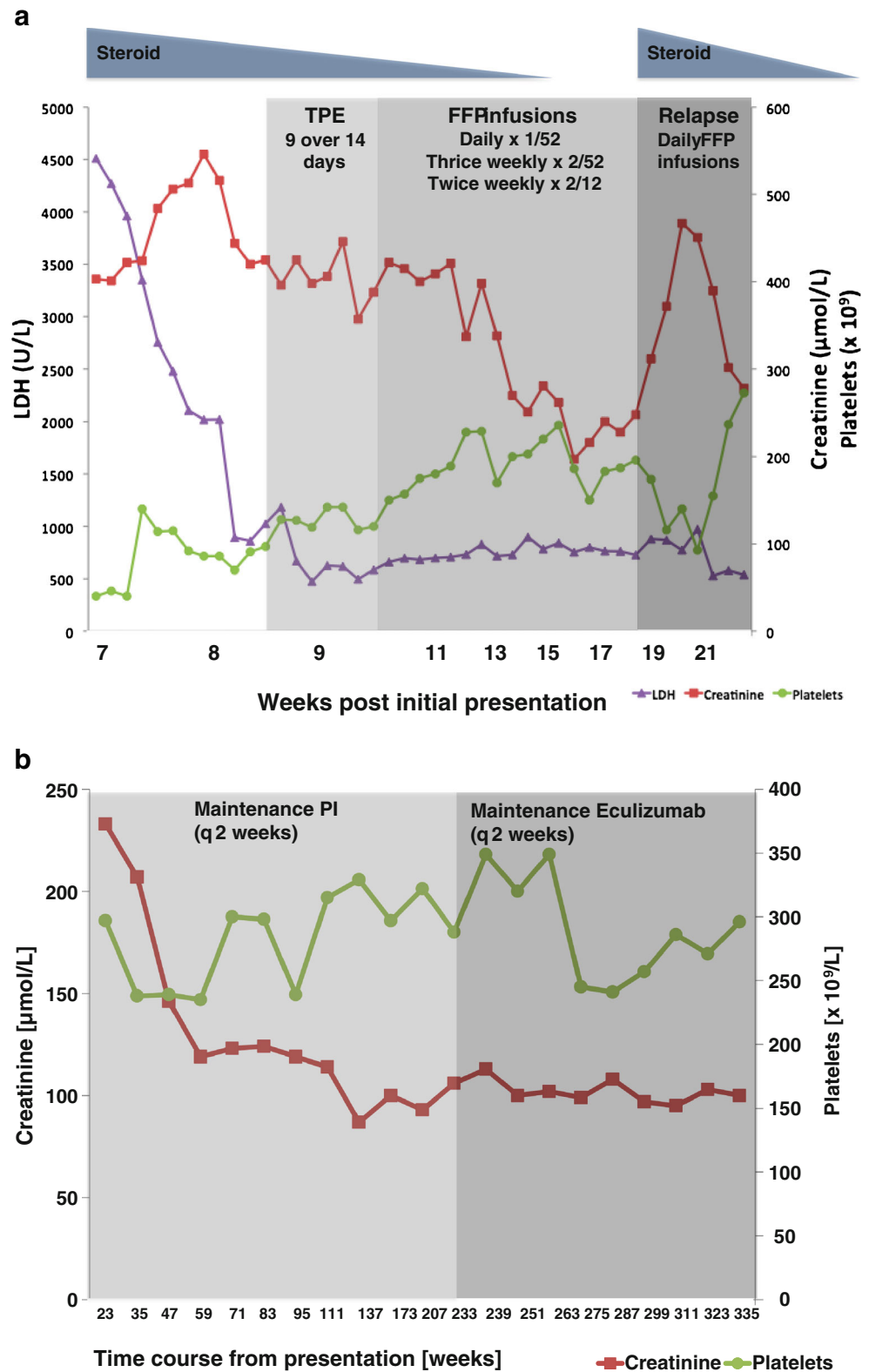
An 8-year-old boy presented with a 3-day history of vomiting, abdominal pain, pallor, lethargy, and decreased fluid intake. He had passed one loose, non-bloody stool, and although his

