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Eculizumab effect on the hemostatic state in patients with paroxysmal nocturnal hemoglobinuria

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ABSTRACT

Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by a hypercoagulable state associated with acute hemolysis. Eculizumab is used to reduce the intensity of intravascular hemolysis in PNH patients.
The hemostatic status of three patients with PNH was assessed during eculizumab treatment by D-dimer assay and the global assays: thromboelastography (TEG), thrombin generation test (TGT), and thrombodynamics (TD). In the state of hemolytic crisis before the therapy D-dimer concentration was increased in two patients accompanied by hypercoagulation changes in TEG parameter angle (α). TD parameter the clot growth velocity (V) revealed hypercoagulability while TGT parameter ETP was within the normal range in all patients.
The lactate dehydrogenase (LDH) activity decreased during the 8 months of eculizumab therapy. The physical health was improved, the frequency of hemolytic crisis decreased. Patients periodically exhibited hypercoagulable state: the mean values $\alpha = 38 \pm 11°$ (with normal range 20–40°), ETP = 1311 ± 442 nM·min (with normal range 800–1560 nM·min), V = 31 ± 4 μm/min (with normal range 20–29 μm/min). During the eculizumab therapy two patients had the repeated clinical manifestation of acute hemolytic crisis, the parameters of the global tests were increased compared to the previous measurement.
The global hemostasis tests TEG, TGT and TD revealed hypercoagulability in patients with PNH during eculizumab therapy.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH), also referred to as Marchiafava–Micheli syndrome, is a rare acquired blood disorder characterized by complement-induced intravascular hemolytic anemia, hemoglobinuria and thrombosis [1–4]. Episodic hemolytic crises are common for PNH patients. Crises usually occur spontaneously; sometimes they are triggered by hypothermia, infection or fatigue [3–7].

The hemostatic system of patients with PNH is characterized by a hypercoagulable state [7–10]; the risk of thrombosis in these patients is 62 times higher than the risk in general population. Thrombosis is the main cause of death in PNH [3–10]. The presumed mechanisms contributing to hypercoagulability in PNH are diverse and include circulating procoagulant microparticles, platelet activation, abnormal expression of adhesion molecules on vascular endothelial cells, chronic hypofibrinolysis, limited bioavailability of nitric oxide, and factor Va (activated by the circulating microparticles, which were derived from complement-lysed abnormal red blood cells) [10–19].

The thrombotic risk in PNH is directly associated with complement-induced intravascular hemolysis. The PNH patients who have increased LDH, abdominal pain, hemoglobinuria occurring during hemolytic crisis are 1.5 times more likely to have thrombosis compared with a standard risk of 30–40% during acute hemolysis [20–22]. However, the underlying mechanisms leading to thrombotic complications during hemolytic crisis in patients with PNH remain unclear [23–26].

Eculizumab is a monoclonal antibody that binds the complement component protein C5, thereby preventing complement-induced lysis of erythrocytes, reducing intravascular hemolysis, hemoglobinuria and transfusion dependence in patients with PNH. In clinical trials in patients with PNH, eculizumab was associated with 3-fold reductions in the frequency of the hemolytic crises, transfusion requirements and thromboembolic events. Additionally, eculizumab was associated with improvements in PNH symptoms, quality of life and survival [27–30].

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Thus, Hillmen et al. [28] showed that the number of thromboses was reduced from 39 to 3 episodes in 195 patients on eculizumab therapy. However, there are few research studies of hemostatic state in patients with PNH during eculizumab therapy and they are inconsistent. On the one hand, the markers of coagulation activation such as D-dimer, TAT-complex, prothrombin fragments F1 + 2 were reported to be significantly reduced during the treatment; on the other hand it was shown that the formation of procoagulant microparticles in patients continued, and moreover, about 20% of patients still marked the increased concentration of D-dimers and prothrombin fragments F1 + 2 during the therapy [29,30]. None of the studies involved the global hemostatic assays which are known to be highly sensitive to hypercoagulable changes. In the present study, in addition to the conventional tests (clotting tests, fibrinogen concentration, D-dimer assay) we used the global methods: thromboelastography (TEG), thrombin generation test (TGT) and thrombodynamics (TD) to monitor the state of the coagulation system in three patients with PNH. The results of three patients with PNH before and during the 8 months of eculizumab therapy showed that, despite the reduction of hemolysis and the frequency of hemolytic crises from 3–6 to 0–1 episodes in 8 months, the global hemostasis tests support the tendency for hypercoagulability in patients with PNH.

