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Cut-off value of red-blood-cell-bound IgG for the diagnosis of Coombs-negative autoimmune hemolytic anemia

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Direct antiglobulin test (DAT)-negative autoimmune hemolytic anemia (Coombs-negative AIHA) is characterized by laboratory evidence of in vivo hemolysis, together with a negative DAT performed by conventional tube technique (CTT) in clinically suspected AIHA patients. The immunoradiometric assay (IRMA) for red-blood-cell-bound immunoglobulin G (RBC-IgG) can be used to diagnose patients in whom CTT does not detect low levels of red cell autoantibodies. We investigated the diagnostic cutoff value of the IRMA for RBC-IgG in Coombs-negative AIHA and calculated its sensitivity and specificity. Of the 140 patients with negative DAT by CTT referred to our laboratory with undiagnosed hemolytic anemia, AIHA was clinically diagnosed in 64 patients (Coombs-negative AIHA). The numbers of Coombs-negative AIHA and non-AIHA patients changed with age and gender. The cutoff values were determined from receiver operating characteristic (ROC) curve according to age and gender. The IRMA for RBC-IgG proved to be sensitive (71.4%) and specific (87.8%) when using these cutoffs. Using these cutoffs for 41 patients with negative DAT referred to our laboratory in 2006, all the pseudonegative cases were treated with steroids before the test. The 31 untreated cases could be grouped using one cutoff value of 78.5 and showed 100% sensitivity and 94% specificity, independent of gender and age. Results indicate that RBC-IgG could become a standard approach for the diagnosis of Coombs-negative AIHA, when measured before treatment. Am. J. Hematol. 84:98–101, 2009. © 2008 Wiley-Liss, Inc.

Introduction

The detection of red-blood-cell-bound immunoglobulin G (RBC-IgG) and complement by direct antiglobulin test (DAT) remains the main serological assay in the diagnosis of autoimmune hemolytic anemia (AIHA) [1]. Several methodologies have been investigated for detection and evaluation of these autoantibodies. DAT by conventional tube technique (CTT) is the method most commonly used in the blood centers and is still considered a gold standard [2]. A positive DAT is almost always seen in association with AIHA [3] and forms the characteristic of the serological diagnosis of AIHA [4,5]. However, it has also been shown that a negative DAT does not exclude the diagnosis of AIHA [5,6] and 1–10% of patients with AIHA have been reported to show a negative DAT [7–9]. These patients, designated "Coombs-negative AIHA" patients, may carry lower numbers of IgG molecules per RBC, yielding a negative tube DAT and in vivo hemolysis [10]. Also, they may have only RBC-IgA autoantibodies or monomeric IgM molecules that induce the clinical and hematological features typical of AIHA [11]. The immunoradiometric assay (IRMA) [12], the complement fixation antibody consumption test [13] and the enzyme-linked antiglobulin test (ELAT) for RBC-IgG, as well as the enzyme-linked immunosorbent assay (ELISA) for IgG eluted from RBCs [14,15] are representative methods to quantitatively detect RBC-IgG. Flow cytometry [16] and the gel test [17,18] are semiquantitative methods. Despite the availability of these sensitive methods, there are no established standard tests for the diagnosis of Coombs-negative AIHA, which often makes it difficult for clinicians to diagnose AIHA in patients with DAT-negative hemolytic anemia. Therefore, the aim of the study was to assess the clinical utility of RBC-IgG levels in the diagnosis of Coombs-negative AIHA patients and to calculate the cutoff values, sensitivity, and specificity after a 1-year follow-up period.

Results

A total of 192 surveys were returned for analysis; a response rate of 78%. The mean age of participants was 51.0 ± 22.9 years (range 0.9–85) and 49% of participants were female. There were no significant differences in age and gender (49.7 ± 27.7 years old and 63% female) between responders and nonresponders to the survey. Of the responders, 144 were DAT-negative. Forty-seven percent of the DAT-negative hemolytic anemia patients (n = 68) were classified as AIHA; 64 had warm-type AIHA and four had cold-type AIHA. Significant differences were found in %Retic (P = 0.03), MCV (P = 0.01), LDH (P = 0.03), IDBIL (P = 0.03), and RBC-IgG (P < 0.0001) levels between the Coombs-negative AIHA and non-AIHA groups. There were no significant differences in age, gender, Hb, and Hp between the two patient groups.

The ROC curves for RBC-IgG levels, using clinical diagnosis as an indicator of AIHA, are shown in Fig. 1. Table I summarizes the AUCs, confidence intervals for AUCs, and likelihood ratios (LRs) for laboratory variables, as well as sensitivities and specificities, which were calculated at the

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optimal cutoff values (maximum point of efficiency curve). RBC-IgG showed the highest AUC values; the other three indices showed low AUC values. These data suggested that only RBC-IgG can effectively distinguish between the Coombs-negative AIHA and non-AIHA patients (see Fig. 2). Use of the cutoff point value (83) showed the sensitivity (70%), specificity (84%), and LR (4.8).

To discover the clinically significant cutoff values, the numbers of Coombs-negative AIHA patients were investigated according to age and gender. In females, there were two groups in the distribution of patients: one in the patients aged less than and one in those aged more than 45 years. In males, there was one peak in the patients aged more than 60 years. Together with the ROC curve for each group, the optimal cutoff values (maximum points of efficiency curve) were calculated for RBC-IgG levels. In females aged less than 45 years and those aged more than 45 years, the optimal cutoff values were 96 and 128, respectively. In males aged less than 60 years and those aged more than 60 years, the optimal cutoff values were 60 and 102, respectively.

Use of these cutoff points for 140 DAT-negative hemolytic anemia patients showed the slightly good sensitivity (71%), specificity (88%), and LR (5.9). Using these cutoff points, 41 cases of DAT-negative hemolytic anemia, which were referred to our laboratory in 2006, were categorized and showed slightly better sensitivity (78%), specificity (94%), and LR (14.1). Interestingly, all the pseudonegative cases had been treated with steroids (see Fig. 3). The 31 untreated cases could be grouped using one cutoff value as 78.5 (Fig. 3b) and showed high sensitivity (100%), specificity (94.1%) and LR [16]. Two patients with non-AIHA hemolytic diseases (drug-induced hemolytic anemia and myelodysplastic anemia) showed positive RBC-IgG, which might suggest the involvement of immunological hemolytic mechanisms [19]. In our laboratory, some patients with myelodysplastic anemia tended to show positive RBC-IgG (data not shown) mechanisms of which are analyzed by our collaborators.

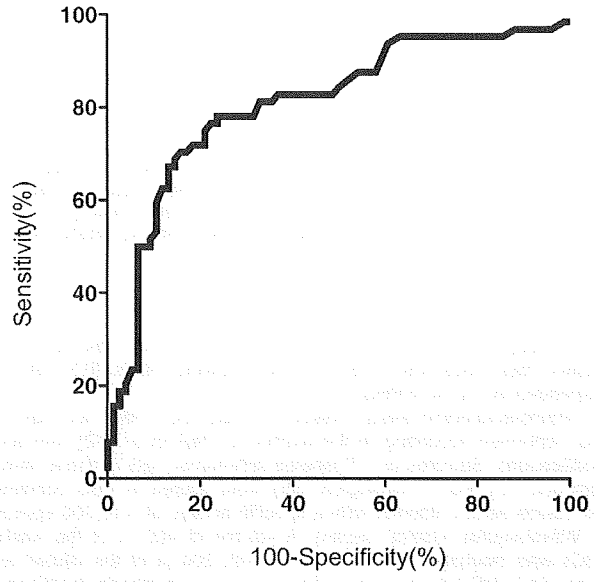

Figure 1. Receiver operating characteristic (ROC) curves for red-blood-cell-bound immunoglobulin G (RBC-IgG) (area under the ROC curves (AUC) 0.81), when the clinical diagnosis of AIHA by the attending doctors after 1-year follow-up was the gold standard.

Discussion

In the management of DAT-negative hemolytic anemia, it is important to distinguish Coombs-negative AIHA patients from other hemolytic anemia, because steroid treatment has major effects on AIHA [6], but steroids have also been associated with several serious side effects [20], which makes clinicians hesitate to use steroids to treat DAT-negative hemolytic anemia patients without diagnosis of AIHA.

In our laboratory, the immunoradiometric assay (IRMA) [12] is used to detect RBC-IgG quantitatively rather than semiquantitative methods such as flow cytometry [16] and gel column [17] for two reasons. First, in our laboratory IRMA has been used since 20 years ago as a central laboratory in Japan and its cost is supported by a grant for research on intractable diseases from the Ministry of Health, Labor and Welfare of Japan. Second, gel column method showed occasionally pseudopositive in some cases, the reason of which has remained unclear and flow cytometry requires normal RBCs as negative control in each measurement. Although the simple methods are desired, quantitative measurements should be used to guarantee their ability to measure subthreshold IgG.

Previously, we reported the value of RBC-IgG (33 ± 13) in 100 healthy Japanese adults and the RBC-IgG required to be DAT-positive (335 ± 72) [21]. Previous studies have reported very similar values [12,22]. The usefulness of RBC-IgG in diagnosis for Coombs-negative AIHA had been reported [23,24]. In previous studies, patients with Coombs-negative AIHA were reported to have abnormal levels of IgG, ranging from 70 to 434 [25] or from 76 to 350 [6]. There are, however, no reports referring to the IgG cutoff value, sensitivity, specificity, and LR. Practically, in our laboratory some non-AIHA samples from patients with hemolysis showed higher values than normal healthy individuals (Figs. 2 and 3), which might suggest the involvement of immunological hemolytic mechanisms [19]. In addition, some Coombs-negative AIHA patients had values very close to the normal range (see Fig. 2). So, in the practical diagnostic procedure, cutoff values must be calculated from RBC-IgG levels of DAT-negative patients with hemolysis rather than from normal RBC-IgG levels of healthy individuals. We have adopted the clinical diagnosis as the gold standard for the diagnosis of Coombs-negative AIHA because there are no established standards and many clinicians have previously clinically diagnosed Coombs-negative AIHA by the presence of hemolysis, denial of other

TABLE I. Area Under the ROC (Receiver Operating Characteristic) Curve (AUC), Confidence Intervals (95%) of AUCs, and Likelihood Ratio for Diagnosing AIHA in Patients With DAT-Negative Hemolytic Anemia (n = 140)

Parameter	AUC ± SE	Confidence interval (95%)	Cutoff point	Sensitivity	Specificity	Likelihood ratio
%Retic (%)	0.61 ± 0.05	0.51–0.71	6.8	54.9	82.8	1.9
MCV (fL)	0.65 ± 0.05	0.54–0.75	94.7	72.6	51.8	1.5
LDH (U/l)	0.62 ± 0.05	0.51–0.73	386	64.7	62.5	1.7
IDBIL (mg/dl)	0.62 ± 0.05	0.52–0.72	1.2	66.7	59.1	1.6
RBC-IgG	0.81 ± 0.04	0.73–0.88	83.0	70.3	84.2	4.8

Cutoff points, sensitivities, and specificities for each test are indicated in maximum points of the ROC curves. Calculated likelihood ratios are based on the cutoff points.

