

A Prospective Study of Cyclosporine A Treatment of Patients with Low-Risk Myelodysplastic Syndrome: Presence of CD55-CD59⁻ Blood Cells Predicts Platelet Response

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Abstract

Although immunosuppressive therapy using antithymocyte globulin or cyclosporine A (CSA) is effective in selected patients with low-risk myelodysplastic syndrome, the response rates reported so far are inconsistent, and the indication of immunosuppressive therapy for myelodysplastic syndrome has not been clearly defined. We treated 20 myelodysplastic syndrome patients (17 refractory anemia cases [RA], 2 RA with excess blasts, and one RA with ringed sideroblasts) with 4 mg/kg per day of CSA for 24 weeks. Among the 19 patients evaluated, 10 showed hematologic improvement; 8 patients showed an erythroid response, 6 showed a platelet response, and one showed a neutrophil response. Most patients with hematologic improvement continued CSA thereafter, and the progressive response was observed until the latest follow-up (median, 30 months). Most toxicities associated with CSA usage were manageable, and no patient had developed acute leukemia up to this point. Short duration of illness, refractory anemia with minimal dysplasia determined by bone marrow morphology, and the presence of paroxysmal nocturnal hemoglobinuria-type cells were significantly associated with the platelet response. A minority of RA patients who did not possess such predictive variables achieved an isolated erythroid response. In conclusion, CSA may be a therapeutic option for patients with RA who do not have adverse prognostic factors.

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1. Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by peripheral cytopenia, morphological dysplasia, and an elevated likelihood of progression to acute leukemia [1]. The international prognostic scoring system

(IPSS) is the most reliable tool for evaluating the risk of leukemic transformation in individuals [2]. According to IPSS, MDS are divided into 4 groups. Complications of bone marrow failure are more likely to influence survival than leukemic transformation in patients with low and intermediate-1 risk categories [3-5]. Therefore, therapeutic approaches for low and intermediate risk patients are mainly aimed at restoring hematopoiesis.

Several studies documented that erythropoietin with or without granulocyte colony-stimulating factor may improve anemia and reduce the requirement of red cell transfusion in approximately 30% of MDS patients [6,7]. However, the median response duration was short (around 2 years), and the

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use of erythropoietin did not increase survival in a controlled trial. According to recent reports, the use of lenalidomide resulted in hematologic improvement (HI), which was defined by International Working Group (IWG) criteria [8], in 56% of enrolled patients, and the response rate was especially high in patients harboring a clonal interstitial deletion involving chromosome 5q31.1 [9,10]. However, the response rate of lenalidomide for MDS patients without chromosome 5q abnormality appears to be less than 50% [11].

Immunosuppressive therapy raises the blood cell count in some MDS patients. Antithymocyte globulin (ATG) leads to a sustained increase in red blood cell, platelet, and neutrophil production in about one third of patients with low-risk MDS, who are not at increased risk of leukemic transformation. A series of phase II trials demonstrated that lasting transfusion independence is obtained in about one third of patients who also achieved a long survival without added risk of leukemic progression [12-14]. Younger age, shorter duration of illness, diagnosis of French-American-British (FAB) refractory anemia (RA), expression of D-related human leukocyte antigen 15 (HLA-DR15), and the presence of a minor clone with the paroxysmal nocturnal hemoglobinuria (PNH) phenotype have been postulated as pretreatment characteristics correlated with ATG responsiveness [12-16]. Since ATG therapy causes severe adverse events, such as serum sickness, patients must be carefully selected for this modality. Cyclosporine A (CSA) also improves cytopenias in selected MDS patients [17-23]. Previously, we collected the results from individual pilot studies investigating CSA treatment for MDS in Japan, and reported that 30 of 50 patients responded to CSA [24]. These promising results prompted us to perform a prospective trial to evaluate the efficacy and safety of CSA for patients with low-risk MDS.

2. Patients and Methods

2.1. Study Design

In May 2001, we initiated an open-labeled, prospective, multicenter, phase II study to evaluate the efficacy and safety of 24-week oral cyclosporine in patients with low and intermediate-1 risk MDS according to IPSS. The primary endpoint was the rate of HI according to the criteria of IWG. Secondary endpoints were the duration of responses beyond 24 weeks and the rate of adverse events. The study was conducted in complete concordance with the declaration of Helsinki and approved by the ethics committees of the participating institutions. Patients fulfilled all of the inclusion criteria: morphologically proven MDS according to FAB classification; IPSS score of less than 1.5; presence of cytopenia (either hemoglobin value less than 10 g/dL, platelet count less than 100,000/ μ L, or neutrophil count less than 1500/ μ L); age range from 18 to 70 years; Zubrod performance status less than 2; and written informed consent. The exclusion criteria were: presence of clinically significant coexisting medical illness; prior history of malignancy or cytotoxic therapy; prior usage of CSA or ATG; and pregnant or lactating women. In all patients registered, the diagnosis of MDS was re-examined by central morphological evaluation in a blinded fashion by independent reviewers (K.T. and Y.Y.) who were not involved

in the treatment of these patients. In addition, peripheral blood samples were subjected to the following analysis: the detection of PNH-type cells, the genetic typing of HLA-DR molecules, and the analysis of abnormally expanded T-cell clones. These tests were not compulsory, and written informed consent was taken independently.

Registered patients initially received 2 mg/kg of body weight twice per day of CSA (Neoral), which was supplied by Novartis Pharma K.K. (Tokyo, Japan). Thereafter, the dose was adjusted to keep the blood trough value at 150 to 200 ng/mL. The response to treatment, adverse events, and blood trough level of CSA were assessed every 2 weeks. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. In cases with more than grade II nonhematologic toxicity or serum creatinine elevation of 1.5 or higher from the baseline, the treatment of CSA was withheld until the patients recovered. Except for treatment during infectious episodes, the use of or corticosteroid was not permitted. If progressive cytopenia or sign of leukemic transformation developed during the course of CSA treatment, patients were considered nonresponders and were allowed to choose alternative therapy. The daily dose, targeted trough level of CSA, and the appropriate treatment period to evaluate the response were based on our previous retrospective survey.

Bone marrow aspiration was performed before starting CSA and just after 24 weeks of therapy. Patients who met the criteria of HI in any of the 3 hematologic lineages at the 24th week received CSA for an additional 8 weeks to confirm the stability of the response. Patients with a sustained response until the 32nd week were regarded as hematologic responders. The treatment of enrolled patients after the response judgment was not identified. The status of the enrolled patients was monitored every 6 months for 36 months.

2.2. Detection of Minor Populations of PNH-Type Cells

Heparinized peripheral blood was drawn from patients, and minor populations of PNH-type cells were detected by high-resolution 2-color flow cytometry, as described previously [25,26]. Identification of the presence or absence of PNH-type cells was performed by S.N., who did not know the clinical response to CSA at the time of each experiment.

2.3. Analysis of Abnormally Expanded T-Cell Clones

RNA isolated from peripheral blood mononuclear cells was converted to double-stranded complementary DNA, and T-cell receptor (TCR) β chain variable region (V β) repertoires were analyzed with an adaptor ligation polymerase chain reaction (PCR)-based microplate hybridization assay [27]. Then, complementarity-determining region 3 size spectratyping was performed [28]. Using peripheral blood mononuclear cells from 4 healthy donors, we confirmed that a normal spectratype was distributed in a Gaussian fashion with 6 to 10 different size classes at 3 nucleotide intervals, as reported previously [29,30]. We defined the spectratype as skewed if more than one oligoclonal or monoclonal pattern was detected.

2.4. Statistical Analysis

Fisher's exact probability test was used to define predictive parameters for responses at the 24th week. All statistical analyses were performed using Stat View software (version 5.0; SAS Institute, Cary, NC, USA).

