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ORIGINAL ARTICLE

Pregnancy outcomes of patients with paroxysmal nocturnal hemoglobinuria treated with eculizumab: a Japanese experience and updated review

Naoyuki Miyasaka[1,14] · Osamu Miura[2,14] · Tatsuya Kawaguchi[3,14] · Nobuyoshi Arima[4,14] · Eriko Morishita[5,14] · Kensuke Usuki[6,14] · Yasuyoshi Morita[7,14] · Kaichi Nishiwaki[8,14] · Haruhiko Ninomiya[9,14] · Akihiko Gotoh[10,14] · Shinsaku Imashuku[11,14] · Akio Urabe[6,14] · Tsutomu Shichishima[12,14] · Jun-ichi Nishimura[13,14] · Yuzuru Kanakura[13,14]

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Abstract Pregnancy with paroxysmal nocturnal hemoglobinuria (PNH) is associated with significant risk of complications, such as life-threatening thrombosis. Recently, eculizumab has come into clinical use and revolutionized the treatment of PNH. However, clinical information regarding eculizumab use for PNH during pregnancy is limited. The present report describes pregnancies with PNH treated with eculizumab that were registered with the Japan PNH study group and reviews the literature. In case 1, the patient received eculizumab throughout pregnancy and delivered a healthy neonate at term, although breakthrough hemolysis occurred at 20 weeks of gestation. In case 2, the patient discontinued eculizumab before pregnancy and developed preeclampsia at 27 weeks of gestation. She received eculizumab and delivered a preterm, but healthy, neonate by cesarean section. In case 3, the patient received eculizumab from 18 weeks of gestation and delivered a healthy neonate at term without any complications. Reports of 11 pregnant women treated with eculizumab were identified in the literature. Of 14 pregnancies, including our own cases, breakthrough hemolysis and preeclampsia occurred in five and two cases, respectively. There were no thrombotic complications, maternal or neonatal deaths, or fetal structural abnormalities. Thus, eculizumab appears to be safe and effective for managing PNH during pregnancy.

Keywords Paroxysmal nocturnal hemoglobinuria · Pregnancy · Eculizumab

✉ Naoyuki Miyasaka
n.miyasaka.gyne@tmd.ac.jp

1 Department of Pediatrics, Perinatal and Maternal Medicine, Graduate School of Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan

2 Department of Hematology, Graduate School of Tokyo Medical and Dental University, Tokyo, Japan

3 Departments of Hematology and Infectious Diseases, Kumamoto University Hospital, Kumamoto, Japan

4 Department of Hematology, Medical Research Institute Kitano Hospital, Osaka, Japan

5 Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan

6 Department of Hematology, NTT Medical Center Tokyo, Tokyo, Japan

7 Division of Hematology and Rheumatology, Department of Internal Medicine, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan

8 Department of Oncology and Haematology, Jikei University Kashiwa Hospital, Kashiwa, Chiba, Japan

9 Division of Clinical Medicine, Faculty of Medicine, University of Tsukuba, Ibaraki, Japan

10 Division of Hematology, First Department of Internal Medicine, Tokyo Medical University, Tokyo, Japan

11 Division of Hematology, Takasago-Seibu Hospital, Takasago, Japan

12 Department of Cardiology and Hematology, Fukushima Medical University, Fukushima, Japan

13 Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan

14 Working Group for Pregnancy in PNH, The Japan PNH Study Group, Tokyo, Japan

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a consequence of the clonal expansion of hematopoietic stem cells that have an acquired somatic mutation in the *PIG-A* gene [1–3]. The affected hematopoietic cells are unusually susceptible to complement-mediated cell lysis, because *PIG-A* mutations lead to the deficiency of glycosyl phosphatidylinositol-anchored proteins (GPI-APs) including the complement-regulatory proteins CD55 and CD59 [4–6]. The clinical manifestation of PNH involves hemolysis with acute exacerbation, cytopenia of varying severity, and a tendency for thrombosis [7–10]. Although PNH has a slight female predominance and is usually diagnosed in a person's early 30s [8, 10], female patients with PNH have historically been discouraged from becoming pregnant because of the increased risk of complications, such as exacerbated hemolytic anemia and life-threatening thromboembolisms [11]. Recently, eculizumab has come into clinical use and revolutionized the treatment of PNH [12, 13]. However, clinical experience and information regarding eculizumab use for pregnant women are limited, and the optimal strategy for the management of women with PNH during pregnancy remains unclear. In response to this issue, the Japan PNH Study Group has organized a working group for pregnancies in PNH (pPNHWG) and has begun to register pregnant patients with PNH in order to share clinical data and pregnancy outcomes. The purposes of this report were to present three pregnancies with PNH registered with the working group and to update the review of pregnancies with PNH treated by eculizumab.

