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Laboratory findings in patients treated with complement factor C3 inhibitor pegcetacoplan

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Abstract

Objectives: Complement inhibitor pegcetacoplan binds to C3 and its activation product C3b. Pegcetacoplan has been approved for the treatment of paroxysmal nocturnal hemoglobinuria. Because pegcetacoplan exerts broad inhibition of the complement cascade its efficacy is also investigated in numerous other diseases caused by complement dysregulation, such as C3 glomerulopathy. Pegcetacoplan causes a number of counterintuitive changes in laboratory results.

Methods: In-depth complement analysis in two patients with PNH and three patients with C3 glomerulopathy, all treated with pegcetacoplan.

Results: C3 levels increase up to 300 % above reference levels. *In vitro* testing showed that this is not a turbidimetric artifact in the C3 immunoassay due to pegcetacoplan-C3 complex formation but appears to be caused by increased half-life of C3 bound to pegcetacoplan. Unbiased mass spectrometric plasma proteome analysis confirmed the dramatic pegcetacoplan-induced increase in circulating C3. Surprisingly, also a three-fold increase of properdin was observed during pegcetacoplan treatment. Serum protein electrophoresis showed an additional band in all patients after pegcetacoplan exposure. This C3-band does not migrate at its expected position because of changes in the mass and charge of C3 bound to pegcetacoplan and should therefore not be misinterpreted as an M-protein. Both *in vitro* experiments and real clinical practice laboratory results demonstrated that pegcetacoplan completely blocked the alternative complement pathway while the classical pathway is affected but remains largely intact.

Conclusions: Because of the increasing use of pegcetacoplan in routine clinical practice, it is important that both clinicians and laboratory specialists are aware of these unexpected therapy-induced laboratory findings.

Keywords: complement inhibition; pegcetacoplan; complement factor C3; properdin; paroxysmal nocturnal hemoglobinuria; C3 glomerulopathy

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Introduction

The complement system is a fundamental component of the innate immune response, comprising a cascade of proteins that, when activated, contribute to pathogen elimination and immune modulation [1]. While essential for host defense, dysregulation or overactivation of the complement system is implicated in a range of pathological conditions [2]. In particular, aberrant activation of the complement cascade plays a central role in the pathogenesis of diseases such as paroxysmal nocturnal hemoglobinuria (PNH), C3 glomerulopathy (C3G), and geographic atrophy secondary to age-related macular degeneration.

Pegcetacoplan is a novel complement inhibitor that targets C3, the central component of all three complement pathways – classical, lectin, and alternative [3]. By binding to

C3 and C3b, pegcetacoplan inhibits the formation of C3 convertases, thereby blocking downstream complement activation. This broad inhibition distinguishes pegcetacoplan from terminal pathway inhibitors such as eculizumab, which acts at the level of C5.

Initially approved for the treatment of PNH in patients who remain anemic despite C5 inhibition, pegcetacoplan has demonstrated efficacy in controlling both intravascular and extravascular hemolysis, addressing that limitation of previous therapies [4]. The approval was based on pivotal trials such as the PEGASUS study, which showed superiority in hemoglobin stabilization and transfusion avoidance compared to eculizumab. More recently, pegcetacoplan has also shown promise in other complement-mediated diseases, including C3G [5] and geographic atrophy [6, 7] where upstream complement blockade may offer therapeutic advantages.

Laboratory findings associated with pegcetacoplan therapy reflect its mechanism of action and clinical impact. In PNH, treatment typically results in increased hemoglobin levels, reduced lactate dehydrogenase, and decreased need for transfusions [8]. Preliminary results of one year pegcetacoplan treatment in C3G (phase 3 VALIANT trial; NCT05067127) indicate a reduction in proteinuria, stabilization of eGFR and decrease in C3 staining in kidney biopsies (presented by dr. Fakhouri at the European Renal Association Congress 2025).

This case-series highlights the impact of pegcetacoplan on laboratory findings of complement testing and possible interferences in other assays.

Materials and methods

Patients

All blood samples were obtained in routine diagnostics from patients who were treated with pegcetacoplan either with an approved indication or off-label, outside the context of a clinical trial. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all individuals included in this study.

Complement factor C3 and C4 levels

The C3 and C4 concentrations were determined in serum by turbidimetry (Cobas 8000 platform, F. Hoffman-La Roche Ltd, Basel, Switzerland). Reference values for C3 are 700–1,500 mg/L and C4 100–400 mg/L.