Materials and methods

Patients

Three patients with PNH from the Department of Rare Diseases, National Research Center for Hematology, were enrolled in the study. Clinical data of the studied PNH patients on admission are shown in Table 1. There was an increased LDH activity and reticulocytosis in all patients. The clinical protocol was approved by the National Research Center for Hematology Ethics Committee.

Therapy

The eculizumab treatment was administered as recommended by the manufacturer; all patients received a low-dose regimen of 600 mg weekly for 4 weeks followed by 900 mg every 2 weeks for the next 8 months. The half-life of eculizumab is 272 ± 82 h; it should be administered at the recommended dosage regimen time points, or within two days of these time points to maintain a constant drug concentration in blood. We assume that during the entire treatment, patients were under continuous action of the drug: it is known that the peak concentration of eculizumab is observed at week 26 (194 ± 76 pg/ml), and the minimal working concentration (35 mg/kg) is reached within an hour after the first administration [31–34]. Patient A was on the anticoagulant therapy with enoxaparin 1.2 mg/day during the first three weeks of eculizumab treatment. There was no any other therapy for other two patients during the eculizumab therapy.

Reagents

Thromboplastin was obtained from Renam, Moscow, Russia; the fluorogenic substrate Z-Gly-Gly-Arg-AMC from Bachem, Bubendorf, Switzerland; phosphatidylcholine (PC) and phosphatidylserine (PS) were obtained from Avanti Chemicals, Ormeau, Australia; Thromborel S, Test Thrombin Reagent, and D-dimer PLUS were obtained from Dade Behring, Germany; and Thrombodynamics kit was from LLC HemaCore, Russia.

Blood collection and plasma preparation

Blood samples were drawn into 9 ml vacuum tubes (Monovette, Sarstedt, Germany) with 0.106 M sodium citrate buffer. The blood samples were processed by centrifugation at 1500 ×*g* for 15 min to obtain platelet-poor plasma (ppp), part of the plasma was subsequently subjected to centrifugation at 10,000 ×*g* for 5 min to obtain platelet-free plasma (pfp) [35].

Clotting time tests, fibrinogen and D-dimer assays

The following tests were performed using fresh ppp samples and the aforementioned reagents: activated partial thromboplastin time (APTT), prothrombin index (PI), thrombin time (TT), fibrinogen and D-dimer concentrations. All tests were performed in the Coagulation Laboratory of the National Research Center for Hematology, using a Sysmex CA-1500 (Sysmex Corporation, Japan) automated analyzer, according to respective manufacturer's instructions.

Thromboelastography

Citrated Native Thromboelastography (TEG) was performed using a TEG 5000 Hemostasis Analyzer System and disposable cups (Haemonetics Corporation, MA, USA). The assays were performed 10 to 30 min after blood collection using citrated blood samples (340 μl) recalcified with 20 μl of 0.2 M $CaCl_2$. The angle alpha (α), the tangent to the thrombloelastographic clotting curve was used for analysis (Fig. 1a).

Thrombin generation test

Sample preparation and experiments were performed as described in [36]. Briefly, ppp was placed in the wells (80 μl/well) of a 96-well flak-bottom micro titer plate. Thereafter, 20 μl of the fluorogenic substrate (the final concentration was 400 μM) and 20 μl thromboplastin (the final concentration was 4 pM) with phospholipid vesicles (the final concentration was 4 μM) were added into each well. Phospholipid vesicles were prepared by extrusion from PC and PS at a percentage ratio of 80:20, as described previously [37]. The kinetics of accumulation of the fluorescing reaction product 7-amino-4-methylcoumarin was recorded for 60 min with a fluorimetric reader (Appliskan; Thermo Fisher Scientific, Finland) ($\lambda_{ex.} = 355$ nm; $\lambda_{em.} = 460$ nm). For all calculations, the program OriginPro 8.0 (OriginLab Corporation, USA) was used. To calculate the area under the thrombin-time curve for a sample, we determined the total amount of thrombin generated in that sample over the period of 50 min endogenous thrombin potential (ETP) (Fig. 1b) [38].