Materials and methods

Four ongoing pregnancies with PNH were registered with pPNHWG between 2011 and 2013. The clinical decisions regarding patient management were made by the attending physicians of each patient in accordance with the Helsinki Declaration, and clinical data were retrospectively obtained after the pregnancies were completed.

Additionally, we searched for cases of patients with PNH in whom eculizumab was used during pregnancy in PubMed and OVID using the following key words: "pregnancy", "paroxysmal nocturnal hemoglobinuria", and "eculizumab". The obtained reports were carefully reviewed, and the clinical data, including age at pregnancy, PNH clone size in granulocytes, lactate dehydrogenase (LDH) level before eculizumab use, anticoagulation therapy before and during pregnancy, duration of eculizumab use during pregnancy, complications during pregnancy and the postpartum period, gestational week at delivery, delivery method, newborn status, and the eculizumab concentration in the maternal blood, umbilical cord blood, and breast milk, were extracted if available.

Results

Among the four pregnancies registered with pPNHWG, one case was already published in the literature [14]. Therefore, we herein report the other three cases.

Case 1

The case 1 patient was diagnosed with severe aplastic anemia at the age of 20 years, which went into complete remission with immunosuppressive therapy. She was found to have a small PNH clone (20 %) at the age of 25 years, which gradually increased in size, and she developed clinically evident hemolytic anemia at the age of 27 years. She became completely dependent on red blood cell (RBC) transfusion at the age of 29 years, with her PNH clone size exceeding 90 %. Regular eculizumab therapy (900 mg every 2 weeks) was initiated at the age of 33 years, and she became transfusion-independent, with her LDH levels remaining within the normal range. She experienced no thrombotic events. She became pregnant at the age of 34 years. In spite of the eculizumab therapy and newly added folic acid supplementation, her hemoglobin level gradually decreased, with reciprocal increases in the LDH and CH50 levels (10–30 %). She had a minor hemolytic episode at 20 weeks of gestation, which was triggered by a common cold and required 2 units of RBC transfusion. As her D-dimer level increased gradually, prophylactic subcutaneous unfractionated heparin (UFH) was initiated at 10,000 U/day at 22 weeks of gestation, increased to 15,000 U/day at 35 weeks, changed to continuous infusion 1 day before delivery, and was stopped 5 h before delivery. She underwent transfusion with 8 units of RBC before delivery, as her hemoglobin (Hb) level decreased to reach 6.9 g/dL. At 37 weeks of gestation, she had a normal spontaneous vaginal delivery of a female infant without any anomalies. The infant weighed 2662 g, with Apgar scores of 8 and 9 at 1 and 5 min, respectively. The postpartum course was uneventful, and breast-feeding was carried out. Subcutaneous heparin was reinitiated 1 day after delivery and continued for 8 weeks. The patient remains healthy on eculizumab continuation, with LDH levels within the normal range and undetectable CH50 levels. Eculizumab was detected in the cord blood at 11.9 μg/mL but was undetectable in the breast milk even immediately after its infusion. At 3 years of age, the child is developing normally. The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy is shown in Fig. 1.