urine was described as being dark in color, there was no decline in urine output noted. On examination, he was pale with icteric sclerae and a few petechiae. Blood pressure was elevated at $119/67 \text{ mmHg}$, and he was clinically hypovolemic. The rest of his examination was unremarkable. Urine dipstick showed protein $>3 \text{ g/L}$ and 3+ blood, and microscopy showed >3 red blood cells/high power field (hpf), occasional white blood cells, and coarse granular casts. Complete blood count revealed a low hemoglobin (67 g/L), thrombocytopenia ($22 \times 10^9/\text{L}$), neutrophil leukocytosis (neutrophils $9.4 \times 10^9/\text{L}$, WBC $13.8 \times 10^9/\text{L}$), and spherocytes and fragments on blood film consistent with a microangiopathic process. Further evidence of hemolysis included a reduced haptoglobin [<0.01 (normal $0.32\text{--}1.98 \text{ g/L}$)], as well as elevated reticulocyte count [329 (normal $10\text{--}100 \times 10^9/\text{L}$)], bilirubin ($38 \mu\text{mol/L}$), AST (176 U/L), and LDH ($6,450 \text{ U/L}$). There was evidence of significant acute kidney injury (urea 57.2 mmol/L , creatinine $472 \mu\text{mol/L}$), mild hyponatremia (sodium 133 mmol/L), borderline hyperkalemia (5 mmol/L), and hyperphosphatemia (3.94 mmol/L).

Initial management was supportive and consisted of fluid resuscitation with normal saline, a packed red blood cell transfusion, and monitoring of his fluid status. Complement studies subsequently revealed a low C3 [0.5 (normal $0.77\text{--}1.43 \text{ g/L}$)] and normal C4. Antineutrophil antibodies (ANA) and antineutrophil cytoplasmic antibodies (ANCA) were negative. Antistreptolysin O (ASO) titer was normal (200 IU/L), and throat swab was negative for beta hemolytic group A *Streptococci*. Verotoxin (Shiga toxin) polymerase chain reaction (PCR) examination of his stool was negative, and no enterohemorrhagic bacteria were detected on stool culture. A spot urine protein/creatinine ratio was elevated at 910 mg/mmol . An abdominal ultrasound showed enlarged and echogenic kidneys.

The patient maintained a good urine output and avoided dialysis. Although his creatinine improved over the first week of admission, his platelets were showing no sign of recovery, his LDH was still markedly elevated ($15,764 \text{ U/L}$), C3 remained low (0.47 g/L), and he required a total of three red blood cell transfusions for anemia. A central line was inserted through his right internal jugular vein (RIJ) on day 9 of admission, and daily ($1.5 \times$) plasma volume exchanges commenced with FFP. There was a good response to TPE in terms of blood parameters, his C3 transiently normalized (0.73 g/L) by day 3, and his creatinine dropped to $91 \mu\text{mol/L}$ after 5 days of TPE. After the first four exchanges, he developed increasing allergic reactions to FFP (one requiring cessation of treatment). He received three further exchanges with predominantly 5 % albumin, but after a total of seven TPEs, a decision was made to transfer him to eculizumab therapy (Fig. 2). He received his first dose of eculizumab (600 mg) on day 16 of admission. He received meningococcal C, Prevnar 13 pneumococcal, and *Haemophilus influenzae* B

Fig. 1 a Clinical course of patient 1 during initial presentation and first major relapse. **b** Clinical course of patient 1 during maintenance plasma infusion (PI) and chronic eculizumab therapy. *FFP*, fresh frozen plasma; *TPE*, therapeutic plasma exchange



vaccinations prior to eculizumab and was additionally placed on penicillin prophylaxis for 14 days. His C3 was low, at 0.69 g/L, platelets $146 \times 10^9/L$, and creatinine 86 μmol/L on

day 16. Four days after eculizumab initiation, his C3 (0.87 g/L), creatinine (67 μmol/L), and platelets ($169 \times 10^9/L$) had normalized. He received weekly eculizumab for 3 weeks

Table 1 Complement studies in patient 1 while established on biweekly eculizumab infusions

Complement study	Normal range	Pre-eculizumab infusion	Posteculizumab infusion
Classical pathway (function)	65–135 %	Undetectable	Undetectable
Alternative pathway (function)	60–140 %	Undetectable	Undetectable
C3d	<40 mU/ml	37	58
SC5b-9	<320 ng/ml	1,316	1,266
C3	0.89–1.87 mg/ml	0.91	0.83
Factor H	284–528 µg/ml	393	362
Anti-factor H	<100 U/ml	129	111