Classical and alternative pathway activity assay

Classical pathway (CP) and alternative pathway (AP) complement activity analyses were performed as previously described by our group [9] and based on Fredrikson et al. [10] and Seelen et al. [11]. For the CP analysis, a Nunc MaxiSorp ELISA plate was coated with 200 µL 3.1 µg/mL rabbit anti-human IgM (Q0333; Dako Agilent, Santa Clara, CA, USA) in 0.05 m carbonate buffer (pH 9.6) and incubated overnight at 4 °C. For the AP analysis, a Nunc MaxiSorp ELISA plate was coated with 8 µg/mL *Salmonella typhosa* LPS (Sigma-Aldrich, St. Louis, MO, USA) (AP) in 120 µL PBS by drying for 2 days at 37 °C. The ELISA plate was blocked for 1 h with 1 % gelatin in Tris-buffered saline (TBS) and only in case of the CP analysis, followed by incubation with 100 µL 4.5 µg/mL human IgM (31-A135; Fitzgerald-fii, Biosynth, UK). Plates were washed 3× with wash buffer (0.05 % Tween 20 in TBS) using a microplate washer (Tecan, Durham, NC, USA). Serum was diluted several times, 50×/75×/150×/300× (CP) in dilution buffer (10 mm Tris, 84 mm NaCl, 0.5 mm $MgCl_2$, 2 mm $CaCl_2$, 0.1 % gelatin) or 5×/7.5×/10×/16×/20× (AP) in dilution buffer (10 mm Tris, 140 mm NaCl, 5 mm $MgCl_2$, 10 mm EGTA, 0.1 % gelatin), added to the wells in duplicate, and incubated for 60 min (CP) or 75 min (AP) at 37 °C. Plates were washed 3× with wash buffer. Mouse aE11 monoclonal anti-C5b-9 (M0777; Dako) was diluted 300× (AP) and 500× (CP) in wash buffer, added to the wells and incubated 1 h at room temperature while shaking at 600 rpm, followed by washing 3× with wash buffer. Alkaline phosphatase-labelled goat anti-mouse IgG (610-1519 Rockland Pottstown, PA USA) was diluted 3,000× (AP) and 5,000× (CP) in wash buffer, added to the wells and incubated 1 h at room temperature while shaking at 600 rpm, followed by washing 3× with wash buffer. P-nitrophenylphosphate (1 mg/mL) in substrate buffer (1 m diethanolamine, 0.5 mm $MgCl_2$ pH 9.8) was added, incubated at 37 °C and the reaction was stopped after 15–20 min by adding 2 m NaOH. The absorbance was measured at 405 nm using a 96-well plate reader (Tecan). Pooled human serum was used as control. Normal reference values for AP are 67–133 %, and normal reference values for CP are 67–149 %.

C3d and C3bBbP measurements

The level of C3d was quantified in EDTA plasma using an ELISA, as previously described [12, 13]. To determine C3d values, pooled activated EDTA plasma of healthy blood donors was used and set to 100 %. This value was calibrated to mg/L using purified C3d containing 10 µg/mL protein [14].

The reference value was determined in a group of 20 healthy volunteers (mean age ± SD; 45.8 ± 11.4). The average C3d value in this group was 4.2 mg/L (SD=1.4) and the C3d upper reference value was set at <8.3 mg/L (average plus 3×SD). As the initial C3 concentration may influence the C3d level and because of intra-individual variations in the concentration of C3, the C3d/C3 ratio is used as the parameter for complement activation. In the same cohort of 20 healthy controls the average C3 concentration was 936 mg/L (SD=181). The average C3d/C3 value 4.7 (SD=1.1) and the C3d/C3 upper reference value was set at <8.1 (average plus 3×SD). The complement activation marker C3bBbP (properdin-stabilized C3 convertase complex) was measured in EDTA-plasma by ELISA based on the protocol described previously [15]. The C3bBbP reference value applicable in diagnostics is <26 CAU/mL (average plus 2xSD of 23 healthy controls).

Mass spectrometry-based protein quantitation

EDTA plasma samples were digested using trypsin as described previously with minor modifications [16]. Samples were analyzed by LC-MS/MS in duplicate. Per measurement an equivalent of ~150 ng total protein was loaded per manufacturer's instructions. Peptides were loaded on an Evosep One liquid chromatography (LC) system using C18 Evotips (Evosep, Odense, Denmark). Peptide separation was performed on a 15 cm C18 column (Evosep Performance, ReproSil-Pur C18, 150 µm I.D., 1.5 µm particle size) using the Evosep 30SPD method. The eluted peptides were analyzed on a timsTOF Pro 2 mass spectrometer (MS) (Bruker Daltonics, Bremen, Germany) operated in the standard data independent acquisition Parallel Accumulation-Serial Fragmentation (dia-PASEF) mode for long gradients. Protein identification and quantification analysis was done with Bruker Proteoscape (BPS, version 2025b, Bruker Daltonics) using TIMS dia-NN [17]. An in-house generated spectral library for plasma proteins was used, and identifications were re-annotated against the canonical Uniprot human protein database (downloaded on 16-8-2024) plus sequences of known contaminants such as keratin and porcine trypsin. The spectral library was generated using LC-MS analyses of a set of 20 healthy subject plasma samples, and of available recombinant complement proteins (CFB, CFD, CFH, CFI, CFP, C2, C3, C4, C5, C6, C7, C8, C9, CFHR1,2,4,5). These samples were analyzed using an identical instrument setup and LC method, but the MS was operated in data dependent data acquisition mode (dda-PASEF). Database searches to identify peptides were performed using ProLuCID in BPS using carbamidomethylation of cysteine as a fixed modification and methionine oxidation as a variable modification. Precursor and fragment mass tolerance was set to 20 and 15 ppm, respectively. The spectral library was generated by combining search results of these dda-PASEF analyses resulting in a spectral library of 11,641 precursors. dia-NN settings for data processing were: 20 ppm precursor tolerance, 15 ppm fragment ion tolerance, carbamidomethylation as fixed modification, oxidation of Methionine as variable modification, maxLFQ intensities were calculated for relative protein quantitation. Match-between-run (MBR) was performed across all measurements to fill-in missing values with an outlier frequency of 0.2, protein grouping was performed on the protein name level, global normalization was performed. MaxLFQ intensities were used to calculate the fold change in plasma protein levels of a patient compared to the sample before treatment.

