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Novel biomarker and easy to perform ELISA for monitoring complement inhibition in patients with atypical hemolytic uremic syndrome treated with eculizumab

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Abstract

Atypical hemolytic uremic syndrome (aHUS) is a devastating disease characterized by thrombus formation in the microvasculature and is associated with complement dysregulation. The recommended treatment is eculizumab, an humanized monoclonal antibody, which binds complement protein C5 and thereby preventing the assembly of the terminal complement complex (TCC, soluble C5b-9) and the generation of C5a, an anaphylatoxin.

The objective of the study was to identify a reliable biomarker, which estimates the degree of complement inactivation under normal and pathophysiological conditions, such as an infection.

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Disclosures

Magdalena Riedl and Therese Jungraithmayr have been on scientific advisory boards for Alexion. Reinhard Würzner has received unrestricted educational grants from Alexion and Eurodiagnostica and has attended meetings organised and financed by these companies (including royalties).

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Here we report on serial measurements of the soluble form of C5b-9, measured in plasma and the TCC capacity (soluble C5b9 after ex-vivo activation, measured in serum) in seven patients with aHUS who were treated with eculizumab. By measuring the latter we were able to assess the maximum possible soluble C5b-9 production under a potent complement trigger. Patients were followed up over a median duration of 3.8 years either on biweekly (q2w) or a three-weekly (q3w) maintenance therapy interval.

As expected, eculizumab treatment resulted in a profound decrease of TCC capacity (median 55, IQR 44–88 AU/ml) compared to baseline (1249, 529–1806 AU/ml, $p < 0.0001$) and there was no significant difference in TCC capacity measurements on q2w vs. q3w maintenance dosing schedule. However, during q3w maintenance significantly more single measurements of TCC capacity above the lower cut-off level were detected (50% on q3w vs. 5% on q2w maintenance, $p < 0.045$). Higher TCC capacity levels were associated with lower eculizumab levels. The TCC capacity may represent a novel and easy to perform parameter to determine level of complement inhibition in patients treated with eculizumab, especially because it identifies the residual capacity of inhibition at pathophysiological stages.

Keywords

Atypical hemolytic uremic syndrome; Thrombotic microangiopathies; Complement activation; Terminal complement complex; Eculizumab

1 Introduction

Atypical hemolytic uremic syndrome (aHUS) is a thrombotic microangiopathy (TMA), leading to thrombus formation in small vessels, hemolytic anemia, thrombocytopenia and renal impairment due to systemic complement activation (Riedl et al., 2014). In 60% of aHUS patients, the disease can be attributed to specific mutations in components or regulators of the alternative complement pathway, such as factor H (Richards et al., 2001), MCP (Fremeaux-Bacchi et al., 2006), factor I (Bienaime et al., 2010), factor B (Goicoechea de Jorge et al., 2007), C3 (Roumenina et al., 2012) and thrombomodulin (Delvaeye et al., 2009). Additionally, autoantibodies against factor H, predominantly in the setting of a *CFHR1/3* deletion, may also cause aHUS (Hofer et al., 2013). Specifically, these alterations result in complement activation with ongoing cleavage of C5 and subsequent excessive levels of the anaphylatoxin C5a and the potentially lytic C5b-9 or terminal complement complex (TCC) (Noris and Remuzzi, 2013). In this scenario, uncontrolled complement activation causes endothelial cell damage, inflammation and a prothrombotic state that manifests clinically as TMA (Markiewski et al., 2007; Noris and Remuzzi, 2009; Noris and Remuzzi, 2013). Recently a mutation in the *DGKε* gene, encoding diacylglycerol kinase ε, a protein involved in cell metabolism has also been linked to aHUS in infants (Lemaire et al., 2013; Bruneau et al., 2015).

Long-term prognosis is devastating with up to 77% of patients with aHUS developing end stage renal disease or death within the first 3 years after diagnosis (Noris and Remuzzi, 2009). Prognosis in aHUS is markedly improved by blocking the terminal complement pathway with eculizumab, a monoclonal anti-C5 antibody (Rother et al., 2007; Legendre et

al., 2013; Licht et al., 2015; Loirat et al., 2016). Two prospective trials and one retrospective analysis of a pediatric cohort have confirmed efficacy and safety of eculizumab in patients with aHUS (Simonetti et al., 2012; Legendre et al., 2013; Licht et al., 2015). However, despite the positive outcome with eculizumab treatment, a standard procedure for monitoring for treatment response and the efficacy of complement inhibition has not been established.

Routinely, C3 levels have been measured to assess complement activation. However, low C3 levels are only reported in about 50% of aHUS patients during active disease and are unable to assess the efficacy of eculizumab treatment (Loirat and Fremeaux-Bacchi, 2011; Cugno et al., 2014; Noris et al., 2014). Previous work by our group and others has shown that SC5b-9 levels are significantly elevated in aHUS patients during a TMA event compared to patients in clinical remission or healthy subjects (Prufer et al., 2006; Cataland et al., 2014).