Thrombodynamics

The Thrombodynamics (TD) spatial clot growth assay was performed using Thrombodynamics Analyzer and Thrombodynamics kit

Table 1
Clinical and demographic data of the studied PNH patients.

Patient	Age, years	Sex	PNH, years	Anticoagulant therapy	Steroids	WBC (10^9), cells/l	Hb, g/l	Platelets (10^9), cells/l	Reticulocytes, ppm	LDH, U/l
Reference values						4.0–9.0	130–160	180–320	2–10	208–378
A[a]	29	M	6	Enoxaparin 1.2 mg/day	No	**2.8**	**54**	178	**101**	**6569**
B[a]	33	M	2	No	No	**3.6**	**53**	**85**	**52**	**1874**
C[a]	27	F	4	No	No	**2.9**	**67**	171	**32**	**3173**

The bold indicates the changes in parameters.
[a] Transfusion dependent patients.

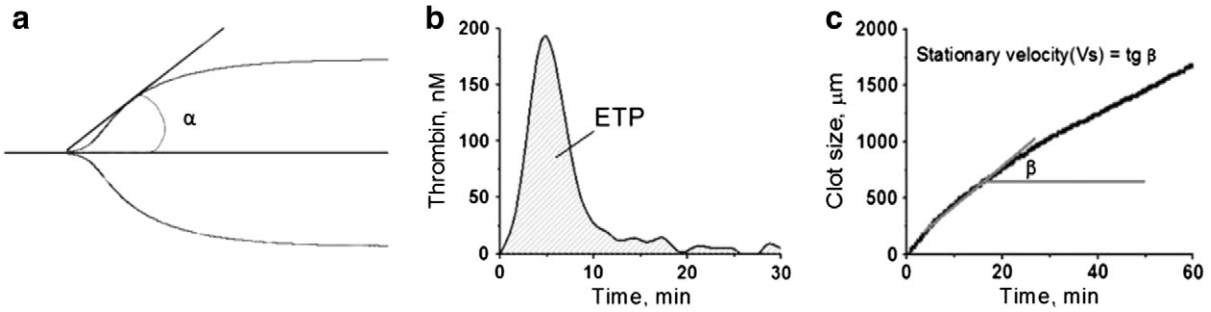

Fig. 1. Global hemostasis assays. Thromboelastographic curve: angle alpha (α) was defined as the angle of the tangent to the curve (a) thrombin generation curve: the endogenous thrombin potential (ETP) was calculated as the area under the curve (b) and clot size dependence on time in the Thrombodynamics assay: clot growth velocity (V) was calculated as the angle of the line that linearized the curve from 15 to 25 min after the start of the clot growth (c).

(HemaCore LLC, Russia). Sample preparation and experiments were performed as described in [35]. Briefly, coagulation is activated in a thin layer of plasma when it is brought in contact with TF immobilized on a plastic surface. Clot formation starts on the activator and propagates into the bulk of plasma where no TF is present. Light scattering by fibrin allows observation of spatial clot formation in a real time by using a time lapse imaging. Fig. 1c shows the curve depending of clot size to time. Based on the clot size dynamics, the clot growth velocity (V) (the mean slope over the period [Tlag + 15 min; Tlag + 25 min]) was calculated.

Reference values for laboratory tests

Reference values for conventional laboratory tests were taken from the respective testing reagents' instructions. To define the normal ranges of TEG, TGT and TD tests were performed with plasma samples obtained from 25, 76 and 40 healthy donors respectively (age 19–76 years, the number of men and women was taken in equal proportions). There were no significant differences between ages and genders for tests. Normal range was calculated as the interval of 5–95 percentiles.

Statistical analysis

The statistical analyses of the differences between the data sets were performed using the Wilcoxon signed-rank test for statistical significance $p < 0.05$. One-way repeated-measures analysis of variance (ANOVA) was used to assess the changes in parameters during the treatment ($p < 0.05$). For all calculations, the program OriginPro 8.0 (OriginLab Corporation, MA, USA) was used.

Table 2
Hematological data of the studied PNH patients on admission.