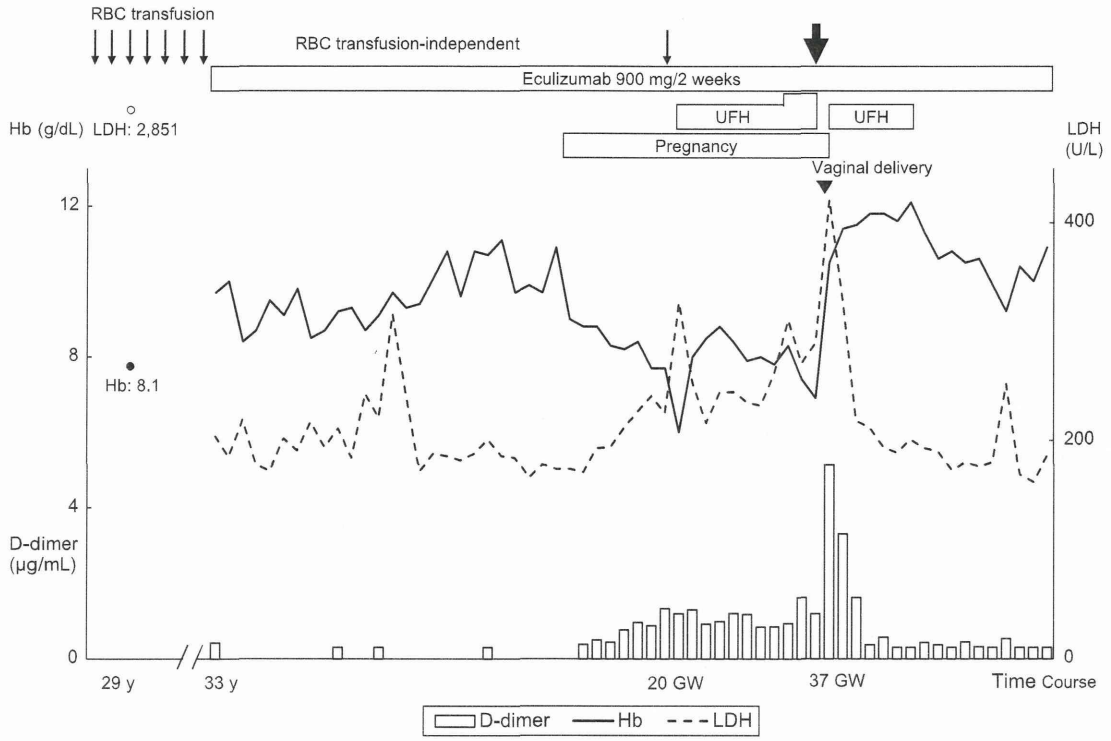

Fig. 1 The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy of case 1

Case 2

The case 2 patient was diagnosed with aplastic anemia-PNH at the age of 17 years and was treated with cyclosporine and RBC transfusions. Her PNH clone size exceeded 70 %, and regular eculizumab therapy was initiated when she was 26 years old. Thereafter, she became transfusion-independent, with LDH levels maintained within the normal range. She experienced no thrombotic events. She discontinued both eculizumab and cyclosporine therapy at the age of 29 years because she desired to become pregnant, which resulted in recurrence of florid intravascular hemolysis (LDH, 1500–2300 U/mL), worsening of anemia and thrombocytopenia (Hb, 5.1 g/dL; platelet counts, 30–50 $\times$ 10^9/L), and RBC transfusion dependence. She became pregnant at the age of 30 years, and prophylactic low-molecular-weight heparin (LMWH) injections were initiated at 21 weeks of gestation because her FDP and D-dimer levels had gradually increased. Danaparoid was also used in lieu of LMWH when her thrombocytopenia transiently worsened. She developed preeclampsia (hypertension and gross proteinuria) at 27 weeks of gestation, and eculizumab was reinstated after the patient provided informed consent. She received additional RBC and platelet transfusions in preparation for termination of pregnancy and underwent emergency cesarean section at 28 weeks of gestation and delivered a healthy newborn weighing 853 g without any anomalies. There was no hemolytic attack or LDH surge during the perioperative period, suggesting a sufficient effect of even a single dose of eculizumab against surgery-induced compliment activation. Pathological examination of the placenta showed infarction and chorangiosis. The patient recovered quickly from the preeclampsia without any complications and was discharged 18 days after surgery. Although the neonate was hospitalized for 3 months in the neonatal intensive care unit due to its immaturity, it developed well without any major complications and was discharged with a body weight of 2670 g. Eculizumab was not detected in either the cord blood or the breast milk. The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy is shown in Fig. 2.