3. Results

3.1. Patient Characteristics at Registration

From May 2001 to April 2004, 22 patients were registered for this protocol. Two patients could not be evaluated because careful follow-up revealed coexisting illness negatively affecting hematopoiesis: smoldering multiple myeloma in one patient and chronic alcoholism in the other. The diagnosis of MDS and the eligibility based on criteria were confirmed in the remaining 20 patients. Primary data for the 20 patients are presented in Table 1. The median age was 52 years old, and 11 patients were male. The median duration of illness before CSA treatment was 5 months (range, 1-168 months). At the time of registration, 19 of 20 patients had anemia (hemoglobin less than 10 g/dL), and 10 patients were transfusion-dependent. Thrombocytopenia with platelet counts of less than 100,000/ μ L was seen in 18 patients, and one patient required regular platelet transfusion. Neutropenia of less than 1500/ μ L was observed in 15 patients. Central morphological review identified that 17 patients (85%) had RA according to FAB classification (FAB-RA). Two other patients had RA with excess of blasts (RAEB) and one had RA with ring sideroblasts (RARS). According to a World Health Organization (WHO) classification system, 17 patients with FAB-RA were categorized either with RA (WHO-RA, eight patients) or refractory cytopenia with multilineage dysplasia (RCMD, 9 patients). Patients with RAEB by FAB classification were diagnosed as RAEB-1 and RARS as RCMD-RS by WHO classification. Bone marrow cellularity was normo- or hyper-cellular in 18 patients. A total of 17 patients had a diploid karyotype. Among 8 patients with WHO-RA, 6 patients had persistent unexplained cytopenia with mild morphological abnormalities. For these patients, central reviewers carefully examined not only smear preparations, but also complete blood counts and biochemical data at diagnosis as well as follow-up periods, as recommended by Yoshida et al [31], and finally diagnosed as RA by WHO classification.

3.2. Hematologic Response

One patient was excluded from HI evaluation; this patient (No. 20) suffered from acute cholecystitis and pneumonia at the 11th week of therapy, and, after full recovery from an infectious episode, he refused further CSA treatment. Two more patients did not complete the 24 weeks of CSA treatment because of grade 4 cytopenia (No. 18), and progressively elevated values of peripheral blood Wilm's tumor gene products (No. 19), which is reportedly predictive of evolution into acute leukemia [32]. Both patients received allogeneic bone marrow transplantation from HLA-matched sibling donors. These 2 patients were regarded as nonresponders.

The therapeutic responses are shown in Table 2. Ten patients (53%) showed HI at the 24th week of therapy, according to IWG criteria, and all responses were continuously observed for 8 successive weeks. Improvement in anemia (HI-E) was observed in 8 of 18 anemic patients. Four of 10 patients became transfusion-independent within 32 weeks. The improvement in thrombocytopenia (HI-P) and neutropenia (HI-N) was observed in 6 of 17 thrombocytopenic (35%) and in one out of 14 (7%) neutropenic patients, respectively.

3.3. Adverse Events within 6 Months of CSA Treatment

Adverse events were assessed in the 20 patients available for evaluation (Table 3). The most common adverse events observed were impaired renal function tests, elevated liver enzymes, and hypomagnesemia, the majority of which were categorized as grade 1 toxicities. One patient required temporal cessation of CSA because of elevated serum creatinine values. Over grade 2 toxicities were documented in 4 patients. A patient with acute cholecystitis and pneumonia was described. One patient (No. 17), who showed therapy-unresponsive severe neutropenia (neutrophil count of less than 200/ μ L), developed fatal pneumonia. Progressive anemia and thrombocytopenia were documented in one patient, respectively. No patient demonstrated increased blast counts in the bone marrow examination performed at the 24th week of therapy.

3.4. Variables Associated with Response

We determined the effect of pretreatment parameters on the probability of response to CSA at the 24th week by univariate analysis. Variables compared with response included age, sex, bone marrow cellularity, pretreatment blood cell counts, transfusion dependence, FAB and WHO classifications, karyotypes, IPSS score values, and genetically typed HLA-DR. As the distribution of patients with platelet and erythroid responses was not similar, patients were also individually analyzed. As shown in Table 4, we could not detect any variables predictive of the overall as well as erythroid response. In contrast, 3 variables were significantly associated with the platelet response: disease duration of less than 4 months, the presence of PNH-type cells, and the bone marrow morphology (judged as RA with minimal dysplasia).

3.5. Follow-up

Among the 20 patients, the follow-up of one patient was lost. In addition, 2 patients who received allogeneic stem cell transplantation were not included in the analysis of the long-term outcome. As shown in Table 2, 16 patients are currently alive without disease progression with a median follow-up of 30 months. In 9 responders, 8 patients maintain hematologic responses with the continuous use of CSA. One patient (No. 16) with an isolated erythroid response refused to continue CSA after 32 weeks and lost the response. Retreatment with CSA was not successful. Another patient who stopped CSA therapy (No. 5) also lost the platelet response, which recovered with the resumption of CSA. She was categorized

Table 1.
Characteristics of Patients*

UPN	Age/Gender	Interval, mt	Red Cell Transfusion	Pretreatment Value†		BM Central Review		Cytogenetics	IPSS Score	PNH-Type Cell	HLA-DR15 (DRB1)
				PLT	Neut	Cellularity	FAB/WHO				
1	18/M	1	No	2.4	612	Hypo	RA5/RA	46, XY	0.5	No	Yes (1501)
2	55/F	1	No	1.6	1308	Normo	RA5/RA	46, XX	0.5	Yes	Yes (1502)
3	61/F	1	Dependent	1.2	906	Normo	RA5/RA	46, XX	0.5	Yes	Yes (1502)
4	31/M	1	No	4.6	957	Hyper	RA5/RA	46, XY	0.5	Yes	Yes (1501)
5	52/F	1	No	1.5	1240	Hyper	RA5/RA	46, XX	0.5	Yes	Yes (1501)
6	31/M	14	No	2.2	1492	Hyper	RA5/RA	46, XY	0.5	No	No
7	40/F	12	Dependent	4.1	420	Hyper	RA/RA	46, XX	0.5	Yes	No
8	52/M	8	No	1.8	1717	Hyper	RA/RA	46, XY	0.5	No	No
9	47/F	5	No	6.4	1820	Hyper	RA/RCMD	46, XX	0.5	No	No
10	27/F	3	No	2.3	1930	Hyper	RA/RCMD	46, XX	0	Yes	No
11	44/M	168	Dependent	22.4	2155	Hyper	RA/RCMD	46, XY	0	NT	NT
12	55/F	1	Dependent	3.6	749	Hyper	RA/RCMD	46, XX	0.5	Yes	Yes (1501)
13	67/F	5	Dependent	1.2	687	Normo	RA/RCMD	46, XX	0.5	No	No
14	55/F	12	Dependent	5.1	1237	Hyper	RA/RCMD	46, XX	0.5	No	Yes (1501)
15	64/M	5	No	6.7	765	Hyper	RA/RCMD	47, XY, +Y, add(9)(q13)	1	No	Yes (1501)
16	50/M	12	Dependent	8.1	340	Hypo	RA/RCMD	47, XY +8	1	No	Yes (1501)
17	50/M	14	Dependent	4.5	70	Hyper	RAEB/RAEB-1	46, XY	1	No	No
18	35/M	2	Dependent	13.2	2153	Hyper	RAEB/RAEB-1	46, XY	0.5	No	No
19	52/M	1	Dependent	2.2	1358	Normo	RARS/RCMDRS	46, XY	0.5	No	Res (1502)
20	63/M	2	No	7.4	901	Hyper	RA/RCMD	46, XY, del20q	0.5	Yes	No

*UPN indicates unique patient number; PLT, platelet count ($\times 10^4/\mu\text{L}$); Neut, neutrophil count (μL); BM, bone marrow; FAB, French-American-British classification; WHO, World Health Organization classification; IPSS, International prognostic scoring system for myelodysplastic syndromes; PNH, paroxysmal nocturnal hemoglobinuria; HLA-DR; D-related human leukocyte antigens; RA, refractory anemia; RCMD, refractory cytopenia with multilineage dysplasia; NT, not tested; RAEB, refractory anemia with excess of blasts; RARS, refractory anemia with ringed sideroblasts; RCMD-RS, RCMD with ringed sideroblasts.

†Interval between diagnosis and enrollment.

‡Average of 2 measurements at least 2 weeks apart.

§Patients showing bone marrow morphology as RA with minimal dysplasia.