Test		Reference values	Patients		
			A	B	C
APPT, sec		25–37	34	38	27
PI, %		80–132	70	69	94
TT, sec		15–19	19	19	17
Fibrinogen, mg/ml		2–4.5	1.4	4	2.2
D-dimer, μg/ml		0–500	**1096**	**3123**	264
TEG	α, deg	20–40	**59.5**	**41.3**	27.9
TGT	ETP, nM[a]min	800–1560	1385	1048	1100
TD	V, μm/min	20–29	**32**	**31**	**32**
LDH, U/l		208–378	**7106**	**1874**	**3173**
Hemoglobin (Hb), g/l		130–160	**40**	**49**	**71**
Platelets, 10^9 cells/l		180–320	**155**	**48**	**122**
Indirect bilirubin, μM		3.4–13.7	**57.7**	12.8	**22.2**
Reticulocytes,%		2–10	**87**	**52**	**32**

The bold indicates the changes in parameters.
[a] Min and max values are performed.

Results

Patients' medical history and admission on hemolytic crisis

The diagnosis of paroxysmal nocturnal hemoglobinuria in all three patients was confirmed by positive sucrose lysis test and Ham's acid hemolysis test, and using immunophenotypic analysis with detecting erythrocyte, granulocyte and monocyte PNH clones. All patients showed a negative dynamics since the first diagnosis: increased incidence of hemolytic crises from 1 a year to 1–2 crises in 3 months, as well as increased transfusion dependence and increased frequency of spastic abdominal pain. On admission to the Department of Rare Diseases of National Research Center for Hematology, all patients were in a state of hemolytic crisis, the state with 37 °C to 40 °C in different patients, reticulocytosis, raised bilirubin and LDH, extremely low hemoglobin, icteric skin and sclera, abdominal pain. A peculiarity of hemolytic crises of patient B is insignificant bilirubinemia (Table 2).

The patients were examined during the first three days from the admission and before the beginning of the treatment. The laboratory data are shown in Table 2. The conventional coagulation tests (APTT, TT, PI and fibrinogen) were in the normal range for all the patients. Patients A and B had increased D-dimer concentration, which is typical for patients with PNH during the hemolytic crisis, whereas for the patient C D-dimer concentration was normal. TEG parameter α detected hypercoagulability in patients A and B; in patient C it was normal. TGT parameter ETP was in the normal range for all the patients. TD parameter V detected hypercoagulability in all patients.

Eculizumab was prescribed according to clinical and laboratory characteristics for all three patients (permanent hemolysis, 1–2

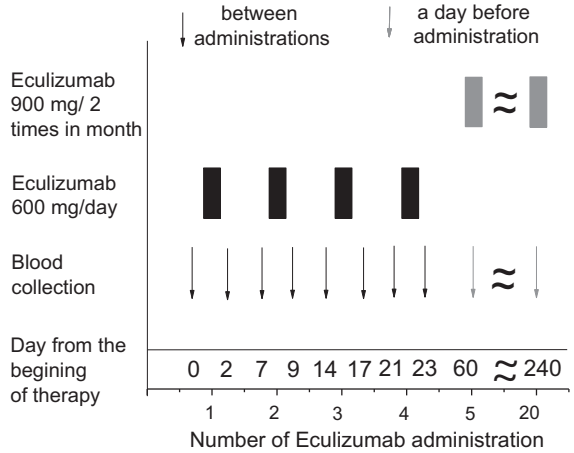

Fig. 2. Schedule of blood sampling during the eculizumab therapy.

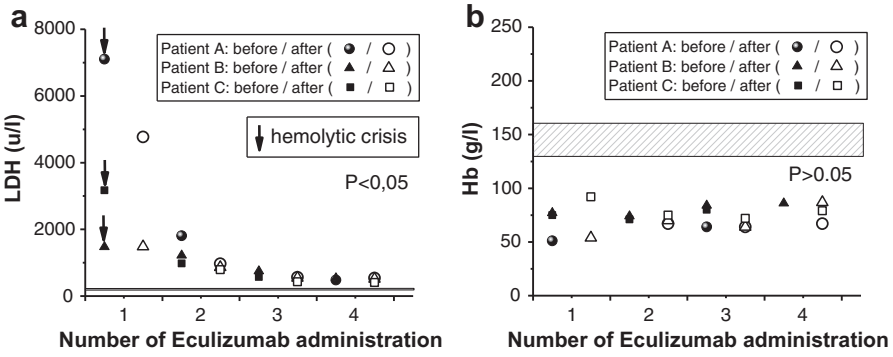

Fig. 3. Effect of eculizumab on hemolysis during the first month of therapy. LDH activity (a) and hemoglobin concentration (b) of the studied PNH patients. Gray shaded area is the normal range. One-way repeated-measures analysis of variance (ANOVA) was used to assess the changes in parameters during the treatment ($p < 0.05$).

episodes of hemolytic crises in 3 months, low hemoglobin (Hb) of 40 g/l, abdominal pain with no sign of thrombosis on ultrasonography, fever, fatigue and headaches, transfusion dependence and thus a high risk of thrombosis).

