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VIII. 研究成果の刊行物・別刷

（主なもの）

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.

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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) <1.8×10^9/L, and platelet <100×10^9/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

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

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).
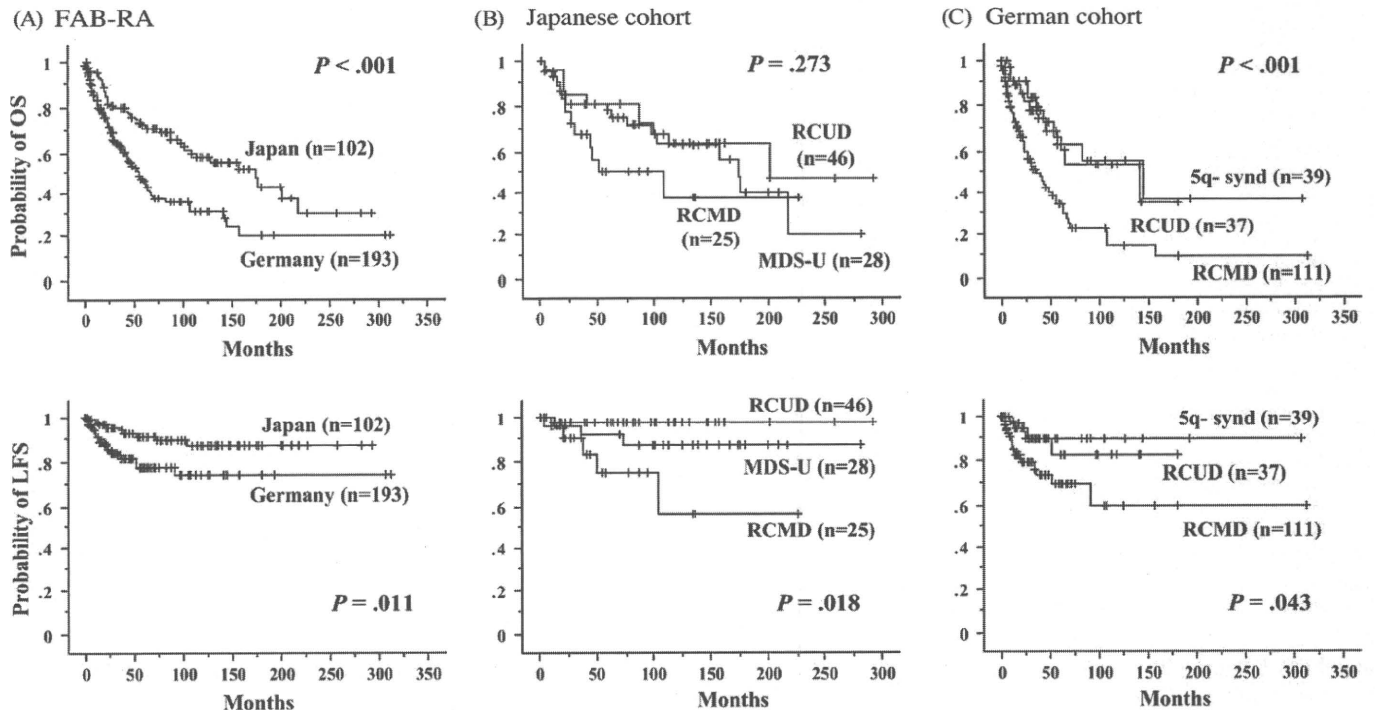