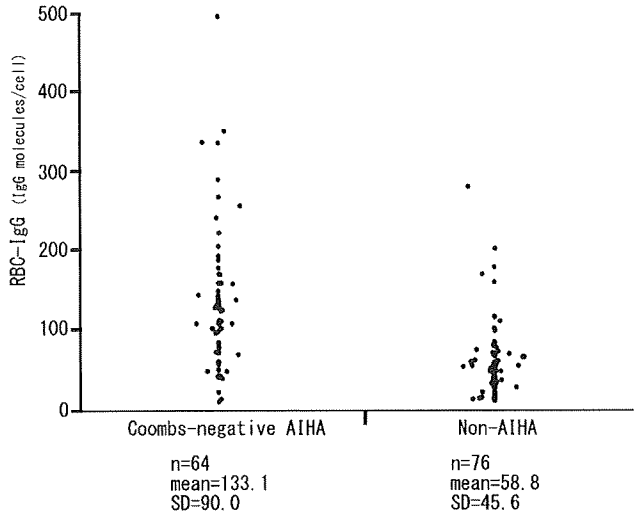

Figure 2. Red-blood-cell-bound IgG (RBC-IgG) of Coombs-negative AIHA and non-AIHA patients between 2003 and 2006. A significant difference was found in RBC-IgG levels ($P < 0.0001$) between the Coombs-negative AIHA and non-AIHA groups.

hemolytic diseases, and responsiveness to steroid treatments with respect of RBC-IgG value [26].

RBC-IgG levels of non-AIHA patients tended to increase with advanced age, especially in females. RBC-IgG levels of Coombs-negative AIHA in females also tended to increase with age and showed a gap at about 45 years of age, which gave two peaks at the ages of 25 and 65 years. In males, there was a smaller increase in RBC-IgG levels with advanced age, but Coombs-negative AIHA was most common in patients aged more than 60 years. These distribution trends were also reported in a previous report in Japanese patients [27]. The report suggested that these tendencies might be attributed to characteristics of AIHA, regardless of DAT-positive or -negative characteristics, and not to the population composition in Japan. In light of these trends, more effective cutoff values could be calculated using age- and gender-stratified analyses. Moreover, exclusion of the treated samples could increase the sensitivity and specificity of RBC-IgG and so it can be considered a standard approach for the diagnosis of Coombs-negative AIHA. Therefore, we propose that the RBC-IgG level should be measured for the diagnosis of Coombs-negative AIHA and the cutoff value used should be 78.5 if RBC-IgG is measured before treatment, and that after treatment the RBC-IgG level might range from the Coombs-negative value to as low as that seen in normal healthy individuals (see Fig. 3).

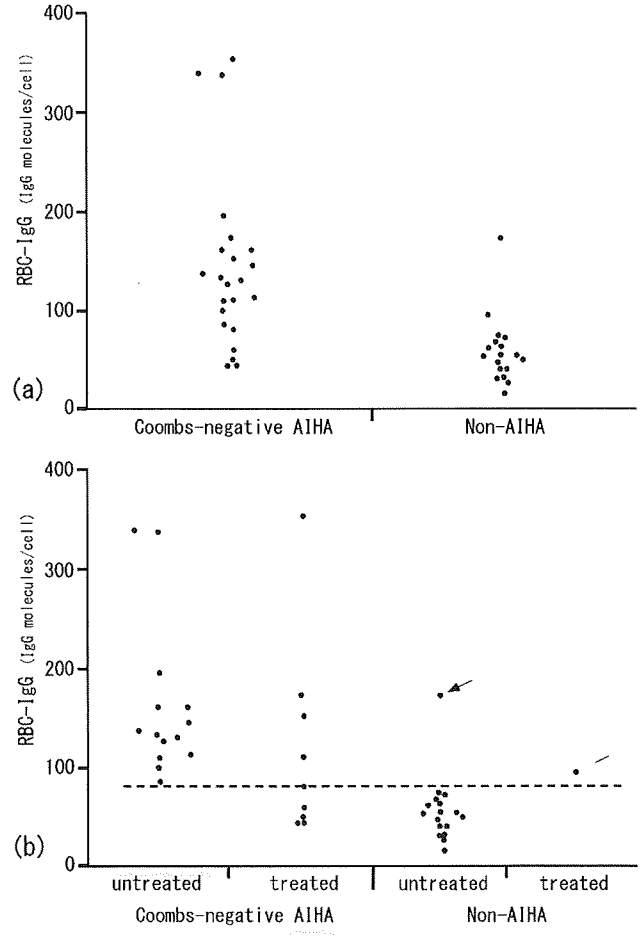

Figure 3. Red-blood-cell-bound IgG (RBC-IgG) of Coombs-negative AIHA and non-AIHA groups in 2006 (a). Each group consists of two subgroups treated or untreated with steroid drugs (b). A dotted line indicates 78.5 IgG molecules per cell. There were two non-AIHA patients with negative DAT, whose RBC-IgG levels were higher than 78.5. The black arrow represents a patient with drug-induced hemolytic anemia and the white arrow indicates a patient with hypoplastic myelodysplastic syndrome.

Materials and Methods

The study was performed over a period of 4 years from 2003 to 2006 at the laboratory of the Center for Community Medicine, Jichi Medical University, Tochigi, Japan, after approval by the Institutional Ethics Panel Committee.

Patients. During a 4-year period, 261 samples from 245 patients were referred to our laboratory for quantitation of RBC-IgG. Of these, 54 (22%) were DAT-positive and 191 (78%) were DAT-negative, as shown by analysis of polyspecific DAT by CTT (Ortho Diagnostics, USA) and monospecific DAT by CTT using anti-IgG and anti-C3d antibodies (Ortho Diagnostics, USA) following manufacturer's instructions.

Sample preparation. Heparinized whole blood (10 ml) samples were collected. The RBC layer was prepared by centrifuging the whole blood at 1,000 rpm for 20 min. The supernatant plasma and buffy coat were discarded. One milliliter samples of packed RBCs were diluted in 10 ml of phosphate-buffered saline (PBS), pH 7.0, 0.15 M. The diluted RBCs were passed through a cotton-wool column to exclude neutrophils and monocytes, according to the method of Jeje et al. [12]. The RBCs were washed four times with PBS, and the resulting RBCs (0.3 ml) were suspended in 0.4 ml of PBS.

Immunoradiometric assay (IRMA) for RBC-IgG. IRMA for RBC-IgG was performed according to the method of Jeje et al. [12] with some modifications. Samples of ^{125}I-labeled anti-human IgG antisera derived from goat (Du Pont, Wilmington, DE) were diluted in PBS containing 3% bovine serum albumin with a specific activity of ~10,000 cpm/200 μl (Wakojunyaku, Osaka, Japan). A volume of 400 μl of the washed RBCs was incubated for 1 h at 37°C with 200 μl of the diluted anti-human IgG. IgG beads were prepared using the methods described by Jeje et al. [12]. Human IgG and beads (SephasorbTM HP) were purchased from Sigma Chemical (St. Louis, MO) and Pharmacia Fine Chemicals (Uppsala, Sweden), respectively. Two hundred microliter samples of IgG beads (2×10^6) were added to the mixture of RBCs and ^{125}I-labeled anti-human IgG and incubated at 37°C for 30 min. The RBCs were lysed by the addition of 80 μl of 20% Triton X-100 (Sigma). The beads were washed four times with 20% Triton X-100-containing PBS, and the radioactivity was measured using a gamma counter (Aroka, Tokyo, Japan). A standard curve was generated using human IgG standards (10–10,000 ng IgG/ml; Sigma). The percent inhibition of binding was plotted against each concentration of IgG. Using the standard curve, RBC-IgG levels were calculated after counting the number of RBCs. Each attending doctor was informed of the RBC-IgG level within 3–10 days of ordering.

Clinical diagnosis questionnaire. At 1 year after referral to our laboratory, follow-up investigations were performed; the attending doctor used a questionnaire to assess the patient's clinical diagnosis. The bases of

clinical diagnosis of Coombs-negative AIHA were in vivo hemolysis (low hemoglobin (Hb) concentration, high percentage of reticulocyte (%Retic), high indirect serum bilirubin (IDBIL) level, high lactate dehydrogenase (LDH) level, low haptoglobin (Hp) level and/or high erythropoiesis level in bone marrow) and exclusion of other anemic icteric diseases without hemolysis (such as megaloblastic anemia, myelodysplastic syndrome, erythroid leukemia, congenital dyserythropoietic anemia, hepatobiliary diseases, and constitutional jaundice). AIHA was diagnosed by measuring the RBC-IgG level, steroid-reactivity and exclusion of alloimmune hemolytic anemia and drug-induced hemolytic anemia.

Statistical analysis. Patients who had negative DAT were divided into AIHA and non-AIHA groups on the basis of clinical diagnosis. The normality of the laboratory variables was analyzed using the Kolmogorov-Smirnov test with Lilliefors significance correction. As most variables were not normally distributed, a Mann-Whitney *U*-test was used to determine the differences between Coombs-negative AIHA and non-AIHA patients. The median range and interquartile range were also calculated for all variables.

The accuracy of the tests for diagnosis of AIHA in DAT-negative hemolytic anemia patients was evaluated using receiver operating characteristic (ROC) curves [28]. By this method, a test that is perfect has 100% sensitivity and no false-positives (1-specificity = 0) and will have an area under the curve (AUC) of 1.0, whereas a test that has no diagnostic value would have an AUC of 0.5. The 95% confidence intervals and LRs were also calculated.