Eculizumab effect on the haemostatic state in patients with PNH during the first month of therapy

All the patients received a low-dose eculizumab regimen of 600 mg weekly for 4 weeks as infusion during 45 min. Blood samples were drawn the day before and 1–2 days after the administration. Blood sampling scheme is represented on Fig. 2.

Patient A had the anticoagulant therapy with enoxaparin 1.2 mg a day prescribed because of persistence of acute hemolysis and high concentration of D-dimers during the first 3 weeks of eculizumab administrations. Blood samples were drawn before the next administration of the anticoagulant in all points except the third administration of eculizumab where blood was drawn 1 h after administration of the anticoagulant, however, this point was excluded from the study.

On the first point before the beginning of the therapy the patients were in a state of hemolytic crisis, revealed increased LDH activity and decreased Hb concentration (45–75 g/l) (Fig. 3). LDH activity was reduced during the first month of eculizumab therapy in all three patients ($p < 0.05$), indicating the decrease of hemolytic activity. A distinctive peculiarity of patient B was that his LDH activity was not decreased immediately after the first eculizumab injection, but gradually reached the upper limit of normal range. Hemoglobin still remained low and did not reach the limit of normal range in all patients, which is typical for patients with PNH during eculizumab therapy [28].

There was no difference between the pre- and post-injection results at all assessment points ($p > 0.05$) during the first month for conventional clotting tests (APTT, TT, PI, fibrinogen), values were in the normal or even in the hypocoagulation area (Tables 3–5). D-dimer concentration was increased only in patient B on the first eculizumab administration and further normalized (α was increased, ETP and V were in normal range in this point). There was no statistically significant difference for the parameters of global hemostatic tests before and after the injection ($p > 0.05$) in all three patients (Tables 3–5). TEG parameter α was periodically increased in all three patients (in 66, 25 and 63% of all assessment points for the patients A, B and C, respectively). TGT parameter ETP was periodically increased in two patients (for patient A in 33% of all assessment points, for patient B in 38% of all assessment points). TD parameter V was in hypercoagulation area periodically in all patients (66%, 12% and 63% of the points for the patients A, B and C, respectively). Thus the hypercoagulability state was identified by two global tests in 9 of 17 assessment points.

Clinical symptoms of hemolytic crisis (hemoglobinuria, weakness, fever, icteric skin and sclera, and spastic abdominal pain) maintained in patients during the first week of therapy. As a side effect of drug administration nausea and headache were noted in two patients (A and C). Patient A also noted aching in the limbs. During the further eculizumab therapy for 4 weeks there was a gradual improvement in physical health in all patients. The clinic of hemolytic crisis and hemoglobinuria disappeared during the first month of eculizumab therapy.

Eculizumab effect on the haemostatic state in patients with PNH during the 2–8 months of therapy

All patients received 900 mg of eculizumab every 2 weeks from the 2nd month for the next 8 months, the infusion during 45 min. Blood samples were drawn on the day of eculizumab administration but before the eculizumab injection 2 times per month for the first 4 months and then once a month (Fig. 2).

Table 3
Laboratory data during the first month of eculizumab therapy for patient A.