In this study we measured SC5b-9 levels, the TCC capacity and eculizumab levels in aHUS patients treated with eculizumab over time. SC5b-9 reflects the non-lytic soluble C5b-9 bound to S-protein/vitronectin in the fluid phase (Hadders et al., 2012). The TCC capacity represents the maximal available SC5b-9 after ex-vivo stimulation (Prufer et al., 2006). Zymosan, a potent activator of the alternative pathway of complement (Unsworth et al., 1993), was added to patient's serum for triggering complement activation (Fig. 1).

The aim of this study was to assess whether the TCC capacity, measured by an easy to perform ELISA, is a good biomarker to predict sufficient complement blockade during eculizumab treatment even under pathophysiological conditions. Therefore we studied aHUS patients treated permanently with eculizumab with two regimens: i) on q2w (every-other-week) maintenance and ii) on q3w (every-third-week) maintenance. TCC capacity levels were compared with circulating eculizumab and eculizumab-C5 complex levels in plasma.

2 Material and methods

2.1 Study design

Since 2002 180 patients with atypical HUS, defined by the triad of thrombocytopenia, hemolytic anemia and reduced renal function have been enrolled in the international aHUS registry (www.hus-online.at). Before enrollment the patient or the guardian gave informed consent. The study was approved by the local hospital ethic commission and is according to the Helsinki Declaration of 1975.

2.2 Patient cohort

From November 2008 to April 2013 serial complement assays were performed in 7 patients with aHUS whilst they were being treated with eculizumab. Table 1 gives a detailed overview of these seven aHUS patients (unpublished, Zimmerhackl et al., 2010; Tschumi et al., 2011). Diagnostic workup included screening for CFH, CFI, MCP, C3, CFB mutations and factor H antibodies.

Six out of seven patients received plasma exchange prior to eculizumab commencement, which was stopped in all before receiving the first eculizumab infusion. The treating physician at each institution determined the dosage and treatment interval.

Meningococcal vaccination was performed in each patient and additional oral antibiotic prophylaxis (penicillin in 3 and cefuroxime in 1 patient) was given to 4 patients. No meningococcal infection was reported. eGFR was calculated with MDRD formula in adults and height-independent Pottel equation in children (Pottel et al., 2012).

2.3 Samples

SC5b-9 levels were measured in EDTA plasma, the TCC capacity in serum. Blood samples were drawn from the patients either before the first eculizumab infusion was administered (prior, baseline) or right before the next eculizumab infusion was given - to determine trough levels, either on q2w or q3w dosing interval. Serum and plasma samples were centrifuged within 1 h, sent on dry ice and stored at −20 °C (<6 months) or at −80 °C (>6 months). The number of samples collected from each patient varies from 1–58 per time period (prior, q2w, q3w). Ninety-eight healthy adult blood donors (without an apparent infection) were used as controls. The median age of the controls was 37 years (IQR: 28–50 years), 46% were male. Data is given as median and interquartile range (IQR), unless indicated otherwise.

2.4 SC5b-9 ELISA and TCC capacity

To determine TCC capacity (maximal possible formation of SC5b-9) 10% baker's yeast (zymosan) solution (Unsworth et al., 1993) was added to serum and incubated over night in a 37 °C warm waterbath (Fig. 1). SC5b-9 and TCC capacity were determined by using a sandwich ELISA based on a neoepitope-specific anti C9 antibody (WU13-15, Hycult Biotech Inc., The Netherlands), in detail described elsewhere (Prufer et al., 2006). In short, ELISA plates were coated overnight with WU13-15, samples were added the next day. A biotinylated C6 antibody was used as a secondary antibody, followed by Streptavidin alkaline phosphatase (Sigma-Aldrich, Vienna, Austria) and p-Nitrophenyl phosphate (Sigma-Aldrich) as a substrate. Absorbance was measured at 405 nm (reference wave length 490 nm). Pooled zymosan activated serum was used as standard curve. AU/ml was calculated using a linear regression curve and log/lin axis scales. A 1/100 dilution of the standard curve was set as 10 AU/ml. Plasma samples were diluted 1:4 and TCC capacity samples 1:100 (trough level)–1:400 (baseline and controls). The concentration of patient samples was calculated using Microsoft Excel.

2.5 Eculizumab levels and eculizumab-C5 complexes

The eculizumab concentration in the serum samples was measured by an ELISA specifically developed for this purpose. To capture eculizumab, the 96-well plates were coated with purified 0.5 μg/well of purified C5 (Calbiochem®, San Diego, CA, USA) diluted in carbonate buffer (pH 9.6) overnight at 4 °C. Serum samples have been diluted in phosphate buffered saline, supplemented with 1% bovine serum albumin (PBS/BSA). Eculizumab detection has been performed using horse radish peroxidase-conjugated goat-antihuman IgG (JacksonImmunoResearch Laboratories, Inc., West Grove, PA, USA). A standard was produced by adding eculizumab (Alexion Pharmaceuticals, Cheshire, CT, USA) to

PBS/BSA in two-fold dilution steps to create a range of 0.8–50 ng/ml. Eculizumab-C5 complexes were detected as described elsewhere (Hallstensen et al., 2015).