Fig. 1. Cumulative overall survival and leukemia free survival of FAB-RA patients who could be classified according to the WHO classification 2008. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) In FAB-RA patients who could be classified according to the WHO classification 2008, 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, $p = 0.043$).
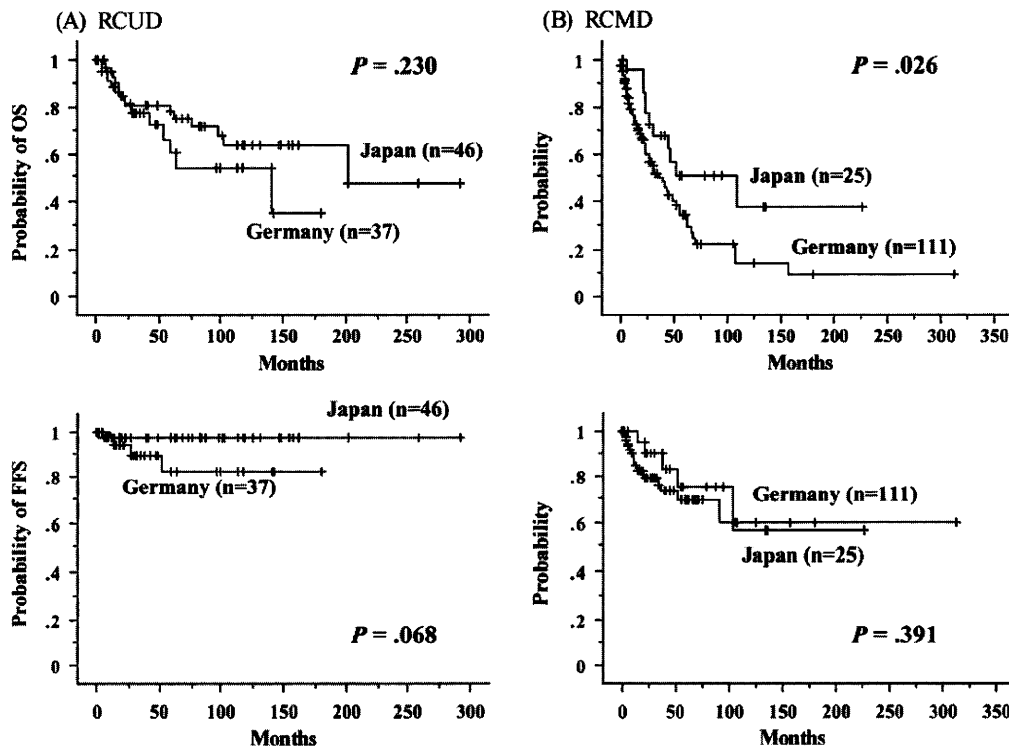
Fig. 2. Comparison of cumulative overall survival and leukemia free survival of RCUD and RCMD between Japanese and German patients. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) The OS of RCUD patients was not significantly different between the two countries (p = 0.230). Japanese 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

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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Low concentration of serum haptoglobin has impact on understanding complex pathophysiology in patients with acquired bone marrow failure syndromes

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Abstract To clarify whether measurement of serum haptoglobin (Hp) has impact on understanding pathophysiology in bone marrow failure (BMF) syndromes, we investigated concentrations of serum Hp by nephelometric procedure in 156 Japanese patients with BMF, including 54 aplastic anemia (AA), 50 paroxysmal nocturnal hemoglobinuria (PNH), and 52 myelodysplastic syndromes (MDS) patients. The frequencies with low concentrations of serum Hp (<42 mg/dL) in PNH patients (98.0%) were significantly higher than those in AA (27.8%; $P < 0.0001$) and MDS (38.5%; $P < 0.0001$) patients. In AA patients, white blood cell (WBC), absolute neutrophil, and platelet counts were significantly decreased in the group (n = 15) with low concentrations of serum Hp than in that (n = 39) with normal concentrations of it, and WBC counts were positively correlated with concentrations of serum Hp, suggesting that WBC counts may affect the concentrations. In MDS patients, hemoglobin concentrations and serum iron were significantly decreased and increased, respectively, in the group (n = 20) with low concentrations of serum Hp than in that (n = 32) with normal concentrations of it, and the values of serum iron were inversely correlated with concentrations of serum Hp, suggesting that ineffective erythropoiesis may affect the concentrations. Several AA and MDS patients with low concentrations of serum Hp

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had Coombs-negative autoimmune hemolytic anemia determined by immunoradiometric assay. In conclusion, several factors in conjunction with pathophysiology contribute to decrease of serum Hp in BMF.

