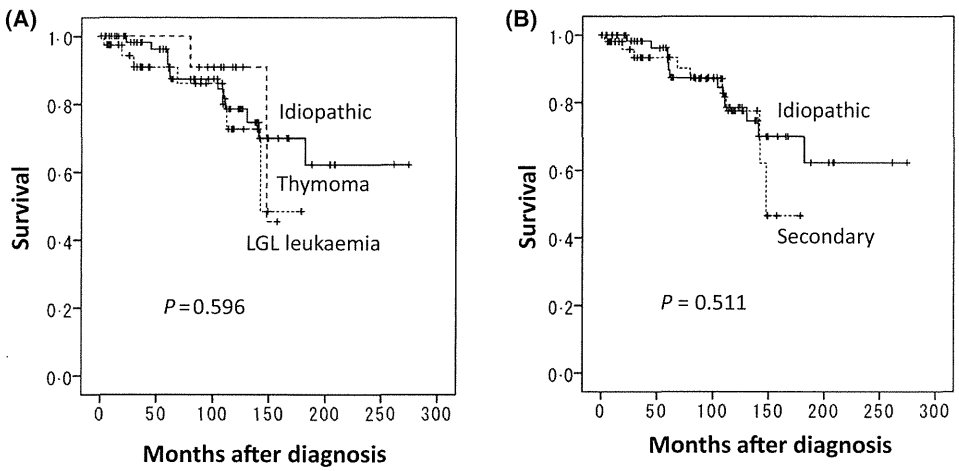

Fig 1. Overall survival of PRCA patients. Kaplan–Meyer estimates of survival for patients with idiopathic (continuous line), thymoma-associated (dotted line), and LGL leukaemia-associated (dashed spaced line) PRCA are shown (A). The overall survival of patients with idiopathic PRCA (continuous line) was also compared to that of patients with secondary PRCA (dotted line) consisting of thymoma-associated and LGL leukaemia-associated PRCA (B). LGL, large granular lymphocyte; PCRA, pure red cell aplasia.

idiopathic PRCA was estimated to be 212·6 months (95% confidence internal: 183·3–242·0). The estimated median overall survival times in thymoma-associated PRCA and LGL leukaemia-associated PRCA were 142·1 and 147·8 months, respectively (Fig 1A). Survival time was not significantly different among the three subtypes of PRCA (P = 0·596), nor was there a significant difference between the idiopathic and secondary forms of PRCA (P = 0·511) (Fig 1B). Given that the average life expectancies for men and women aged 65 years were 18·88 and 23·97 years, respectively, according to the 2009 report of the Ministry of Health, Labour and Welfare of Japan (www.mhlw.go.jp/english/wp/wp-hw5/dl/23010102e.pdf), the life expectancy of patients with acquired chronic PRCA patients to be shorter than the general population of Japan.

Cause of death and risk factors

Twenty-two deaths were reported, and the outcome was unknown in two patients. The leading causes of death were infection and organ failure (Table I). The status of PRCA on the last observations was reported in 15 out of these 22 patients, and 11 patients were not in remission. The causes of death in three patients in remission, two of whom were still taking oral ciclosporin, included lymphoma (N = 1) and pneumonia (N = 1), and the cause of death was not determined in the remaining patient (data not shown).

Potential risk factors for death in the three subtypes of PRCA were tested, including age, gender, aetiology and response to induction therapy, and the response to induction therapy was identified as a risk factor for death in PRCA patients (P = 0·002) (Table II). Observational periods were not different depending upon the outcome.

Next, we examined whether relapse of anaemia would be attributed to death in patients who showed a haematological response after immunosuppressive therapy. Potential risk factors for death in patients with CR or PR were tested, including age, gender, aetiology and relapse of anaemia, and we identified relapse of anaemia as a risk factor for death (P < 0·001) (Table III). Relapse of anaemia was not known in one patient. Refractoriness to induction immunosuppressive therapy and relapse of anaemia after successful immunosuppression were risk factors for death in idiopathic, thymoma-associated, and LGL leukaemia-associated PRCA.

Table II. Risk factors for death in idiopathic, thymoma-associated and LGL leukaemia-associated PRCA.

Category and variable	Alive (N = 102)	Death (N = 22)	P value
Age, years (median)	18–89 (59·1)	31–82 (57·8)	0·696
Observational period, months	0–274 (86·3)	3–182 (85·6)	0·962
Gender			
Male	39	12	0·131
Female	63	10	
Aetiology			
Idiopathic	58	13	0·755
Thymoma-associated	32	7	
LGL leukaemia-associated	12	2	
Idiopathic vs. secondary			
Idiopathic	58	13	0·698
Secondary	44	9	
Response to induction therapy			
CR	49	4	0·002
PR	38	8	
NR	9	9	

LGL, large granular lymphocyte; PCRA, pure red cell aplasia; CR, complete remission; PR, partial remission; NR, no response.

Table III. Risk factors for death in responders.

Category and variable	Alive (N = 87)	Death (N = 12)	P value
Age, years: range (median)	18–89 (60·0)	31–73 (58·0)	0·756
Observational period, months: range (median)	4–274 (98·3)	45–181 (112·2)	0·102
Gender			
Male	35	6	0·404
Female	52	6	
Aetiology			
Idiopathic	53	7	0·903
Thymoma-associated	23	3	
LGL leukaemia-associated	11	2	
Idiopathic vs. secondary			
Idiopathic	53	7	0·941
Secondary	34	5	
Relapse of anaemia			
Yes	31	12	<0·001
No	55	0	
Unknown	1	0	

LGL, large granular lymphocyte.