JMP 7.0.1 for Macintosh (SAS Institute, Cary, NC) and GraphPad Prism 4.0c for Macintosh (GraphPad Software, San Diego, CA) were the statistical software used.

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ORIGINAL ARTICLE

Lenalidomide is active in Japanese patients with symptomatic anemia in low- or intermediate-1 risk myelodysplastic syndromes with a deletion 5q abnormality

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Abstract Lenalidomide is an immunomodulatory agent recently reported to be effective in the treatment of transfusion-dependent anemia due to low- or intermediate-1 risk myelodysplastic syndromes (MDS) associated with a deletion 5q (del 5q) cytogenetic abnormality. We conducted a multicenter, single-arm clinical trial to evaluate the safety and efficacy of lenalidomide in Japanese patients with anemia in low- or intermediate-1 risk MDS associated with the del 5q cytogenetic abnormality. Eleven patients (5 with transfusion-dependent anemia; 6 with transfusion-independent symptomatic anemia) received once daily oral administrations of 10 mg of lenalidomide for 21 consecutive days in a 28-day treatment cycle. The efficacy was assessed by the IWG criteria. At an interim analysis after ≥24 weeks of therapy, hemoglobin increase was noted in all 11 patients, with a median increase of 6.0 g/dL (range, 0.9–10.9) from the baseline. All transfusion-dependent patients achieved

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transfusion independence. Histopathologic and cytogenetic improvement was also noted. Neutropenia and thrombocytopenia were the most common adverse events related to lenalidomide. The adverse events were manageable, and no patients experienced serious adverse events or adverse events requiring treatment discontinuation. The results indicate that lenalidomide can be a useful agent for treating Japanese patients with anemia associated with low- or intermediate-1 risk MDS with the del 5q cytogenetic abnormality.

Keywords Lenalidomide · Myelodysplastic syndromes · Chromosome 5q deletion

1 Introduction

Myelodysplastic syndromes (MDS) are a group of acquired hematologic malignancies characterized by peripheral cytopenias and dysplastic morphology together with normocellular or hyperplasic bone marrow [1]. Anemia is the most common clinical manifestation of MDS, which may influence the quality of life of patients. MDS affects approximately 5 of 100,000 people and is more common in middle-aged and elderly populations [2]. Furthermore, MDS progresses to acute leukemia over a period of several months to several years in 20–40% of the entire MDS population.

The administration of erythropoiesis-stimulating agent (ESA) alone or in combination with granulocyte colony-stimulating factors can alleviate MDS-associated anemia, although the benefits of the treatments are generally limited to patients with low serum erythropoietin concentration with lesser need for red blood cell (RBC) transfusions [3, 4]. Therefore, supportive RBC transfusion is the mainstay of treatment for symptomatic anemia in MDS [5]. However, long-term transfusion therapy may cause iron-overload disorder, which may lead to organ damage most commonly involving the liver, heart and pancreas [6]. Thus, concomitant administration of iron-chelators is necessary, which is an additional burden for the patients.

Lenalidomide is an immunomodulatory compound originally developed by Celgene Corporation. Lenalidomide has a variety of pharmacologic actions, including stimulation of T lymphocytes and natural-killer cells, inhibition of angiogenesis, suppression of tumor-cell growth, regulation of stem cell differentiation, and suppression of inflammation and hyperalgesia [7]. A US-clinical study evaluated the effect of lenalidomide in patients with transfusion-dependent anemia of low- or intermediate-1-risk MDS, having a cytogenetic abnormality in the long arm of chromosome 5 (del 5q), by the International Prognostic Scoring System (IPSS) [8]. In the US clinical study MDS-003, ninety-nine (67%) of 148 patients achieved transfusion independence. The median increase in the blood hemoglobin concentration from the baseline was 5.4 g/dL. Cytogenetic response was observed in 62 (73%) of 85 patients in whom chromosomal examinations were performed; the abnormal bone marrow cells detected at the baseline were absent in 38 patients [9]. Lenalidomide at a 10 mg per day starting daily dosage was well tolerated with predictable and manageable adverse events. The most common adverse events were granulocytopenia and thrombocytopenia requiring temporarily withholding lenalidomide therapy with or without resumption at a reduced daily dosing.

Lenalidomide has been approved by the US Food and Drug Administration (FDA) as a treatment for transfusion-dependent anemia in low- or intermediate-1-risk MDS with del 5q. The National Comprehensive Cancer Network (NCCN) clinical practice guidelines for MDS (Version 1, 2009) [10] recommend the use of lenalidomide for the treatment of anemia associated with MDS. The incidence of MDS with del 5q is quite rare in Japan [11] and less frequent than in the West, as has also been suggested in other Asian countries [12]. Of 425 Japanese MDS patients registered in the Japan National Research Group on Idiopathic Bone Marrow Failure Syndromes, 50 cases (11.8%) had chromosome 5 abnormalities and the estimated rate of MDS with del 5q and 5q- syndrome among MDS patients was 8.4 and 1.3%, respectively. Lenalidomide is not yet in clinical use in Japan.

We conducted a multicenter, single-arm, open-label clinical trial to assess the efficacy and safety of lenalidomide treatment in Japanese patients with anemia in low- or intermediate-1-risk MDS with del 5q.

2 Design and materials

2.1 Study design and treatment

The study design is illustrated in Fig. 1. The study was a multicenter, single-arm, open-label study. The treatment cycle consisted of 28 days: the patients received 10 mg of lenalidomide once daily for 21 consecutive days followed by a drug holiday for 7 days. On occurrence of dose-limiting adverse events the dose was reduced or the treatment interrupted according to the protocol. Treatment with lenalidomide was to be continued for a maximum of 156 weeks until disease progression or disease recurrence after a response to the treatment. The treatment was terminated if there was no response at 32 weeks after the initiation of treatment. The study protocol was approved by IRB.

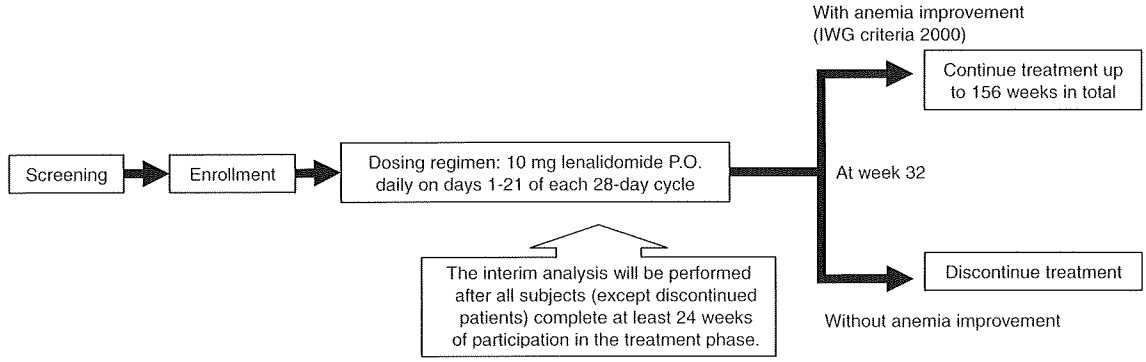

Fig. 1 Study design. This trial was designed as a multicenter, single-arm, open-label study. A treatment cycle consisted of 28 days: the patients received 10 mg lenalidomide once daily for 21 consecutive days and had drug holiday for 7 days. Treatment with lenalidomide was to be continued for a maximum of 156 weeks until disease progression or recurrence after a response to the treatment. The treatment was terminated if there was no response in terms of the efficacy end points at 32 weeks after the initiation of treatment

2.2 Patients

The study subjects were MDS patients aged 20 years or more who met all of the following criteria: del 5q with or without additional chromosomal abnormalities; low- or intermediate-1 risk MDS by the IPSS score; transfusion-dependent anemia or symptomatic anemia with a blood hemoglobin concentration of less than 10.0 g/dL [12]. Written informed consent was obtained from all the patients before enrollment in the study.

The patients were excluded from the study if they met any of the following criteria: pregnancy or lactation; neutrophil count of less than 750/μL, platelet count of less than 50,000/μL, serum creatinine level of greater than 2.5 mg/dL, or serum AST or ALT levels greater than 3 times the upper normal limit; a history of deep venous thrombosis or pulmonary embolism; or treatments of MDS immediately before the initiation of the study, including chemotherapeutics, immunomodulators, antithymocyte globulin, erythropoiesis-stimulating factors and stem cell transplantation.

2.3 Clinical and laboratory evaluations

The efficacy end points of the study were improvement in anemia, duration of the improvement, change in blood hemoglobin concentration, cytogenetic response and bone marrow response. The criteria of improvement in anemia in this study followed that of the International Working Group (IWG) 2000 criteria [13] and were defined as transfusion independence lasting for 8 consecutive weeks after the treatment in a transfusion-dependent anemic patient requiring at least 4.5 units (equivalent to 2 units in Western countries) of RBC transfusion in the 8 weeks preceding lenalidomide treatment, or a greater than 2.0 g/dL increase from the baseline in the blood hemoglobin concentration that lasted for 8 consecutive weeks after the treatment in a transfusion-independent symptomatic anemic patient requiring less than 4.5 units of RBC transfusion preceding initiation of lenalidomide treatment. Morphologic and cytogenetic examinations of the bone marrow were performed using aspiration/biopsy specimen. Cytogenetic analysis was performed with both conventional G-banding and fluorescence in situ hybridization (FISH) procedure. For G-banding analysis, chromosomes were pretreated by trypsin, stained with Giemsa and karyotyped according to the recommendation of the ISCN (2005) [14]. For intrerphase analysis, the BAC clone containing *EGR1* gene (Vysis LSI EGR1 (5q31) SpectrumOrange probe; Abott Molecular) was used as a FISH probe. FISH signals were evaluated in 100 nuclei per slides. Separate panels of independent reviewers assessed the cytogenetic response, the bone marrow response and reanalyzed the IPSS classification.

The safety evaluation consisted of assessments of the adverse events in terms of terminology, frequency, severity as graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 3.0 and relationship with lenalidomide treatment.