1 Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is included in the acquired bone marrow failure (BMF) syndromes, which are manifested by aplastic or hypoplastic BM and clonal hematopoiesis, with aplastic anemia (AA) and myelodysplastic syndromes (MDS) [1]. Several researchers reported that 15–88.6% of untreated and/or treated AA patients have glycosylphosphatidylinositol (GPI)-deficient hematopoietic cells, as do 10–23% of MDS patients [2]. More recently, some studies by a highly sensitive flow cytometric assay indicated that a minor population of PNH-type cells is an immunologic marker in BMF, and that AA and MDS patients with this population frequently respond to immunosuppressive therapy (IST), including antithymocyte globulin (ATG) and cyclosporine A (CyA) [3, 4]. Then, international-PNH interest group (I-PIG) proposed that PNH is classified into 3 subcategories: classic PNH (subcategory A), PNH in the setting of another specified BM disorder (subcategory B), and subclinical PNH [5]. These subcategories are determined chiefly with clinical symptoms of PNH, such as visible hemoglobinuria and thrombosis, past history of AA and MDS and their hematologic and laboratory findings, and hematologic and laboratory findings of hemolysis, including decrease of serum haptoglobin (Hp).

Hp is a plasma a_2-sialoglycoprotein synthesized primarily by hepatocytes, which binds free hemoglobin through the formation of high-affinity complexes [6], leading to elimination of free hemoglobin by endocytosis and degradation in macrophages [7]. Also, Hp is a positive acute-phase protein and is characterized by a molecular heterogeneity with 3 major phenotypes: Hp 1-1, Hp 2-2, and heterozygous Hp 2-1 [8, 9]. So far, it is known that concentration of serum Hp is influenced according to various disorders and/or pathophysiologies. Decreased concentrations of serum Hp may be observed in disorders associated with hemolytic anemia, including PNH, ineffective erythropoiesis, which is recognized in MDS, liver disease, hereditary ahaptoglobinemia, and with pregnancy and estrogen therapy, whereas increased concentrations of it may be present in any of diseases in which concentrations of acute-phase reactants are increased, such as infections and malignancies [10, 11]. However, it is unclear what the frequencies of BMF syndromes patients with low concentrations of serum Hp are and over what percentages of GPI-deficient erythrocytes in patients with PNH undergo the decreased concentrations of serum Hp.

In the present study, to clarify impact of serum Hp on pathophysiology in patients with BMF, we investigated expressions of GPI-anchored proteins on erythrocytes, granulocytes, and monocytes of peripheral blood (PB) on the cell surfaces by flow cytometry and quantified concentrations of serum Hp by the nephelometric procedure in 156 Japanese patients with acquired BMF syndromes, including 54 AA, 50 PNH, and 52 MDS patients. In addition, we quantified IgG bound to erythrocytes on the cell surfaces by the immunoradiometric assay (IRMA) in 15 AA and 17 MDS patients with low levels of serum Hp.

2 Materials and methods

2.1 Patients

PB samples were taken from 156 Japanese patients with BMF syndromes, including 54 AA, 50 PNH, and 52 MDS patients, after obtaining informed consent and approval from the Institutional Human Research Committee.

The diagnosis and grading of the severity of AA were based on the criteria of the International Agranulocytosis and Aplastic Anemia Study Group [12] and that of Frickhofen et al. [13], respectively. The diagnosis of PNH was made on the basis of the history, clinical and laboratory findings, and the results of CD59 expression on erythrocytes and granulocytes as determined by flow cytometry. A patient with over 1% of CD59⁻ erythrocytes and granulocytes was judged to have PNH erythrocytes and granulocytes, respectively [14, 15]. The subcategory of PNH was determined according to the classification of I-PIG [5]. The diagnosis and phenotypes of MDS were determined from PB cell and BM cell cytomorphology and chromosomal findings after excluding other disorders presenting with pancytopenia according to the French-American-British (FAB) [16] and the World Health Organization (WHO) criteria [17] at diagnosis and/or at the time of examination. The clinical, hematologic, and laboratory findings and treatment, requirements of red blood cell (RBC) transfusion, and past or present complications at the time of examination in our patients are summarized in Tables 1 and 2, respectively.