In order to confirm that the response to induction therapy and relapse of anaemia were associated with death, we analysed the survival time of PRCA patients. Because of the data availability, 98 previously untreated patients and 76 patients with relapsed PRCA were included in this analysis. Treatment response and relapse of anaemia are time-dependent covariates, and these factors were analysed as a risk for survival following study entry using the Mantel-Byar method to avoid the guarantee time bias. The time between the date for treatment response and relapse of anaemia was also treated as guarantee time and we analysed the survival time in patients, once they had achieved a response, by the same method. As shown in Fig 2, a significant difference in survival was demonstrated between the patients in response to induction immunosuppressive therapy and those with NR. There was also a significant difference between the patients remaining in remission and relapsing PRCA patients.

Re-induction therapy for relapsing PRCA

The efficacy of remission induction therapy as an initial treatment and re-induction therapy for subsequent relapse was previously reported in detail (Sawada *et al*, 2007; Fujishima *et al*, 2008; Hirokawa *et al*, 2008). In the present study, we updated the data regarding the treatment and response of relapsing PRCA. Immunosuppressants were administered in 38 patients (23 ciclosporin, 6 cyclophosphamide, 8 corticosteroid, 1 antithymocyte globulin) and anabolic steroid was administered in one patient for the relapse of anaemia (Table IV). Haematological response was obtained in 69·6% of patients with idiopathic PRCA, 50·0% of patients with thymoma-associated PRCA and 80·0% of patients with LGL leukaemia-associated PRCA. In total, 65·8% of patients with relapsing PRCA responded to immunosuppressive therapy. Response of relapsing PRCA to immunosuppressive therapy was inferior to those of previously untreated (treatment-naïve) patients, except for those with LGL leukaemia-associated PRCA (P = 0·679).

Discussion

There were three major findings of the present study. First, the survival time was not significantly different among

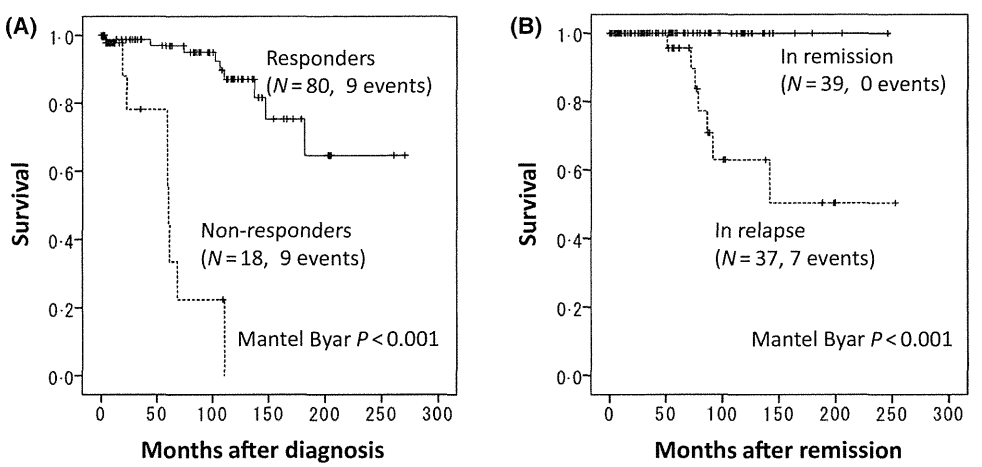

Fig 2. Outcome of pure red cell aplasia patients according to the treatment response and relapse of anaemia (Mantel-Byar estimations). (A) Probability of survival in patients responding to the treatment and those not responding to the treatment. (B) Probability of survival in those patients who achieved and remained in remission and those who achieved remission but eventually relapsed. These curves do not represent usual Kaplan–Meyer estimates, because treatment response and relapse of anaemia are time-dependent covariates. Please note that graphical representation of Mantel-Byar estimates should thus not be interpreted as curves for comparing pre-defined patient groups.

Table IV. Efficacy of immunosuppressive therapy for relapsing PRCA.

	Responder, *n* (%)	Non-responder, *n*	*P* value
Idiopathic			
Untreated (N = 62)	58 (93·5)	4	0·010
1st relapse (N = 23)	16 (69·6)	7	
Thymoma-associated			
Untreated (N = 34)	30 (88·2)	4	0·029
1st relapse (N = 10)	5 (50·0)	5	
LGL leukaemia-associated			
Untreated (N = 14)	12 (85·7)	2	0·679
1st relapse (N = 5)	4 (80·0)	1	
Total			
Untreated (N = 110)	100 (90·9)	10	<0·001
1st relapse (N = 38)	25 (65·8)	13	

LGL, large granular lymphocyte; PCRA, pure red cell aplasia.

patients with idiopathic, thymoma-associated, and LGL leukaemia-associated PRCA. This was also true when patients with idiopathic PRCA were compared with patients with a secondary form of PRCA (P = 0·511). However, the life expectancy of acquired chronic PRCA patients appears to be shorter than the general population of Japan; average life expectancies for men and women aged 65 years were 18·88 and 23·97 years, respectively, according to the 2009 report of the Ministry of Health, Labour and Welfare of Japan (www.mhlw.go.jp/english/wp/wp-hw5/dl/23010102e.pdf).

Second, infection and organ failure, but not progression of thymoma or LGL leukaemia, were the leading causes of death. In addition, refractoriness to induction immunosuppressive therapy and relapse of anaemia after successful immunosuppression were risk factors for death. These findings suggest that management of infectious complications and treatment of post-transfusion iron overload may contribute to the improvement of the treatment outcome of acquired chronic PRCA patients receiving immunosuppressive therapy. In fact, one report demonstrated that iron chelation therapy improved haematopoiesis in transfusion-dependent patients with thymoma-associated PRCA, although the mechanism was uncertain (Kojima *et al*, 2013). A similar effect of iron chelation therapy on haematopoiesis has been described in aplastic anaemia patients (Oliva *et al*, 2010; Lee *et al*, 2013).