Table 2.
Outcome of Treatment with Cyclosporine A (CSA)*

UPN	HI at 24th Week (Duration of CSA before Achieving HI)			Therapy after Study Period	Present Status
	HI-E	HI-P	HI-N		
1	Major (8 wks)	Minor (6 wks)	No	Unknown	Lost follow-up at 6 mo
2	Minor† (6 wks)	Minor† (4 wks)	No	CSA	Alive at 30 mo, continuous response
3	Minor† (24 wks)	Minor† (24 wks)	Major (6 wks)	CSA	Alive at 36 mo, continuous response
4	Major (2 wks)	Major (4 wks)	No‡ (18 mo)	CSA	Alive at 36 mo, continuous response
5	No‡ (24 mo)	Minor† (20 wks)	No	CSA	Alive at 36 mo, continuous response
6	No	No	No	Androgen	Alive at 24 mo
7	No‡ (24 mo)	No‡ (10 mo)	No	CSA	Alive at 24 mo, continuous response
8	No	No	—	Observation	Alive at 36 mo
9	No	No	—	Observation	Alive at 36 mo
10	—	Minor (4 wks)	—	CSA	Alive at 36 mo
11	Minor† (22 wks)	—	—	CSA	Alive at 24 mo, continuous response
12	No	No	No	Observation	Alive at 30 mo
13	Major (16 wks)	No‡ (14 mo)	No‡ (14 mo)	CSA	Alive at 24 mo, continuous response
14	Major (18 wks)	No	No	CSA	Alive at 30 mo, continuous response
15	No	No	No	Observation	Alive at 36 mo
16	Major (8 wks)	No	No	mPSL, androgen	Alive at 30 mo, lost response
17	No	No	No	Observation	Died at 7 mo
18	No	—	—	Allo-SCT	WT-1 value elevated
19	No	No	No	Allo-SCT	Grade 4 cytopenia developed
20	NE	NE	NE	Androgen	Cyclosporine withheld due to infection, alive at 18 mo

*UPN indicates unique patient number; HI, hematologic improvement according to the response criteria from the International Working Group; HI-E, erythroid response; HI-P, platelet response, HI-N, neutrophil response; mPSL, methyl-prednisolone; allo-SCT, allogeneic stem cell transplantation.
†Maximal hematologic response turned out to be the most major until the latest follow-up.
‡Hematologic response was obtained until the latest follow-up.

as HI-E as well. Hematologic responses were durable and progressive. Figure 1 shows the kinetics of hematologic increments in patients who showed responses before the latest follow-up. Except for one patient (No. 10), a minor response at the 24th week turned out to be the most major response at the time of the latest follow-up. The patient (No. 10) has had a continued minor response for 36 months. In addition, many responders gained further responses that were not attained by patients treated with CSA for 24 weeks. Until now, 2 erythroid, one platelet, and 2 neutrophil responses have been further documented. In addition, one patient (No. 7), who was judged as a nonresponder at the 24th week, continued to take CSA thereafter, and obtained platelet and erythroid responses at the 10th and 24th months, respectively.

3.6. Analysis for the Presence of Minor T-Cell Clones

To gain insights into the mechanism of CSA-induced hematological responses, the presence or absence of minor T-cell clones was examined. We could examine the pretreatment T-cell repertoires in 13 patients, and samples drawn at the 24th week of CSA therapy were also available in 6 of them. Before CSA treatment, 11 patients showed skewed complementarity-determining region 3 spectratypes, and 2 patients displayed normal patterns. The presence or absence as well as the number of minor T-cell clones did not correlate with therapeutic outcomes (data not shown). As shown in Figure 2, the comparison of TCR-Vβ spectratypes between those obtained at pretreatment and after 24 weeks of CSA demonstrated that abnormally expanded minor T-cell clones

present before CSA therapy persisted with a similar frequency even in CSA responders.

4. Discussion

In this prospective trial, 10 of 19 patients available for evaluation (53%) showed HI within 24 weeks of CSA therapy, and their responses lasted for at least 2 years if CSA was continuously administered. Reports documenting the efficacy of CSA against low-risk MDS are limited. In addition, most reports were single center experiences or a retrospective survey. Only 3 multicenter prospective trials exist, which reported

Table 3.
Adverse Events Observed within 6 Months of Cyclosporine A Treatment

	Grade According to CTC Version 2.0				
	0	1	2	3	4
Cardiovascular	19	1	0	0	0
Infectious	16	0	2	1*	1†
Renal	9	10	1	0	0
Hepatic	8	10	1	1*	0
Gastrointestinal	17	2	1	0	0
Metabolic	8	11	1	0	0
Cutaneous	17	3	0	0	0
Neutropenia	18	0	1	1†	0
Thrombocytopenia	18	1	0	1	0
Anemia	19	0	0	0	1

*†Adverse events developed in the same patients.

Table 4.
Pretreatment Variables Associated with Hematologic Response at 24th Week*

	Overall Response			Erythroid Response			Platelet Response		
	Responder	Nonresponder	P	Responder	Nonresponder	P	Responder	Nonresponder	P
Age, y			.81			.96			1
More than 50	5	4		4			3	5	
50 or younger	5	5		4	6		3	6	
Disease duration			.83			1			.009
4 mo or longer	4	5		4	5		0	8	
Less than 4 mo	6	4		4	5		6	3	
Karyotype			1			1			.51
Normal	9	8		7	9		6	9	
Abnormal	1	1		1	1		0	2	
PNH-type cells			.33			.64			.03
Yes	5	2		3	3		5	2	
No	4	7		4	7		1	9	
HLA DRB1 1501			.33			.35			.64
Yes	5	2		4	3		3	4	
No	4	7		3	7		3	7	
RA with minimal dysplasia			.44			.71			.03
Yes	5	2		4	3		5	2	
No	5	7		4	7		1	9	

*See Table 1 for abbreviations.

exceedingly high CSA response rates. Janasova demonstrated a response rate of 82% (14/17) [17], Dixit showed that 14 of 19 patients (74%) gained HI according to IWG criteria [19], and Chen reported the response rate at 62.5% (20/32) [23]. Although Atoyebi reported unsuccessful outcomes in 6 patients with FAB-RA and RARS [22], inconsistent results are inevitable in small-sized studies, as documented in studies using ATG [12,33]. The response rate of 53% in our study is consistent with previous reports as well as our retrospective survey [24]. Low-risk MDS, especially FAB-RA, is highly diverse in both clinical presentations and pathophysiology. In addition, a recent report from Matsuda demonstrated that the frequency of WHO-RA among FAB-RA is much higher in Japanese than in German patients [34,35]. Thus, comparison of response rates in patients of different ethnic background seems to have limited value. Rather, the predictive variables of the CSA response, which can be universally applicable, should be elucidated and compared between trials. We found that 3 variables: the presence of PNH-type cells, short duration of illness (less than 4 months), and the diagnosis of RA with minimal dysplasia, were significantly associated with the platelet response at the 24th week. In contrast, we could not find any predictive variables for overall and erythroid responses. The predictive value of having HLA-DRB1 1501, which was associated with the CSA response in our retrospective survey, was not confirmed in this study.

To our surprise, hematologic responses were durable and progressive after the 24th week of evaluation. Six additional HI were documented in four patients after the 24th week. RA patients with minimal dysplasia or PNH-type cells gathered into one cohort (RAminiD/PNH cohort) (UPN 1-7, 10, 12, 20) had an elevated probability of multi-lineage responses. HI was obtained in 7 of 9 patients, and 6 of 7 responses were multilineage. In particular, the platelet response was almost restricted in this cohort. Some patients in the other cohort, who showed neither the feature of RA

with minimal dysplasia nor PNH-type cells (No. 8, 9, 11, 13-19), also gained a hematologic response to CSA (hematologic response rate of 4/10). However, most responses were restricted to the erythroid lineage. The significance of the presence of PNH-type cells in the prediction of the response

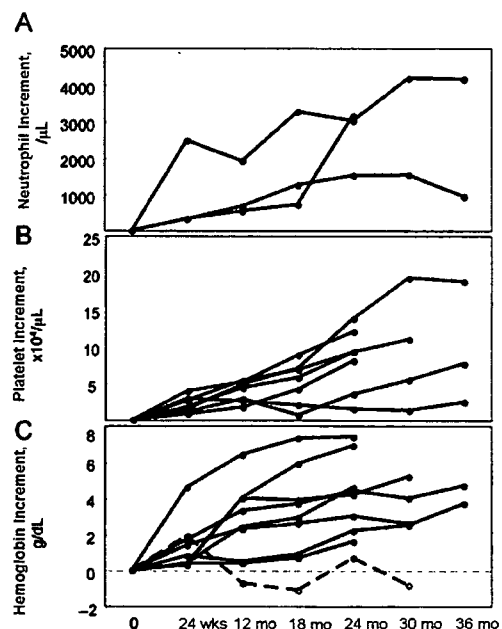


Figure 1. Hematologic increments in patients who showed a response up to the latest follow-up. A, Increments in neutrophil counts from baseline values in three patients who showed neutrophil responses. B, Increments in platelet counts in 7 responders. C, Increments in hemoglobin values in 9 responders. One patient who lost the response after the 12th month is indicated as a broken line.