At diagnosis, the findings of BM aspiration and BM biopsy revealed that 54 of 54 AA patients, 18 of 50 PNH patients, and 17 of 52 MDS patients had hypocellular BM, and only one of 50 PNH patients and 18 of 52 MDS patients showed abnormal karyotypes by chromosomal analysis using BM cells, although chromosomal karyotypes

Table 1 Clinical, laboratory, and hematologic findings at the time of examination in 156 patients with BMF

	AA (n = 54)	PNH (n = 50)	MDS (n = 52)	P*
Age (years)	53.5 ± 18.6	44.5 ± 16.6	64.2 ± 14.8	MDS [AA, \0.01; MDS [PNH, \0.001; AA [PNH, \0.05
Sex (female:male)	30:24	23:27	21:31	n.s.
Duration of illness (months)	80.8 ± 93.3	89.3 ± 84.8	43.5 ± 47.1	AA [MDS, \0.05; PNH [MDS, \0.01
WBC ($\times 10^9$/L)	3.24 ± 1.26	3.74 ± 1.87	4.52 ± 5.79	n.s.
ANC ($\times 10^9$/L)	1.77 ± 1.01	2.34 ± 1.85	2.28 ± 2.70	n.s.
ALC ($\times 10^9$/L)	1.17 ± 0.56	1.02 ± 0.60	1.34 ± 0.66	n.s.
RBC ($\times 10^{12}$/L)	2.84 ± 1.05	2.94 ± 0.87	2.87 ± 0.79	n.s.
Hb (g/L)	95.2 ± 34.2	93.9 ± 28.3	97.6 ± 25.0	n.s.
Reticulocyte count ($\times 10^9$/L)	46.1 ± 24.1	146.2 ± 228.6	60.3 ± 30.2	PNH [AA, \0.005; PNH [MDS, \0.001
Platelet count ($\times 10^9$/L)	86.8 ± 79.1	136.6 ± 83.5	113.8 ± 101.9	PNH [AA, \0.02
MCV (fL)	101.0 ± 10.9	97.3 ± 9.8	102.9 ± 9.4	MDS [PNH, \0.03
CD4/CD8[a]	1.56 ± 1.07	1.44 ± 0.84	1.59 ± 0.92	n.s.
CD59⁻ erythrocytes (%)[b]	0.14 ± 0.18	42.96 ± 32.71	0.10 ± 0.13	PNH [AA, \0.001; PNH [MDS, \0.001
CD59⁻ granulocytes (%)[b]	0.97 ± 3.38	57.12 ± 39.56	1.04 ± 4.52	PNH [AA, \0.001; PNH [MDS, \0.001
CD48⁻ monocytes (%)[b]	1.11 ± 3.75	60.53 ± 37.78	0.49 ± 1.41	PNH [AA, \0.001; PNH [MDS, \0.001
AST (U/L)	23.6 ± 14.3	55.1 ± 44.1	22.9 ± 12.7	PNH [AA, \0.001; PNH [MDS, \0.001
ALT (U/L)	27.7 ± 27.9	20.4 ± 10.4	21.8 ± 20.6	n.s.
LDH (IU/L)	203 ± 56	1069 ± 882	234 ± 85	PNH [AA, \0.001; PNH [MDS, \0.001
Serum iron (lg/dL)	152.3 ± 77.7	91.1 ± 69.2	110.6 ± 57.6	AA [PNH, \0.0001; AA [MDS, \0.01
TIBC (lg/dL)	279.6 ± 61.8	325.4 ± 69.0	287.4 ± 58.5	PNH [AA, \0.005; PNH [MDS, \0.01
Serum ferritin (ng/mL)	1659 ± 5018	141 ± 234	318 ± 534	AA [PNH, \0.03
Creatinine (mg/dL)	1.1 ± 1.8	0.8 ± 0.5	0.8 ± 0.6	n.s.
Occult blood of urine (?:−)	8:46	24:26	6:46	PNH [AA, \0.0005; PNH [MDS, \0.0001