Test		Reference values	1 administration		2 administration		3 administration		4 administration		P[a]
			Before	After	Before	After	Before	After	Before	After	
Patient A											
APPT, sec		25–37	35	40	41	40	–	–	38	39	0.29
PI, %		80–132	66	73	57	76	–	–	87	87	0.83
TT, sec		15–19	16	18	17	18	–	–	17	16	0.22
Fibrinogen, mg/ml		2–4.5	2.3	1.6	1.6	1.3	–	–	2	2	0.59
D-dimer, μg/ml		0–500	497	65	97	62	–	–	100	88	0.07
TEG	α, deg	18–40	**61.4**	26.4	**52.4**	**–**	**–**	**–**	**53.2**	**49.3**	0.29
TGT	ETP, nM[a]min	800–1560	1275	**1891**	1026	**2175**	–	–	1213	1424	0.14
TD	V, μm/min	20–29	**35**	28	29	**30**	–	–	**37**	**34**	0.29
LDH, U/l		208–378	**7106**	**4771**	**1809**	**971**	**–**	**565**	**–**	**–**	0.86
Hemoglobin (Hb), g/l		130–160	51	–	–	67	64	64	–	–	0.37

The bold indicates the changes in parameters.
[a] The differences between the data sets before and after administration were performed using the Wilcoxon signed-rank test.

Table 4
Laboratory data during the first month of eculizumab therapy for patient B.

Test		Reference values	1 administration		2 administration		3 administration		4 administration		P[a]
			Before	After	Before	After	Before	After	Before	After	
Patient B											
APPT, sec		25–37	38	39	37	52	36	41	37	37	0.11
PI, %		80–132	87	97	105	92	93	94	94	93	1.00
TT, sec		15–19	18	17	18	19	17	17	16	17	1.00
Fibrinogen, mg/ml		2–4.5	2.5	3.1	2.5	2	2.1	2	2.1	2.2	0.66
D-dimer, μg/ml		0–500	244	**861**	229	115	138	144	91	79	0.89
TEG	α, deg	18–40	39.3	**40.7**	**53.7**	38.7	34.9	39.8	34	39.7	0.47
TGT	ETP, nM[a]min	800–1560	**2922**	1433	1385	1498	**1650**	1387	1277	**1700**	0.89
TD	V, μm/min	20–29	27	25	27	24	27	25	23	**30**	0.88
LDH, U/l		208–378	**1472**	**1486**	**1219**	**865**	**749**	**546**	**536**	**516**	0.89
Hemoglobin (Hb), g/l		130–160	77	54	74	70	84	64	86	87	0.31

The bold indicates the changes in parameters.
[a] The differences between the data sets before and after administration were performed using the Wilcoxon signed-rank test.

There were no changes ($p > 0.05$) in conventional clotting tests (APTT, TT, PI and fibrinogen), values were in the normal or even in hypocoagulation area during the eculizumab therapy (Table 6). The dynamics of global coagulation assay parameters during 8 months on therapy remained similar to those on the first month of eculizumab therapy. TEG parameter α was periodically increased in all three patients (in 55, 10 and 60% of all assessment points for the patients A, B and C, respectively). TGT parameter ETP was also periodically increased in all three patients (in 25, 40 and 10% of all assessment points for the patients A, B and C, respectively). TD parameter V was in hypercoagulation area periodically in all patients (55%, 30% and 60% of all assessment points for the patients A, B and C, respectively) (Fig. 4). Thus the hypercoagulation

Table 5
Laboratory data during the first month of eculizumab therapy for patient C.

Test		Reference values	1 administration		2 administration		3 administration		4 administration		P[a]
			Before	After	Before	After	Before	After	Before	After	
Patient C											
APPT, sec		25–37	29	30	32	27	30	32	29	31	0.88
PI, %		80–132	81	98	84	98	93	97	88	85	0.11
TT, sec		15–19	16	18	17	18	15	17	17	16	0.22
Fibrinogen, mg/ml		2–4.5	2.4	2	1.6	2.3	2	1.8	2	2	1.00
D-dimer, μg/ml		0–500	214	250	270	273	282	229	217	103	0.89
TEG	α, deg	18–40	37.3	**44.4**	**43.4**	**41.9**	28.5	**45.4**	**48**	26.8	1.00
TGT	ETP, nM[a]min	800–1560	–	1205	–	1284	1145	1071	1201	1392	0.49
TD	V, μm/min	20–29	**31**	**35**	**31**	**30**	27	**33**	29	25	0.66
LDH, U/l		208–378	**3173**	**–**	**980**	**791**	573	430	–	406	0.19
Hemoglobin (Hb), g/l		130–160	75	92	71	75	80	72	–	79	0.72

The bold indicates the changes in parameters.
[a] The differences between the data sets before and after administration were performed using the Wilcoxon signed-rank test.