The pharmacokinetics of lenalidomide in Japanese patients was also evaluated. The concentrations of unchanged lenalidomide in plasma on the first 5 days of treatment were used to calculate the pharmacokinetic parameters, including the maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), area under the plasma concentration–time curve (AUC), elimination half-life ($t_{1/2}$) and total clearance (CL/*F*).

2.4 Statistical analysis

Since the present study was a single-arm, open-label study, only descriptive analyses were performed.

3 Results

3.1 Patients

Fourteen patients were screened; 11 patients received the lenalidomide treatment, and 3 patients were found ineligible for the study. This report presents the interim analysis results from the data obtained until the data cutoff date (15 October 2008), when all 11 patients had received the study treatment for at least 24 weeks. The median duration of the study at the data cutoff date was 48.1 weeks (range, 28.1–56.1 weeks). None of the patients were withdrawn from the study.

The demographics of the patients (4 males and 7 females) treated with lenalidomide are listed in Table 1. The average age of the patients was 71.8 years. The median duration of the disease was 1.9 years; 2 and 9 patients had low- and intermediate-1-risk MDS by the IPSS score, respectively. 5 patients had transfusion-dependent anemia requiring at least 4.5 units (equivalent to 2 units in Western countries) of RBC transfusion in the 8 weeks before treatment, and 6 patients had transfusion-independent symptomatic anemia requiring less than 4.5 units of RBC transfusion in the same period. The FAB subtype classification was refractory anemia (RA) in 9 patients and RA with excess blasts (RAEB) in 2 patients. According to the WHO classification, 8 patients had 5q-syndrome, 2 patients had RAEB-1 and 1 patient had refractory cytopenia with multi-lineage dysplasia (RCMD). A total of 7 patients had received treatment for MDS, 4 patients each receiving alfacalcidol and menatetrenone.

The median amount of blood transfusion at the baseline was 8.0 units for patients with transfusion-dependent anemia and 1.0 unit for those with transfusion-independent symptomatic anemia. The median blood hemoglobin concentration at the baseline was 7.1 g/dL for patients with transfusion-dependent anemia and 6.4 g/dL for those with transfusion-independent symptomatic anemia.

3.2 Anemia improvement

Anemia improvement was observed in all the 11 patients who were treated with lenalidomide (Table 2). The 5 patients with transfusion-dependent anemia at the baseline had increased blood hemoglobin concentration after the treatment, and achieved transfusion independence; the median blood hemoglobin concentration increased from 7.1 g/dL at the baseline to 12.7 g/dL with treatment, with a median increase of hemoglobin of 6.0 g/dL from the baseline. In the 6 patients with transfusion-independent symptomatic anemia, the median blood hemoglobin concentration increased from 6.4 g/dL at the baseline to 12.7 g/dL, with a median increase of hemoglobin of 5.3 g/dL from the baseline. The median time to the anemia improvement was 6.3 weeks (range, 3.1–31.1 weeks). The improvement persisted in all the patients on the data cutoff date. The median duration until the interim analysis was 41.0 weeks (range, 17.1–46.1 weeks).

The changes in blood hemoglobin concentrations of the patients who received lenalidomide are shown in Fig. 2. The median blood hemoglobin concentration was 7.0 g/dL at the baseline, and 12.7 g/dL at the maximum during the

Table 1 Patient characteristics

Subject no.	Age/sex	Height (cm)	Weight (kg)	Duration of MDS (years)	IPSS[a] Group (independent review)	FAB classification of MDS	WHO classification of MDS	Baseline hemoglobin (g/dL)	Baseline transfusion dependency	Baseline transfusion units[b]
0020001	73/male	162.0	58.4	2.7	Intermediate-1	RAEB	RAEB-1	5.50	Yes	8
0040001	64/female	151.0	61.2	0.2	Intermediate-1	RA	5q-syndrome	6.05	No	4
0040002	83/female	144.5	44.5	4.2	Intermediate-1	RA	5q-syndrome	6.60	No	0
0040003	76/female	147.5	50.6	0.9	Intermediate-1	RA	5q-syndrome	6.25	No	2
0050001	68/male	170.5	74.9	2.1	Low	RA	5q-syndrome	4.70	No	2
0060001	72/female	146.0	42.6	1.0	Intermediate-1	RA	5q-syndrome	8.00	Yes	8
0080001	74/female	151.0	52.8	4.8	Intermediate-1	RAEB	RAEB-1	7.10	Yes	12
0080002	69/female	160.3	63.0	1.8	Intermediate-1	RA	5q-syndrome	9.10	No	0
0080003	65/female	155.7	56.7	0.6	Intermediate-1	RA	5q-syndrome	6.95	Yes	6
0090001	79/male	164.4	51.2	2.1	Intermediate-1	RA	RCMD	7.55	No	0
0090002	67/male	159.2	64.9	1.9	Low	RA	5q-syndrome	7.80	Yes	6

MDS myelodysplastic syndromes, *IPSS* International Prognostic Scoring System, *FAB* French–American–British, *WHO* World Health Organization classification, *RAEB* refractory anemia with excess blasts, *RA* refractory anemia, *RCMD* refractory cytopenia with multi-lineage dysplasia

[a] IPSS Group = sum of marrow blast + karyotype + cytopenia score

[b] RBC transfusion units within 56 days before the start of the study drug. In Japan, 1 unit of RBC contains 200 mL of whole blood

anemia improvement period; the median change from the baseline was 6.0 g/dL.

3.3 Cytogenetic response

The cytogenetic responses are shown in Table 3. All 11 patients treated with lenalidomide had abnormal metaphases with del(5q) at baseline. Abnormal metaphases were eliminated at the time of the completion of cycle 6 (on day 169) in three of 10 evaluable patients. Furthermore, no 5q abnormality was detectable by interphase FISH in two patients (0050001 and 0090001) on day 169. Additional cytogenetic abnormality, which had not been observed at baseline was detected in two patients (0040002 and 0080002) during the treatment.

Table 2 Anemia improvement with lenalidomide

Response	No. of patients	Responder *n* (%)
Overall improvement in anemia	11	11 (100.0)
RBC transfusion independence and increase in hemoglobin level of ≥1 g/dL[a]	5	5 (100.0)
Increase in hemoglobin level of >2 g/dL[b]	6	6 (100.0)
Hemoglobin (g/dL)		
Baseline[c] median (range)	11	7.0 (4.7–9.1)
Response[d] median (range)	11	12.7 (8.9–15.6)
Change median (range)	11	6.0 (0.9–10.9)

[a] Transfusion independence and an increase in hemoglobin of at least 1 g/dL from the baseline for 8 consecutive weeks for patients who were RBC transfusion dependent at baseline

[b] >2 g/dL increase in hemoglobin without transfusion from the baseline for 8 consecutive weeks for patients who were RBC transfusion independent with hemoglobin <10 g/dL at baseline

[c] The baseline hemoglobin concentration was the mean of the two most recent hemoglobin measurements prior to the start of lenalidomide treatment

[d] The response hemoglobin concentration was the maximum value during improvement in anemia

3.4 Bone marrow response

The bone marrow cellularity normalized at cycle 6 in 5 patients: 2 patients with a hypercellular bone marrow and 3 patients who had a hypocellular bone marrow before treatment. On the other hand, 2 patients with normocellular bone marrow before the treatment had hypocellular bone marrows at cycle 6.

No patients had a change in terms of worsening in the FAB or WHO subtype. The number of BM erythroblasts increased with treatment. Therefore, the proportion of BM myeloblasts declined to less than 5% in 2 patients. In 2 patients, the FAB subtype changed from RAEB at the baseline to RA. By the WHO subtype, 2 patients with RAEB-1 at the baseline changed to 5q deletion syndrome in one patient and RCMD in the other.

3.5 Adverse events and dose adjustment

Adverse events were reported in all 11 patients treated with lenalidomide. The grade 3 or higher lenalidomide-related adverse events were neutropenia ($n = 10$), leukopenia ($n = 6$), lymphopenia ($n = 3$), thrombocytopenia ($n = 1$), and hypertension ($n = 1$) (Table 4). None of the patients had grade 3 or higher hemorrhagic adverse events or infections, or discontinued the treatment because of adverse events. A serious compression fracture was reported in 1 patient, which was considered to be unrelated to lenalidomide treatment. Clinically significant changes were not observed in any other safety parameters, including laboratory tests, vital signs, and ECG. A total of 8 patients had

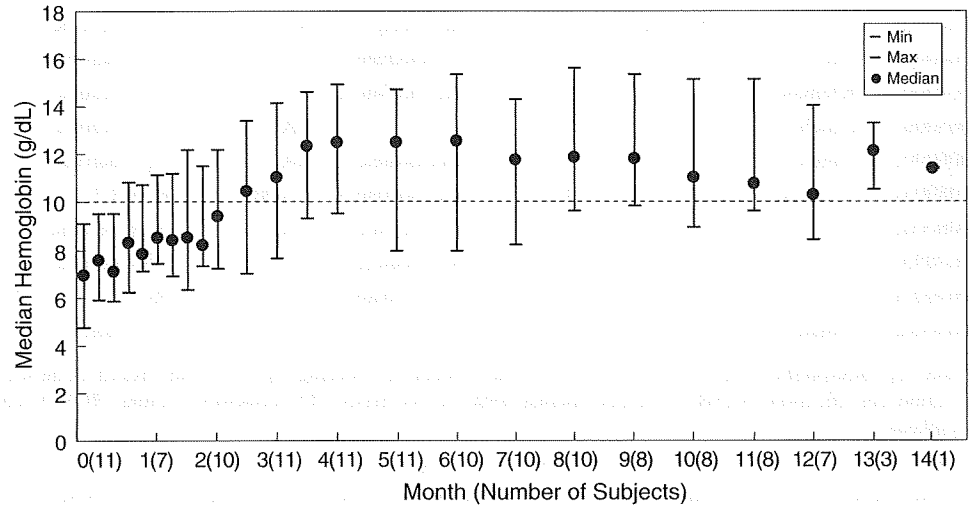

Fig. 2 Hemoglobin levels over treatment time. The median hemoglobin concentration was 7.0 g/dL at the baseline, and 12.7 g/dL at the maximum during the anemia improvement period; the median change from the baseline was 6.0 g/dL

Table 3 Cytogenetic response, as assessed by interphase FISH and G-banding procedure