WBC indicates white blood cell count, ANC absolute neutrophil count, ALC absolute lymphocyte count, RBC red blood cell (RBC) count, Hb concentration of hemoglobin, MCV mean corpuscular volume, AST aspartate aminotransferase, ALT alanine aminotransferase, LDH lactate dehydrogenase, TIBC total iron-binding capacity, n.s. not significant

* Statistical significance was examined as described in the Sect. 2. The values of contents, except for sex and occult blood of urine, are expressed as the mean ± SD. The age of MDS patients was significantly higher than that (49.7 ± 18.1 years) of HV (n = 43), but there were no differences in sex ratio among AA patients, PNH patients, MDS patients, and HV (female:male = 19:24)

[a] CD4/CD8 was examined in 40 of 54 AA, 36 of 50 PNH, and 27 of 52 MDS patients

[b] The proportions of CD59⁻ erythrocytes, CD59⁻ granulocytes, and CD48⁻ monocytes, which were determined by the single-color analysis as described in the Sect. 2, from HV were 0.04 ± 0.05% (range 0–0.17%), 0.06 ± 0.06% (range 0–0.22%), and 0.11 ± 0.08% (range 0–0.35%), respectively

in 6 AA and 2 PNH patients were not determined because of lack of mitotic cells. In AA patients, non-severe, severe, and very severe patients were 46.3 or 83.3%, 44.4 or 16.7%, and 9.3 or 0% at diagnosis or at the time of examination, respectively. Thirteen of 50 PNH patients were included into AA-PNH syndrome [18], and 5 of 50 PNH patients had pathophysiology of MDS at diagnosis and/or at the time of examination. According to the criteria of I-PIG, 47 of 50 PNH patients were classified into the subcategories A and B: 18 and 29 patients were included in the subcategories A and B, respectively. However, 3 PNH patients were not classified into any subcategories because they had normocellular or hypercellular BM, although they had pancytopenia, some thin findings of hemolysis in conjunction with lower proportions of GPI-negative erythrocytes, no clinical features of PNH, such as visible

hemoglobinuria and thrombosis, and no past history of AA or MDS. Subsequently, 2 PNH patients frequently received packed RBC transfusions because of continuously severe hemolysis or BMF. Fifty-two MDS patients included 42 refractory anemia (RA), 3 RA with ringed sideroblasts (RARS), one RA with excess of blasts (RAEB), 5 chronic myelomonocytic leukemia (CMML), and one unclassified patients according to the criteria of the FAB and 22 RA, 19 refractory cytopenia with multilineage dysplasia (RCMD), one RARS, two RCMD-RS, one RAEB-1, one MDS unclassified, one 5q- syndrome, and 5 MDS/myeloprolif-erative disorders patients according to those of the WHO at diagnosis and at the time of examination.

As controls, after informed consent PB samples were obtained from 43 Japanese healthy volunteers (HV, female: male = 19:24; mean age, 49.7 years; range 24–85 years),

Table 2 Treatment, requirements of blood transfusion, and past or present complications at the time of examination in 156 patients with BMF

	AA (n = 54)	PNH (n = 50)	MDS (n = 52)
Treatment			
IST (CyA)	23	9	5
Prednisolone	11	19	6
Androgens	18	6	2
Cytokines (Epo or G-GSF)	3	1	0
Ara-C and HU	0	0	1
Warfarin	0	6	0
None or transfusion alone	12	18	38
Requirements of blood transfusion			
Packed RBC	15	16	8
Complications			
?:-	19:35	19:31	20:32
Carcinoma[a]	2	1	7
Gastro-intestinal tract diseases[b]	0	3	3
Cholelithiasis	0	3	0
Type-C chronic hepatitis	1	0	1
Heart-vascular diseases[c]	9	5	6
Renal diseases	3	5	2
Thyroid diseases	1	2	0
Acute infection	0	3	1
Neurological diseases	2	0	0
Collagen disease or autoantibodies	1	0	4[d]
Iron deficiency anemia	2	8	0
Thrombosis	0	2	0
Others	2	3	2