Despite undetectable 50% hemolytic complement (CH50) and aminoglycoside phosphotransferase (APH)50, suggesting full complement inhibition, elevated pre-eculizumab soluble terminal complement complex (SC5b-9) levels speak in favor of sustained complement activation. This may be explained by decreased eculizumab concentrations toward the end of the treatment interval. The effect of eculizumab on plasma levels of the activation products complement 3d (C3d) and SC5b-9 assessed 1 h after treatment has to be interpreted with care, as eculizumab-induced complement inhibition may not have been fully apparent within the first 24 h after treatment considering the half-life of complement activation products (CAP). Note that complement factor H (CFH) autoantibodies remain positive

(induction phase) prior to converting to biweekly infusions (maintenance phase) (Fig. 2). Further complement studies were performed both pre- and post-eculizumab infusions on two separate occasions (at 5 and 6 months) during biweekly maintenance therapy. High pre-eculizumab soluble terminal complement complex (SC5b-9) levels, possibly indicating emerging complement activation toward the end of the treatment cycle, were significantly lowered upon eculizumab reinfusion (Table 2) [16]. Whereas in-depth complement workup detected no mutations in *CFH*, *CFI*, *CFB*, *MCP/CD46*, *THBD/CD141*, *CFHR5*, or *C3* genes, and ADAMTS13 activity was within normal range, autoantibodies to CFH were positive (Table 2), and a homozygous deletion of *CFHR3/CFHR1* was confirmed by MLPA. Interestingly, we observed a positive impact of eculizumab on anti-CFH titers. However,

C3 levels remained low, most probably due to continuous CAP dysregulation via the anti-CFH autoantibodies and/or a transiently positive C3 nephritic factor (C3NeF) (Table 2).

Discussion

We describe the safe and effective use of the complement C5 inhibitor, eculizumab, in maintaining a disease-free state in the presence of ongoing anti-CFH autoantibodies in a previously plasma-therapy (infusion)-dependent DEAP-HUS patient. In another patient, plasma therapy (exchange)—although clinically successful—was hampered by allergic reactions to FFP and had to be replaced by eculizumab, which maintained

Fig. 2 Clinical course of patient 2. TPE, therapeutic plasma exchange

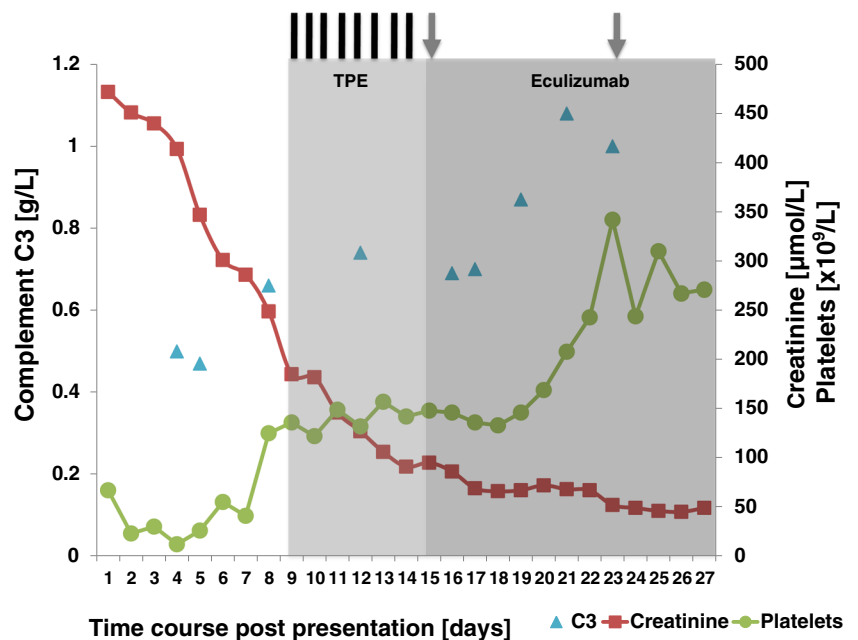


Table 2 Complement studies in patient 2 pre- and posteculizumab infusion performed 1 month apart while established on biweekly eculizumab infusions for 2 months