Case 3

The case 3 patient was diagnosed with mild aplastic anemia and iron deficiency at the age of 25 years, which was treated with oral iron supplementation. She became pregnant at the age of 29 years, and noticed red urine in the morning at 10 weeks of gestation. Laboratory studies showed hemolytic anemia (Hb, 7.8 g/dL) with elevated LDH (2300 U/mL). After physicians detected a large PNH clone (81 %), her diagnosis was changed to

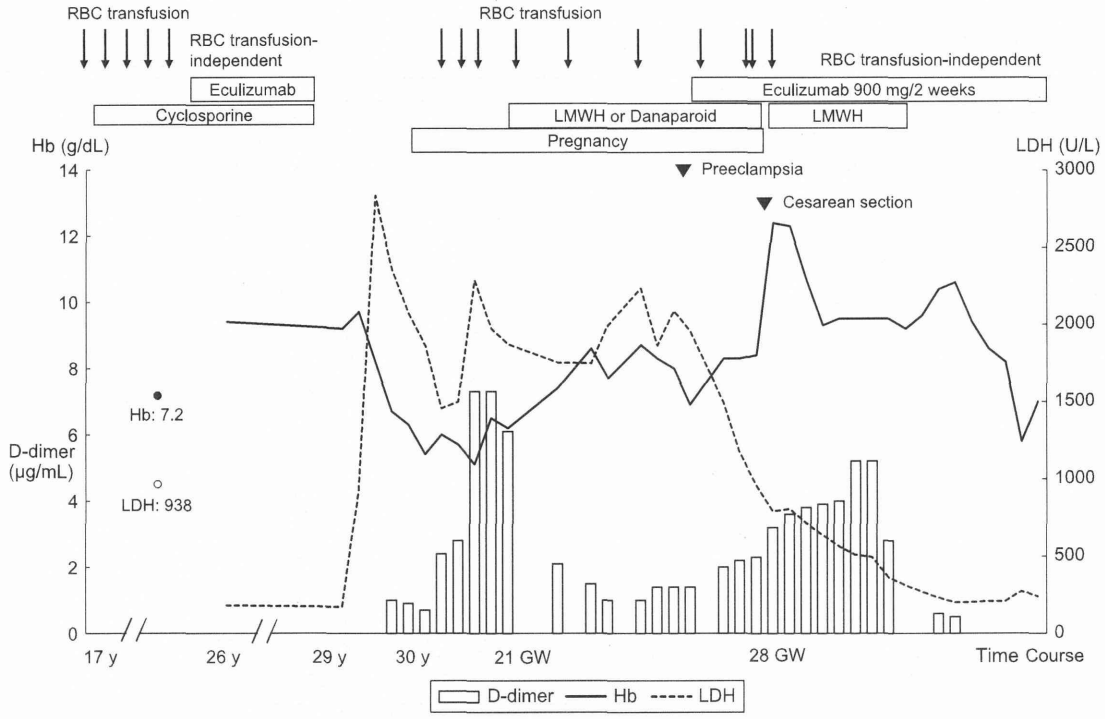

Fig. 2 The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy of case 2

PNH. After the patient provided informed consent and underwent meningococcal vaccination, she began regular eculizumab therapy (900 mg every 2 weeks) and prophylactic UFH injection at 18 weeks of gestation. The proteinuria disappeared and the level of LDH decreased within a few weeks (Fig. 3). However, the anemia did not improve and the level of LDH remained higher (average, 358 U/mL) than normal. Although the doses of eculizumab and UFH were not changed, D-dimer was kept to <1.5 μg/mL throughout the pregnancy. She had a normal spontaneous vaginal delivery at 40 weeks of gestation of a female infant without any anomalies weighing 3110 g. Pathological examination of the placenta showed decidual vasculopathy, suggesting incomplete remodeling of the spiral arteries. Subcutaneous heparin was reinitiated 12 h after delivery and continued for 5 weeks. Because the postnatal bleeding was heavy, the patient received a one-time RBC transfusion and was discharged with her neonate 6 days after delivery. The patient remains well on eculizumab continuation, with LDH levels within the normal range and CH50 levels at the minimum limit of detection. Eculizumab was detected in the cord blood at 15.0 μg/mL but was undetectable in the breast milk even immediately after its infusion. At 3 years of age, the child is developing normally. The time course of the changes in Hb, LDH, and D-dimer after pregnancy is shown in Fig. 3.