The counts, presented in treatment or complications item, partially include the duplex ones

Epo erythropoietin, G-CSF granulocyte colony-stimulating factor, Ara-C cytosine arabinoside, HU hydroxyurea

[a] Carcinoma in the past history included colon cancer in 2 AA and 2 MDS patients, early gastric cancer in 3 MDS patients, oral cavity carcinoma in one PNH patient, lung cancer in one MDS patient, and prostatic carcinoma in one MDS patient. One MDS patient complicated both colon cancer and early gastric cancer

[b] Gastro-intestinal tract diseases include colon polyp, gastric ulcer, colitis, perforation of intestine, dysphagia, and gastric spasm

[c] Heart-vascular diseases include congenital or acquired heart diseases, hypertension, and diabetes mellitus

[d] Direct Coombs' test in one MDS patient was positive at diagnosis, but it disappeared by treatment of prednisolone until the time of this examination

436 Japanese HV (female:male = 277:159; mean age, 51.6 years; range 18–80 years), or 20 Japanese HV (female:male = 10:10; mean age, 49.9 years; range 24–79 years) for analysis of CD59 expressions on erythrocytes and granulocytes and of CD48 expression on monocytes by flow cytometry, for analysis of concentrations and haplotypes of serum Hp, or for analysis of IgG bound to erythrocytes, respectively.

2.2 CD59 expressions on erythrocytes and granulocytes and CD48 expression on monocytes by flow cytometry

Immunofluorescent staining and flow cytometric analysis of CD59 expression on erythrocytes were performed using a mouse monoclonal antibody to CD59 (3E1; IgG1), as described previously [14, 19]. Immunofluorescent staining and flow cytometric analysis of CD59 expression on granulocytes and of CD48 expression on monocytes were performed using mouse monoclonal antibodies to CD59 labeled with fluorescein isothiocyanate (H19; IgG2a, j; RD PharMingen, San Diego, USA) and CD48 labeled with fluorescein isothiocyanate (14-57; IgG1; Immunotech, Marseille, France), respectively, according to the previous method [20, 21] with a slight modification of the method of Wang et al. [3]. Irrelevant monoclonal antibodies of the same subclasses were used as negative controls [14, 19–21].

2.3 Concentrations and haplotypes of serum Hp

The concentrations of serum Hp in PB from all patients with BMF syndromes and from 436 HV were determined by the nephelometric procedure, developed by Van Lente et al. [22], with some modifications according to the protocol [23] using the Behring Nephelometer II (BN II; Dade Behring Marburg GmbH, Marburg, Germany). An aliquot of 210 I L of solution mixture, including 10 I L of sample diluted with phosphate buffer, 40 I L of N antiserum to human Hp (Dade Behring Marburg GmbH), 80 I L of buffer for sample, and 80 I L of buffer for the reagent, in each patient was applied to the nephelometer. Below 42 mg/dL of serum Hp were evaluated as low concentrations of serum Hp in this study, as described in Sect. 3. The haplotypes of serum Hp were examined by the method of Kirch and Genth [24] using polyacrylamide gel electrophoresis with a slight modification in the patients and 436 HV.

2.4 IgG bound to erythrocytes on the cell surfaces

Quantification of RBC-associated IgG was examined according to the IRMA developed by Jeje et al. [25] with some modifications, as previously described [26, 27], in 15 and 17 patients with AA and MDS, respectively, who showed low levels of serum Hp. Only one of 15 AA and 2 of 17 MDS patients examined were dependent on RBC transfusions at the time of examination. Seventeen MDS patients included one of CMML, 2 of RARS, and 14 of RA according to the criteria of the FAB. Unfortunately, 2 MDS patients were dead due to acute pneumonia before this