Table 6
Laboratory data during the 2–8 months of eculizumab therapy.

Test	Reference values	Months of eculizumab therapy										
		2		3		4		5		6	7	8
Patient A												
APPT, sec	25–37	39	36	35	38	35	38	***38***[a]	35	32	32	34
PI, %	80–132	79	81	84	57	80	130	***56***	86	78	81	81
TT, sec	15–19	18	17	16	18	16	17	***19***	16	18	18	17
Fibrinogen, mg/ml	2–4.5	1.5	1.8	1.9	1.7	1.7	2	***1.9***	1.9	1.9	2.1	2.2
D-dimer, μg/ml	0–500	88	68	46	59	65	79	–	85	73	97	100
Patient B												
APPT, sec	25–37	37	40	39	40	35	37	–	***42***	38	38	36
PI, %	80–132	102	101	104	101	101	70	–	***84***	91	97	83
TT, sec	15–19	18	17	17	17	18	18	–	***20***	17	17	16
Fibrinogen, mg/ml	2–4.5	1.9	2	2.9	2	2.2	2.5	–	***2.1***	2.2	1.9	2.5
D-dimer, μg/ml	0–500	85	71	**515**	71	–	–	–	***88***	76	62	65
Patient C												
APPT, sec	25–37	35	32	29	30	33	33	–	29	29	30	27
PI, %	80–132	88	97	96	99	91	93	–	92	94	83	100
TT, sec	15–19	17	19	16	15	18	17	–	16	18	17	17
Fibrinogen, mg/ml	2–4.5	2.2	1.9	2.7	2.1	2.1	2.2	–	2.3	2	2.4	2.1
D-dimer, μg/ml	0–500	159	126	159	106	–	–	–	276	174	167	135

The bold indicates the changes in parameters. And italics indicates the point of hemolytic crisis.
[a] Points of crisis are in bold italics.