Third, the haematological responses of patients with relapsing PRCA to immunosuppressive therapy were inferior to those of untreated PRCA patients. To our knowledge, this may be the first time that formal evidence regarding the inferior response to therapy in relapsing PRCA patients compared to untreated patients has been demonstrated. The poor response in patients with relapsing PRCA provides an additional important suggestion that preventing relapse of anaemia may be crucial to avoid subsequent treatment failure in chronic PRCA. Sawada *et al* (2007) previously showed that 86% of patients with idiopathic PRCA who had responded to ciclosporin relapsed within 1·5–40 months after discontinuation of maintenance therapy. Thus, maintenance therapy appears to be required for most patients responding to immunosuppressive treatment. Given that ciclosporin has nephrotoxicity as a major limiting side effect, its dosage should be carefully reduced to the minimum level required for maintenance of haematological response (Sawada *et al*, 2008).

Although it is still unknown why relapsing PRCA is more refractory to treatment, several explanations are possible. One possibility is the evolution of pathogenic T lymphocyte clones; there is accumulating evidence for clonal T cell expansion in acquired chronic PRCA (Masuda *et al*, 1997, 1999; Handgretinger *et al*, 1999; Go *et al*, 2001; Fujishima *et al*, 2006). During treatment, pathogenic T cell clones may proliferate with or without acquiring neoplastic features. This hypothesis is testable by longitudinal measurement of the T cell clone size in a quantitative manner by using a clonal marker such as *STAT3* mutations (Fasan *et al*, 2013; Ishida *et al*, 2013; Jerez *et al*, 2013). Another explanation may be the emergence of clonal haematopoiesis during immunosuppressive therapy. We know that PRCA may precede the diagnosis of myelodysplasia (Garcia-Suarez *et al*, 1998; Wang *et al*, 2007; Inui *et al*, 2012), as is the case with idiopathic aplastic anaemia (Socie *et al*, 1993). Morphological and cytogenetic examination of bone marrow should be advised if immunosuppression fails to achieve response.

In conclusion, refractoriness to induction immunosuppressive therapy and relapse of anaemia may be risk factors for death in idiopathic, thymoma-associated, and LGL leukaemia-associated PRCA. Effective maintenance therapy and the management of infectious complications are crucial for improving the prognosis of chronic acquired PRCA. The role of iron chelation therapy in refractory or relapsed patients should be explored in another prospective cohort study with the appropriate number of patients (Takatoku *et al*, 2007; Suzuki *et al*, 2008).

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Authorship statement

M.H. designed the study, collected and analysed data and wrote the manuscript; K.S. and N.F. designed the study, collected and interpreted data and helped to write the manuscript; M.T., M.B., K.D., H.T., S.N., A.U., S.F., Y.Y., F.K., K.O., K.S., A.M., M.K., A.A., N.K., H.H., M.O., K.O., M.K. collected and analysed data.

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Conflict of interest statement

All authors have no conflict of interest to report.

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ORIGINAL ARTICLE

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

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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

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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.