2.6 Statistics

Statistical analysis was performed using Mann–Whitney-*U* test and Kruskal-Wallis as indicated. The values used for q2w and q3w maintenance were taken 1 month after. Graphpad Prism 6 was used for data analysis and for preparation of figures. A p-value < 0.05 was considered statistically significant.

2.7 Determination of cut-off level for TCC capacity

The reference interval for TCC capacity was calculated using IBM SPSS software version 21 as follows (Lumsden and Mullen, 1978; Zurakowski et al., 1999; Katayev et al., 2010): The IQR of TCC capacity in controls was calculated and the upper (75th percentile + (1.5 × IQR)) and lower (25th percentile −(1.5 × IQR)) limits were determined. All values above or below these percentiles (n = 3) were considered as outliers and removed. As TCC capacity did not show a normal distribution (Kolmogorov-Smirnov $p < 0.0001$), a natural log-transformation was applied before calculating the 2.5th percentile to determine the lower cut-off level for TCC capacity in controls. Values were converted back to the original units by calculating the antilog of the logarithmically transformed values. The lower cut-off level for TCC capacity was calculated as 150 AU/ml.

3 Results

3.1 No difference of SC5b-9 levels under eculizumab therapy

Prior to receiving eculizumab patients showed an elevated SC5b-9 concentration compared to controls (2.5, IQR 1.9–3.9 AU/ml vs. 1.2, IQR 0.8–1.8 AU/ml; $p < 0.02$). At this time point, 6/7 patients received plasma exchange. SC5b-9 levels remained unchanged upon transition from plasma exchange to eculizumab q2w and q3w maintenance therapy (2.3, IQR 1.6–3.8 vs. 2.3, IQR 1–16.3, n.s.).

3.2 Terminal complement complex (TCC) capacity decreases under eculizumab therapy

At baseline, prior to the first eculizumab infusion, the TCC capacity showed a median level of 1249 AU/ml (IQR 529–1806 AU/ml) compared to healthy controls with a median of 505 AU/ml (IQR 290–856 AU/ml; $p = 0.09$). A significant decline ($p < 0.0001$) was observed under eculizumab treatment, with a median of 55 AU/ml (44–88 AU/ml) during biweekly administration, indicating inhibition of terminal complement activation (Fig. 2A). This was observed as early as after the first infusion of eculizumab.

After treatment extension to a three-week maintenance schedule (q3w) an overall trend to an increase of the TCC capacity was observed (209 AU/ml, IQR 106–510 AU/ml, $p = 0.06$) (Fig. 2A). Furthermore, a significant higher number of single measurements above the cut-off level (150 AU/ml, 2.5th percentile of healthy controls) was detected in patients with the q3w dosing regime (50%, IQR 26–77% on q3w vs. 5%, IQR 0–12% on q2w; $p = 0.02$, Mann–Whitney-*U*-test) (Fig. 3). This was seen in patient #1 and #2 (detailed in Fig. 2B and

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C), but also #4 and #6 already after the first three-week interval and in patient #7 three months later with an intercurrent illness (not detailed).

3.3 Eculizumab levels decrease over time on q3w dosing

In 3 patients we measured eculizumab levels and eculizumab-C5 complexes in addition to the TCC capacity. Eculizumab levels and eculizumab-C5 complexes decreased over time when patients were on q3w dosing (Fig. 4, Table 2). In patient #6 we had samples available before the 1st and 2nd q3w dosage. Increased TCC capacity was seen at 3 weeks, prior to the 1st dosage and then again 2 weeks later. Higher TCC capacity values were associated with lower eculizumab levels and eculizumab-C5 complexes (Fig. 4A). In one patient with high TCC capacity and low eculizumab levels (the same patient #6) we were able to detect lysis of rabbit erythrocytes, indicating that the non-inhibited C5b-9 can be inserted in cell membranes and induce cell damage (Fig. 4B).

3.4 One aHUS patients develops a TMA episode under q3w dosing

Seven months after commencing eculizumab treatment patient #2 was switched to a three weeks interval, which resulted in an immediate increase of TCC capacity (from median 63 AU/ml at q2w to 297 AU/ml, maximum 642 AU/ml), indicating less complement inhibition at day 21, prior to next eculizumab infusion (Fig. 2B). Twenty-eight months later the girl suffered from a TMA event, triggered by an upper respiratory tract infection. Platelets decreased to 108×10^9/l, LDH was elevated and eGFR decreased from 52 to 33 ml/min/1.73 m^2. A re-induction and a q2w administration of eculizumab as maintenance therapy (TCC capacity 78 AU/ml) were able to normalize platelet counts (218×10^9/l) and increase eGFR to 56 ml/min/1.73 m^2.