Subject no.	No. of nuclei with one EGR1 signal[a]			No. of abnormal chromosomes with del (5q)[b]			
	Screening	Day85	Day169	Karyotype	Screening	Day 85	Day 169
0020001	74	25	ND	46,XY,del(5)(q13q33)	19	11	ND
0040001	51	10	4	46,XX,del(5)(q13q33)	8	2	2
0040002	72	0	7	46,XX,del(5)(q13q33)	17	1	3
				46,idem,add(11)(q13)	0	1	0
0040003	83	11	4	46,XX,del(5)(q13q33)	20	5	0
0050001	67	3	0	46,XY,del(5)(q22q31)	8	1	0
0060001	37	33	65	46,XX,del(5)(q31q35)	19	5	11
0080001	77	57	23	46,XX,del(5)(q13q33), inv(9)(p12q13)	20	13	5
0080002	41	6	5	46,XX,del(5)(q13q33)	14	0	1
				46,XX,del(5)(q13q33), del(20)(q11.2q13.3)	0	1	4
0080003	22	5	53	46,XX,del(5)(q13q33)	9	1	14
0090001	23	10	0	46,XY,del(5)(q13q33)	7	0	0
				46,XY,der(5)del(5)(q13q33)t(5;?)(q35;?)	9	6	0
0090002	60	13	49	46,XY,del(5)(q15q33)	19	3	13

ND no data

[a] With interphase FISH in each patient, 100 nuclei were analyzed

[b] With G-banding analysis in each patient, 20 metaphase cells were analyzed

Table 4 Treatment-related adverse events with NCI CTCAE grades 3 or 4

System organ class/ preferred term[a]	Initial dose (10 mg) ($N = 11$) n (%)
Subjects with at least one NCI CTCAE grade 3 or 4-related adverse event	11 (100.0)
Blood and lymphatic system disorders	11 (100.0)
Neutropenia	10 (90.9)
Leukopenia	6 (54.6)
Lymphopenia	3 (27.2)
Thrombocytopenia	1 (9.0)
Vascular disorders	1 (9.0)
Hypertension	1 (9.0)

NCI CTCAE National Cancer Institute Common Terminology Criteria for Adverse Events

[a] Preferred terms and system organ classes are coded using the MedDRA dictionary (version 10.0). A subject with multiple occurrences of an adverse event is counted only once in the preferred term category

reduced lenalidomide dosages from 10 mg daily to 5 mg daily (21 consecutive days). On the data cutoff, lenalidomide dosage for these 8 patients was 5 mg daily. The reason for the dose reduction was neutropenia (≥grade 3; $n = 8$). Furthermore, administration was temporarily interrupted in 8 patients due to AEs. The reasons for the dose interruption were neutropenia (≥grade 3, $n = 8$) and abnormal laboratory value of TSH (grade 2, $n = 1$).

3.6 Pharmacokinetic evaluation

Blood lenalidomide pharmacokinetics was evaluated during the first five days of therapy. Serum specimens were successfully collected in 6 patients.

The mean C_{max} values were 136 ng/mL at day 1 and 149 ng/mL at day 4 of lenalidomide treatment. The mean AUC_{τ} values were 866.5 ng h/mL at day 1 and 877.9 ng h/mL at day 4. The ratios of the C_{max} and AUC_{τ} values at day 4 to those at day 1 were 1.16 and 1.04, respectively. The results did not suggest drug accumulation after repeated administrations. The t_{max} values were 2.52 h at day1 and 2.93 h at day 4 of the treatment, with no significant change. Other pharmacokinetic parameters at day 4 were also similar to those at day 1; the $t_{1/2}$ values were 3.26 and 3.57 h, and the CL/F values were 189.8 and 189.9 mL/min at day 1 and day 4, respectively.

4 Discussion

Chronic anemia compromises the quality of life in MDS patients. In addition, chronic blood transfusion therapy may lead to complications such as iron-overload disorder and cardiac failure. The need for blood transfusions also correlates with the risk of progression to acute myeloid leukemia, resulting in a poor prognosis in patients with lower-risk MDS [5].

In this study, lenalidomide treatment resulted in rapid increase in the hemoglobin (median time to anemia

improvement was 6.3 weeks) in all Japanese patients with anemia in low- or intermediate-1-risk MDS with del 5q, and transfusion independence in patients who were transfusion dependent at baseline, demonstrating that lenalidomide can effectively improve anemia in Japanese MDS patients. Moreover, the effect of lenalidomide was persistent in all the patients at the data cutoff date (median duration of the study, 48.1 weeks; range, 28.1–56.1 weeks). The median duration of improvement in anemia was not estimable.

The effect of anemia improvement observed in the present study compare favorably with the results from the US pivotal clinical study MDS-003 [9].

Of note is not only the achievement of transfusion independence in patients with RBC transfusion-dependent anemia, but also the increase of blood hemoglobin concentration to at least 10 g/dL in this study. In most western countries, the threshold for receiving an RBC transfusion for anemia associated with MDS is a blood hemoglobin concentration less than 10 g/dL [15]. In Japan, in contrast, RBC transfusion is generally avoided whenever possible, initiated only when the blood hemoglobin concentration is below 7 g/dL. Such a clinical practice is also apparent in the present study, as patients with blood hemoglobin concentration as low as 4.7 g/dL at baseline had not been heavily transfused. Improvement of anemia in Japanese MDS patients not receiving RBC transfusion, thus, is as clinically important as successful achievement of independence from RBC transfusion in western countries.

Histopathologic findings confirmed improvement of the dysplasia of megakaryocytes, paralleling the improvement in anemia from lenalidomide. Moreover, cytogenetic evaluation demonstrated improvement of chromosome abnormality with del(5q) in 10 patients on day 85, which suggests that the cytogenetic response and the Hb increase run parallel, although a relation between maintenance of the anemia improvement and the cytogenetic response was not observed.

In the cytogenetic evaluation, a new complex karyotype, del(20q),del(5q), was detected in one patient. The incidence of del(5q) accompanied with del(20q) was reported in 8% [16]. Considering more than a 2-year history of MDS in this patient, it would be likely that a progression to this complex karyotype was the natural course of MDS. The del(5q) accompanied with del(20q) is presumed to be less sensitive to lenalidomide than the isolated del(5q). It may be considered that the del(5q),del(20q) became obvious in this study, since the original del(5q) abnormality disappeared due to the lenalidomide treatment. In these 2 years, lenalidomdie-inducing risks of clonal evolution and AML progression have been discussed, but several recent studies reported that the risk of AML progression for patients who received lenalidomide was similar to those in the historical data [17, 18].

In a previous US study MDS-003, approximately half of the patients reported lenalidomide-related neutropenia and thrombocytopenia of grade 3 or higher (by the Common Toxicity Criteria of the National Cancer Institute, Version 2.0), which were the most common reasons for the dose adjustment of lenalidomide [9]. In the present study, the adverse events, most notably neutropenia, did not lead to treatment discontinuation, and were manageable with dose reduction, dose interruption or symptomatic treatment.

Lenalidomide was absorbed with maximum plasma concentrations occurring at approximately 3 h following oral administration and then rapidly eliminated from plasma ($t_{1/2}$ was less than 4 h and CL/F was about 190 mL/min). Multiple dose (10 mg × 4 days) did not cause accumulation of lenalidomide (C_{max}/AUC ratios were 1.16 and 1.04 for day1 and day4, respectively). Other PK parameters were similar between day 1 and day 4. It was also reported that lenalidomide was rapidly adsorbed and eliminated in healthy volunteers. Moreover, multiple dosing did not result in drug accumulation [19].

Oral lenalidomide confers less burden on the patients than transfusion therapy and can improve the quality of life. The results of the present study indicate that lenalidomide can be a useful drug for the treatment of MDS with del(5q) in Japanese patients as well. In the absence of approved ESA for MDS, such as erythropoietin, lenalidomide is potentially a new therapeutic option of great clinical significance for the treatment of anemia in Japanese patients with del(5q) MDS.

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Differences in the distribution of subtypes according to the WHO classification 2008 between Japanese and German patients with refractory anemia according to the FAB classification in myelodysplastic syndromes

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ABSTRACT

We reported the different clinical features between Japanese and German refractory anemia (RA) patients in FAB classification. We re-analyzed the clinical features by WHO classification revised in 2008. The frequencies of refractory cytopenia with unilineage dysplasia (RCUD) and myelodysplastic syndrome-unclassified (MDS-U) with pancytopenia in Japanese patients were higher than in German patients ($p < 0.001$). Refractory cytopenia with multilineage dysplasia patients showed the most unfavorable prognosis in both countries. The higher frequencies of MDS-U with pancytopenia and RCUD in Japanese patients may influence the different clinical characteristics between Japanese and German FAB-RA patients.

1. Introduction

Myelodysplastic syndromes (MDS) are acquired clonal stem cell disorders characterized by ineffective hematopoiesis with myelodysplasia [1] and are associated with a high risk of progression to acute leukemias [2]. MDS are very heterogeneous in terms of their morphology, clinical features, and survival [3]. There are several reports indicating possible differences in clinical features between Western MDS types and Eastern MDS types [4–9]. The median age of MDS patients in Korea and Thailand were reported to be 57 [8] and 56 [7], respectively. On the other hand, large MDS studies from Western countries showed a median or mean age of 68–73 years [10–13]. We have reported that the clinical features of refractory anemia with excess of blasts (RAEB) or RAEB in transformation (RAEB-t) according to the French–American–British (FAB) classification [14] seemed to be similar between Japanese and Western patients [15]. However, previous reports [5,15] indicated that Japanese MDS patients have a lower frequency of refractory anemia with ringed sideroblasts (RARS) according to the FAB classification and a higher frequency of refractory anemia according to the FAB classification (FAB-RA) than the Western International Prognostic Scoring System (IPSS) study [10], and we reported that the clinical and laboratory features of Japanese FAB-RA patients apparently differ from those of German patients after a precise morphologic consensus (FAB classification: concordance rate, 98.4%; κ, 0.94; $p < 0.001$; prior World Health Organization (WHO) classification (WHO classification 2001) [16]: concordance rate, 83.8%; κ, 0.73; $p < 0.001$) [17]. That was the first comparison report between Western and Eastern FAB-RA patients after confirming morphological consensus. Japanese FAB-RA patients were younger, showed more severe cytopenia(s), a lower frequency of abnormal karyotypes, a lower frequency of MDS with isolated del(5q) (5q- syndrome), and a more favorable prognosis in terms of the overall survival (OS) and leukemia free survival (LFS) in our previous study.

