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Sensitive, reliable and easy-performed laboratory monitoring of eculizumab therapy in atypical hemolytic uremic syndrome

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ABSTRACT

Complement C5 inhibitor eculizumab treatment in atypical hemolytic uremic syndrome is effective, but associated with high costs. Complement inhibition monitoring in these patients has not been standardized. In this study we evaluated novel functional assays for application in routine follow-up.

We documented that the Wieslab® complement screen assay showed a sensitivity of 1–2% of C5 activity by adding purified C5 or normal human serum to a C5 deficient serum. All the patient samples obtained during the treatment course, were completely blocked for terminal complement pathway activity for up to four weeks after the eculizumab infusion. Levels of complexes between eculizumab and C5 were inversely correlated to the complement activity ($p = 0.01$). Moreover, titrating serum from eculizumab-treated patients into normal serum revealed that eculizumab was present in excess up to four weeks after infusion.

Thus, we demonstrate sensitive, reliable and easy-performed assays which can be used to design individual eculizumab dosage regimens.

1. Introduction

The atypical form of the hemolytic uremic syndrome (aHUS) has a poor prognosis with up to 50% of cases progressing to end stage renal disease and up to 25% of lethal outcomes in the acute phase. Complement dysregulation leading to glomerular endothelial cell damage is considered to be a central element in aHUS etiology [1,2].

The complement system, a part of the innate immune system, can be activated through three pathways: the classical (CP), the lectin (LP), and the alternative (AP) [3,4]. Currently, genetic variants in AP are identified in 50–60% of aHUS patients. Furthermore, the presence of autoantibodies against factor H or genetic variants of thrombomodulin are associated with aHUS pathogenesis [5–17].

Eculizumab (Soliris®) is a monoclonal antibody that binds to C5 and prevents its cleavage into C5a and C5b, thereby completely blocking the formation of terminal complement complex (C5b-9). It is currently approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of paroxysmal nocturnal hemoglobinuria (PNH) and aHUS and has opened a new era in the treatment of these diseases [18–23]. However, the optimal or individualized treatment schedule has not yet been established due to lack of reliable and easy routine tests to monitor the treatment.

The current therapy scheme is split into two phases: the initial phase (up to four weeks for patients with ≥40 kg body mass), followed by the maintenance phase. For pediatric patients (<18 years old and <40 kg), dosage regimen is adjusted to their body mass; the other patients receive the same amount of the drug according to the standardized adult treatment schedule. The present EMA and FDA guidelines recommend a life-long therapy with eculizumab infusions every two weeks (three weeks for the smallest infants) in the maintenance phase to prevent aHUS relapses. This recommendation has been questioned in

Abbreviations: aHUS, hemolytic uremic syndrome; CP, classical pathway; LP, lectin pathway; AP, alternative pathway; FDA, Food and Drug Administration; EMA, European Medicines Agency; PNH, paroxysmal nocturnal hemoglobinuria; C5D, C5 deficient; NHS, normal human serum; EDTA, ethylenediamineteraacetic acid; MBL, mannose-binding lectin; sC5b-9, soluble C5b-9; E–C5, eculizumab and C5 complexes.

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clinical practice, mainly due to the following concerns: a certain, though small, risk of meningococcal infection and sepsis [24,25], possible immunological complications in the long term, including development of neutralizing antibodies, and the extremely high cost of this treatment.

Although approval of eculizumab signified new hope for the aHUS patients, available reports describing pharmacokinetic and pharmacodynamics of this drug remain limited. Thus, in this study we aimed to establish a monitoring regimen for patients treated with eculizumab using novel and reliable complement assays that can be used routinely to follow the complement activity in these patients with high degree of sensitivity and specificity. This approach presents a highly relevant tool to develop individual treatment protocols for patients receiving complement inhibition therapy.

2. Materials and methods

2.1. Study population

All nine patients included in the study received eculizumab treatment at the Departments of Pediatric Nephrology and Nephrology of the Radboud University Medical Center as recommended by the current EMA and FDA guidelines. In several patients the treatment was discontinued or longer intervals between infusions were chosen as indicated.

The genetically C5 deficient (C5D) donor, included in the study as a control, has previously been described [26]. A normal human serum (NHS) pool was made using serum samples from 20 healthy adult volunteers. Purified human C5 was obtained from Quidel (San Diego, CA).

The study was approved by the institutional review board of the Radboud University Medical Center and was performed in accordance with the appropriate version of the Declaration of Helsinki. Informed consent of all patients and/or their parents as well as of the healthy volunteers was obtained before analysis.