Complement study	Normal range	Pre-eculizumab 1	Posteculizumab 1	Pre-eculizumab 2	Posteculizumab 2
Classical pathway (function)	65–135 %	Undetectable	Undetectable	Undetectable	Undetectable
Alternative pathway (unction)	60–140 %	37	12	Undetectable	Undetectable
C3d	<40 mU/ml	54	52	60	53
SC5b-9	<320 ng/ml	314	260	1525	730
C3	0.89–1.87 mg/ml	0.41	0.40	0.45	0.40
Factor H	284–528 µg/ml	343	292	316	286
Factor I	21–46 µg/ml	31	25	35	31
Anti-factor H	<100 U/ml	547	482	316	201
C3 nephritic factor	Negative	Low positive	Low positive	Negative	Negative

Pre- and posteculizumab 1 Although undetectable 50% hemolytic complement (CH50) and low-level functional activity of the complement alternative pathway (CAP) indicate an incomplete inhibition by eculizumab before and even postapplication, normal soluble terminal complement complex (SC5b-9) plasma concentrations speak in favor of sufficient inhibition of the terminal complement sequence in vivo. Increased complement 3d (C3d) at both time points of sampling reflect a still ongoing complement activation, e.g., due to the presence of anti-complement factor H (anti-CFH) autoantibodies, leading to CAP activation. This is not unexpected, as the C5 inhibitor eculizumab does not interfere directly with the early activation process via either of the three pathways up to C3. *Pre- and posteculizumab 2 (1 month later)*: Although systemic total complement function [50% hemolytic complement and aminoglycoside phosphotransferase (CH50, APH50)] is abolished, elevated C3d and SC5b-9 indicate robust complement activation. Although reduced posteculizumab SC5b-9 concentrations speak in favor of successful treatment, low C3 and still elevated levels of both SC5b-9 and C3d indicate ongoing complement activation. Again, as the second (post) sample was obtained only after 1 h, the full inhibitory effect of eculizumab may be masked by the fact that pre-existing complement activation products may not have been fully metabolized

this patient in remission despite ongoing presence of antibodies and evidence of complement activation.

DEAP-HUS

Autoantibodies [immunoglobulin G (IgG)1 and 3 subtypes] to CFH were first described in association with aHUS in three patients [7]. The CFH autoantibodies bind to the short consensus repeats 19–20 (SCR 19–20) in the C-terminal-recognition region of CFH, a known “hot spot” for aHUS-associated mutations in the *CFH* gene, containing—among others—the binding sites for C3b, heparin, and endothelial cells. Thus, antibodies—similar to *CFH* mutations—result in failure of regulation of the CAP C3 convertase (C3bBb) on the endothelial surface [17]. Recognizing a correlation between the presence of CFH autoantibodies and the homozygous deletion of genes for CFH-related proteins *CFHR3* and *CFHR1* (*CFHR3/CFHR1*) caused by nonallelic homologous recombination on chromosome 1q32—a phenomenon initially described as an independent susceptibility factor for aHUS [18]—a novel subgroup of aHUS was defined as deficiency of CFHR proteins and CFH autoantibody-positive HUS, or DEAP-HUS [8].

Whereas genetic predisposition is not fully understood, *CFHR3/CFHR1* deficiency was found in up to 93 % of patients with autoimmune aHUS, suggesting a causal role for the development of these antibodies [19]. Furthermore, a regulatory function of CFHR1 on the C5 convertase has recently been recognized via which CFHR1—when

deficient—may also contribute to aHUS pathogenesis [20]. Similarly, *CFHR3* has regulatory effects on the C3 convertase of complement [21].

CFHR3 and *CFHR1* lie in contiguous regions on chromosome 1q32, downstream of *CFH*, and sequence homologies render this region at risk of chromosomal recombination, often affecting both *CFHR3* and *CFHR1* together. However, it has recently been suggested that deficiency of *CFHR1* rather than *CFHR3* seems to be a precursor to the development of CFH autoantibodies [9], and so far, only five patients with autoantibody-mediated aHUS but not lacking *CFHR3/CFHR1* have been described [9, 22]. In addition, mutations in *CFH*, *CFI*, *MCP/CD46*, and *C3* have been detected in five of 13 patients with *CFHR3/CFHR1* deletion and autoantibody-mediated aHUS, suggesting a “multiple-hit” mechanism to the etiology of autoimmune aHUS in at least some of these patients [22]. Further corroboration of a genetic multiple-hit hypothesis comes from the recent publication of the European Working Party on Complement Genetics in Renal Diseases, in which certain risk haplotypes, especially in *CFH* and *MCP/CD46*, screened for in 795 patients across multiple cohorts predicted a worse prognosis [6].