MDS subtypes in the WHO classification 2001 [16] was revised in 2008 (WHO classification 2008) [18]. Refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT) were combined into refractory cytopenia with unilineage dysplasia

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(RCUD) in the WHO classification 2008. The diagnosis of MDS-unclassified (MDS-U) according to the WHO classification 2008 can be made in the following instances:

1. Patients with the findings of RCUD or refractory cytopenia with multilineage dysplasia (RCMD) but with 1% blasts in the peripheral blood (PB) (PB blasts type).
2. Cases of RCUD which are associated with pancytopenia (RCUD/pancytopenia type).
3. Patients with cytopenia(s) with 1% or fewer blasts in the PB and fewer than 5% in the bone marrow (BM), unequivocal dysplasia in <10% of the cells in one or more myeloid lineages, and who have cytogenetic abnormalities (cytogenetic abnormalities type).

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification because of 1% blasts in the PB. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification because of unequivocal dysplasia. Thus, FAB-RA patients are classified as RCUD, RCMD, MDS with isolated del(5q) (5q- syndrome) or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008. In the present study, we re-analyzed in detail the clinical features of Japanese and German FAB-RA patients by using revised MDS subtypes in the WHO classification 2008.

2. Patients and methods

The dataset of consecutive patients with primary FAB-RA of our previous study [17] (total 728 consecutive patients: Japan, 131 cases; Germany, 597 cases) were used for the present retrospective analysis. Japanese patients of this dataset were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital or affiliated hospitals between April 1976 and January 1997. German patients were diagnosed at the Department of Hematology, Oncology and Clinical Immunology of the Heinrich-Heine University between January 1973 and December 2002. Patients who had previously been treated with anti-neoplastic drugs or ionizing radiation were excluded from the study. Patients without the available necessary data for the WHO classification 2008 were excluded from the present study. Cytogenetic analyses were performed with a trypsin-Giemsa banding technique on BM cells from aspirates. Ordinarily 20–30 metaphases were examined. Cytogenetic aberrations were grouped according to the IPSS publication [10]. Thresholds for cytopenia(s) were defined as those of the IPSS (hemoglobin (Hb) <10.0 g/dL, absolute neutrophil count (ANC) $<1.8 \times 10^9$/L, and platelet $<100 \times 10^9$/L). Criteria for dysplasia were defined as those of a previous German report [19]. Hypoplastic BM was defined as <30% cellular in patients <60 years old, or <20% cellular in patients ≥60 years old [20]. If hypoplastic BM and certain dysplasia more than 10% in one or more of major myeloid cell lines were present, a diagnosis of hypoplastic MDS was made. Patients were reclassified according to the definition of WHO classification 2008 for MDS subtyping by using PB and BM findings, morphologic findings, and cytogenetic findings of the previous dataset [17]. Comparisons of the clinical features at the time of diagnosis and OS and LFS were analyzed by using the dataset of our previous study [17]. OS was measured from the date of diagnosis until death due to any cause, the date of stem cell transplantation, or until the last patient contact. LFS was measured from the date of diagnosis until the date of diagnosis of acute leukemia. This study was approved by the Institutional Review Board of Saitama International Medical Center, Saitama Medical University, Saitama, Japan.

2.1. Statistical methods

The chi-square test and the nonparametric Mann–Whitney test were used to compare the proportions of patients and continuous data, respectively. The Kaplan–Meier method was used to generate the estimate of cumulative probabilities of OS and LFS. The difference in the cumulative probabilities within subcategories of patients was compared using a two-sided log-rank test. A two-sided p value of <0.05 was considered to be statistically significant. All statistical analyses were performed with the use of StatView (version 5.0, SAS Institute, Cary, NC).

3. Results

3.1. Comparison of frequencies of subtypes according to the WHO classification 2008 between Japanese and German FAB-RA patients

A total of 295 patients (Japan, 102 cases; Germany, 193 cases) could be classified according to the WHO classification 2008. A total of 433 patients (Japan, 29 cases; Germany, 404 cases) could not be classified according to the WHO classification 2008 due to a deficit of either cytogenetic data or adequate peripheral blood data, and 427 patients presented without available cytogenetic findings (Japan, 29 cases; Germany, 398 cases). There were 6 patients (Germany, 6 cases) without any data of peripheral blood.

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification due to unequivocal dysplasia. Therefore, patients with MDS-U (PB blasts type) or with MDS-U (cytogenetic abnormalities type) were not included in the previous dataset. Because the previous dataset used in the present study was that of FAB-RA patients, dysplasia existed in at least one lineage and the frequency of blasts in PB was <1% in all patients. Therefore, all MDS-U patients in the present study were diagnosed as RCUD/pancytopenia type. Most Japanese FAB-RA patients were classified as RCUD, RCMD, or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008 (Table 1A). Most German FAB-RA patients were classified as RCUD, RCMD, or 5q- syndrome (Table 1B). The frequency of RCUD in Japanese FAB-RA patients (45%) was significantly higher than that in German FAB-RA patients (19%) ($p < 0.001$). The frequency of patients with bicytopenia in Japanese RCUD patients was 59%, but that in the German RCUD patients was only 19%. Among 46 Japanese RCUD patients, number of patients with single cytopenia was 17 cases (37%) including 2 RA, 4 RN and 11 RT cases. Among 37 German RCUD patients, number of patients with single cytopenia was 22 cases (59%) including 7 RA, 11 RN and 4 RT cases. Frequency of RT was 2% of German FAB-RA patients. The frequency of RT of Japanese FAB-RA patients (11%) was higher than that of German FAB-RA patients. The frequency of MDS-U in Japanese FAB-RA patients (29%) was significantly higher than that in German FAB-RA patients (3%) ($p < 0.001$). The frequency of RCMD in Japanese FAB-RA patients (25%) was significantly lower than in German FAB-RA patients (58%) ($p < 0.001$). The frequency of 5q- syndrome in Japanese FAB-RA patients (3%) was significantly lower than in German FAB-RA patients (20%) ($p < 0.001$) (Table 1C).

3.2. Comparison of clinical and laboratory features at the time of diagnosis between Japanese and German patients could be classified according to the WHO classification 2008

The age of patients in RCUD, MDS-U and RCMD subtypes did not differ between the two countries. The MDS-U (RCUD/pancytopenia type) subtype was younger than other subgroups in Japanese patients. The gender ratios in the RCUD

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Table 1
Laboratory features at the time of diagnosis in FAB-RA patients who could be classified according to the WHO classification 2008.

	RCUD	MDS-U	RCMD	5q- synd
(A) Japanese patients, $n = 102$				
Patients = n (%)	46(45)	28(29)	25(25)	3(3)
Gender (male/female)	28/18	12/16	11/14	2/1
Age (years)	57(16–86)	51(15–82)	63(16–88)	60(59–74)
Neutrophils ($\times 10^9$/L)	1.89 (0.44–4.69)	1.10 (0.26–1.77)	1.28 (0.05–10.24)	0.73 (0.50–2.54)
Hemoglobin (g/dL)	10.2 (3.0–14.3)	6.9 (4.2–9.1)	8.2 (2.9–14.0)	6.3 (4.6–10.8)
Platelets ($\times 10^9$/L)	41(4–246)	29(7–98)	50(13–390)	207(134–212)
Abnormal karyotype = n (%)	12(26)	6(21)	9(36)	3(100)
Hypoplastic bone marrow = n (%)	3(7)	3(11)	0(0)	0(0)
(B) German patients, $n = 193$				
Patients = n (%)	37(19)	6(3)	111(58)	39(20)
Gender (male/female)	20/17	1/5	80/31	14/25
Age (years)	62(20–80)	56(19–59)	63(15–86)	62(32–78)
Neutrophils ($\times 10^9$/L)	1.92 (0.36–8.72)	1.41 (0.48–1.50)	1.60 (0.21–19.40)	1.95 (0.61–6.78)
Hemoglobin (g/dL)	11.0 (5.2–15.4)	9.4 (5.5–9.8)	9.2 (5.1–16.9)	8.7 (3.0–12.2)
Platelets ($\times 10^9$/L)	128(2–840)	33(10–90)	102(9–999)	250(28–1540)
Abnormal karyotype = n (%)	12(32)	3(50)	47(42)	39(100)
Hypoplastic bone marrow = n (%)	3(8)	2(33)	13(12)	5(13)
	Japan vs Germany			
(C) Comparison between Japanese and German patients				
(1) RCUD patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.532$			
Age (years)	$p = 0.150$			
Neutrophils ($\times 10^9$/L)	$p = 0.466$			
Hemoglobin (g/dL)	$p = 0.087$			
Platelets ($\times 10^9$/L)	$p < 0.001$			
Abnormal karyotype (%)	$p = 0.526$			
Hypoplastic bone marrow (%)	$p = 0.782$			
(2) MDS-U patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.239$			
Age (years)	$p = 0.557$			
Neutrophils ($\times 10^9$/L)	$p = 0.821$			
Hemoglobin (g/dL)	$p = 0.036$			
Platelets ($\times 10^9$/L)	$p = 0.752$			
Abnormal karyotype (%)	$p = 0.150$			
Hypoplastic bone marrow (%)	$p = 0.156$			
(3) RCMD patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.007$			
Age (years)	$p = 0.401$			
Neutrophils ($\times 10^9$/L)	$p = 0.494$			
Hemoglobin (g/dL)	$p = 0.016$			
Platelets ($\times 10^9$/L)	$p = 0.030$			
Abnormal karyotype (%)	$p = 0.561$			
Hypoplastic bone marrow (%)	$p = 0.072$			
(4) 5q- synd patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.290$			
Age (years)	$p = 0.920$			
Neutrophils ($\times 10^9$/L)	$p = 0.144$			
Hemoglobin (g/dL)	$p = 0.370$			
Platelets ($\times 10^9$/L)	$p = 0.188$			
Abnormal karyotype (%)	N/A			
Hypoplastic bone marrow (%)	$p = 0.509$			