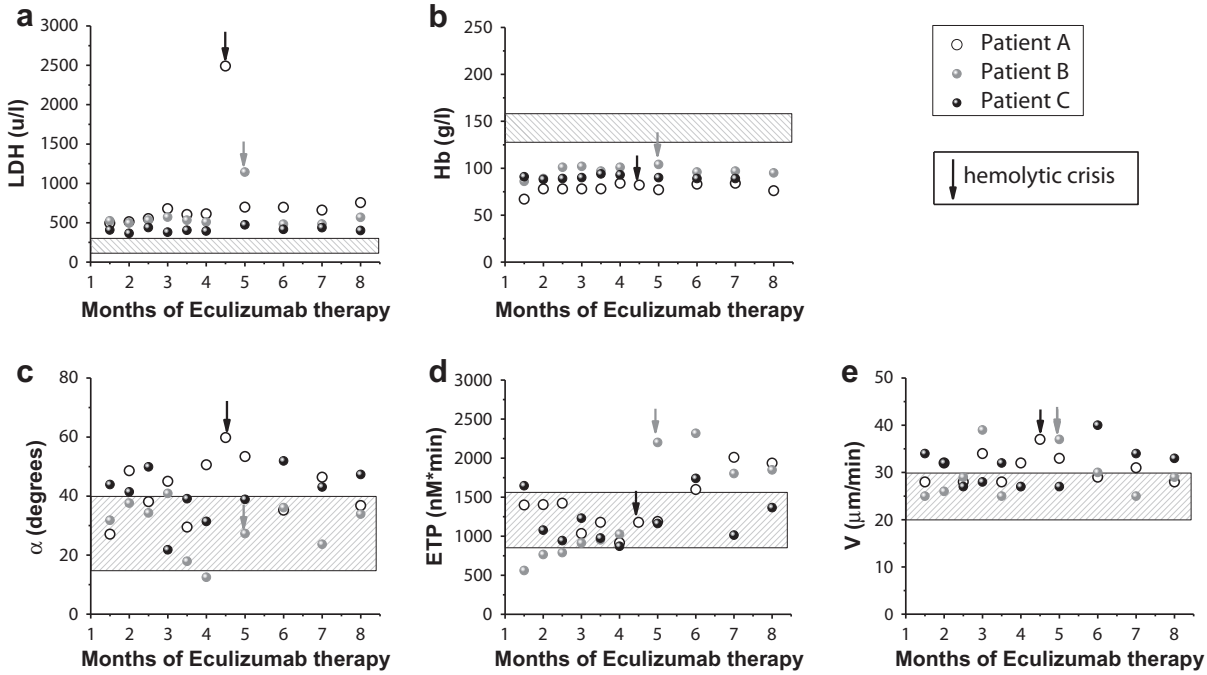

Fig. 4. Effect of eculizumab on hemolysis and hemostasis changes during the first month of therapy. LDH activity (a), hemoglobin concentration (b), TEG parameter α (c), TGT parameter ETP (d), and TD parameter V during the 8 months of eculizumab therapy. Gray shaded area is the normal range. Two hemolytic crises occurred during the studied period: in patient A (black arrow) and in patient B (gray arrow).

state was identified by two global tests in 10 of 20 assessment points and by three global tests in 3 points for all patients. Patient B had the increased D-dimer concentration (Table 6) on the third month of eculizumab therapy, while all of the global test parameters were in the normal range.

Patients A and B had repeated hemolytic crisis on the 5th month of eculizumab therapy with increased LDH activity, bilirubinemia, reticulocytosis, hemolyzed blood plasma, the positive urine hemosiderin and the positive direct Coombs test with growing titer. The TEG parameter α was increased in patient A and in patient B it was in the normal range. ETP in TGT conversely were in normal range in patient A and increased in patient B, V was increased in both patients. Interestingly, even in the absence of hypercoagulation as indicated by the global tests, the comparison with the previous point indicates the increase of clotting in 1.5–2 times by all global hemostasis assays for both patients (Fig. 4).

Discussion

The study of the effect of eculizumab treatment on the hemostatic state of three patients with PNH for 8 months showed that the incidence of hemolytic crises in patients was reduced from 3–6 to 0–1 for half a year of treatment.

There was statistically significant ($p < 0.05$) decrease in LDH activity during the eculizumab therapy in all three patients, which agrees well with the literature data [27–32]. Typically, the reduction in LDH activity is associated with the reduction of hemolysis, one of the thrombogenic mechanisms in PNH, leading to the reduction of thrombotic risk and hypercoagulability. However, we have not detected significant changes in the parameters of hemostasis during the 8 months of therapy. Thus, conventional clotting tests (APTT, TT, PI, fibrinogen concentration) were in the normal range on admission and during all eculizumab treatment in all the patients. D-dimer concentration rose slightly twice in patient B and was in normal range throughout the therapy in patients A and C. Despite the continuous therapy with eculizumab (drug retains its activity within 2 weeks from administration [31,32]) the parameters of global tests TEG, TGT and TD were periodically in the hypercoagulation area in all three patients. This confirmed the concept of multifactorial hypercoagulation disorders in patients with PNH. The hypercoagulability during the eculizumab therapy agrees well with the literature data. Thus, Weitz et al. [30] have shown that the increased production of factor Xa remained and the formation of procoagulant microparticles continues despite the eculizumab therapy. 20% of patients with PNH under eculizumab treatment indicated the increased risk markers associated with thrombotic risk: D-dimer concentration, prothrombin fragment F1 + 2, and plasminogen activator inhibitor activity [29].

Global hemostatic tests TEG, TGT and TD revealed the significant increase in the values of parameters α, ETP and V in two patients with repeated hemolytic crisis on the eculizumab therapy, followed by deterioration of physical condition of the patients. This agrees well with the literature data about hypercoagulability in patients with PNH with acute hemolysis [19–21].

It is known that not all the patients with PNH are significantly affected by the eculizumab therapy. Approximately 9% of patients completely retained hemolytic activity, and 20% had partial improvement, hemolytic crises occurred despite the ongoing therapy, but their frequency decreased, which correlates well with the condition of our patients [31,39–43]. Despite the 85% reduction in the risk of thrombosis during eculizumab therapy, thrombotic complications in patients still happen [28,30,32].

In our study the reduction of hemolysis markers and transfusion dependence, improvement in physical health and thus in quality of life were shown in three patients with PNH under eculizumab therapy. But this study also shows that this therapy does not lead to the complete disappearance of crisis episodes in two of the three patients with PNH with the increased parameters of TEG, TGT and TD. This may be the reason for continuing preventive anticoagulant therapy during eculizumab treatment.

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