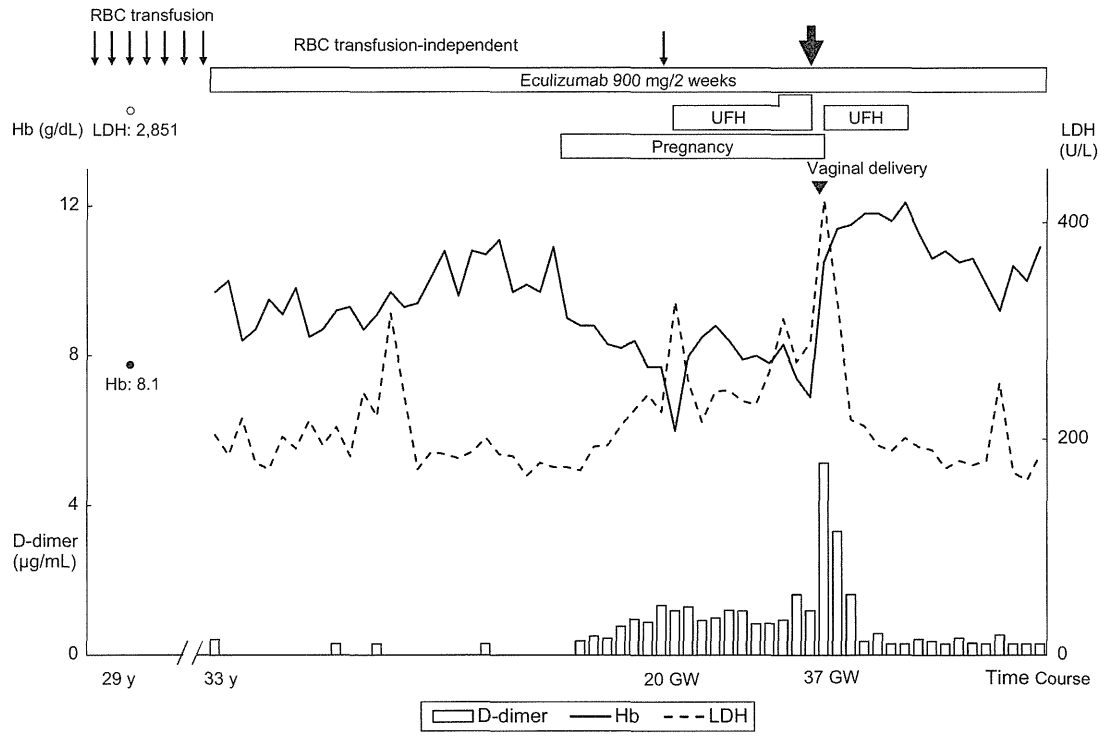

Fig. 1 The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy of case 1

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

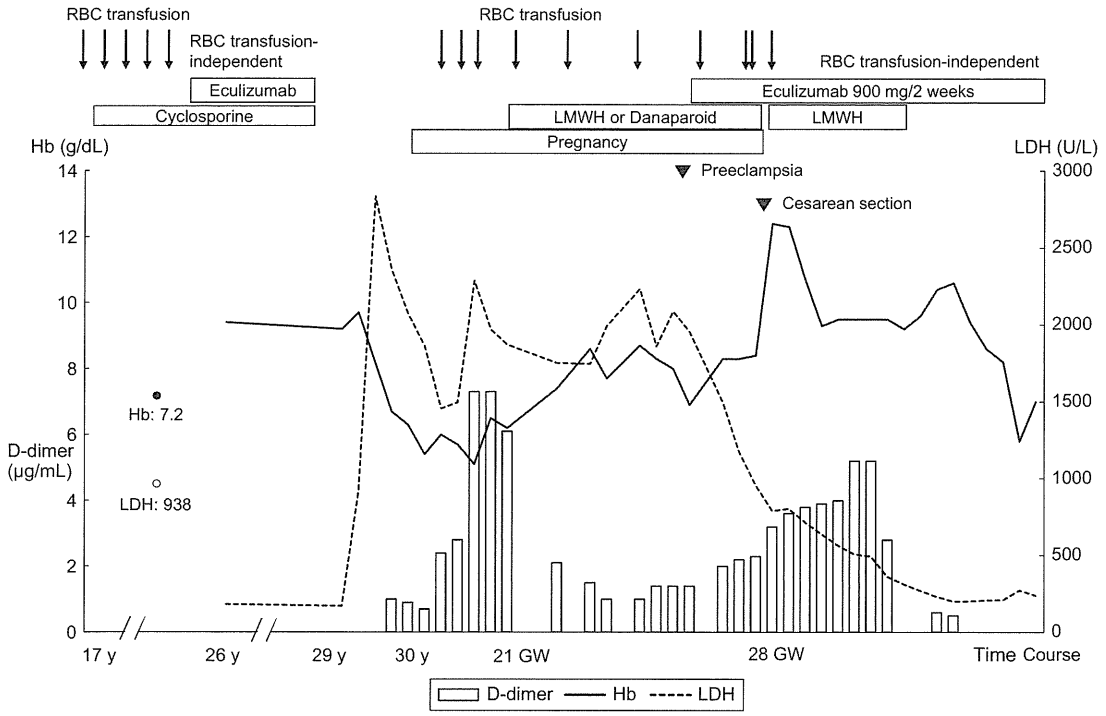

Fig. 2 The time course of the changes in Hb, LDH, and D-dimer before and after pregnancy of case 2

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

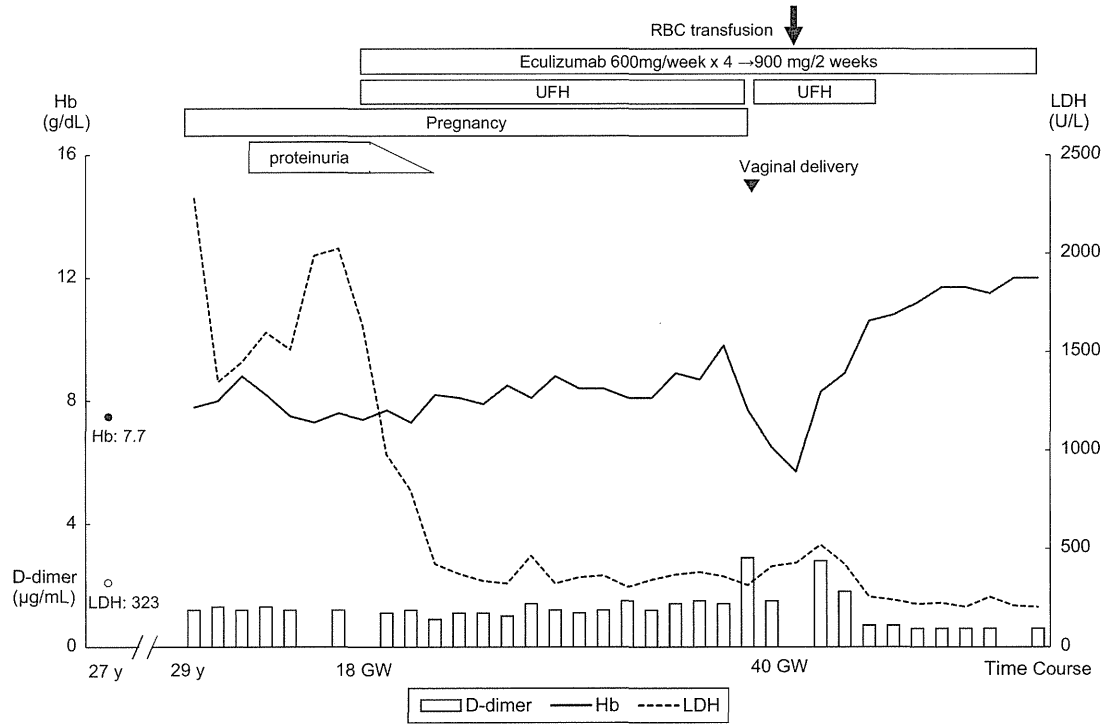

Fig. 3 The time course of the changes in Hb, LDH, and D-dimer after pregnancy of case 3

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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ORIGINAL ARTICLE

Proposal of criteria for dyserythropoiesis in the diagnosis of myelodysplastic syndromes