More recent studies on the pathobiology of the autoantibodies have shed more light on their pathogenicity [23, 24]. The autoantibodies affect CFH binding to pentraxin 3, a protein family, including C-reactive protein (CRP) that normally aids CFH binding to cell surfaces and enhances endothelial cell protection against complement-mediated

cytotoxicity [23]. It is now known that the antibodies do not just affect CFH binding to C3b on the cell surface but have many effects on CFH function, both in the fluid phase and on the surface, including: (i) reduced CFH binding to C3(H₂O) in the fluid phase, (ii) reduced binding of CFH to C3b, (iii) reduced CFH dissociation of the C3bBb complex (decay activity), and (iv) altered cofactor activity [24].

Treatment concepts

The principal treatment concept for complement-mediated aHUS hinges on restoring proper CAP regulation by removing circulating factors or replacing deficient or defective regulatory proteins, and to date, it centers on the prompt initiation of TPE using FFP or virus-inactivated, pooled plasma (SD-plasma; Octaplas®), as outlined in evidence-based consensus guidelines [25, 26]. Ongoing management can be achieved with prophylactic plasma infusions to maintain remission [27], but complications are multiple and include primary or secondary treatment failure, allergic reactions, and access-related infections or thrombosis [28]. There are no consensus guidelines for managing DEAP-HUS, but treatment concepts can be developed following those for other autoimmune diseases. TPE will remove CFH autoantibodies and simultaneously—with plasma as replacement fluid—enhance the circulating CFH pool, which contributes to CAP regulation and reduces the antibody load via specific binding. In fact, Strobel et al. proposed using the CFH autoantibody-binding capacity of the CFHR1 C-terminus, which is almost identical to the CFH C-terminus, as a potential treatment tool in DEAP-HUS patients [29]. However, as none of these strategies are capable of blocking ongoing antibody production, a treatment strategy limiting antibody synthesis would be useful, especially after the initial acute management, to prevent recurrence.

In keeping with this concept several immunosuppressive agents have been employed and allowed termination of TPE or plasma infusions, including prednisone, intravenous immunoglobulin (IVIG), azathioprine, MMF, cyclophosphamide, and rituximab (Table 3) [7, 9, 11, 14, 15, 22, 30–32]. Cyclophosphamide was used in three DEAP-HUS patients at a dosage of 1 g/1.73 m² for five pulses in one and 0.5 g/1.73 m² for two pulses in the other two patients and resulted in a sustained reduction in CFH autoantibodies without recurrence of aHUS or the ongoing need for TPE [15]. In the large series reported by Dragon-Durey et al., plasma exchange and immunosuppression, when used in combination from disease onset, had a favorable outcome in eight of nine patients [10].

The successful use of rituximab as an antibody-depleting strategy in a plasmatherapy-dependent patient with DEAP-HUS has been reported. Dosage employed was 375 mg/m² weekly for 4 weeks, and this effected a sustained reduction in

CFH IgG autoantibodies and normal C3 for a 4-month period [14]. Rituximab was also used in another child, but there was a recurrence 3 months later despite complete depletion of CD19- and CD20-positive cells [15]. A similar experience was reported by Skerka et al. in which rituximab actually failed to lower CFH autoantibody titers and to prevent recurrence despite depleted CD19 and CD20 cells [32]. CFH antibodies disappeared in one patient but were unaffected in another two DEAP-HUS patients, as reported by Dragon-Durey et al. [10]. Why there is a variable effect on the antibody titers despite confirmed CD19 and CD20 B-cell depletion is uncertain, and close monitoring of titers is warranted. It must also be noted that an exclusive immunosuppressive strategy fails to take into account the potential contribution of a lack of the C5 convertase inhibitor CFHR1 [18, 20] or the possibility of other mutations in complement regulatory proteins that might be contributing to the pathogenesis of aHUS in some patients, as suggested by the findings of Moore et al. [22].