4 Discussion

Over the last years, eculizumab became the recommended treatment of choice for most patients with aHUS (Loirat et al., 2016). There is an increasing need to quantify the level of complement inhibition in patients treated with eculizumab. This is the first analysis studying the TCC capacity – in contrast to SC5b-9 - to determine level of treatment induced C5 inhibition.

As eculizumab blocks the generation of the terminal pathway, detecting SC5b-9 seemed to be an ideal marker for disease monitoring. However, in this study we were not able to see a difference in plasma levels of SC5b-9 during treatment with eculizumab. This is in line with other reports, which were not able to show that treatment with eculizumab alters the SC5b-9 levels (Mache et al., 2009; Davin et al., 2010; Weitz et al., 2011; Radhakrishnan et al., 2012; Noris et al., 2014). Complement inhibition resulted in a profound decrease of the TCC capacity in all patients during eculizumab treatment (q2w maintenance). We thus analyzed complement activity by determining the TCC capacity in patients receiving eculizumab via a q2w or q3w dosing interval.

A q2w maintenance dosing schedule was described as sufficient for blocking terminal complement activation (Legendre et al., 2013), and delayed next dosing was associated with hemolysis and deterioration of renal function in one patient (Chatelet et al., 2010). Earlier

reports already indicated that termination of treatment can lead to TMA recurrence (Nurnberger et al., 2009; Legendre et al., 2013). This is not unexpected as the elimination half-life of eculizumab was reported as 11 ± 3 days and in a pediatric cohort as 14.5 days. (Rother et al., 2007; Greenbaum et al., 2016). In paroxysmal nocturnal hemoglobinuria (PNH), another complement disorder, it was recommended that eculizumab dosing interval should not be expanded beyond 17 days (Nakayama et al., 2016). TCC capacity levels of patients on q3w maintenance therapy were more frequently above the lower cut-off level of healthy controls than patients on q2w maintenance. Some levels were as high as levels detected in healthy controls, indicating lack of full terminal complement inhibition over the course of q3w dosing. The elevated TCC capacity was associated with clinical TMA or possible ongoing renal damage. One patient demonstrating higher TCC capacity levels had a TMA recurrence. Three additional patients showed a decline or persistently impaired eGFR even after initial eGFR improvement when maintenance therapy was switched from q2w to q3w, the latter also being associated with elevated TCC capacity levels. Furthermore, we were able to show that high TCC capacity levels are associated with low circulating eculizumab concentration and reduced eculizumab-C5 complexes.

Other complement assays to determine complement activation have been studied in patients with aHUS on eculizumab treatment. Using more global functional assays, such as APH50 and CH50 or the Complement system Screen WIESLAB, others reported an absence of total complement activity of classical and alternative pathway after eculizumab administration (Zuber et al., 2012; Cugno et al., 2014; Volokhina et al., 2015). Volokhina and co-workers reported a complete complement blockade for up to 4 weeks after the last eculizumab dosage. In their study eculizumab-C5 complexes decreased only when dosage interval extended 5 weeks (Volokhina et al., 2015). Compared to our study they followed their patients up to dose 16 (<12 months) and discontinued then and some of the eculizumab-C5 complexes were measured in serum. We however, have a follow-up of patients on chronic q3w dosing for up to 40 months. Noris and colleagues found CH50 not helpful for eculizumab monitoring and employed C5b-9 deposition on endothelial cells as monitoring tool (Noris et al., 2014). However, the latter test needs confocal microscopy and is not an easy assay to perform.

The assay we propose here, TCC capacity, however is easy to perform, as it is ELISA based. Additionally it reflects TCC levels after maximal stimulation, mimicking a patient on eculizumab experiencing a complement triggering condition, like an infection. Infections are known to trigger aHUS, possibly via induction of complement activation (Loirat and Fremeaux-Bacchi, 2011; Fremeaux-Bacchi et al., 2013). One patient in our cohort with incomplete complement inhibition suffered from a TMA episode following an infection. During q2w maintenance despite being challenged with intercurrent illness and infections, no TMA episode/decline in renal function was observed in our cohort or reported in literature (except DGKε – associated HUS) (Lemaire et al., 2013; Loirat et al., 2016).

Eculizumab therapy has clearly improved long-term outcome in patients with aHUS. Up to now, no reliable and comprehensively studied tool to monitor complement blockade in this patient cohort is available. Although only measured in a small cohort, the TCC capacity might be a good parameter to determine grade of complement inhibition in these patients,

especially if patients experience a complement triggering event such as an infection. Further studies are needed to address the question to which extent the terminal pathway has to be blocked to prevent ongoing terminal pathway activation and subsequent TMA and to compare the available tools.