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Abstract The percentage manifesting dysplasia in bone marrow needed to qualify as significant is ≥10 % in each lineage. However, detailed analyses of this threshold have not been reported. Here, we analyzed dyserythropoiesis (dysE) in 109 myelodysplastic syndromes (MDS) patients with 21 immune thrombocytopenia (ITP)/12 hemolytic anemia (HA) patients as a control. In present study, mild megaloblastic erythroblasts were specifically named 'red cell with abnormal chromatin clumping (RCACC)'. RCACC ≥10 % in erythroblasts was observed in 29 % of ITP patients and 58 % of HA patients. The numbers of MDS patients with RCACC in erythroblasts <10, 10–19 and ≥20 % were 1, 3, and 105, respectively. We analyzed dysE criteria according to the WHO classification (original WHO dysE). Most of our MDS patients (98 %) had original WHO dysE ≥20 %. The ITP patients with original WHO dysE ≥10 % was 48 %, and there were no ITP patients had original WHO dysE ≥20 %. Sixty-seven percent of HA patients had original WHO dysE ≥10 %, and three patients (25 %) had original WHO dysE ≥20 %. Raising the threshold of the original WHO dysE from 10 to 20 or 30 % may provide more suitable criteria. If RCACC is not included in dysE criteria, we think that '10 %' is a suitable threshold for the determination of dyserythropoiesis.

Keywords Myelodysplastic syndromes · Diagnosis · Dysplasia · Dyserythropoiesis

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Introduction

Myelodysplastic syndromes (MDS) are very heterogeneous in terms of their cytomorphology, clinical features, and survival rates [1]. The French–American–British (FAB) classification for the diagnosis of MDS was proposed in 1982 [2]. The third and fourth World Health Organization (WHO) classifications of tumors of hematopoietic and lymphoid tissues [3, 4] were proposed in 2001 and 2008, respectively. To confirm a diagnosis of MDS in the absence of certain cytogenetic/molecular abnormalities in patients with less than 5 % blasts, the identification of dysplasia in any one of the three major cell lineages (erythroid, granulocytic, and megakaryocytic) is necessary. According to the WHO guidelines, the required percentage of cells manifesting dysplasia in the bone marrow to qualify as significant is 10 % or over in one or more hematopoietic cell lineages. However, detailed analyses of the threshold have not been published, to our knowledge.

Dysplastic changes are observed in many primary or acquired hematologic disorders, e.g., vitamin B_{12} deficiency [5], folate deficiency [6], autoimmune diseases [7], viral infection [8], and aplastic anemia (AA) [9, 10]. Dysplastic changes in healthy subjects were described by Bain [11]. Parmentier et al. reported that in the bone marrow (BM) of healthy BM donors, clinically significant dyserythropoiesis (dysE) (≥10 %) was found in 34 % of the 120 donors [12]. However, assessments of dysplastic changes are subjective. Some past studies [12, 13] have

already made clear that the investigator concordance rates regarding dysE were poor. In addition, although megaloblastic change is a dysplastic form according to the WHO classification, the assessment of mild megaloblastic change is especially difficult.

We conducted a detailed cytomorphologic analysis of refractory anemia (RA) according to the FAB classification (FAB-RA) [13]. In the present study, to create dysE criteria that are suitable for diagnosing MDS, we analyzed dysE using the dataset from our prior study [14], and BM slides from both MDS patients without increased blasts and patients with certain immune thrombocytopenic purpura (ITP)/hemolytic anemia (HA).

Methods

Patients

We used the dataset of Japanese patients (100 cases) from our previous study [14]. The patients had FAB-RA except for 5q-syndrome. The patients were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital, or affiliated hospitals between April 1976 and January 2002. The chromatin of one of the patients could not be examined in detail because of poor marrow preparations. The cases of an additional 10 MDS patients without increased blasts diagnosed at Saitama Medical University International Medical Center were included in the present study.

Patients with disorders other than MDS (e.g., AA, paroxysmal nocturnal hemoglobinuria, megaloblastic anemia, autoimmune hemolytic anemia, anemia of chronic disorders, large granular lymphocytic leukemia, hairy cell leukemia, chronic liver disorders, and hypersplenism) were excluded from the study, and 21 certain ITP/12 certain HA patients were included for morphologic controls. Gamma-globulin bolus therapy, prednisolone or *Helicobacter pylori* sanitization therapy was effective for these ITP patients. Laboratory findings and/or responses of treatment were fit to HA. The final patient series was comprised of 109 patients with MDS (58 males, 51 females, median age 56 years, range 15–88 years), 21 patients with ITP (10 males, 11 females, median age 60 years, range 15–81 years) and 12 patients with HA (6 males, 6 females, median age 43 years, range 26–80 years) including 9 autoimmune hemolytic anemia (AIHA) and 3 hereditary spherocytosis (HS) patients. This study was approved by the Institutional Review Board of the Saitama Medical University International Medical Center.

Cytomorphologic study

We performed microscopy examinations using standard methods: BM Wright–Giemsa (WG), or May–Giemsa (MG), Prussian blue and periodic acid-Schiff (PAS) stained films, and for peripheral blood (PB), WG or MG stained films.