Ecuzumab

There is strong evidence from murine models for the pivotal role of C5 in the pathogenesis of aHUS [33]. Ecuzumab is a humanized murine monoclonal IgG that binds with high affinity to complement C5, thus preventing its cleavage and the formation of terminal complement components, including the membrane attack complex (MAC), SC5b-9 [34]. Although originally developed for treating rheumatoid arthritis and various nephritides [35], reports of its success in paroxysmal nocturnal hemoglobinuria (PNH) [36] and subsequently aHUS defined its current role [37]. Since the original report by Nuernberger [37], ecuzumab has successfully been used as rescue therapy in plasma-unresponsive aHUS [38–40] and in the peritransplant period, when it can actually negate the need for plasmatherapy and prevent or rescue posttransplant recurrence [41–44].

We observed that ecuzumab works in maintaining remission in a plasmatherapy-dependent DEAP-HUS patient over the longer term (patient 1) and, more acutely, in a patient already showing a response to plasma exchange but who had allergic reactions to FFP. Moving forward, we envisage that the role of ecuzumab in DEAP-HUS may be fourfold, namely: (i) in the acute phase, (ii) with relapses, (iii) as maintenance therapy, and (iv) in the peritransplant setting. Ecuzumab can effectively block the terminal complement cascade and arrest the damage associated with the presence of anti-CFH autoantibodies, as it does in the other forms of genetic aHUS. In DEAP-HUS, reducing the antibody load with an immunosuppressive agent likely also plays an important role. However, until a full screen for all known mutations in complement regulatory proteins associated with aHUS other than the *CFHR3/CFHR1* deletion is performed, a

Table 3 Review of the clinical management of deficiency of complement factor H-related (CFHR) proteins and CFH autoantibody-positive hemolytic uremic syndrome (DEAP-HUS) patients

Study	No. patients	Treatments employed	Recurrence	Outcome
Dragon-Durey et al. [7]	3	TPE + azathioprine (1 patient), plasma infusions + IVIG (1 patient), TPE + steroids (1 patient)	Yes; in all 3 patients	ESKD in 1 patient (after a second relapse)
Kwon et al. [14]	1	Rituximab (375 mg/m ² /week×4 weeks) pretransplantation in a known anti-CFH patient achieved sustained low antibody titres where TPE + azathioprine failed, thus allowing transplantation	No recurrence in allograft at 2 years follow up	Stable transplant
Le Quintrec et al. [11]	1	TPE for aHUS recurrence in a 5th renal transplant patient also on prednisone, MMF, and tacrolimus	Yes; post 3 previous transplants	TPE dependent
Lee et al. [30]	3	TPE, plasma infusions, prednisone, azathioprine, + IVIG (1 patient), TPE, plasma infusions + prednisone (1 patient), plasma infusions (1 patient)	Yes; in all 3 patients	ESKD in 1 patient
Abarrategui-Garrido et al. [9]	7	TPE alone (2 patients), TPE, plasma infusions + prednisone (1 patient), TPE + azathioprine (1 patient), plasmapheresis with 5 % albumin (patient died), no treatment in 2 patients who presented with ESKD		ESKD in 3 patients, 1 death (myocarditis with 1st presentation)
Boyer et al. [15]	3	TPE, plasma infusions, rituximab (relapsed postrituximab), steroids + cyclophosphamide (1 patient), TPE, plasma infusions + cyclophosphamide (1 patient), TPE, steroids, + cyclophosphamide (cyclophosphamide dose between 2 and 5 pulses of 0.5–1 g/1.73 m ²)	Yes; 1 patient	Normal renal outcome (max. follow-up 3 years in 1 patient)
Moore et al. [22]	13	TPE, biweekly TPE required in 2 patients	3 patients	ESKD 6 patients (3 of whom transplanted with no recurrence)
Strobel et al. [31]	3	TPE (all 3 patients), MMF (2 patients), rituximab (1 patient)—375 mg/m ² ×3 doses at 3 week intervals—unsuccessful, cyclophosphamide (1 patient)×3 pulses, immune adsorption (abandoned because of allergic reaction)	Yes; 1 patient	CKD 1, ESKD 1
Skerka et al. [32]	2	Plasma infusions (1 patient), TPE, MMF, + rituximab (autoantibody titer not reduced despite depleted CD19 and CD20 cells)	2 patients	ESKD in 2 patients