In conclusion, therapeutic complement inhibition can be determined by measuring the novel biomarker TCC capacity by an easy to perform ELISA, which can indicate successful complement inhibition even in pathophysiological conditions. The cost of eculizumab has led to several patients receiving the drug in off label extended intervals. Our study indicates that in some patients with sufficient complement inhibition even under triggering conditions treatment intervals can be delayed and money saved. Other patients, however, could suffer from these too optimistically extended intervals and are then at risk for ongoing organ damage, a sudden TMA episode or progressive decline in renal function. This state of being “at risk”, however, can be assessed, as in this case complement inhibition would not be sufficient when tested for the TCC capacity.

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Glossary

aHUS	atypical hemolytic uremic syndrome
APH50	Alternative complement activity
CFB	Complement factor B
CFH	Complement factor H
CFI	Complement factor I
CH50	Total complement activity
CKD	Chronic kidney disease
DGKε	Diacylglycerol kinase ε
eGFR	Estimated Glomerular Filtration Rate
LDH	Lactate dehydrogenase

q2w	Dosing every 2 weeks
q3w	Dosing every 3 weeks
SC5b-9	C5b-9 bound to S protein
SCR	Short consensus repeat
TCC	Terminal complement complex
TMA	Thrombotic microangiopathy

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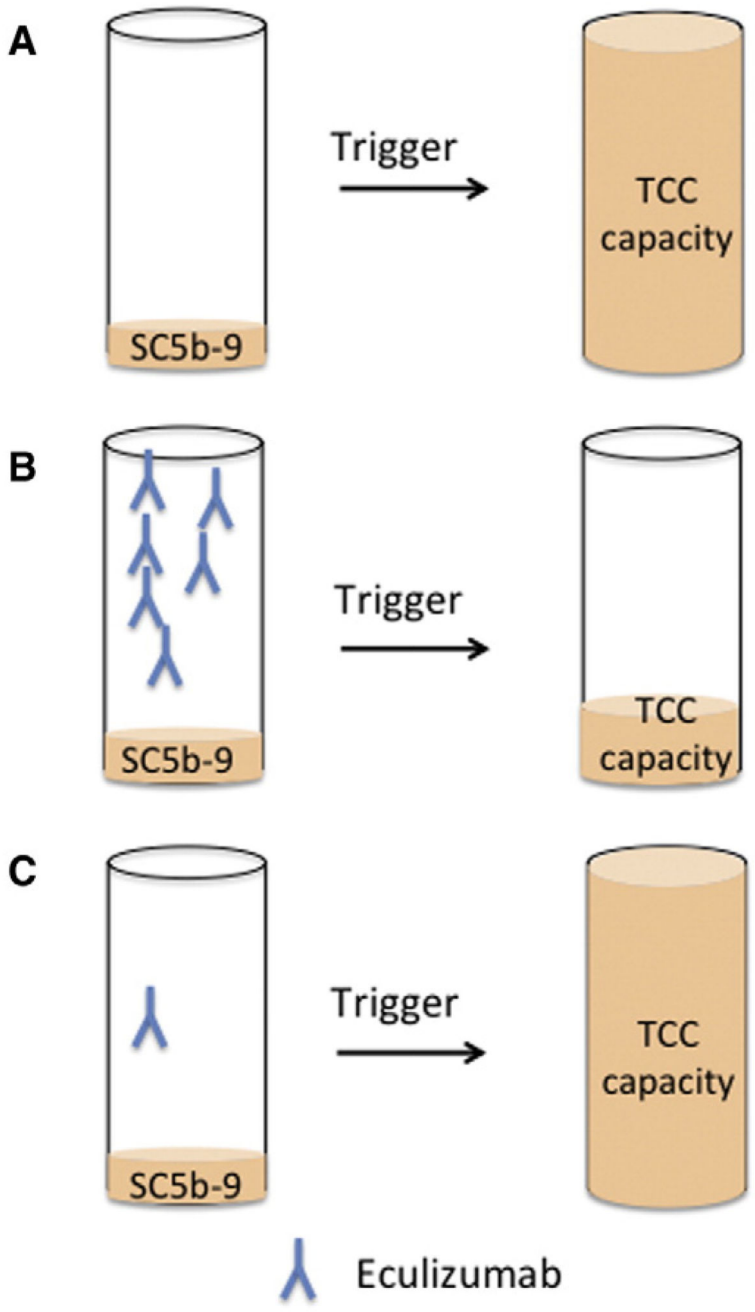

Fig. 1.

Principles of TCC capacity measurement with and without eculizumab therapy. Zymosan activates the alternative complement pathway and uses all available C5 (and other complement proteins) to form SC5b-9. Eculizumab binds to C5 and therefore prevents the formation of SC5b-9. Only free C5 can be activated and will contribute to SC5b-9 levels. (A) High TCC capacity values are expected when patient is not treated with eculizumab. (B) Very low levels of TCC capacity are expected when eculizumab blockade is sufficient. (C) When the eculizumab concentration is at its lower level, the strong in vitro trigger will lead

to an increase of TCC capacity, i.e. the patient is at potential risk in case of an infection, despite a controlled low SC5b-9 concentrations in the native sample.