Results

Case descriptions

To illustrate the impact of pegcetacoplan treatment on routine laboratory test results, this case-series describes five patients who initiated therapy at our academic center in 2024 or 2025. Table 1 provides an overview of the patient characteristics. Two patients were diagnosed with PNH who initially responded well to eculizumab. Both patients remained anemic despite C5 inhibition and were switched to pegcetacoplan because of persisting anemia caused by C3-mediated extravascular hemolysis.

Three patients received off label use of pegcetacoplan because of treatment resistant dense deposit disease, which is a subtype of C3G. As outlined in Table 1, prior to pegcetacoplan these patients received two to four other lines of treatment.

Laboratory findings

Patients with increased complement activation generally have a low C3 secondary to complement consumption. In response to successful treatment with complement inhibitors, the C3 levels normalize. In all patients who started pegcetacoplan, we indeed observed a fast increase in C3 levels. Strikingly, in all patients, C3 concentrations increased to values far above the reference values (Figure 1). For patients 2 and 4, frequent C3 monitoring was performed in the first months of pegcetacoplan treatment. Based on the slope of C3 levels in these two patients, we conclude that the C3

Table 1: Patient characteristics.

Patient	Diagnosis	Treatment history
1. Female, 77 years	Paroxysmal nocturnal hemoglobinuria	1st line: eculizumab for 12 years, stopped because of anemia due to extravascular hemolysis; 2nd line: pegcetacoplan.
2. Female, 14 years	C3 glomerulopathy (dense deposit disease)	1st line: prednisone; 2nd line: mycophenolate mofetil, prednisone; 3rd line: rituximab; 4th line: pegcetacoplan (which was stopped after 8 months because of decline in kidney function); 5th line: methylprednisolone and dialysis.
3. Female, 45 years	Paroxysmal nocturnal hemoglobinuria	1st line: eculizumab for 6 years, stopped because of anemia due to extravascular hemolysis; 2nd line: pegcetacoplan. Switched to 3rd line ravulizumab after 1 year because of progressive cytopenia.
4. Female, 35 years	C3 glomerulopathy (dense deposit disease)	1st line: mycophenolate mofetil, prednisolone, cyclophosphamide. 2nd line: mycophenolate mofetil, prednisone; 3rd line: tacrolimus, prednisolone; 4th line: anti-factor D, prednisone. 5th line: pegcetacoplan.
5. Male, 44 years	C3 glomerulopathy (dense deposit disease) after two kidney transplantations	1st kidney transplant: mycophenolate mofetil, tacrolimus; 2nd kidney transplant: 1st line mycophenolate mofetil, tacrolimus and prednisolone; 2nd line: pegcetacoplan (add-on).

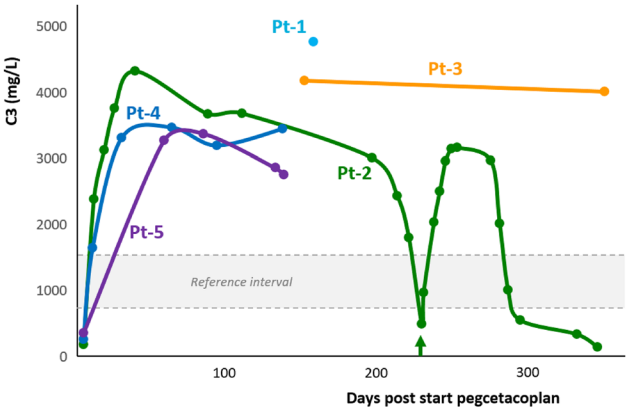

Figure 1: C3 dynamics in response to pegcetacoplan treatment. Each patient is identified by a unique color. All five patients reach C3 serum concentrations far above the normal reference value (indicated by the grey-shaded area). The green arrow indicates the timepoint in which pegcetacoplan is replaced by methylprednisolone treatment.

increase to levels above 3,000 mg/L is observed within 3 weeks after start of pegcetacoplan.