TPE therapeutic plasma exchange, IVIG intravenous immunoglobulin, MMF mycophenolate mofetil, aHUS atypical HUS, CFH complement factor H, CKD chronic kidney disease, ESKD end-stage kidney disease

treatment concept of blocking complement via eculizumab rather than focusing on CFH autoantibodies alone is likely safest. Also, because relapses are frequent, occurring in up to 58 % of patients and predominantly within the first 6 months after first presentation [10], continuing eculizumab therapy at least for this time period seems rational. Measuring antibody titers has been suggested as having a possible role in monitoring and long-term management of these patients [10, 45]. Where immunosuppressive agents fail to reduce antibody titers or while awaiting depletion in anti-CFH autoantibodies, eculizumab could also be employed as maintenance therapy. A careful wean off eculizumab could be considered once antibody titers are depleted, with close monitoring for relapses using hematological (hemoglobin and platelets) and biochemical (LDH and haptoglobin) parameters of hemolysis and measures of complement activation (C3 and SC5b-9), especially during intercurrent illnesses.

Abandoning a treatment concept of definite complement control must be carefully considered, in light of the risk imparted by the presence of additional—known or as yet undiscovered—aHUS causing mutations that may coexist

with the *CFHR3/CFHR1* deletion. As these mutations could trigger aHUS via a “multiple-hit” phenomenon, continued eculizumab as a definitive treatment strategy could also be justified, and discontinuing therapy in these patients could expose them to a relapse with its inherent consequences.

Monitoring treatment outcome

Measuring CFH autoantibody titers is still experimental, there is no standardized assay as yet, and the level at which these antibodies turn pathogenic in an individual are not known [46]. Issues pertaining to antibody measurement, as well as utilization of antibody titers for diagnosis, prognosis, and management, has been a longstanding problem in other autoimmune diseases, such as in systemic lupus erythematosus (SLE) [47–49], ANCA-positive vasculitis [50], and antiglomerular basement membrane (anti-GBM) antibody-mediated disease [50]. On the other hand, antiphospholipase A2 receptor antibodies (anti-PLA2R) in membranous nephropathy are not only useful in establishing the diagnosis but also in monitoring treatment response [51,

52]. Whereas CFH autoantibody titers have been suggested in recent practice guidelines to aid risk stratification for kidney transplantation in aHUS patients [53], such guidelines are still lacking for the primary disease. Monitoring response to therapy by measuring ongoing complement activity, such as CH50, APH50, SC5b9, and the C3 split product C3d, as performed in our patients (see Tables 1 and 2), remains experimental and needs to be prospectively studied and validated. Finally, a recent study suggests that measuring the circulating autoantibody–CFH immune complexes may correlate better with disease activity in DEAP-HUS [24].

Conclusion

DEAP-HUS represents a unique subgroup of complement-mediated aHUS. As the role for eculizumab in the treatment of aHUS (and STEC-HUS) becomes more clearly defined, its use in managing DEAP-HUS needs to be considered. Given the high rate of early relapse, the possible coexistence of both known and unknown complement-gene mutations, the possible pathogenic role of CFHR1 as a CAP regulator, and the experimental nature of measuring and using CFH autoantibodies to guide management, we feel that a complement-directed therapy is crucial in DEAP-HUS. We propose that eculizumab be used acutely to arrest the complement-mediated damage and that it be continued for at least 6 months considering the high rate of recurrence in DEAP-HUS during this time frame—and certainly until a full complement abnormality screen has been completed. Immunosuppressive agents targeted at B cells and ongoing antibody production can also be added. However, results of using agents such as rituximab have not been as encouraging as might have been hoped. Although limitations to measuring CFH autoantibody titers exist, antibody testing may become a valuable tool for monitoring treatment response. Thus, it may be possible to wean patients off maintenance therapy with eculizumab once antibody levels are depleted and perhaps if other markers of complement activation, such as SC5b-9, remain normal. It must be appreciated that there is currently not enough evidence to define a final treatment algorithm for DEAP-HUS, and the optimal combination of immunosuppression, antibody depletion, and complement control needs to be prospectively studied in a larger cohort of DEAP-HUS patients [54].

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