Values for presentation characteristics are given as median and range where applicable. N/A, not applicable; RCUD, refractory cytopenia with unilineage dysplasia; MDS-U, MDS-unclassified; RCMD, refractory cytopenia with multilineage dysplasia; 5q- synd, MDS with isolated del(5q).

and MDS-U subtypes were not significantly different between the two countries. The frequency of male patients in Japanese RCMD subgroup was significantly lower than that in German RCMD subtype. Japanese patients had significantly lower platelet counts than German patients in both the RCUD and RCMD subtypes. Japanese MDS-U (RCUD/pancytopenia type) and RCMD patients showed significantly lower Hb concentrations than German MDS-U (RCUD/pancytopenia type) and RCMD patients. Japanese RCUD patients showed a tendency towards lower Hb concentrations than German RCUD patients. The ANC did not differ significantly between the two countries in RCUD, MDS-U (RCUD/pancytopenia type), and RCMD patients (Table 1). The frequency of cytogenetic abnormalities in the Japanese FAB-RA patients was significantly lower than in German patients ($p < 0.001$) (Tables 1 and 2). The frequencies of cytogenetic abnormalities in the RCUD, MDS-U (RCUD/pancytopenia type), and RCMD subtypes were not significantly different between the two countries (RCUD, $p = 0.526$; RCMD, $p = 0.561$; MDS-U (RCUD/pancytopenia type), $p = 0.150$). The frequency of isolated del(5q) in Japanese FAB-RA patients was significantly lower than in German patients

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Table 2
Cytogenetic findings at the time of diagnosis in FAB-RA patients who could be classified according to the WHO classification 2008.

	RCUD	MDS-U	RCMD	5q- synd	Total
(A) Japanese patients, $n = 102$					
Patients = n	46	28	25	3	102
Good	37(80.4%)	23(82.1%)	16(64.0%)	3(100%)	79(77.5%)
Normal	34(73.9%)	22(78.6%)	16(64.0%)	0(0%)	70(68.6%)
−Y	0	1	0	0	1
del(5q)	0	0	0	3	3
del(20q)	3	0	0	0	3
Intermediate	8(17.4%)	3(10.7%)	4(16.0%)	0	15(14.7%)
Poor	1(0.2%)	2(7.2%)	5(20.0%)	0	8(7.8%)
Complex (≥3 abnormalities)	0	1	4	0	5
Chromosome 7 anomalies	1	1	1	0	3
(B) German patients, $n = 193$					
Patients = n	37	6	111	39	193
Good	27(73.0%)	3(50.0%)	72(64.9%)	39(100%)	141(73.1%)
Normal	25(67.6%)	3(50.0%)	64(57.7%)	0(0%)	92(47.7%)
−Y	2	0	2	0	4
del(5q)	0	0	0	39	39
del(20q)	0	0	6	0	6
Intermediate	4(10.8%)	2(33.3%)	23(20.7%)	0	29(15.0%)
Poor	6(16.2%)	1(16.7%)	16(14.4%)	0	23(11.9%)
Complex (≥3 abnormalities)	5	0	9	0	14
Chromosome 7 anomalies	1	1	7	0	9

Good indicates normal, −Y, del(5q), del(20q); poor, complex (≥3 abnormalities) or chromosome 7 anomalies; intermediate, other abnormalities not listed in good and poor subgroups.

($p < 0.001$) (Table 2). The most frequent cytogenetic aberration in the intermediate cytogenetic risk according to the IPSS publication was trisomy 8 (4 German RCMD cases, 3 Japanese RCUD cases, 1 Japanese MDS-U case). The frequencies of hypoplastic BM were not significantly different between the two countries in the RCUD and MDS-U (RCUD/pancytopenia type) subtypes. In the RCMD subtype, there were no Japanese patients presenting with findings concordant with hypoplastic BM. However, the frequency of German RCMD patients with hypoplastic BM was 12% (Table 1).

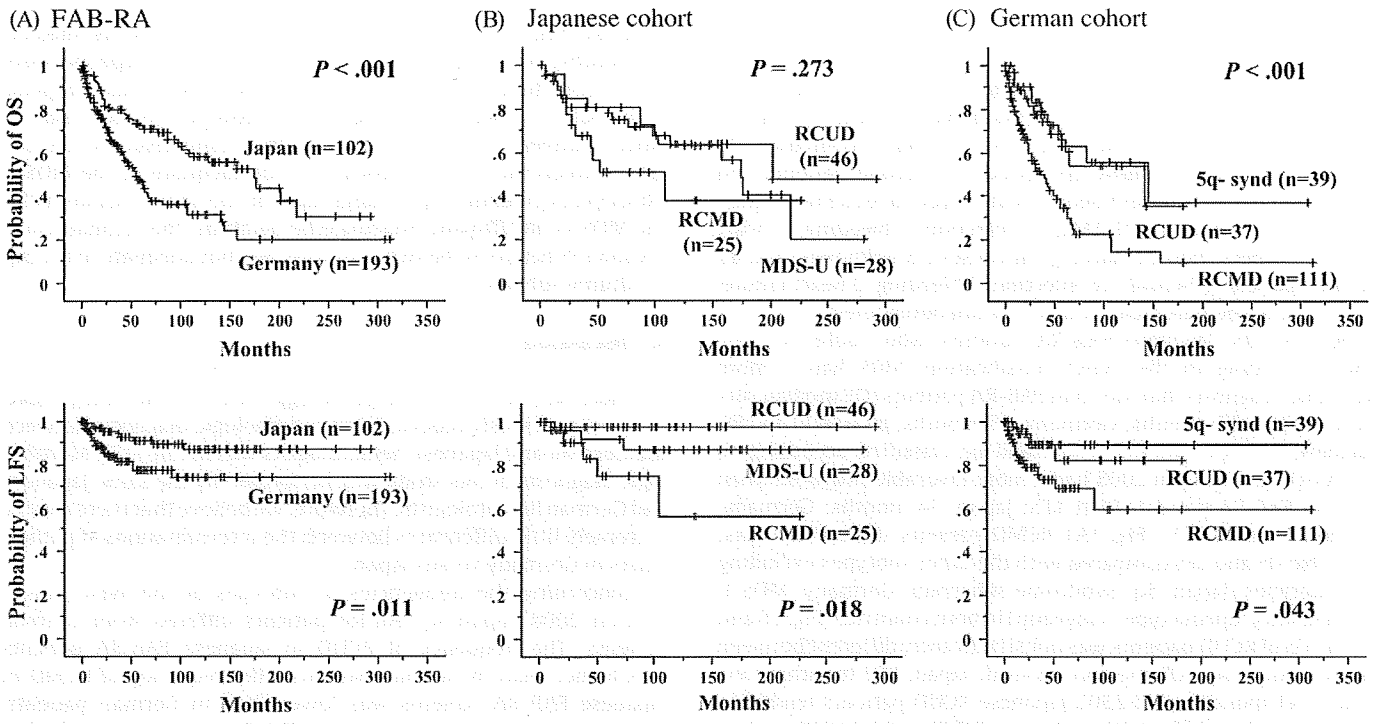

Fig. 1. Cumulative overall survival and leukemia free survival of FAB-RA patients who could be classified according to the WHO classification 2008. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) In FAB-RA patients who could be classified according to the WHO classification 2008, Japanese patients had a more favorable OS than German patients ($p < 0.001$). Japanese patients had a more favorable LFS than German patients ($p = 0.011$). (B) In Japanese FAB-RA patients who could be classified according to the WHO classification 2008, RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding a rare subtype (5q- syndrome subtype). RCUD patients showed more favorable OS and LFS than RCMD patients (OS, $p = 0.128$; LFS, $p = 0.004$). MDS-U (RCUD/pancytopenia type) patients tended to show more favorable OS and LFS than RCMD patients (OS, $p = 0.218$; LFS, $p = 0.137$). (C) In German FAB-RA patients who could be classified according to the WHO classification 2008, RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding a rare subtype (MDS-U (RCUD/pancytopenia type) subtype). RCUD patients showed more favorable OS and LFS than RCMD patients (OS, $p = 0.003$; LFS, $p = 0.075$). 5q- syndrome patients showed more favorable OS and LFS than RCMD patients (OS, $p = 0.002$; LFS, $p = 0.043$).

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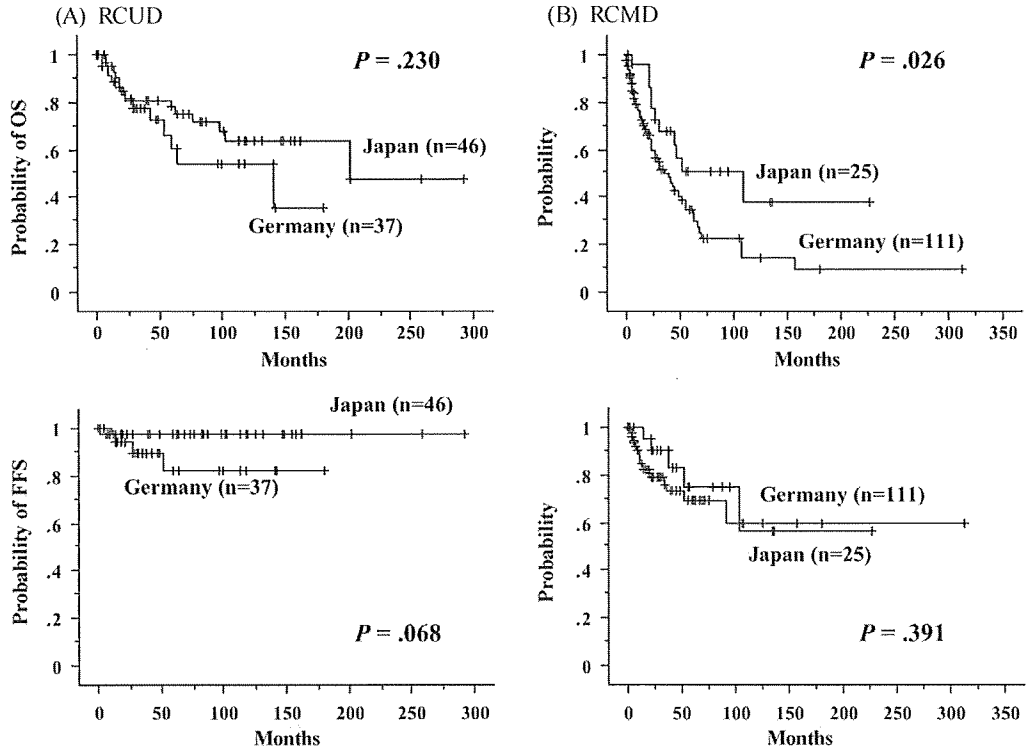

Fig. 2. Comparison of cumulative overall survival and leukemia free survival of RCUD and RCMD between Japanese and German patients. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) The OS of RCUD patients was not significantly different between the two countries ($p = 0.230$). Japanese RCUD patients tended to show a more favorable LFS than German RCUD patients ($p = 0.068$). (B) Japanese RCMD patients showed a more favorable OS than German RCMD patients ($p = 0.026$). The LFS of RCMD patients was not significantly different between the two countries ($p = 0.391$).