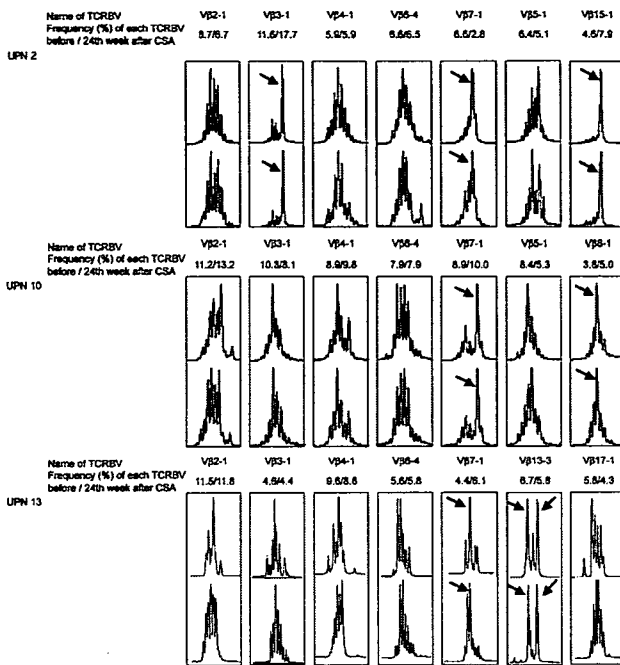


Figure 2. Representative T-cell receptor β chain variable region (TCR-V β) spectratype before and after cyclosporine A (CSA) therapy. The spectratypes listed were the eight frequent V β families; the frequency was defined by microplate hybridization assay. The name of TCR V β , the relative frequency of each V β , and spectratypes are shown. All patients shown here were CSA responders.

to immunosuppressive therapy against aplastic anemia as well as MDS has been reported [15,25,26]. A retrospective survey demonstrated that RA patients harboring PNH-type cells showed less pronounced morphologic abnormality, rare progression into acute leukemia, a higher incidence of HLA-DR15, and higher CSA response rate [26]. The results of this study are in agreement with previous observations.

Moldrem and Kochenderfer showed that MDS patients exhibited a skewed TCR-V β repertoire, indicating the presence of a clonal T-cell population. The clonal population was diminished only in responders to immunosuppressive therapy and ATG [36,37], which is consistent with clinical findings that ATG produces long-lasting HI without additional therapy. In this study, we also detected the presence of abnormally expanded T-cell clones in most MDS patients. However, CSA administration for 24 weeks did not affect expanded T-cell clone frequencies, even in responders. Thus, CSA does not appear to eliminate pathologic T-cells, but inhibits their marrow-suppressive function, thus bringing about a CSA-dependent hematological response, as reported in aplastic anemia [38].

In addition to its promising effects, CSA has a limited toxicity profile and can be safely administered in outpatient clinics. CSA produced less severe adverse events than ATG or lenalidomide, which caused effects defined as over grade 2 toxicities. Stadler reported that the administration of horse- or rabbit-derived ATG produced higher than grade 2 toxicities in 23 of 35 MDS patients [13]. Higher than grade 2

neutropenia (65%) or thrombocytopenia (53%) was documented in a phase I trial of lenalidomide [9]. Most of the adverse events seen in CSA-treated patients were slightly elevated liver enzymes or marginally impaired renal function tests (elevated creatinine or potassium values); dose reduction or interruption of CSA was rarely needed.

In conclusion, the use of CSA was associated with HI in selected patients with FAB-RA without severe adverse events. Patients harboring minor populations of PNH-type cells or showing minimal dysplastic features have an elevated likelihood of recovering from thrombocytopenia and anemia. Erythropoiesis is generally restored in patients who do not have PNH-type cells. As the hematologic response is CSA-dependent, long-term outcomes, including the possibility of accelerating leukemic transformation, must be carefully observed.

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ORIGINAL ARTICLE

Improvement of criteria for refractory cytopenia with multilineage dysplasia according to the WHO classification based on prognostic significance of morphological features in patients with refractory anemia according to the FAB classification

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In the criteria of refractory cytopenia with multilineage dysplasia (RCMD) according to the WHO (World Health Organization) classification, the frequency threshold concerning dysplasia of each lineage was defined as 10%. To predict overall survival (OS) and leukemia-free survival (LFS) for patients with refractory anemia (RA) according to the French-American-British (FAB) classification, we investigated prognostic factors based on the morphological features of 100 Japanese and 87 German FAB-RA patients, excluding 5q-syndrome. In the univariate analysis of all patients, pseudo-Pelger–Huet anomalies $\geq 10\%$ (Pelger+), micromegakaryocytes $\geq 10\%$ (mMgk+), dysgranulopoiesis (dys G) $\geq 10\%$ and dysmegakaryopoiesis (dys Mgk) $\geq 40\%$ were unfavorable prognostic factors for OS and LFS (OS; $P < 0.001$, LFS; $P < 0.001$). The prognostic effects of the morphological features were similar in both Japanese and German patients. However, dys Mgk $\geq 10\%$ was not correlated with OS and LFS. In the multivariate analysis, mMgk+ and dys Mgk $\geq 40\%$ were adverse prognostic factors for OS for all patients, and dys G $\geq 10\%$ and dys Mgk $\geq 40\%$ were adverse prognostic factors for LFS for all patients. On the basis of the present analysis, we propose the following modified morphological criteria for RCMD. Modified RCMD should be defined as FAB-RA, excluding 5q-syndrome with dys G $\geq 10\%$, dys Mgk $\geq 40\%$ or mMgk+.

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Keywords: myelodysplastic syndromes; refractory anemia; refractory cytopenia with multilineage dysplasia; WHO classification; prognosis

Introduction

Myelodysplastic syndromes (MDSs) 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 MDSs are very heterogeneous in terms of their morphology, clinical features and survival.³ Refractory anemia (RA) according to the French-American-British (FAB) classification is generally classified as a low-risk group.⁴ The International Prognostic Scoring System (IPSS) was

reported to be useful for assessing prognosis in MDS patients according to the FAB classification.⁵ According to the WHO (World Health Organization) classification,⁶ most FAB-RA patients are re-classified into refractory cytopenia with multilineage dysplasia (RCMD) or WHO-RA. It was reported that RCMD patients showed a more unfavorable prognosis than WHO-RA patients.^{7–9} The criteria for RCMD include a uniform threshold of 10% for dysplasia in each lineage. However, the impact of this threshold on prognosis has not been fully assessed. Concerning the individual forms of dysplasia, we have previously reported that pseudo-Pelger–Huet anomalies (Pelger) and micromegakaryocytes (mMgk) were significantly correlated with overall survival (OS) and leukemia-free survival (LFS) in FAB-RA patients.^{10,11} Here, we report the impact of the threshold for dysplasia in each lineage and the individual dysplasias on the prognosis of FAB-RA patients.

Patients and methods

Patients

A total of 200 patients (Japan, 100 cases; Germany, 100 cases) with a diagnosis of primary FAB-RA were selected randomly. Patients who had previously been treated with antineoplastic drugs or ionizing radiation were excluded from the analysis. Japanese patients were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital or affiliated hospitals in Japan between April 1976 and January 2002. German patients were diagnosed at the Department of Hematology, Oncology and Clinical Immunology of the Heinrich-Heine University in Germany between January 1973 and December 2002. Thirteen FAB-RA patients with isolated del(5q) (5q-syndrome; all were German patients) were excluded from the analysis. This retrospective analysis was performed in 100 Japanese and 87 German FAB-RA patients. Age, sex and cytogenetic findings of the patients at the diagnosis are summarized in Table 1.