In routine diagnostics, it is very rare to measure C3 concentrations substantially above the upper reference value of 1,500 mg/L. More specifically, there are no known complement disorders or other diseases associated with increased C3 values. We performed an *in vitro* pegcetacoplan spike-in experiment to investigate whether pegcetacoplan-induced C3 increase over time is caused by an actual increase in the concentration of circulating C3 or whether it is a turbidimetric artifact caused by pegcetacoplan-C3 complex formation influencing light scatter. For this, pegcetacoplan was spiked into healthy control serum in concentrations ranging from 0 to 1,000 µg/mL. This concentration-range covers the expected pegcetacoplan levels observed in patients treated with pegcetacoplan [18]. Table 2 shows that the baseline C3 concentration in this sample was 975 mg/L. The measured C3 concentration was not influenced when pegcetacoplan was spiked-in. The minor C3 variation between samples is caused by technical variation of the turbidimetric C3 assay itself. These data clearly demonstrate that pegcetacoplan binding to C3 does not influence the measured C3 concentration.

Functional complement testing showed that spiked-in pegcetacoplan completely abolished the alternative complement pathway (AP). Pegcetacoplan also had a dose-dependent effect on the classical complement pathway (CP). However, even when pegcetacoplan was titrated at 1,000 µg/mL, which is above its physiological concentration [18], the CP is still close to normal. These *in vitro* experiments are in line with literature regarding the impact of pegcetacoplan on functional complement tests in preclinical studies [8].

Interestingly, the strongly increased C3 values in all patients give rise to an additional band when serum protein electrophoresis was performed (Figure 2A). This unique band must not be misinterpreted as an M-protein. Immunofixation electrophoresis (IFE) in fact demonstrated that

Table 2: Effect of spiked-in pegcetacoplan on complement measurements.

Concentration pegcetacoplan	C3 mg/L (ref 700–1,500)	AP % (ref 67–133)	CP % (ref 67–149)
0 µg/mL	975	94	105
50 µg/mL	984	82	92
200 µg/mL	999	23	86
400 µg/mL	961	<20	77
1,000 µg/mL	999	<20	65

the band represented C3 protein (Figure 2B). It is important to note that the C3 band in all five patients migrates in the γ-region because it is bound to pegcetacoplan which affects electrophoretic migration. In individuals not exposed to pegcetacoplan, C3 migrates in the β-region (Figure 2C).

The shift in electrophoretic migration of the C3-pegcetacoplan also allows this technique to study the binding kinetics of pegcetacoplan to C3. When pegcetacoplan is spiked-in at a concentration of 200 µg/mL in a healthy control serum, almost all C3 is shifted below the red dotted line, which indicates the migration pattern of unbound circulating C3 (Figure 2D). At a pegcetacoplan concentration of 400 µg/mL, all C3 is bound. This complete binding coincides with a sharp drop in the alternative pathway (AP = <20 %) as shown in Table 2. Remarkably, even when all C3 is bound to pegcetacoplan, the classical pathway is still within normal range (CP=77 %), which suggests that the alternative complement pathway is dependent on unbound C3 while the classical complement pathway is still largely functional with pegcetacoplan bound to C3.

The data of the *in vitro* experiments mentioned above are in line with the complement pathway test-results obtained during routine diagnostics in the five patients described in this case-series. Pegcetacoplan mainly impacts the alternative pathway and consequently a complete absence of AP activity was observed in most patients (Table 3). The impact on the classical pathway was minimal, with CP activity values that were either normal or slightly decreased. Pegcetacoplan further caused a strong inhibition of complement activation which was measured with the complement activation marker C3d. The C3d/C3-ratio is widely used as a more precise indicator of complement activation, because it accounts for interindividual variability in C3 levels. Pegcetacoplan-induced decline of the C3d/C3-ratio is extremely pronounced and is evidently strongly influenced by the high C3 titers.

How to use C3 as biomarker of disease activity in patients treated with pegcetacoplan

Complement C3 levels can be a useful biomarker for monitoring disease relapse. Reduced C3 levels are a surrogate marker for complement activity and can precede disease flare-ups [19]. The clinical value of monitoring C3 in patients treated with pegcetacoplan is more complex because all these patients have high C3 values, the absolute C3 concentration will not easily fall below the C3 reference values. However, an absolute decline of C3 should alert the clinician to consider that the underlying cause could possibly be a disease flare-up or poor medication adherence. In our case-series, patient two experienced a sharp decrease in C3. This decrease in C3 coincided with increasing creatinine levels in a period in which the patient also experienced a viral infection. It turned out that the strong C3 decline was caused by treatment nonadherence of the patient, followed by a rapid increase in C3 while on hospital controlled pegcetacoplan injections. Compared to the kidney biopsy that was taken before the start of pegcetacoplan, a reduced C3 staining intensity (from 3+ to 1–2+) and only minimal endocapillary proliferation was observed. Chronic injury was clearly increased (from 10 % to 30 % sclerosed glomeruli) with 65 % interstitial fibrosis. Electron microscopy showed an unchanged massive amount of dense deposits. Overall, these paghology-findings suggested no signs of C3G disease relapse but did demonstrate chronic renal injury. Pegcetacoplan was discontinued due to end stage kidney failure. It is interesting to look at the C3 kinetics in this patient after pegcetacoplan was stopped. Instead of reaching steady state low C3-levels, the C3 first peaked to levels again far above reference values which can be explained by the long pegcetacoplan half-life of 8.6 days [18]. After pegcetacoplan clearance, a rapid decrease of C3 levels was observed. In