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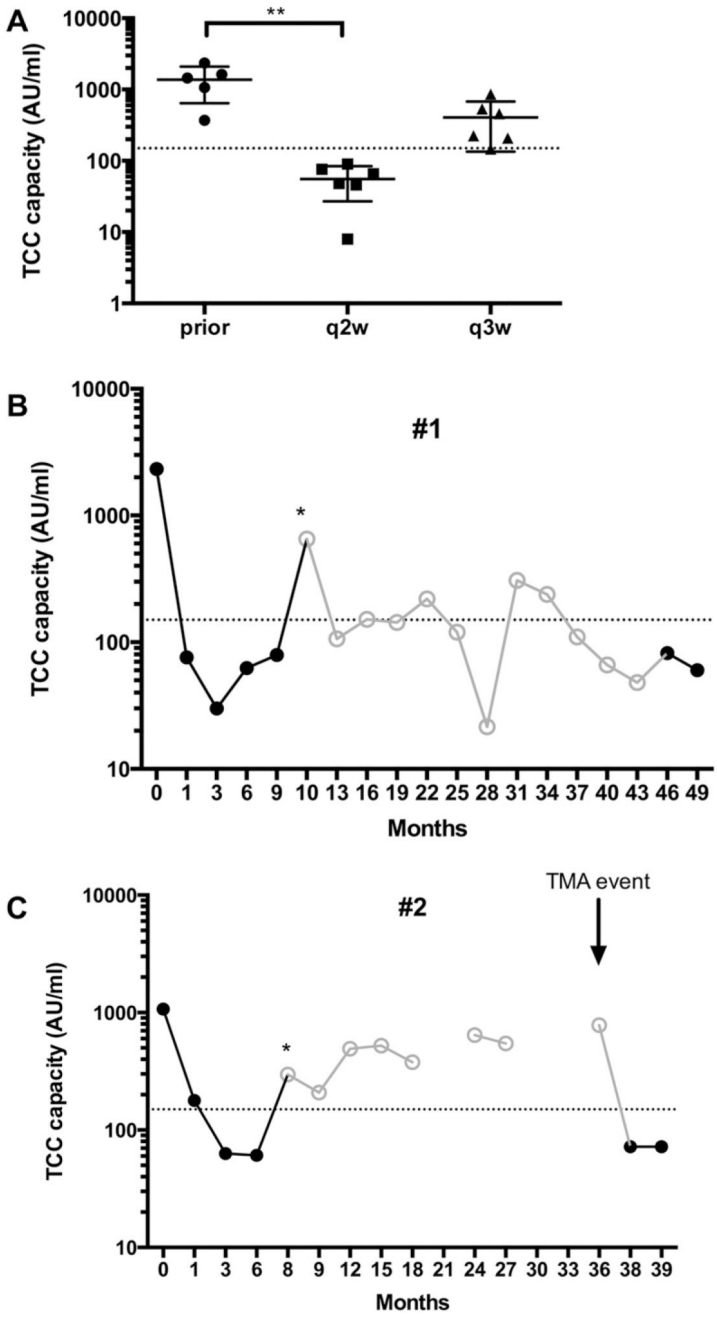

Fig. 2.

TCC capacity levels decreases under eculizumab therapy. (A) TCC capacity levels (logarithmic scale) are displayed for patients at different time points: prior eculizumab, on q2w maintenance and q3w maintenance therapy. A significant decrease compared to TCC levels prior eculizumab therapy was observed for q2w maintenance levels. **$p < 0.05$ (Kruskal-Wallis). (B) TCC capacity levels over time in patient #1. (C) TCC capacity levels over time in patient #2. Open dots/line in grey indicate q3w maintenance interval. Dotted line indicates lower cut-off level of 150 AU/ml. Asterisk indicates switch to q3w

maintenance. q2w, maintenance dosing biweekly; q3w, maintenance dosing every three weeks.