2.2. Sample collection

Whole blood was allowed to clot at room temperature for 30–45 min (serum samples) or immediately placed on ice (ethylenediamineteraacetic acid (EDTA) plasma samples). Serum and EDTA plasma samples were prepared by centrifugation within 1 h after whole blood collection (10 min, 2500 ×*g*, 4 °C), aliquoted and stored at −80 °C. Urine samples were collected and centrifuged (10 min, 2500 ×*g*, 4 °C), supernatants were aliquoted and stored at −80 °C.

2.3. Assays to detect complement activity

The Wieslab® complement system screen (Euro Diagnostica AB, Malmö, Sweden) was used according to the manufacturer's protocol. This kit detects serum complement activity through CP, LP and AP with C5b-9 as common detection system for all three pathways. Due to the high degree of mannose-binding lectin (MBL) deficiency in the population, which gives a low LP activity, we included only the CP and AP assays of this kit in the present study. These assays were tested for sensitivity using a C5 deficient serum and were used to analyze serum samples from patients before, during and after treatment with eculizumab. In addition, serum samples from eculizumab-treated patients were diluted in NHS and analyzed in these assays.

The soluble C5b-9 (sC5b-9) levels in urine samples were measured using electroluminescent epitope assay as described before [27].

2.4. Assay to detect eculizumab–C5 complexes

The eculizumab and C5 (E–C5) complexes were detected in serum and urine samples using an enzyme-linked immunosorbent assay as described in detail previously [28]. In several cases serum samples were not available, then the E–C5 complexes were measured in EDTA plasma, as indicated in footnote of Table 2.

2.5. Statistical analysis

Statistical analyses were performed using one-way ANOVA and unpaired two-tailed t-test. Spearman's rank test was used for correlation testing.

3. Results

3.1. Characterization of the patient group

Nine patients were included in the study, eight with aHUS (P1–8) and one with STEC-HUS (P9) (Table 1). The STEC-HUS patient was diagnosed by detection of antibodies against serotype O157. This patient received a single eculizumab infusion, based on the severe clinical symptoms and non-bloody diarrhea at presentation. Six patients (P1–P5 and P9) were receiving eculizumab therapy during the time of the study, of whom four started during the study period and therefore had a baseline sample included (P1, P3, P4, P9). Therapy of three patients (P6, P7, and P8) was already discontinued and only one single sample from each of these was collected (Table 2). The dosage regimen was in general applied according to the EMA and FDA guidelines (every week initially and then every second week on maintenance), but in patients P1, P4, and P5 the intervals between the eculizumab infusions were extended late in the maintenance phase from three to six weeks. From the patients that discontinued therapy or received eculizumab treatment with extended time intervals, none suffered from aHUS recurrence.

3.2. Sensitivity of the complement activity assay to detect functionally active C5

A useful assay to monitor the complement inhibitory effect of eculizumab requires a high degree of sensitivity for C5 activity at low C5 concentrations in order to find the optimal time-point for the next infusion. Therefore, we first analyzed the sensitivity of the Wieslab® complement system screen test with respect to detection of small amounts of free and functionally active C5. For this purpose we used serum from a C5 deficient (C5D) individual, added purified C5 protein or NHS as C5 source, at increasing concentrations, and measured recovery of complement activity (Fig. 1). C5D serum supplemented with purified C5 at two-fold steps (1 μg/mL–512 μg/mL) showed recovery of complement activity, as measured both by the CP and AP assay, at levels of approximately 10 μg C5/mL serum (Fig. 1A and B). C5D serum was then supplemented with NHS (1%–100% of the total volume) and recovery activity was measured (Fig. 1C and D). Detectable recovery started already at 1–2% of added NHS in both the CP and the AP assay, indicating that both these assays are highly sensitive to detect even trace amounts of C5 present.

Table 1
Patients receiving eculizumab treatment that were included in the study.

Patient number	aHUS genetic change	Gender (F/M)	Age at time of study (years)
P1	Factor H: c.1778T > A, p.Leu593Stop	F	28
P2	Unknown	M	2
P3	Unknown	M	9
P4	Unknown	M	12
P5	Factor H: c.2572T > A, p.Trp858Arg	F	22
P6	Factor H: c.2120delC	F	44
P7	C3: c.481C > T, p.Arg161Trp	F	32
P8	Factor B: c.967A > G, p.Lys323Glu	F	21
P9[a]	Unknown	F	13

[a] STEC-HUS patient.