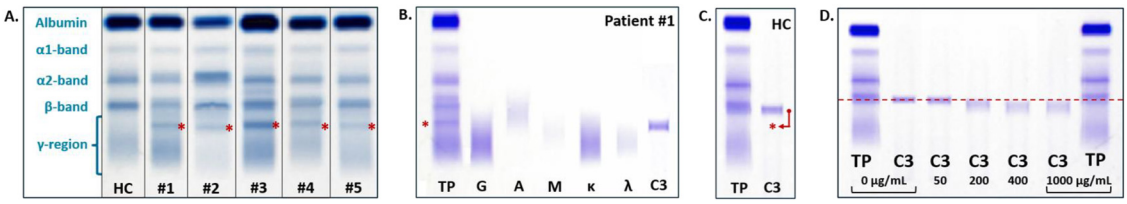

Figure 2: Electrophoresis data of patients treated with pegcetacoplan. (A) After pegcetacoplan exposure, serum protein electrophoresis shows a distinctive new band in the γ-region in all five patients (illustrated with red asterix). (B) Characterization with immunofixation electrophoresis (IFE) demonstrates that this new band it is not an M-protein. Anti-C3 reagents proof that the band is composed of C3 (IFE of one representative patient is shown). (C) In healthy controls not exposed to pegcetacoplan, C3 migrates in the β-region. The C3 bound to pegcetacoplan affects its electrophoretic migration. (D) IFE of pegcetacoplan spiked in serum further illustrates the shift in migration. The concentrations indicate concentration of pegcetacoplan in each analysis. G, IgG; A, IgA; M, IgM; κ, kappa; λ, lambda; TP, total protein.

Table 3: Overview of complement diagnostics in patients treated with pegcetacoplan.

	C3 mg/L (ref 700–1,500)		C3d mg/L (ref <8.3)		C3d/C3-ratio (ref <8.1)		AP % (ref 67–133)		CP % (ref 67–133)		C3bBbP CAU/mL (ref <26)	
Patient	**Before**	**After[a]**	**Before**	**After[a]**	**Before**	**After[a]**	**Before**	**After[a]**	**Before**	**After[a]**	**Before**	**After[a]**
1.	n.t.	4,766	n.t.	n.t.	n.t.	n.t.	25	n.t.	<10	95	n.t.	n.t.
2.	180	4,863	36	12	200	4	29	30	50	63	12	65
3.	n.t.	4,100	n.t.	n.t.	n.t.	n.t.	n.t.	29	<10	68	n.t.	n.t.
4.	259	3,358	28	5	109	2	n.t.	<20	n.t.	48	14	45
5.	355	3,320	10	5	29	1	n.t.	<20	n.t.	76	39	52

[a]If more values are available, the average plateau-value is reported. n.t. = not tested.

patient two, C3 levels decreased to reference value only two months after pegcetacoplan was stopped (Figure 1).

Broad overview of changes in the complement system during pegcetacoplan exposure

To generate an unbiased overview of the effect of pegcetacoplan on other components of the complement system we performed a plasma proteome analysis. Filtering for complement proteins that were detected in all samples as the sole protein in a protein group, allowed us to investigate the dynamics of 36 complement proteins in the blood of three patients before and during exposure to pegcetacoplan. Of all 36 complement proteins, the most affected complement factor is C3, which increased on average more than ten-fold upon pegcetacoplan exposure (Table 4). Strikingly, we also observed a strong increase in circulating properdin. The three-fold increase in properdin is a surprising result since pegcetacoplan does not directly bind properdin. Three months after pegcetacoplan was stopped in patient 2, both C3 and properdin levels normalized to levels observed prior to pegcetacoplan exposure (data not shown). Mass spectrometric proteomics data cannot differentiate between free properdin and properdin as part of the C3bBbP complex. We therefore performed additional C3bBbP analyses and found that C3bBbP increases on average three-fold after patients start pegcetacoplan treatment (Table 3). We did not observe strong changes in any of the other complement factors in the plasma proteome analysis (Table 4).