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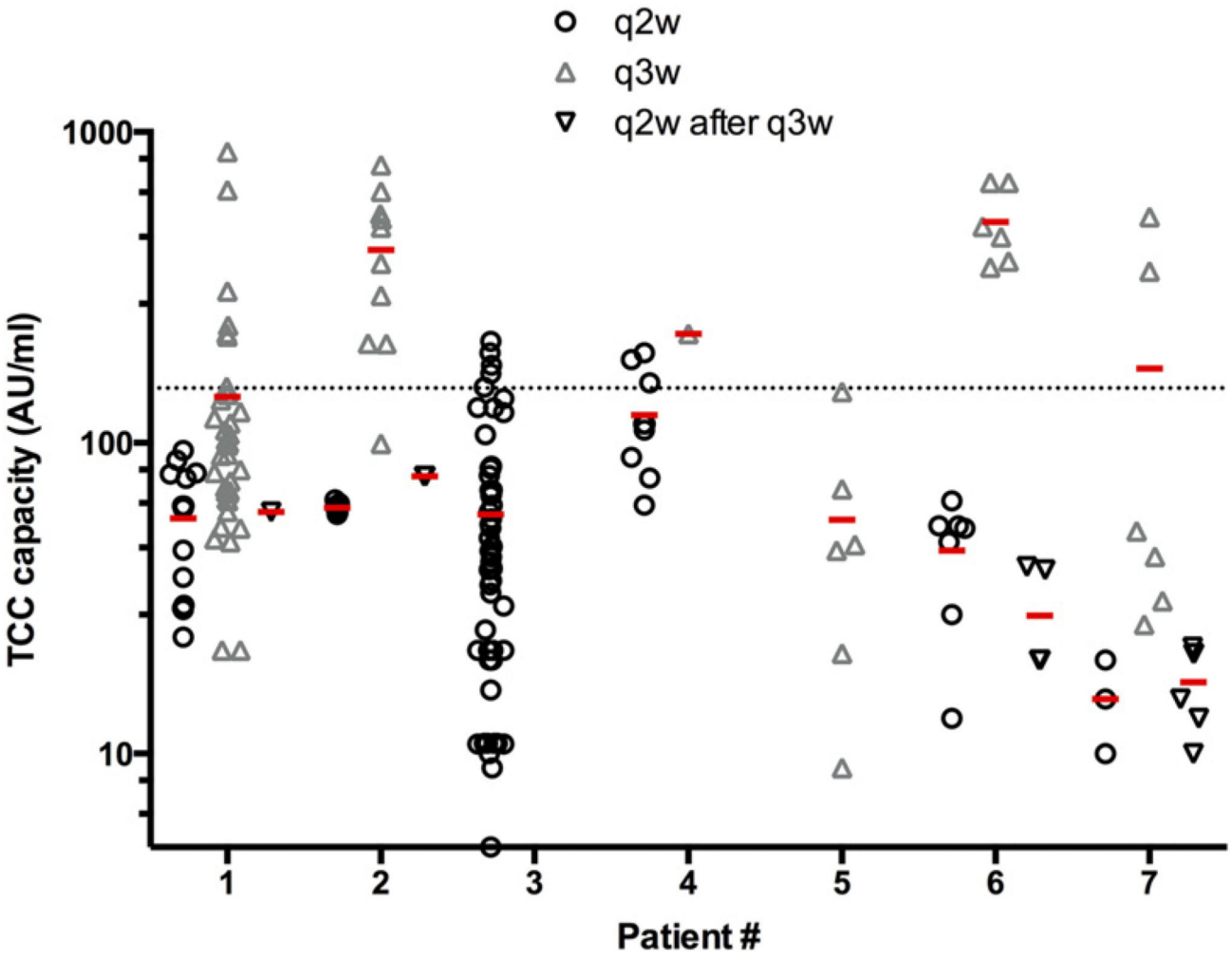

Fig. 3.

Significant more single measurements above the cut-off level when on q3w maintenance therapy. For each patient (x-axis) single measurements under q2w maintenance (black circle), q3w maintenance (grey triangle) and after re-intensification (q2w after q3w maintenance, black triangle) are displayed on the y-axis (logarithmic scale). Significant more values above the cut-off level of 150 AU/ml (dotted line) were observed under q3w maintenance. The red line indicates the mean. q2w, maintenance dosing biweekly; q3w, maintenance dosing every three weeks.