In our previous study [14], we performed a detailed cytomorphologic analysis. We limited the dysplasias to only the dysplasias described in the third edition WHO classification [3], as follows: dysplasias of the nucleus in erythroid-lineage cells were defined as nuclear budding, internuclear bridging, karyorrhexis, multinuclearity, or megaloblastoid changes. Dysplasias of the cytoplasm in erythroid-lineage cells were defined as ring sideroblasts, vacuolization, or PAS positivity (diffuse or granular). At least 200 erythroblasts in BM were examined in each patient. Ring sideroblasts were defined as erythroblasts with at least five siderotic granules covering at least one-third of the circumference of the nucleus. In the present study, we defined megaloblastoid changes as a form of the lowest priority, e.g., multinuclearity with megaloblastoid changes was judged as multinuclearity. In the present study, we defined the cut-off level for dyserythropoiesis (dysE) as 10 % according to the WHO classification [3], and we defined erythroblast with large blocks of chromatin separated by clear zones was named 'red cell with abnormal chromatin clumping (RCACC)' in the present study. RCACC have the nuclei showing irregular chromatin blocks which give coarse and dense chromatin appearance. According to the description of WHO classification, RCACC may be classified to megaloblastic changes. However, typical megaloblastic nuclei show even size of chromatin blocks which gives granular appearance. RCACC is shown as dysplastic red cell form with coarse chromatin in American Society of Hematology (ASH) Image Bank [15, 16]. However, the evaluation of this form is very difficult, and the concordance rate of RCACC by the observers may be poor. We defined that the dysE criteria according to the WHO classification (original WHO dysE) included RCACC. Moreover, we used two methods to evaluate dysE: one as original WHO dysE, and the other as dysE excluding RCACC from the original WHO dysE (strict dysE).

The microscopic examinations were performed by at least two experienced hematologists. Before each microscopic examination, the hematologists conferred regarding the microscopic morphology. When at least one of the two hematologists determined RCACC of erythroblasts, the cell was judged to be RCACC.

Results

Dysplastic form types in erythroblasts

Megaloblastic change including RCACC was the most frequent dysplastic change of erythroblasts in not only the

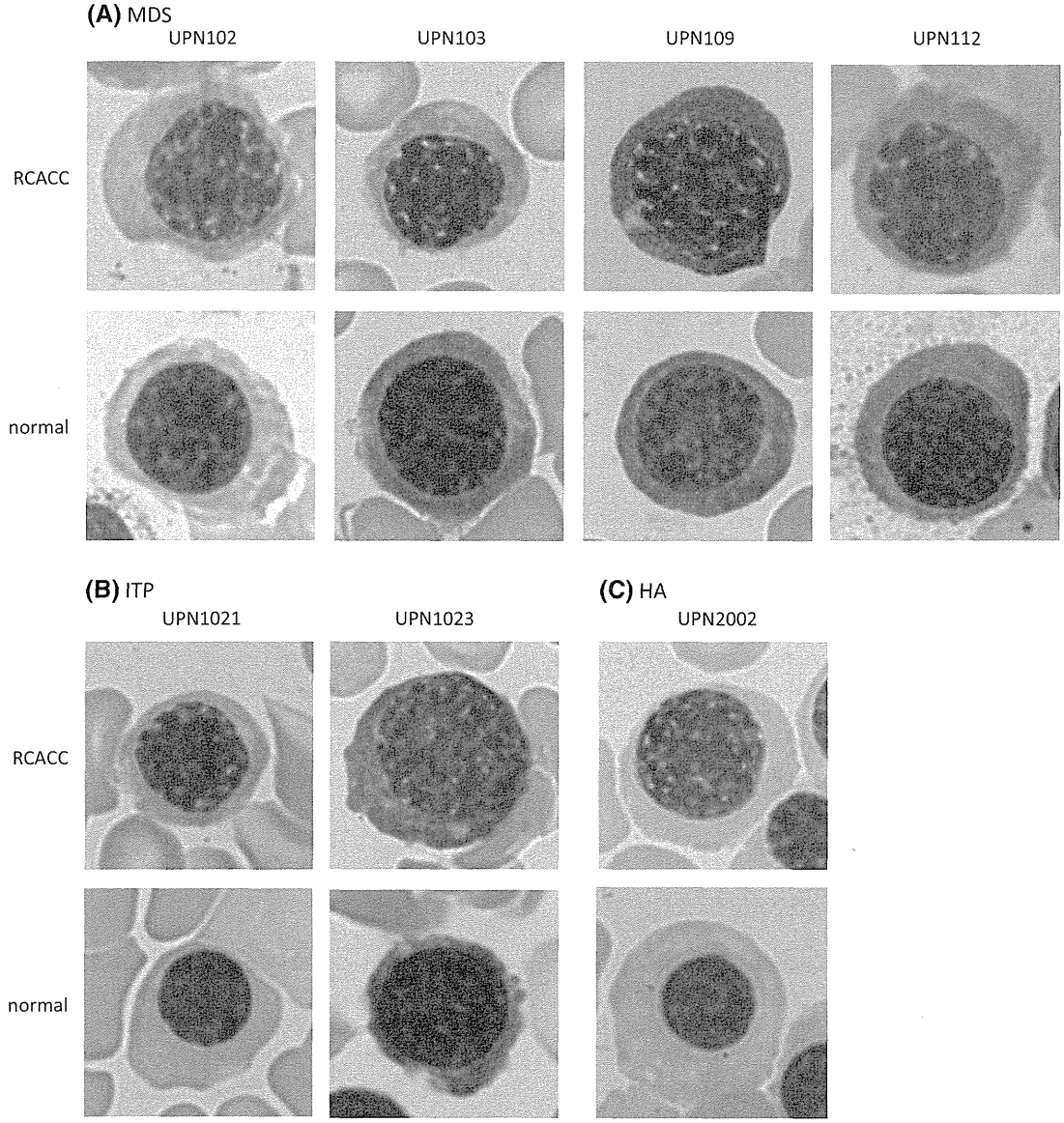

Fig. 1 RCACC (red cell with abnormal chromatin clumping) and normal erythroblasts. **a** MDS patients. **b** ITP patients. **c** hemolytic anemia patients. *Upper* RCACC, *Lower* normal erythroblast of maturation level same as *upper* RCACC

MDS cases but also the ITP/HA cases. The second most frequent dysE form was budding.