Discussion

Pegcetacoplan has obtained worldwide approval for subcutaneous use in PNH [4] and intravitreal use in geographic atrophy in age-related macular degeneration [6]. Next to that, pegcetacoplan is gaining new indications in nephrology. The VALIANT phase 3 study reported a substantial reduction in proteinuria and favorably safety profile of pegcetacoplan for patients with C3G and immune complex membranoproliferative glomerulonephritis (IC-MPGC) [20]. FDA approval was granted in July 2025 for pegcetacoplan in treatment of these rare complement-mediated kidney diseases. This approval represents a pivotal shift from non-specific immunosuppression towards disease-modifying therapy for C3G patients. When administered in patients, pegcetacoplan causes several counterintuitive changes in laboratory results. Because of the increased use of pegcetacoplan in routine clinical practice, it is important that both clinicians and laboratory specialists are aware of these therapy-induced laboratory findings.

Subcutaneous pegcetacoplan 1,080 mg twice weekly results in rapid steady state serum concentrations and robust biomarker response within 4 weeks after treatment initiation in PNH [18]. This is in line with the fast decrease of the alternative pathway complement hemolytic activity and the normalization of complement activation biomarkers that we observed in this case-series. In this report we show that within three weeks after initiation of pegcetacoplan therapy, C3 levels increase to unprecedented high levels of 2–3 times the upper reference limit. *In vitro* experiments showed that the increased C3 levels are not caused by a turbidimetric artifact in the C3 immunoassay due to pegcetacoplan-C3 complex formation. Normal C3 half-life is approximately three days [21]. We hypothesize that the C3 bound to pegcetacoplan increases the half-life of C3 to the effective half-life of pegcetacoplan, which was shown to be 8.6 days in patients with PNH [18]. This three-fold increase in C3 half-life can explain the C3 levels ranging from 3,000–5,000 mg/L we observed in our case-series.

For laboratory specialists it is important to realize that pegcetacoplan impacts serum protein electrophoresis results. The combination of the unprecedented high C3 levels and the electrophoretic shift of the C3-pegcetacoplan

Table 4: Fold change of plasma complement proteins during pegcetacoplan treatment.

Uniprot protein description	Fold-change[a] during vs. before pegcetacoplan			Average fold-change[a]
	Patient 2	Patient 4	Patient 5	
Complement C3	17.6	9.5	6.0	11.0
Properdin	4.6	2.5	2.4	3.2
Complement factor B	1.4	1.2	1.7	1.4
Complement C5	1.5	1.0	1.5	1.3
Complement component C9	1.1	1.0	1.9	1.4
Complement component C8 gamma chain	1.4	1.0	1.5	1.3
Complement factor H-related protein 5	1.4	1.0	1.4	1.3
Complement component C8 beta chain	1.3	1.0	1.3	1.2
Complement factor H-related protein 4	1.2	1.0	1.4	1.2
Complement component C8 alpha chain	1.2	1.0	1.3	1.2
Mannan-binding lectin serine protease 1	1.4	1.1	0.9	1.1
Vitronectin	1.1	1.1	1.1	1.1
Complement component C6	1.2	0.9	1.2	1.1
Ficolin-3	1.1	1.1	1.0	1.1
Complement C1r subcompon-like protein	1.2	0.9	1.1	1.1
Complement factor H	1.1	1.0	1.0	1.1
Complement component C7	1.2	1.0	1.0	1.1
Complement C1r subcomponent	1.1	1.0	1.0	1.0
Complement factor H-related protein 2	1.0	1.0	1.1	1.0
Mannose-binding protein C	1.3	0.6	1.3	1.1
Complement C1s subcomponent	1.1	1.0	1.0	1.0
Plasma protease C1 inhibitor	1.1	0.9	1.1	1.0
Clusterin	1.1	0.9	0.9	1.0
Complement factor I	1.0	0.8	1.2	1.0
Complement C1q subcomponent subunit B	1.0	1.0	1.0	1.0
Complement C1q subcomponent subunit A	1.0	1.0	0.9	1.0
Complement factor H-related protein 1	0.8	1.0	1.1	1.0
Complement C1q subcomponent subunit C	1.1	1.0	0.9	1.0
Complement C2	1.0	0.9	1.0	1.0
Mannan-binding lectin serine protease 2	1.1	0.9	1.0	1.0
Complement C4-A	1.0	0.8	1.1	1.0
Complement C4-B	1.0	0.8	1.1	1.0
Complement factor D	0.9	1.0	1.0	0.9
Ficolin-2	1.1	1.0	0.7	0.9
C4b-binding protein beta chain	0.8	0.8	0.8	0.8
C4b-binding protein alpha chain	0.8	0.9	0.9	0.8

[a]Fold change was calculated as the complement protein ratio during: before pegcetacoplan treatment. An average fold change of 1.0 indicates that pegcetacoplan does not affect the plasma concentration of this complement factor. Increase in protein expression more than two-fold are indicated in green.

complex consistently produces an additional electrophoretic band, which may be misinterpreted as an M-protein [22]. Given that M-protein testing may be requested during the course of pegcetacoplan treatment – for instance, to evaluate potential M-protein–associated renal comorbidities – [23] awareness of this phenomenon is essential to avoid diagnostic misinterpretation of a monoclonal gammopathy.