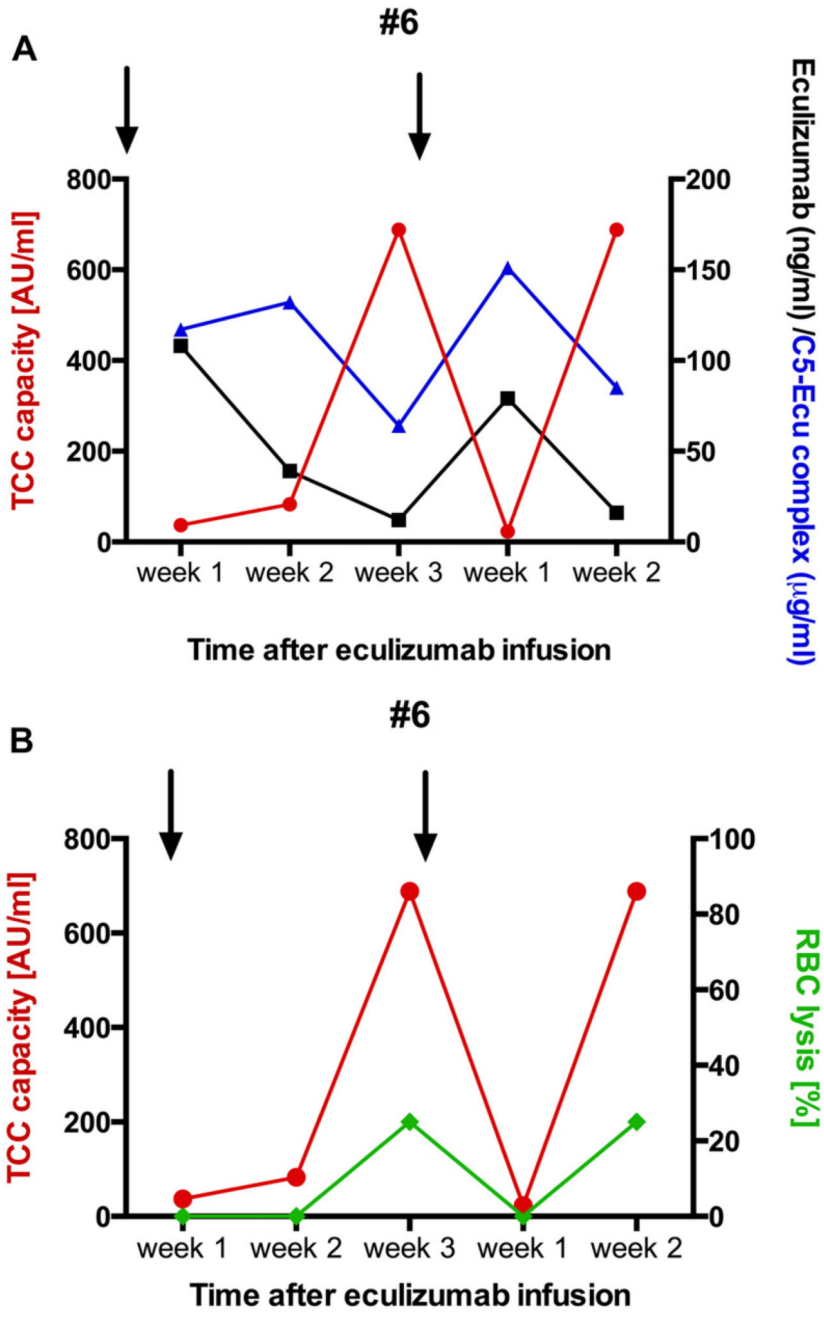

Fig. 4.

TCC capacity, eculizumab and E-C5 complexes in a patient after transition to q3w. (A) TCC capacity (red, left x-axis), eculizumab (black, right x-axis) and eculizumab-C5 complexes (blue, right x-axis) were measured in patient #6 for the first 5 week after treatment extension to q3w. TCC capacity levels are high 3 weeks and 2 weeks after eculizumab infusion, respectively. Concomitantly eculizumab levels and E-C5 complexes decreased. Arrows

indicated eculizumab infusion. (B) High TCC capacity levels are associated with lysis of rabbit RBCs, indicating that serum samples can form C5b-9 complexes to lyse RBCs.

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Table 1

Overview of patient cohort.

	Age at disease onset	Age at eculizumab therapy onset	Genetics	PE before eculizumab	Dosing of eculizumab
#1	4 years (Zimmerhackl et al., 2010)	10 years (Nov 08)	CFH mutation (SCR20)	10 days	900 mg once 600 mg q2w/q3w
#2	1.2 years	4 years (June 09)	CFH mutation (SCR20)	Chronic PE treatment	600 mg once 300 mg q2w/qw3w
#3	25 years	25 years (June 09)	CFH mutation (SCR20)	Intensive PE treatment (for 1 month)	900 mg induction 1200 mg q2w
#4	7.5 years (Tschumi et al., 2011)	8.5 years (June 09)	CFH mutation (SCR10)	Chronic PE treatment (3 ×/ week)	600 mg q2w/q3w
#5	1.5 years	2 years (May 10)	Polymorphism CFH	Chronic PE treatment (12 in total)	600 mg for induction 300 mg q2w/q3w
#6	32 years	33 years (April 11)	Several polymorphisms in C3, CFB, CFH and CFI	Daily PE treatment	900 mg induction 1200 mg q2w/q3w
#7	22 years	31 years (Jan 12)	C3 mutation	No	900 mg induction 1200 mg q2w/q3w

CFB, complement factor B; CFH, complement factor H; CFI, complement factor I; q2w, maintenance dosing biweekly; q3w, maintenance dosing every three weeks; PE, plasma exchange; SCR, short consensus repeats.

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Table 2

TCC capacity, eculizumab levels and E-C5 complexes in two patients.

	TCC capacity (AU/ml)	E level (ng/ml)	E-C5 complex (μg/ml)	Interval	Time
#1	30	547	166	q2w	3.5 months after start
	651	622	168	q3w	Prior 2nd dose on q3w
	137	116.5	119	q3w	17 months on q3w
	307	68	90	q3w	30 months on q3w
#2	65.5	161	143	q2w	4 weeks after start
	297	39	100	q3w	Prior 1st dose on q3w
	524	19.5	85	q3w	6 months on q3w, infection
	78	89	93	q2w	2 months on q2w, 2 months after TMA event

q2w, maintenance dosing biweekly; q3w, maintenance dosing every three weeks; E, eculizumab;