Degree of megaloblastic change of erythroblasts

In the group of 109 MDS patients, RCACC of erythroblasts was the most frequent subtype of megaloblastic erythroblasts (Fig. 1a). RCACC in erythroblasts was observed in not only MDS but also ITP/HA cases (Fig. 1b, c). RCACC in erythroblasts ≥10 % was observed in six (29 %) of the 21 ITP and 7 (58 %) of the 12 HA patients. There was no ITP patient with RCACC in erythroblasts ≥20 %. In HA patients, 2 patients had RCACC in erythroblasts ≥20 %. Among the MDS patients, the numbers of patients with RCACC in erythroblasts <10, 10–19 and ≥20 % were 1, 3 and 105, respectively (Fig. 2).

Dyserythropoiesis according to the WHO classification (original WHO dysE)

All 109 of the MDS patients had original WHO dysE ≥10 %, whereas of the 21 ITP patients, 10 (48 %) patients had original WHO dysE ≥10 %. Eight (67 %) of the HA patients had original WHO dysE ≥10 %. Most of the MDS

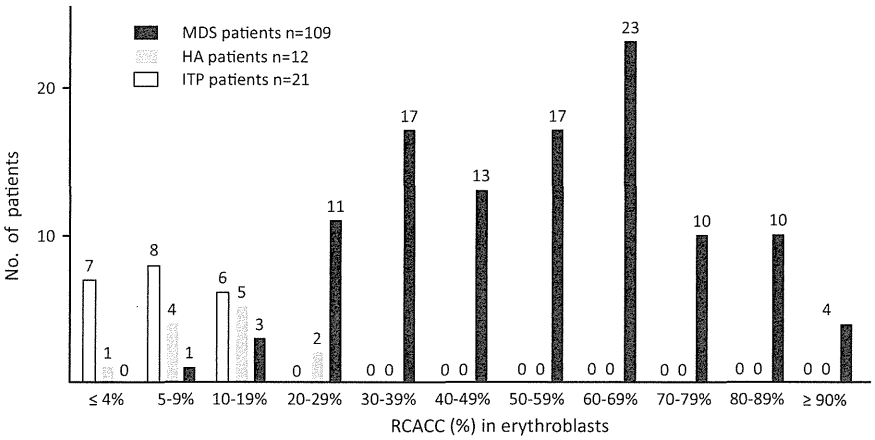

Fig. 2 RCACC (red cell with abnormal chromatin clumping) and normal erythroblasts. RCACC ≥10 % was observed in 29 % of the 21 ITP patients. No ITP patients had RCACC ≥20 %. RCACC ≥10 % was observed in 17 % of the 12 HA patients. Two HA patients had RCACC ≥20 %. In contrast, RCACC ≥20 % was observed in 96 % of the 109 MDS patients

Table 1 Original WHO dyserythropoiesis (dysE) and strict dysE

		Original WHO dysE		No of patients (%)	
		≥30 %	29–20 %	19–10 %	>10 %
MDS ($n = 109$)					
Strict dysE	≥10 %	76 (69.7 %)	1(0.9 %)	0 (0.0 %)	–
No. of patients (%)	>10 %	27 (24.8 %)	3 (2.8 %)	2 (1.8 %)	0 (0.0 %)
ITP ($n = 21$)					
Strict dysE	≥10 %	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	–
No. of patients (%)	>10 %	0 (0.0 %)	0 (0.0 %)	10 (47.6 %)	11 (52.4 %)
Hemolytic anemia ($n = 12$)					
Strict dysE	≥10 %	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	–
No. of patients (%)	>10 %	1 (8.3 %)	2 (16.7 %)	5 (41.7 %)	4 (33.3 %)

Original WHO dysE dysE criteria according to the WHO classification, *Strict dysE* dysE excluding 'red cell with abnormal chromatin clumping (RCACC)' from the original WHO dysE

patients (98 %) had original WHO dysE ≥20 %. None of the ITP patients had original WHO dysE ≥20 %. Three HA patients had original WHO dysE ≥20 % (Table 1; Fig. 3). No ITP patients and HA had WHO dysG and/or DysMgk ≥10 %.

Dyserythropoiesis excluding RCACC from original WHO dysE (strict dysE)

Three ITP patients (14 %) showed strict dysE ≥5 %, but no ITP patient showed strict dysE ≥10 %. One HA patient (8 %) showed strict dysE ≥5 %, but no HA patient showed strict dysE ≥10 %. In contrast, 25 (23 %) MDS patients showed strict dysE 5–9 %, and 77 (71 %) showed strict dysE ≥10 % (Table 1; Fig. 4).

Discussion

Morphologic dysplastic changes are not specific findings for MDS patients, and are also observed in many primary or acquired hematologic disorders, and even healthy subjects. Indeed, Parmentier et al. reported that healthy donors (34 %) showed 10 % or over dyserythropoietic changes [12]. Della Porta et al. reported that dysE according to the WHO 2008 criteria was detected in 312 of their MDS subjects (99 %), and the median number of dysplastic erythroid cells was 53 % (range 10–94 %); they also reported that 131 (69 %) of their patients with non-clonal cytopenia including ITP showed dysplastic erythroid cells ≥10 % [14]. ITP patients have thrombocytopenia and BM with increased megakaryocytes. Distinguishing between MDS and ITP is not easy. Our present ITP patients showed almost the same results as those of the healthy donors examined by Parmentier et al. [12] and the patients with non-clonal cytopenia including ITP examined by Della Porta et al. [17]. Megaloblastic change predominated in the healthy donors in this report.