Updated review

Five single-case reports [14–18] and one report of a series of six cases [19] were found in the literature. The clinical characteristics and pregnancy outcomes of these patients together with those of our own cases are summarized in Table 1. The mean age of the patients was 30.2 years, and the mean PNH clone size in granulocytes was 77.7 %. LDH levels before eculizumab use exceeded 1000 in all cases. Therapeutic or prophylactic anticoagulation therapy was prescribed during pregnancy in all cases but one. Eculizumab was used only early in gestation and was ceased after the patient learned she was pregnant in three cases; it was initiated in the second or third trimester of pregnancy in four cases; and it was used during the entire pregnancy in seven cases. Eculizumab infusion at a higher dose or shorter interval than those of the standard protocol (900 mg every 2 weeks) was performed in four cases due to breakthrough hemolysis. Breakthrough hemolysis requiring blood transfusion occurred in five cases, whereas no thromboembolic complications or maternal death have been reported. Preeclampsia was found in two cases, and the pregnancies were terminated by cesarean section at 28 weeks gestation in both cases. Preterm delivery was observed in three cases, of which two were due to preeclampsia and the other due to a twin pregnancy. All newborns were healthy and without any structural anomalies. Eculizumab was detected in the cord

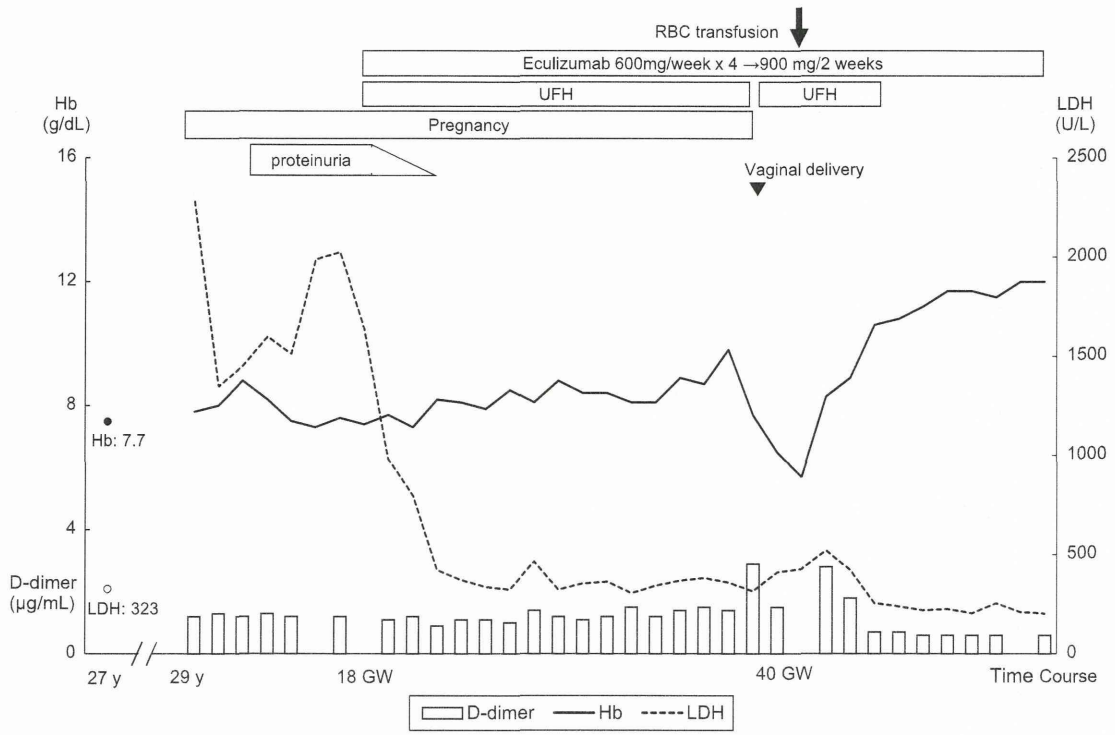

Fig. 3 The time course of the changes in Hb, LDH, and D-dimer after pregnancy of case 3

blood in 4 of 8 samples measured, although the concentration was much lower than the therapeutic level. Eculizumab was not detected in any sample of breast milk obtained from six patients, except for one sample for which maternal blood contamination could not be completely excluded.

Discussion

We presented three cases of PNH in which pregnancies were successfully managed with eculizumab therapy. In cases 1 and 2, PNH had been diagnosed and eculizumab therapy was already initiated before the women became pregnant. Eculizumab therapy was continued throughout the pregnancy in case 1, whereas it was discontinued before the patient became pregnant and reinstated after the onset of preeclampsia and just before cesarean section in case 2. In case 3, the diagnosis of PNH was made after the patient became pregnant and eculizumab therapy was initiated in the second trimester of pregnancy.