Morphological study

Microscopical examinations were performed using standard methods (bone marrow (BM) Wright-Giemsa (WG) or May-Giemsa (MG), Prussian blue and periodic acid-Schiff stained films and peripheral blood (PB) WG or MG stained films). PB and BM differential counts were performed on 100 and 500 cells, respectively. Evaluations of the BM cellularity and number

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Table 1 Results of morphological analysis and univariate analysis of OS and LFS in patients with FAB-RA, excluding 5q-syndrome

Variable	No. of Patients	Percentile of OS			Percentile of LFS			
		(months)			(months)			
		75%	50%	P-value	90%	75%	50%	P-value
All patients (n = 187)								
<i>Age (years)</i>								
Older than 60	96	21	59	<0.001	23	104	NR	0.053
60 or younger	91	68	202		53	NR	NR	
<i>Sex</i>								
Male	103	23	102	0.230	22	NR	NR	0.311
Female	84	44	NR		51	NR	NR	
<i>Neutrophils</i>								
<i>Dys G</i>								
≥10%	41	20	33	<0.001	10	25	52	<0.001
<10%	122	54	175		104	NR	NR	
<i>Pelger</i>								
Positive*	26	16	29	<0.001	13	25	36	<0.001
Negative	153	31	158		52	NR	NR	
<i>Dys G/Pelger</i>								
Dys G ≥10% with Pelger positive*	26	16	29	0.114	13	25	36	0.455
Dys G ≥10% without Pelger positive*	15	20	52		10	28	NR	
<i>Megakaryocytes</i>								
<i>Number of Mgc</i>								
Normo/increased	154	23	109	0.083	25	NR	NR	0.046
Decreased	29	88	NR		NR	NR	NR	
<i>Dys Mgc</i>								
≥10%	129	23	98	0.254	23	104	NR	0.101
<10%	25	23	176		NR	NR	NR	
<i>Dys Mgc</i>								
≥40%	75	20	42	<0.001	14	36	NR	<0.001
<40%	79	88	217		NR	NR	NR	
<i>mMgc</i>								
Positive**	25	12	23	<0.001	13	25	51	<0.001
Negative	158	44	158		38	NR	NR	
<i>Dys Mgc/mMgc</i>								
Dys Mgc ≥40% with mMgc positive**	24	12	23	<0.001	13	22	51	0.034
Dys Mgc ≥40% without mMgc positive**	51	26	76		20	38	NR	
<i>Chromosome (IPSS)</i>								
Good	129	52	158	<0.001	74	NR	NR	<0.001
Int	35	20	NR		14	NR	NR	
Poor	23	7	27		4	22	31	
Japanese patients (n = 100)								
<i>Age (years)</i>								
Older than 60	42	20	44	<0.001	14	51	NR	0.005
60 or younger	58	56	157		NR	NR	NR	
<i>Sex</i>								
Male	53	29	176	0.802	25	NR	NR	0.702
Female	47	52	175		51	NR	NR	
<i>Neutrophils</i>								
<i>Dys G</i>								
≥10%	17	22	29	<0.001	11	25	38	<0.001
<10%	79	88	176		104	NR	NR	
<i>Pelger</i>								
Positive*	12	27	31	0.003	22	25	38	0.010
Negative	87	52	176		74	NR	NR	
<i>Dys G/Pelger</i>								
Dys G ≥10% with Pelger positive*	12	27	31	0.724	22	25	38	0.481
Dys G ≥10% without Pelger positive*	5	5	20		4	4	NR	

Table 1 Continued

Variable	No. of Patients	Percentile of OS			Percentile of LFS			
		(months)		P-value	(months)			P-value
		75%	50%		90%	75%	50%	
Megakaryocytes								
Number of Mgc								
Normo/increased	81	31	175	0.411	37	NR	NR	0.234
Decreased	19	88	NR		NR	NR	NR	
Dys Mgc								
$\geq 10\%$	69	29	157	0.439	25	NR	NR	Not available
$< 10\%$	12	59	176		NR	NR	NR	
Dys Mgc								
$\geq 40\%$	38	23	52	0.001	22	38	NR	0.003
$< 40\%$	43	176	217		NR	NR	NR	
mMgc								
Positive**	12	22	23	< 0.001	14	25	51	< 0.001
Negative	88	62	176		74	NR	NR	
Dys Mgc/mMgc								
Dys Mgc $\geq 40\%$ with mMgc positive**	12	22	23	< 0.001	14	25	51	0.019
Dys Mgc $\geq 40\%$ without mMgc positive**	26	42	106		37	NR	NR	
Chromosome (IPSS)								
Good	76	62	175	0.076	104	NR	NR	< 0.001
Int	15	19	NR		NR	NR	NR	
Poor	9	29	38		4	25	37	
German patients (n = 87)								
Age, y								
Older than 60	54	23	108	0.921	28	NR	NR	0.712
60 or younger	33	16	68		13	NR	NR	
Sex								
Male	50	20	54	0.137	20	NR	NR	0.308
Female	37	29	158		36	NR	NR	
Neutrophils								
Dys G								
$\geq 10\%$	24	16	36	0.040	10	20	52	< 0.001
$< 10\%$	43	26	136		53	NR	NR	
Pelger								
Positive*	14	12	20	0.012	5	13	31	< 0.001
Negative	66	23	68		52	NR	NR	
Dys G/Pelger								
Dys G $\geq 10\%$ with Pelger positive*	14	12	20	0.072	5	13	31	0.177
Dys G $\geq 10\%$ without Pelger positive*	10	43	65		10	52	NR	
Megakaryocytes								
Number of Mgc								
Normal/increased	73	16	54	0.159	23	53	NR	Not available
Decreased	10	44	108		NR	NR	NR	
Dys Mgc								
$\geq 10\%$	60	16	52	0.412	20	52	NR	0.419
$< 10\%$	13	22	NR		NR	NR	NR	
Dys Mgc								
$\geq 40\%$	37	12	29	0.001	13	28	53	0.001
$< 40\%$	36	65	NR		NR	NR	NR	
mMgc								
Positive**	13	10	16	< 0.001	13	31	31	0.044
Negative	70	26	136		28	NR	NR	
Dys Mgc/mMgc								
Dys Mgc $\geq 40\%$ with mMgc positive**	12	9	16	0.019	5	13	31	0.489
Dys Mgc $\geq 40\%$ without mMgc positive**	25	20	43		20	28	53	
Chromosome (IPSS)								
Good	53	44	136	< 0.001	52	NR	NR	< 0.001
Int	20	26	65		13	NR	NR	
Poor	14	5	9		5	5	31	

Abbreviations: FAB, French-American-British; IPSS, International Prognostic Scoring System; LFS, leukemia-free survival; OS, overall survival; RA, refractory anemia.

Pelger positive*: the presence of 10% or more Pelger among 200 mature neutrophils.

mMgc positive**: the presence of 10% or more mMgc among 25 or more megakaryocytes.

of megakaryocytes were performed using the specimens of BM trephine biopsy and/or clot section.

We held two meetings on BM morphology at the Heinrich-Heine University, as reported previously.¹² At the first joint review, we mainly discussed the evaluation of dysplasia and diagnosis using the training slides. After the first joint review, the Japanese and German groups evaluated the detailed morphological analysis separately in each country. After this separate review, the second joint review meeting for morphological consensus was performed. The observers were blinded to the clinical and laboratory data, including cytogenetics, until finishing this joint review meeting for morphological consensus.

In the present study, we limited dysplasias to only dysplasias described in the WHO classification⁶ as follows. Dysplasias of the nucleus in erythroid lineage cells were defined as budding, bridging, internuclear, karyorrhexis, multinuclearity or megakaryoblastoid change. Dysplasias of the cytoplasm in erythroid lineage cells were defined as ringed sideroblasts, vacuolization or PAS positivity (diffuse or granular). Concerning granulocytes, dysplasias were defined as small size, nuclear hypo-segmented mature neutrophils, hypersegmentation, hypogranularity or pseudo-Chediak-Higashi granules. Dysplasias of megakaryocytes were defined as micromegakaryocytes, non-lobulated nuclei or multiple widely separated nuclei. A morphological study was performed in detail. A minimum of 25 megakaryocytes, 200 erythroblasts and 200 neutrophils in BM were examined in each patient. The cutoff levels for dyserythropoiesis (dys E) and dysgranulopoiesis (dys G) were defined as 10% according to the WHO classification. Dysmegakaryopoiesis (dys M_{gk}) was evaluated with two cutoff levels, 10% according to the WHO classification or 40% according to data previously reported from the German group.^{7,13} Patients with decreased megakaryocytes were excluded from the evaluation of dys M_{gk}. Two distinct dysplastic changes, Pelger and mM_{gk}, were also evaluated. We defined hypo-segmented mature neutrophils with strikingly clumpy chromatin as 'Pelger', and mono- or binucleated megakaryocytes with a size equal to or smaller than promyelocytes as 'mM_{gk}'. Positivity for Pelger (Pelger+) was defined as the presence of 10% or more Pelger among 200 mature neutrophils. Positivity for mM_{gk} (mM_{gk}+) was defined as the presence of 10% or more mM_{gk} among 25 or more megakaryocytes. Patients with decreased megakaryocytes were judged to be negative for mM_{gk} (mM_{gk}-). The final morphological evaluation was based on the consensus among the Japanese and German groups by joint review.