In this case-series it is shown that in real-world clinical practice, pegcetacoplan causes strong and persistent inhibition of complement activation, and the alternative complement pathway activity is completely blocked in most patients. Pegcetacoplan has no meaningful impact on the classical complement pathway activity, despite the fact that all circulating C3 is bound to pegcetacoplan, which is in line with previous observations in clinical trials involving pegcetacoplan [5, 8]. Pegcetacoplan binds to C3, thereby preventing cleavage of C3 into C3a and C3b, but pegcetacoplan also binds to C3b, preventing C3-convertase activity in the alternative pathway amplification, which is in line with a clear inhibition of the alternative pathway activity assay (AP = <20 %). It is currently unknown why the classical complement pathway is largely unaffected by pegcetacoplan. Remaining classical pathway activity may be explained by a lack of full C5-convertase activity inhibition. Pre-formed C3b or a low level of C3b formation can provide classical pathway C5-convertase complex formation that may provide C5-convertase activity and formation of the membrane attack complex. In addition, Mannes et al. showed that C4b alone can, to some extent, recruit and prime C5 for consecutive proteolytic activation, thereby demonstrating a C3-bypass mechanism of C5 activation [24].

In many patients with complement-mediated disorders, C3 is utilized as biomarker to monitor disease activity and

therapeutic responses. As C3 monitoring may guide clinicians in their patient management, it is important to recognize that pegcetacoplan affects the utility of C3 in disease monitoring. This is due to two main factors: first, individual baseline levels of C3 are significantly higher; second, the extended half-life of C3 (bound to pegcetacoplan) slows its decline, making it a less timely indicator of disease relapse. As a result, it takes much longer in patients who receive pegcetacoplan for the C3 value to drop below critical values that are suggestive for disease relapse. Overall, more real-world data are needed to determine how dynamic C3 test-results can be interpreted and applied in the management of patients receiving pegcetacoplan. Pegcetacoplan has a half-life of approximately 8.6 days [18]. Consequently, even after discontinuation, the drug remains systemically available for over a month and will continue to influence C3 kinetics during this period.

Data obtained in an unbiased analysis of complement proteins in the blood confirmed that pegcetacoplan mainly induced increased C3 concentrations. Next to that we observed a three-fold increase in circulating properdin during treatment with pegcetacoplan. Properdin stabilizes the C3 convertase (C3bBb) complex, which enhances and prolongs complement activation [25]. We found that the increase in C3bBbP levels after pegcetacoplan exposure contributes to the net increase in properdin. As far as we know, no published data are available on the effect of pegcetacoplan on properdin. No known mechanisms suggest that pegcetacoplan directly affects properdin binding, synthesis, secretion, or degradation. Since the C3bBbP increase does not result in increased alternative pathway activation in patients who receive pegcetacoplan, we hypothesize that pegcetacoplan binds C3bBbP via C3(b). This functionally blocks C3bBbP activity and at the same time increases the half-life of the C3bBbP complex which explains why this complex is increased after pegcetacoplan exposure. We did not observe strong changes in any of the other 34 complement factors that we analyzed. A limitation here is however that we only analyzed three patients. To exclude that pegcetacoplan has small but significant effects on complement factors other than C3 and properdin, plasma protein expression needs to be studied in larger cohorts.

In this case-series we showed that C3 levels increase up to 300 % above reference levels when patients are treated with pegcetacoplan. This is caused by the long half-life of the C3-pegcetacoplan complex and complicates the use of C3 as biomarker to monitor disease. Pegcetacoplan further induces a three-fold increase of properdin, which is in part attributed to an increase in C3bBbP complexes. Functionally, pegcetacoplan treatment completely blocks the alternative pathway while the classical pathway is affected but remains largely intact. Because of the increasing use of pegcetacoplan in routine clinical practice, it is important that both clinicians and laboratory specialists are aware of these therapy-induced laboratory findings.

Research ethics: This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).
Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
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Data availability: The data that support the findings of this study are available from the corresponding author, JFMJ, upon reasonable request.