Pregnancy outcomes of patients with PNH before eculizumab came into clinical use and its pathophysiological consideration

Fieni et al. reviewed 43 cases of pregnancies in women with PNH published in English between 1965 and 2005 together with their own case [11] and reported that minor complications occurred in 79 % of cases, major complications occurred in 35 % of cases, and maternal mortality was 12 % during pregnancy and the postpartum period. The most frequent minor complications were exacerbated anemia and thrombocytopenia, and RBC and/or platelet transfusion was required in 63 % of patients. The representative major complications associated with maternal death were thrombotic diseases, such as Budd–Chiari syndrome, cerebral sinus thrombosis, and pulmonary thromboembolisms. Since prophylactic anticoagulation therapy was undertaken only in 14 % of cases, the authors emphasized the importance of prophylactic anticoagulation therapy. On the other hand, de Guiber summarized 25 pregnancies with PNH in France between 1978 and 2008 [20], and reported that thrombotic events occurred in 20 % and that maternal mortality was 8 % although prophylactic anticoagulation therapy was undertaken in 72 % of patients. In addition, hypertensive disorders including preeclampsia, eclampsia, and HELLP syndrome (hemolysis, elevated liver enzyme, and low platelet count) were observed in 14 and 20 % in each report, respectively, which were significantly higher than those in the normal population (3–5 %). Furthermore, heavy uterine bleeding during parturition and in the postpartum period was observed in 5 and 12 % of cases in the two reports.

Table 1 Summary of patient characteristics and pregnancy outcomes when eculizumab was used during pregnancy

Case number	Reference	Maternal age at pregnancy (years)	PNH granulocyte clone size (%)	LDH at baseline (IU/L)	Anticoagulation therapy		Eculizumab use in pregnancy	Complications in pregnancy	Gestational week at delivery (weeks)	Delivery method (indication)	Newborn status [birth weight (g)]	Eculizumab level (μg/mL)		
					Before pregnancy	During pregnancy						Maternal blood	Cord blood (days after last dose)	Breast milk (days after last dose)
1	Danilov et al. [14]	34	30	1500	Therapeutic heparin	Therapeutic heparin	From 30 weeks and postpartum	Thrombocytopenia, RBC/PLT transfusion	36	C-section (twin, breech)	Healthy (2919 and 2199)	NM	NM	NM
2	Kelly et al. [18]	25	92.9	2376	Warfarin	Therapeutic heparin	Up to 5 weeks	None	Not stated	Not stated	Healthy (not stated)	NM	NM	NM
3	Kelly et al. [18]	22	95.8	2014	Not known	Not known	Up to 14 weeks	Postpartum pyrexia of unknown origin	Not stated	Not stated	Healthy (not stated)	NM	NM	NM
4	Kelly et al. [18]	26	87.5	1263	Not known	Therapeutic heparin	Up to 4 weeks	None	Not stated	Not stated	Healthy (not stated)	NM	NM	NM
5	Kelly et al. [18]	27	99.7	10,300	No	Prophylactic heparin	Entire pregnancy (increased from 28 weeks) and postpartum	Breakthrough hemolysis, RBC transfusion	Term	Normal vaginal	Healthy (not stated)	116.1	Undetected	Undetected (1, 2, 3, 9, 10 days after delivery)
6	Kelly et al. [18]	35	97.6	1616	No	Therapeutic heparin	From 27 weeks (weekly) and postpartum	Postpartum hemorrhage	35	C-section (twin)	Healthy (2400 and 2000)	80.5	19.2/14.4	NM
7	Kelly et al. [18]	28	98.1	2642	Warfarin	Therapeutic heparin	Entire pregnancy and postpartum	Preeclampsia	28	C-section (preeclampsia)	Healthy (900)	63.2	Undetected	NM