Cytogenetics

Cytogenetic analysis was 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.

Statistical analysis

Patients were followed from the date of diagnosis until June 2004 for the Japanese and July 2003 for the German patients. Prognosis was evaluated by OS and LFS. OS was measured from the date of diagnosis until death owing to any cause, until 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. Univariate analysis of sex, age category, each morphological parameter and cytogenetic subgroups according to IPSS on prognosis was evaluated with cumulative probabilities using the Kaplan–Meier method and

compared using a log-rank test. Multivariate analysis was performed with several significant parameters from univariate analysis. The interaction between parameters was also examined. The effects of parameters were evaluated as hazard ratios and their 95% confidence intervals. Continuous data were compared using the nonparametric Mann–Whitney test, and proportions were compared using the χ^2 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, USA) or SAS software (version 8.2, SAS Institute).

Results

Morphological analysis

In the 187 cases reviewed, we evaluated suitable marrow preparations for the detailed assessments of myelodysplasia. The results of morphological analysis are summarized in Table 1. All patients showed dys E \geq 10%. Some marrow preparations could not be examined in detail. Especially concerning the frequency of dys G, 24 cases could not be evaluated, because the observation of granules of neutrophils was difficult owing to the poor staining condition of the films. Most of the patients with mM_{gk}+ had dys M_{gk} \geq 40%. Of the patients with dys M_{gk} < 40%, only one patient had mM_{gk}+. However, even with this patient it was judged that the frequency of dys M_{gk} was 36%.

Univariate analysis of the effects of each parameter on OS and LFS

Follow-up periods ranged from 1 to 292 months (median 43 months). During the follow-up period, 79 patients died and 24 patients transformed to acute leukemia. Japanese FAB-RA patients aged 60 years or less had a more favorable prognosis than German FAB-RA patients aged 60 years or less in OS (*P* = 0.001). Table 1 shows the univariate analysis of the effects of each parameter on OS and LFS. In the analysis of all 187 patients, Pelger+, mM_{gk}+, dys G \geq 10% and dys M_{gk} \geq 40% were significant adverse prognostic factors for OS and LFS. In a separate analysis for each country, there was no prognostic difference regarding Pelger+, mM_{gk}+, dys G \geq 10% or dys M_{gk} \geq 40% between the Japanese and German patients. When cases with dys G \geq 10% were divided into dys G \geq 10% with or without Pelger+, the prognosis of dys G \geq 10% with Pelger+ was not significantly different from that of dys G \geq 10% without Pelger+ on OS and LFS in all patients and in the patients of each separate country. When cases with dys M_{gk} \geq 40% were divided into dys M_{gk} \geq 40% with or without mM_{gk}+, the effect on the prognosis of dys M_{gk} \geq 40% with mM_{gk}+ was greater than that of dys M_{gk} \geq 40% without mM_{gk}+ on OS and LFS in all patients. In a separate analysis for each country, the prognosis of patients showing dys M_{gk} \geq 40% with mM_{gk}+ was worse than that of patients with dys M_{gk} \geq 40% without mM_{gk}+ regarding OS in patients of both countries and LFS in Japanese patients. Cytogenetic subgroups according to IPSS significantly affected OS and LFS in all patients. In a separate analysis for each country, they significantly affected OS in German patients and LFS in patients of both countries. Age > 60 years significantly affected OS in all patients. In a separate analysis for each country, age > 60 years significantly affected OS and LFS in only Japanese patients.

Table 2 Multivariate Cox hazard analysis of parameters for overall and leukemia-free survival in patients with FAB-RA, excluding 5q-syndrome

Characteristic	OS			LFS		
	Model A HR (95% CI)	Model B HR (95%CI)	Model C HR (95% CI)	Model A HR (95% CI)	Model B HR (95% CI)	Model C HR (95% CI)
<i>All patients</i>	<i>n</i> = 136	<i>n</i> = 136	<i>n</i> = 136	<i>n</i> = 136	<i>n</i> = 136	<i>n</i> = 136
Country, German (vs Japan)	1.1 (0.6–2.0)	1.3 (0.8–2.3)	1.1 (0.6–2.0)	1.1 (0.4–3.0)	1.2 (0.5–3.1)	0.9 (0.4–2.3)
Age, older than 60 years (vs 60 years or younger)	1.7 (0.9–3.1)	1.8 (1.0–3.1)*	2.1 (1.1–3.7)*	1.6 (0.6–4.0)	1.6 (0.6–3.9)	3.0 (1.1–8.3)*
Sex, male (vs female)	1.6 (0.9–2.7)	1.3 (0.7–2.3)	1.1 (0.6–2.0)	2.4 (0.9–6.2)	2.3 (0.8–6.2)	2.0 (0.7–5.4)
Dys G ≥ 10% (vs < 10%)	2.1 (1.1–3.8)*	1.6 (0.7–3.7)	1.5 (0.6–3.4)	5.3 (2.0–14)*	5.4 (1.5–19)*	6.3 (1.7–23)*
Pelger, positive (vs negative)		1.2 (0.5–3.2)	1.2 (0.5–3.1)		0.8 (0.2–3.0)	0.7 (0.2–2.6)
Dys Mlgk ≥ 40% (vs < 40%)	2.7 (1.5–5.0)*	1.9 (1.0–3.8)*	1.9 (0.9–3.7)	5.6 (1.6–20)*	5.0 (1.3–18)*	3.9 (1.0–15)*
mMgk, positive (vs negative)		3.6 (1.8–7.4)*	3.1 (1.4–6.7)*		2.0 (0.7–6.0)	0.9 (0.2–3.2)
Chromosome (IPSS), low			1			1
Chromosome (IPSS), intermediate			2.4 (1.2–4.9)*			3.1 (0.9–11)
Chromosome (IPSS), poor			2.5 (1.1–5.7)*			10.5 (2.7–40)*
<i>Japanese patients</i>	<i>n</i> = 78	<i>n</i> = 78	<i>n</i> = 78	<i>n</i> = 78	<i>n</i> = 78	<i>n</i> = 78
Age, older than 60 years (vs 60 years or younger)	6.3 (2.7–15)*	5.9 (2.4–14)*	6.1 (2.5–15)*	5.4 (1.5–20)*	5.0 (1.3–20)*	7.3 (1.7–30)*
Sex, male (vs female)	1.1 (0.5–2.3)	1.1 (0.5–2.4)	1.0 (0.5–2.1)	1.5 (0.4–5.0)	1.7 (0.5–6.6)	1.9 (0.4–7.6)
Dys G ≥ 10% (vs < 10%)	3.5 (1.4–8.8)*	4.9 (1.2–19)*	4.6 (1.1–19)*	5.1 (1.2–23)*	11.1 (1.5–82)*	9.6 (1.1–83)*
Pelger, positive (vs negative)		0.5 (0.1–2.1)	0.7 (0.1–3.0)		0.2 (0.1–2.1)	0.2 (0.1–2.3)
Dys Mlgk ≥ 40% (vs < 40%)	2.1 (0.9–4.5)	1.8 (0.8–4.2)	2.1 (0.9–5.2)	4.9 (1.0–25)*	4.2 (0.8–24)	4.4 (0.7–28)
mMgk, positive (vs negative)		1.7 (0.6–5.3)	1.2 (0.4–4.1)		2.1 (0.4–11)	1.0 (0.1–7.6)
Chromosome (IPSS), low			1			1
Chromosome (IPSS), intermediate			2.8 (0.9–8.6)			2.7 (0.2–31)
Chromosome (IPSS), poor			1.4 (0.4–4.8)			6.7 (1.2–37)*
<i>German patients</i>	<i>n</i> = 58	<i>n</i> = 58	<i>n</i> = 58	<i>n</i> = 58	<i>n</i> = 58	<i>n</i> = 58
Age, older than 60 years (vs 60 years or younger)	0.5 (0.2–1.1)	0.7 (0.3–2.0)	1.1 (0.4–3.0)	0.5 (0.1–1.7)	0.3 (0.1–1.6)	3.1 (0.2–53)
Sex, male (vs female)	1.4 (0.6–3.5)	1.3 (0.5–3.2)	1.1 (0.4–2.7)	3.4 (0.8–14)	3.8 (0.8–18)	2.1 (0.4–10)
Dys G ≥ 10% (vs < 10%)	1.9 (0.8–4.5)	1.7 (0.5–5.7)	1.6 (0.5–5.1)	12.8 (2.3–72)*	18.0 (1.8–181)*	28.5 (2.1–380)*
Pelger, positive (vs negative)		1.1 (0.3–4.1)	0.9 (0.2–3.4)		0.8 (0.1–5.0)	0.5 (0.1–3.7)
Dys Mlgk ≥ 40% (vs < 40%)	3.6 (1.3–9.9)*	2.5 (0.8–8.0)	2.0 (0.6–6.2)	7.3 (0.9–60)	10.1 (1.0–105)*	4.4 (0.4–48)
mMgk, positive (vs negative)		2.4 (0.8–7.4)	2.5 (0.8–7.6)		0.5 (0.1–3.4)	0.4 (0.1–3.0)
Chromosome (IPSS), low			1			1
Chromosome (IPSS), intermediate			2.9 (1.0–8.8)*			4.7 (0.7–34)
Chromosome (IPSS), poor			4.4 (1.2–16)*			59.6 (0.9–4165)

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval; IPSS, International Prognostic Scoring System; OS, overall survival; LFS, leukemia-free survival.