References

1. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010; 11:785–97.
2. Mastellos DC, Hajishengallis G, Lambris JD. A guide to complement biology, pathology and therapeutic opportunity. Nat Rev Immunol 2024;24:118–41.
3. Ricklin D, Reis ES, Mastellos DC, Gros P, Lambris JD. Complement component C3 – the “Swiss Army Knife” of innate immunity and host defense. Immunol Rev 2016;274:33–58.
4. Hillmen P, Szer J, Weitz I, Roth A, Hochsmann B, Panse J, et al. Pegcetacoplan versus eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med 2021;384:1028–37.
5. Dixon BP, Greenbaum LA, Huang L, Rajan S, Ke C, Zhang Y, et al. Clinical safety and efficacy of pegcetacoplan in a phase 2 study of patients with C3 glomerulopathy and other complement-mediated glomerular diseases. Kidney Int Rep 2023;8:2284–93.
6. Heier JS, Lad EM, Holz FG, Rosenfeld PJ, Guymer RH, Boyer D, et al. Pegcetacoplan for the treatment of geographic atrophy secondary to age-related macular degeneration (OAKS and DERBY): two multicentre, randomised, double-masked, sham-controlled, phase 3 trials. Lancet 2023;402:1434–48.
7. Patel SB, Heier JS, Chaudhary V, Wykoff CC. Treatment of geographic atrophy: an update on data related to pegcetacoplan. Curr Opin Ophthalmol 2024;35:64–72.
8. Wong RSM, Pullon HWH, Amine I, Bogdanovic A, Deschatelets P, Francois CG, et al. Inhibition of C3 with pegcetacoplan results in normalization of hemolysis markers in paroxysmal nocturnal hemoglobinuria. Ann Hematol 2022;101:1971–86.
9. Langereis JD, van der Molen RG, de Kat Angelino C, Henriet SS, de Jonge MI, Joosten I, et al. Complement factor D haplodeficiency is associated with a reduced complement activation speed and diminished bacterial killing. Clin Transl Immunol 2021;10:e1256.

10. Fredrikson GN, Truedsson L, Sjoholm AG. New procedure for the detection of complement deficiency by ELISA. Analysis of activation pathways and circumvention of rheumatoid factor influence. J Immunol Methods 1993;166:263–70.
11. Seelen MA, Roos A, Wieslander J, Mollnes TE, Sjoholm AG, Wurzner R, et al. Functional analysis of the classical, alternative, and MBL pathways of the complement system: standardization and validation of a simple ELISA. J Immunol Methods 2005;296:187–98.
12. Branten AJ, Kock-Jansen M, Klasen IS, Wetzels JF. Urinary excretion of complement C3d in patients with renal diseases. Eur J Clin Invest 2003; 33:449–56.
13. Dhooge PPA, Runhart EH, Li CHZ, de Kat Angelino CM, Hoyng CB, van der Molen RG, et al. Systemic complement activation levels in Stargardt disease. PLoS One 2021;16:e0253716.
14. Kauffmann RH, Herrmann WA, Meyer CJ, Daha MR, Van Es LA. Circulating IgA-immune complexes in Henoch-Schonlein purpura. A longitudinal study of their relationship to disease activity and vascular deposition of IgA. Am J Med 1980;69:859–66.
15. Bergseth G, Ludviksen JK, Kirschfink M, Giclas PC, Nilsson B, Mollnes TE. An international serum standard for application in assays to detect human complement activation products. Mol Immunol 2013;56:232–9.
16. Willems E, Alkema W, Keizer-Garritsen J, Suppers A, van der Flier M, Philipsen R, et al. Biosynthetic homeostasis and resilience of the complement system in health and infectious disease. EBioMedicine 2019;45:303–13.
17. Demichev V, Szyrwiel L, Yu F, Teo GC, Rosenberger G, Niewienda A, et al. dia-PASEF data analysis using FragPipe and DIA-NN for deep proteomics of low sample amounts. Nat Commun 2022;13:3944.
18. Crass RL, Smith B, Adriaens S, Chapel S, Langdon G. Population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses of pegcetacoplan in patients with paroxysmal nocturnal hemoglobinuria. Drugs R D 2024;24:563–73.
19. Nilsson B, Ekdahl KN. Complement diagnostics: concepts, indications, and practical guidelines. Clin Dev Immunol 2012;2012:962702.
20. Bomback AS, Daina E, Remuzzi G, Kanellis J, Kavanagh D, Pickering MC, et al. Efficacy and safety of pegcetacoplan in kidney transplant recipients with recurrent complement 3 glomerulopathy or primary immune complex membranoproliferative glomerulonephritis. Kidney Int Rep 2025;10:87–98.
21. Pangburn MK, Schreiber RD, Muller-Eberhard HJ. Formation of the initial C3 convertase of the alternative complement pathway. Acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. J Exp Med 1981;154:856–67.
22. McCudden CR, Jacobs JFM, Keren D, Caillon H, Dejoie T, Andersen K. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. Clin Biochem 2018;51:72–9.
23. Bridoux F, Nasr SH, Arnulf B, Leung N, Sirac C, Jaccard A. Renal manifestations of MGUS. Hematol Am Soc Hematol Educ Program 2024;2024:489–98.
24. Mannes M, Dopler A, Zolk O, Lang SJ, Halbgebauer R, Hochsmann B, et al. Complement inhibition at the level of C3 or C5: mechanistic reasons for ongoing terminal pathway activity. Blood 2021;137: 443–55.
25. Blatt AZ, Pathan S, Ferreira VP. Properdin: a tightly regulated critical inflammatory modulator. Immunol Rev 2016;274:172–90.