Table 1 continued

Case number	Reference	Maternal age at pregnancy (years)	PNH granulocyte clone size (%)	LDH at baseline (IU/L)	Anticoagulation therapy		Eculizumab use in pregnancy	Complications in pregnancy	Gestational week at delivery (weeks)	Delivery method (indication)	Newborn status [birth weight (g)]	Eculizumab level (μg/mL)		
					Before pregnancy	During pregnancy						Maternal blood	Cord blood (days after last dose)	Breast milk (days after last dose)
8	Marasca et al. [15]	34	69	Not stated	No	Prophylactic heparin	Entire pregnancy and postpartum	None	38	Normal vaginal	Healthy (3430)	NM	NM	NM
9	Ando et al. 2014 [13]	37	56	2300	No	No	Entire pregnancy and postpartum	None	37	C-section (breech)	Healthy (2428)	NM	16.9 (5 days)	10.1/undetected (2/4 days)
10	Sharma et al. [16]	32	90	Not stated	No	Prophylactic heparin	Entire pregnancy (increased from 30 weeks) and postpartum	Breakthrough hemolysis, RBC transfusion	36	C-section (elective)	Healthy	11 before increase 100 after increase	Undetected	Undetected
11	Patriquin et al. [17]	30	23	1000–2000	No	Prophylactic heparin	Entire pregnancy (increased from 2nd trimester) and postpartum	Breakthrough hemolysis, RBC transfusion	36	C-section (placenta previa)	Healthy	NM	NM	NM
12	Our case 1	34	96	2200	No	Prophylactic heparin	Entire pregnancy and postpartum	Breakthrough hemolysis, RBC transfusion	37	Normal vaginal	Healthy (2662)	NM	11.9 (10 days)	Undetected (0 and 4 days)
13	Our case 2	30	71	2300	No	Prophylactic heparin	From 27 weeks and postpartum	Preeclampsia	28	C-section (preeclampsia)	Healthy (830)	103.2	Undetected (3 days)	Undetected (1 day)
14	Our case 3	29	81	2300	No	Prophylactic heparin	From 18 weeks and postpartum	Postpartum hemorrhage	40	Normal vaginal	Healthy (3110)	101.8	15.0 (9 days)	Undetected (1 day)

PNH paroxysmal nocturnal hemoglobinuria, *LDH* lactate dehydrogenase, *C-section* cesarean section, *NM* not measured

When considering the pathophysiology of pregnancies with PNH, two important pregnancy-related physiological changes in women should be taken into account, i.e. complement activity and the balance between coagulation and anticoagulation systems. Tedder et al. reported that CH50, C3, C4, C6, and C7 increased and C1 inhibitor decreased throughout pregnancy [21], indicating that the complement system was activated during pregnancy. Because PNH cells are unusually susceptible to complement activation, intravascular hemolysis is exaggerated, resulting in an increased requirement of blood transfusion during pregnancy. As for the coagulation systems, pregnancy is associated with increased levels of factors VII, VIII, X, XII, XIII, and von Willebrand factor. The circulating fibrinogen level doubles in pregnancy, while protein S and tissue plasminogen activator are decreased [22]. These findings suggest that the balance between pro- and anticoagulation systems and fibrinolysis is shifted towards a hypercoagulable state during pregnancy. Although the mechanisms responsible for thrombus formation in PNH is complex, elevated extracellular free hemoglobin, excessive platelet activation, and a physiological prothrombotic state during pregnancy appear to operate synergistically and cause serious thrombotic complications [23]. As is known in the anti-phospholipid syndrome (APS), thrombophilic conditions in early gestation are associated with insufficient implantation and placental ischemia, which lead to recurrent miscarriage, fetal growth restriction (FGR), and pregnancy-induced hypertension, specifically early-onset preeclampsia (less than 32 weeks gestation) [24], which might explain why hypertensive disorders are increased in pregnancies with PNH. Furthermore, vascular endothelial dysfunction caused by intravascular hemolysis forms a vicious cycle together with vasospasm and microthrombus formation, which leads to thrombotic microangiopathy (TMA), including thrombocytopenia and HELLP syndrome [25]. Although hemorrhagic delivery appears to be associated with anticoagulation therapy, it might be partly caused by uterine muscle dysfunction due to PNH-related smooth muscle dystonia [26].