In the present study, mild megaloblastic change was given specific name as 'RCACC'. In our results, RCACC was the most frequent erythroid dysplastic change in not only the MDS groups but also the ITP and HA group. Parmentier et al. also reported that ring sideroblasts were not found in healthy objects [12]. Our present findings support their report. In addition, assessments of mild megaloblastic change are subjective. Bennett noted that 'Distinguishing mild megaloblastic change from dysplastic erythroid precursors continues to be a challenge' [18]. In our morphology conferences, the poor discrimination between normal and

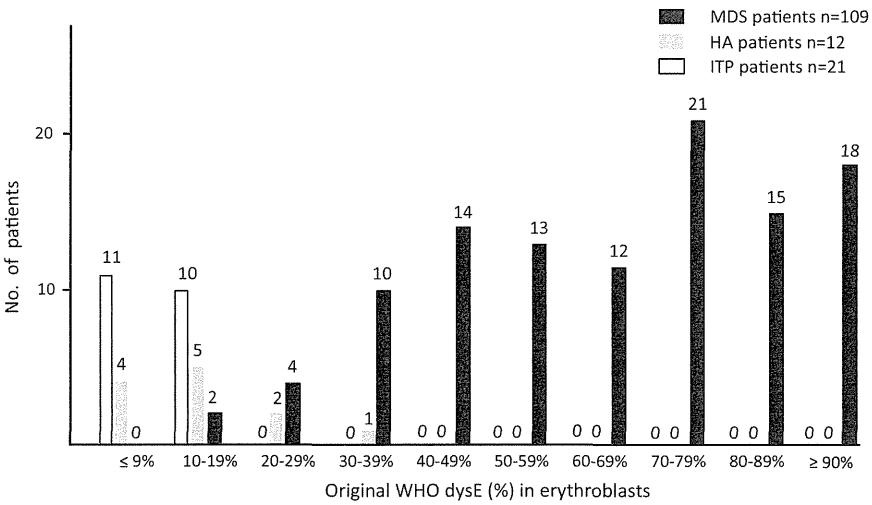

Fig. 3 Original WHO dysE (dyserythropoiesis according to the WHO classification). Among the ITP patients, patients with original WHO dysE ≥10 % were 48 %. Among the HA patients, patients with original WHO dysE ≥10 % were 67 %. Most of the MDS patients (98 %) had original WHO dysE ≥20 %. None of the ITP patients had original WHO dysE ≥20 %. Only 1 HA patient had original WHO dysE ≥20 %

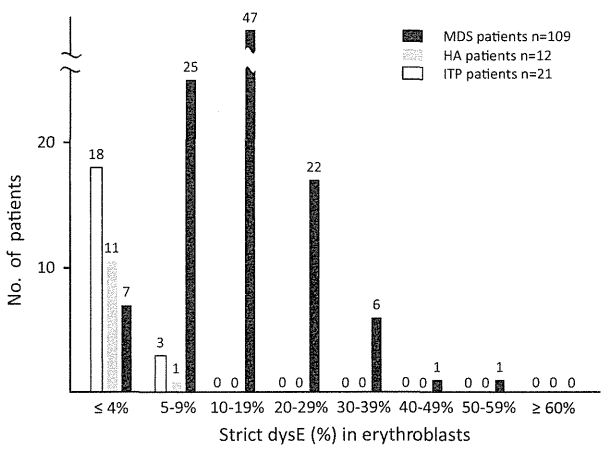

Fig. 4 Strict dysE (dyserythropoiesis excluding RCACC from original WHO dysE). Among the MDS patients, 71 % had strict dysE ≥10 %, whereas no ITP or HA patients had strict dysE ≥10 %. No MDS patients had strict dysE ≥60 %

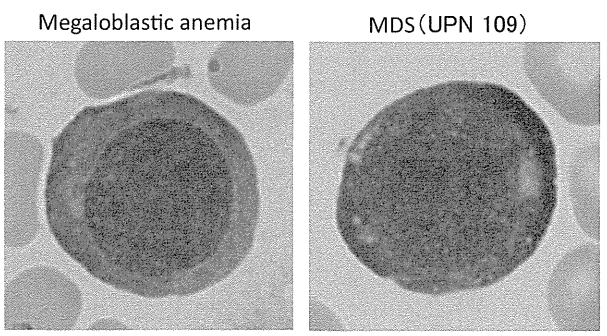

Fig. 5 Megaloblastic changes. Nuclei of typical megaloblastic changes show even size of chromatin blocks which gives granular appearance

dysplastic was related to poor inter-observer concordance. In contrast, inter-observer concordances of dysplastic forms excluding mild megaloblastic change were higher than that of mild megaloblastic change (data not shown). RCACC and megaloblastic changes are similar morphologic forms (Fig. 5). Nuclei of typical megaloblastic changes show even size of chromatin blocks which gives granular appearance. In contrast, RCACC have the nuclei showing irregular chromatin blocks which give coarse and dense chromatin appearance. By enough experience to judge RCACC in MDS, we think that physician can distinguish RCACC from typical megaloblastic changes. However, the mechanism of their formation is unknown. RCACC may be the later phase of megaloblastic changes. RCACC and megaloblastic changes may not have the relations.

Diagnoses of MDS in the absence of certain cytogenetic/molecular abnormalities in patients with <5 % blasts are done mainly by cytomorphologic examination. DysE (≥10 %) in BM was found in healthy BM donors [12]. We expect that the cause may be an overestimation of megaloblastoid change. In the present study, when at least one of two observers concluded that there was RCACC of erythroblasts, we decided to judge the cell as RCACC. Thus, the judgment of RCACC in this study was not strict. All 109 MDS patients in our study had original WHO dysE ≥10 %.

Our 21 patients with ITP achieved a hematological response after gamma-globulin bolus treatment, prednisolone, or *Helicobacter pylori* sanitization therapy, and their diagnoses of ITP was thus certain. Similarly, the diagnosis of HA is certain by laboratory findings and the effectiveness for the treatment. In our study of dysE in the BM of the ITP patients, original WHO dysE ≥10 % was found in 48 % of the cases. Moreover, original WHO dysE ≥10 % was found in 67 % of the HA cases. These