Model A included country category, age, sex, dichotomized dysgranulopoiesis and dysmegakaryopoiesis.
 Model B included country category, age, sex, dichotomized dysgranulopoiesis, Pelger, dichotomized dysmegakaryopoiesis and micromegakaryocytes.
 Model C included country category, age, sex, dichotomized dysgranulopoiesis, Pelger, dichotomized dysmegakaryopoiesis, micromegakaryocytes and cytogenetic findings.
 *Statistically significant hazard ratio.

Multivariate analysis of the effects of each parameter on OS and LFS

As a next step, we performed a multivariate analysis based on our results obtained in univariate analysis. Table 2 shows the multivariate analysis of the effects of each parameter on OS and LFS. We analyzed parameters in all patients and in patients of each country separately. Model A included the country, age category, sex, dys G category and dys Mlgk category. Model B included the country, age category, sex, dys G category, Pelger, dys Mlgk category and mMgk. Model C included parameters of model B and cytogenetic findings. In model A, dys G ≥ 10% and dys Mlgk ≥ 40% were significantly associated with an adverse prognosis regarding OS and LFS in all patients. In Japanese patients, dys G ≥ 10% was a significant adverse prognostic factor for OS and LFS, and Mlgk ≥ 40% was a significant adverse prognostic factor for LFS. In German patients, dys G ≥ 10% was

a significant adverse prognostic factor for LFS, and Mlgk ≥ 40% was a significant adverse prognostic factor for OS. In model B, dys Mlgk ≥ 40% and mMgk + were significantly associated with an adverse prognosis regarding OS, and dys G ≥ 10% and dys Mlgk ≥ 40% were significant adverse prognostic factors for LFS in all patients. In Japanese patients, dys G ≥ 10% was a significant adverse prognostic factor for OS and LFS. In German patients, dys G ≥ 10% and dys Mlgk ≥ 40% were significant adverse prognostic factors for LFS. In model C, mMgk + and cytogenetic subgroups were significantly associated with an adverse prognosis regarding OS, and dys G ≥ 10%, dys Mlgk ≥ 40% and cytogenetic subgroups were significant adverse prognostic factors for LFS in all patients. In Japanese patients, dys G ≥ 10% was a significant adverse prognostic factor for OS, and dys G ≥ 10% and cytogenetic subgroups were significant adverse prognostic factors for LFS. In German patients,

cytogenetic subgroups were a significant adverse prognostic factor for OS, and dys G $\geq 10\%$ was a significant adverse prognostic factor for LFS. Age > 60 years was a significant adverse prognostic factor for OS and LFS of all models in Japanese patients. In contrast, age > 60 years was not a significant adverse prognostic factor in German patients.

Proposal for morphological criteria for RCMD

Regarding OS, RCMD patients who were diagnosed using a uniform threshold of 10% for dys G and dys M_{gk} according to the original WHO classification did not show a worse prognosis than WHO-RA patients ($P=0.111$) (Figure 1). This finding indicates that the morphological criteria for RCMD of the original WHO classification may be insufficient for assessing the prognosis. Dys M_{gk} $\geq 10\%$ was not correlated with OS and LFS. However, the frequency of dys M_{gk} was correlated with prognosis in FAB-RA patients, excluding 5q-syndrome. Patients with dys M_{gk} $\geq 70\%$ or dys M_{gk} of 40–70% showed a more unfavorable prognosis than patients with dys M_{gk} of 10–40% or dys M_{gk} $< 10\%$ (OS, $P < 0.001$; LFS, $P < 0.001$). Patients with dys M_{gk} $\geq 70\%$ had a more unfavorable prognosis than patients with dys M_{gk} of 40–70% (OS, $P=0.003$; LFS, $P=0.114$). However, there was no prognostic difference between the

patients with dys M_{gk} of 10–40% and patients with dys M_{gk} $< 10\%$ (OS, $P=0.277$; LFS, $P=0.881$). (Figure 2) Most of the patients with mM_{gk} + had dys M_{gk} $\geq 40\%$. Of the patients with dys M_{gk} $< 40\%$, only one patient had mM_{gk} +. The prognostic effect of mM_{gk} + might relate to the prognostic difference between patients with dys M_{gk} $\geq 40\%$ and patients with dys M_{gk} $< 40\%$. To clarify this point, we compared the OS and LFS between patients showing dys M_{gk} $\geq 40\%$ without mM_{gk} + (dys M_{gk} $\geq 40\%$ /mM_{gk} -) and patients with dys M_{gk} $< 40\%$. However, patients with dys M_{gk} $\geq 40\%$ /mM_{gk} - had a more unfavorable prognosis than patients with dys M_{gk} $< 40\%$ (median survival: dys M_{gk} $\geq 40\%$ /mM_{gk} -, 76 months; dys M_{gk} $< 40\%$, 217 months; $P=0.001$, 10%; LFS: dys M_{gk} $\geq 40\%$ /mM_{gk} -, 20 months; dys M_{gk} $< 40\%$, not reached; 25% LFS: dys M_{gk} $\geq 40\%$ /mM_{gk} -, 38 months; $P < 0.001$). In addition, dys M_{gk} $\geq 40\%$ and mM_{gk} + were independent adverse prognostic factors for OS in the model B of multivariate analysis.

We attempted to modify the original WHO criteria for RCMD. Except for 5q-syndrome, the WHO classification for the MDS category does not include cytogenetic findings. Therefore, we excluded the cytogenetic findings from the parameters for RCMD. Based on uni- and multivariate analyses, we propose modified morphological criteria for RCMD, as shown in the following. FAB-RA patients, excluding 5q-syndrome, are re-classified into RCMD or WHO-RA. Category A is defined as dys E $\geq 10\%$. Categories B1, B2 and B3 are defined as dys G $\geq 10\%$, dys M_{gk} $\geq 40\%$ and mM_{gk} +, respectively. RCMD is diagnosed when category A and any other category B are present. WHO-RA is defined as FAB-RA other than RCMD. Of the 173 present patients who were suitable for a detailed assessment of dysplasia, our FAB patients, excluding 5q-syndrome, were re-classified into 89 modified WHO-RA and 84 modified RCMD patients according to our modified morphological criteria. Frequency of the 'poor risk karyotype' according to IPSS in the modified WHO-RA (5%) was lower than that in the modified RCMD (20%) ($P=0.002$). In contrast, the frequency of the 'good risk karyotype' in the modified WHO-RA (80%) was higher than that in modified RCMD (61%) ($P=0.006$). In the OS, modified RCMD patients were significantly more unfavorable than modified WHO-RA patients (Figure 3a). For patients aged 60 years or less, the OS of the modified RCMD patients was significantly more unfavorable than that of the modified WHO-RA patients. And, for those older than 60 years, the modified

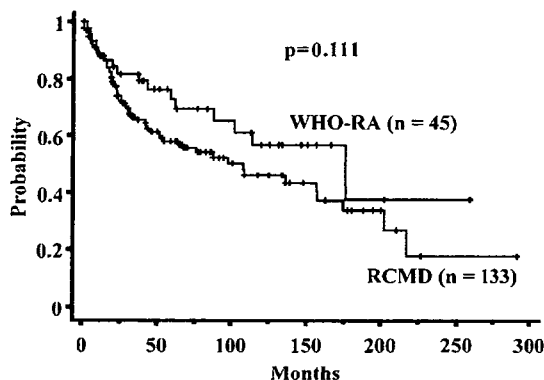


Figure 1 Cumulative overall survival and leukemia-free survival of WHO-RA and RCMD patients according to the original WHO classification. In overall survival, the RCMD patients, according to original WHO classification, did not show a more unfavorable prognosis than the original WHO-RA patients ($P=0.111$).