Pregnancy outcome of the patient with PNH treated with eculizumab

Eculizumab is a humanized monoclonal antibody directed against the terminal complement protein C5, and it inhibits complement-mediated cell lysis [27]. According to clinical trials in PNH patients, eculizumab stabilizes hemoglobin levels; reduces intravascular hemolysis, thrombotic events, and transfusion requirements; and improves quality of life [12, 28]. As eculizumab inhibits intravascular hemolysis itself, it can be expected to decrease the risk of complications during pregnancy and the postpartum period in women with PNH. As shown in the table, pregnancy outcomes of patients with PNH taking eculizumab during pregnancy were almost favorable. Specifically, it is noteworthy that no thromboembolic complications or maternal death has been reported. However, breakthrough hemolysis frequently occurred during pregnancy, and the eculizumab infusion protocol had to be changed by reducing the interval or increasing the dose because of breakthrough hemolysis in four cases. These findings appear to be associated with the relative shortage of eculizumab because of increased complement levels and dilution effects by increased plasma volume during pregnancy. This idea is supported by the fact that CH50 during pregnancy was more elevated than that postpartum in case 1, in which the schedule of eculizumab infusion was not changed throughout the pregnancy. The relatively elevated LDH levels in our cases 1 and 3 during pregnancy also suggested that regular doses of eculizumab infusion was insufficient for pregnant women with PNH. Sharma et al. measured serum eculizumab concentration in a pregnant woman and reported that it was much lower than the therapeutic level [17]. Therefore, it might be necessary to monitor lactate dehydrogenase (LDH) and/or CH50 levels and accordingly adjust the dosage and/or interval of eculizumab infusion. Preeclampsia was found in two cases and both pregnancies were terminated by cesarean section at 28 weeks' gestation. In our case 2, cessation of eculizumab before pregnancy might have been associated with the development of preeclampsia because preeclampsia is partly caused by insufficient implantation due to maternal micro-vascular dysfunction [29]. However, in the other case, preeclampsia occurred despite the continuation of eculizumab, suggesting that PNH is associated with an increased risk for preeclampsia even on eculizumab therapy.

Eculizumab is a hybrid of IgG4 and IgG2, and the latter is known to be less capable of crossing the placenta [30]. However, among eight cases in which eculizumab concentration in the cord blood was measured, it was detected in four cases. Although the concentration was substantially lower than the therapeutic level and no obvious impairment of the complement function in the newborn was reported [31], this level may not be negligible for the fetus. Therefore, long-term follow-up of the infants and accumulation of clinical data are necessary. Nonetheless, it should be noted that no anomalies or adverse events have been reported thus far in neonates whose mothers have received eculizumab, even in the cases in which eculizumab was administered during the first trimester of pregnancy.

Optimal anticoagulation therapy during pregnancy in eculizumab era

Although the prevention of thrombotic complications during pregnancy is essential, it is not clear whether the use of anticoagulants is mandatory for patients receiving

eculizumab therapy. Theoretically, if intravascular hemolysis is completely inhibited by eculizumab, the risk of thromboembolic complications would become as low as that in non-PNH pregnancies. In fact, prophylactic anticoagulant therapy was initiated from the second trimester and no thrombotic event occurred in our cases 1 and 3. However, the half-life of eculizumab is relatively long (10–12 days) and subtle adjustment of its dosage and/or interval is not easy. Therefore, realistically, complementary anticoagulant therapy seems necessary, along with close monitoring of thrombotic markers, such as the D-dimer level. Conversely, eculizumab could reduce the need for anticoagulants, which will in turn help decrease heparin-associated complications, such as hemorrhagic deliveries.

In conclusion, it appears that eculizumab can be safely used during pregnancy, and eculizumab use in combination with prophylactic anticoagulation therapy can reduce the risks of thrombotic complications and maternal death. These findings are in consistent with the recently published data from a relatively large number of pregnant patients with eculizumab treatment [32]. However, risks of breakthrough hemolysis and preeclampsia remain even in patients treated with eculizumab. In order to reduce the requirement for blood transfusion during pregnancy, adjustment of the dosage and/or interval of eculizumab infusion by monitoring LDH and/or CH50 levels might be necessary.

Several questions remain unanswered. Should eculizumab be used for all pregnant patients with PNH, or should it be used only in selected patients based on, for example, LDH levels and/or PNH clone size? Should eculizumab be initiated in the first trimester of pregnancy or the second trimester? Is there any adverse effect during development of the child who was exposed to eculizumab during the fetal period? Further study is necessary to answer these questions and to develop an evidence-based guideline for the management of pregnancies with PNH.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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