3.3. Prognosis

Follow-up periods ranged from 1 to 292 months (median, 78 months) in Japanese FAB-RA patients who could be classified according to the WHO classification 2008. Follow-up periods in German patients ranged from 0 to 313 months (median, 23 months). During the follow-up period, 9 Japanese patients and 27 German patients progressed to acute myeloid leukemia (AML). Forty Japanese patients (9 AML, 15 infection, 7 bleeding, 1 heart failure, 2 others (non-hematological causes), 6 unknown) and 81 German patients (24 AML, 16 infection, 7 bleeding, 2 heart failure, 5 others (non-hematological cause), 27 unknown) died.

For the OS, Japanese FAB-RA patients who could be classified according to the WHO classification 2008 had a more favorable prognosis than German FAB-RA patients (OS median survival: Japan, 117 months; Germany, 55 months; $p < 0.001$). In LFS, Japanese FAB-RA patients who could be classified according to the WHO classification 2008 had a more favorable prognosis than German FAB-RA patients (10% LFS: Japan, 74 months; Germany, 14 months; $p = 0.011$) (Fig. 1A). RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding rare subtypes (Japan, 5q -syndrome subgroup; Germany, MDS-U (RCUD/pancytopenia type) subgroup) in both countries (Fig. 1B and C). The OS of RCUD patients was not significantly different between the two countries (OS median survival: Japan, 202 months; Germany, 141 months; $p = 0.230$). Japanese RCUD patients tended to show a more favorable LFS than German RCUD patients (LFS median survival: Japan, more than 292 months; Germany, 27 months; $p = 0.068$) (Fig. 2A). Japanese RCMD patients showed a more favorable OS than German RCMD patients (OS median survival: Japan, 109 months; Germany, 36 months; $p = 0.026$). The LFS of RCMD patients was not significantly different between the two countries (10% LFS: Japan, 38 months; Germany, 10 months; $p = 0.391$) (Fig. 2B). Follow-up periods ranged from 1 to 282 months (median, 114 months) in Japanese MDS-U (pancytopenia type) patients. In contrast, follow-up periods ranged from 15 to 46 months (median, 31 months) in German MDS-U (RCUD/pancytopenia type) patients. In addition, there were only 6 German MDS-U (RCUD/pancytopenia type) patients. Because of the short follow-up periods and the small number of German patients, the comparison of OS and LFS between the two countries was not adequate in the MDS-U (RCUD/pancytopenia type) subgroup. For the same reasons as for the MDS-U (RCUD/pancytopenia type) subtype, the comparison of OS and LFS between the two countries was not adequate in the 5q- syndrome subtype.

4. Discussion

There was no centralized pathology review in this study. However, we previously reported that morphologic diagnosis between the German and Japanese hematologists was in line [17]. Morphologic diagnosis of this study was performed by the same Japanese and German hematologists. Therefore, we believe that there may be extremely little differences between the interpretations of pathologists in Germany versus Japan.

Concerning the frequencies of subtypes of the WHO classification 2008, Japanese FAB-RA patients differed from German patients. The frequency of RCUD in Japanese FAB-RA patients was higher than in German patients. The frequency of RCMD in Japanese FAB-RA patients was lower than in German patients. The frequency of RT of Japanese FAB-RA patients was higher than that of German patients. The frequency of 5q- syndrome in Japanese FAB-RA patients was lower than in German patients. Morel et al. [21] and Greenberg et al. [10] reported that the frequencies of isolated del(5q) in patients with all MDS subtypes were 4.7% and 5.9%, respectively. Several reports have already indicated that MDS with isolated del(5q) is rare in Japanese patients. Toyama et al. [5] and Matsushima et al. [6] (Toyama

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et al., 2.0%; Matsushima et al., 1.5%) reported that Japanese MDS patients had a lower frequency of isolated del(5q) than patients in Western reports. Most interestingly, the frequency of MDS-U (RCUD/pancytopenia type) in Japanese FAB-RA patients was significantly higher than in German FAB-RA patients. It is suggested here that the frequencies of each MDS subtype cannot be solely judged by the results of the present study. However, in the previous consecutive dataset [17] of the present study including the patients classified according to the WHO classification 2008, the frequency of Japanese FAB-RA patients with pancytopenia (35.1%) was significantly higher than in German patients (13.1%) ($p < 0.001$). Therefore, it is very likely that the frequency of the MDS-U (RCUD/pancytopenia type) subtype in Japanese patients is higher than that in German patients. We believe that the different frequencies of RCUD and MDS-U (RCUD/pancytopenia type) between two countries are noticeable and important for discussing the differences in clinical features between these two countries.

Japanese FAB-RA patients were younger than German FAB-RA patients in our previous study [17]. In contrast, the age of Japanese patients was not significantly different from that of German patients in the RCUD, MDS-U and RCMD subgroups in the present study. However, the comparison of age in the present study is problematic. Cytogenetic findings are necessary for a diagnosis according to the WHO classification 2008. Therefore, patients in the previous data set without available cytogenetic data were excluded from the present study. In German patients with advanced age, the frequency of patients where cytogenetic examinations were performed was low. In German patients, the age of patients without available cytogenetic data (median, 74 years) was significantly higher than in patients with available cytogenetic data (median, 63 years) ($p < 0.001$). In contrast, the age of Japanese patients without available cytogenetic data (median, 60 years) was not significantly different from Japanese patients with available cytogenetic data (median, 56 years) ($p = 0.542$). The age of German patients without available cytogenetic data (median, 74 years) was significantly higher than that of Japanese patients without available cytogenetic data (median, 60 years) ($p < 0.001$). Therefore, it was considered that the age of German patients in the present study was not representative. MDS-U (RCUD/pancytopenia type) patients (median, 51 years) tended to be younger than FAB-RA patients excluding the MDS-U (RCUD/pancytopenia type) subtype (median, 58 years) in Japanese patients. The German MDS-U (RCUD/pancytopenia type) patients also tended to be younger than other subtypes.

We previously reported that Japanese FAB-RA patients showed more severe cytopenia(s) [17]. The MDS-U (RCUD/pancytopenia type) subtype showed more severe cytopenia(s) in the present study. The frequency of MDS-U (RCUD/pancytopenia type) in Japanese patients was higher than that in German patients. The high frequency of the MDS-U (RCUD/pancytopenia type) subtype in Japanese patients may largely influence the unique characteristics (younger age and more severe cytopenia(s)) of the Japanese FAB-RA patients that were clarified by our previous report [17].

We reported that the frequency of cytogenetic abnormalities in Japanese FAB-RA patients were lower than in German patients in previous study [17]. The cause of this finding was the low frequency of 5q- syndrome in Japanese FAB-RA patients.

We reported that Japanese FAB-RA patients presented with a favorable overall OS and LFS in previous study [17]. The OS and LFS of Japanese and German FAB-RA patients who could be classified according to the WHO classification 2008 in the present study were similar to our previous report. Several guidelines [22–24] have been published in Western countries. To adapt these Western guidelines to Asian patients, some modifications may be required, taking into account ethnic differences. Nevertheless, no difference was found in LFS between Japanese and German RCMD patients, Japanese RCMD patients showed a more favorable OS than German RCMD patients. It was reported that transfusion dependency was an adverse prognostic factor in MDS patients [3]. Most Japanese patients with Hb concentrations lower than 7.0 g/dL had received red cell transfusion. In contrast, most German patients with Hb concentrations lower than 9.0 g/dL had received red cell transfusion. This difference in threshold for the induction of transfusion between the two countries may influence the different OS between the two countries. The frequency of German patients with Hb concentrations lower than 9.0 g/dL (41%) was higher than that of Japanese RCMD patients with Hb concentrations lower than 7.0 g/dL (28%). In fact, RCMD patients with Hb concentrations lower than 9.0 g/dL tended to show a more unfavorable OS than RCMD patients with Hb concentrations of 9.0 g/dL or more in German patients (OS median survival: Hb lower than 9.0 g/dL, 30 months; Hb at least 9.0 g/dL, 48 months; $p = 0.054$).

Reports of several Eastern countries showed consistently unique characteristics of Eastern MDS, like young age, and a low frequency of RARS and 5q- syndrome [5,8,9,15] and the absence of a prognostic impact of cytopenia [7,8,17], although environmental factors differ between the countries. Therefore, we consider that there are genetic differences between East and West, rather than environmental factors.

In conclusion, the frequency of RCUD and MDS-U (RCUD/pancytopenia type) in Japanese patients was higher than in German patients. In particular, MDS-U (RCUD/pancytopenia type) patients occupied approximately 30% among Japanese FAB-RA patients, but MDS-U was rare (3%) in German patients. Concerning the age at the time of diagnosis, the MDS-U (RCUD/pancytopenia type) subtype was apparently younger than other subgroups in Japanese patients. The cytopenia(s) of the MDS-U (RCUD/pancytopenia type) subtype were more severe than in the RCUD and RCMD subtypes in Japanese patients. RCMD patients showed the less favorable OS and LFS than the other subtypes in both countries. The frequency of RCMD in Japanese patients was lower than that in German patients. We believe that the different frequencies of MDS subtypes according to the WHO classification 2008 between Japanese and German FAB-RA patients underlie the different clinical characteristics of FAB-RA patients between the two countries.

Conflict of interest statement

The authors reported no potential conflict of interest.

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