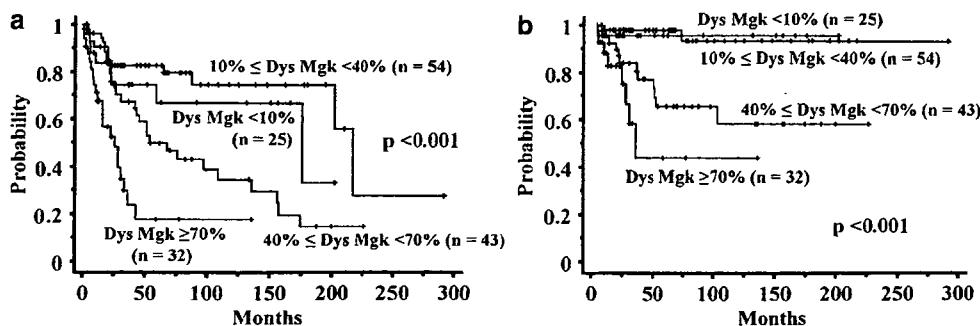


Figure 2 Cumulative overall and leukemia-free survival and frequency of dysmegakaryopoiesis in patients with FAB-RA, excluding 5q-syndrome. (a) Overall survival (OS). (b) Leukemia-free survival (LFS). (a) Patients with dys M_{gk} $\geq 70\%$ or dys M_{gk} of 40–70% showed a more unfavorable OS than patients with dys M_{gk} of 10–40% or dys M_{gk} $< 10\%$ ($P < 0.001$). Patients with dys M_{gk} $\geq 70\%$ had a more unfavorable OS than patients with dys M_{gk} of 40–70% ($P=0.003$). There was no prognostic difference between patients with dys M_{gk} of 10–40% and patients with dys M_{gk} $< 10\%$ ($P=0.277$). (b) Patients with dys M_{gk} $\geq 70\%$ or dys M_{gk} of 40–70% showed a more unfavorable LFS than patients with dys M_{gk} of 10–40% or dys M_{gk} $< 10\%$ ($P < 0.001$). The LFS of patients with dys M_{gk} $\geq 70\%$ tended to be worse than that of patients with dys M_{gk} of 40–70% ($P=0.114$). There was no prognostic difference between patients with dys M_{gk} of 10–40% and patients with dys M_{gk} $< 10\%$ ($P=0.881$).

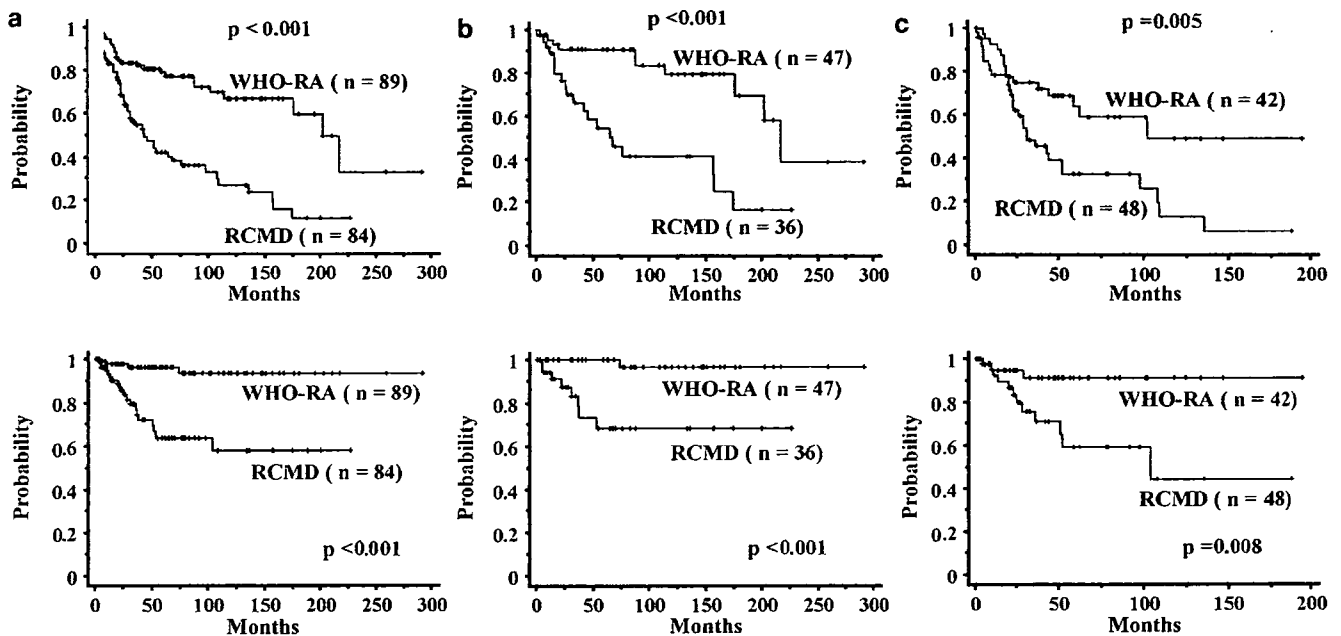


Figure 3 Cumulative overall survival and leukemia-free survival of WHO-RA and RCMD patients according to the modified WHO classification. (Top) Overall survival (OS). (Bottom) Leukemia-free survival (LFS). (a) Among all patients with FAB-RA, excluding 5q-syndrome, the modified RCMD patients had a more unfavorable prognosis than the modified WHO-RA patients (OS, $P < 0.001$; LFS, $P < 0.001$). (b) In patients aged 60 years or younger, the modified RCMD patients had a more unfavorable prognosis than the modified WHO-RA patients (OS, $P < 0.001$; LFS, $P < 0.001$). (c) In patients aged older than 60 years, the modified RCMD patients had a more unfavorable prognosis than the modified WHO-RA patients (OS, $P = 0.005$; LFS, $P = 0.008$).

RCMD patients show a more unfavorable OS than the modified WHO-RA patients (Figure 3b-c). In the LFS, the modified RCMD patients were significantly more unfavorable than the modified WHO-RA patients (Figure 3a). For patients aged 60 years or less, the LFS of modified RCMD patients was significantly more unfavorable than that of the modified WHO-RA patients. For those older than 60 years, the modified RCMD patients show a more unfavorable LFS than the modified WHO-RA patients (Figure 3b-c).

Discussion

The patients in the present study were selected randomly, and the number of patients was smaller than our previous study.¹² However, the clinical features of Japanese patients were different from those of German patients as in the previous study. Japanese patients in present study were significantly younger than German patients (median age: Japan, 56 years; Germany, 62 years; $P = 0.026$). Japanese patients had lower absolute neutrophil counts (median: Japan, $1.39 \times 10^9/l$; Germany, $1.82 \times 10^9/l$; $P = 0.069$), lower hemoglobin concentrations (median: Japan, 8.2 g/dl; Germany, 10.3 g/dl; $P < 0.001$), lower platelet counts (median: Japan, $34 \times 10^9/l$; Germany, $108 \times 10^9/l$; $P < 0.001$), and a lower frequency of cytogenetic abnormalities (Japan, 27%; Germany, 64%; $P < 0.001$) than German patients.

Previous reports from our Japanese and German MDS study group, as well as other investigators, confirmed that WHO-RA patients had a more favorable prognosis than RCMD patients.^{7-9,12} In our previous report,¹² the concordance rate of morphological diagnosis according to the WHO classification between Japanese and German hematologists was 83.8%, and a significant concordance was achieved while using the WHO

classification (κ 0.73, $P < 0.001$). Therefore, we believe that the evaluation of the frequency of dysmyelopoiesis is comparable between Japanese and German hematologists. Moreover, the present final evaluations concerning dysplasia were reached by consensus among the Japanese and German groups by a joint review. We believe that the WHO classification based on morphological features is useful, at least, in our Japanese and German groups, if the morphological features reflect the prognosis. However, the previous study¹² was performed according to the criteria of a prior report from Germany.⁷ The threshold of dys M_{gk} in this report was defined as 40%. In the criteria of RCMD according to the original WHO classification, the threshold of frequency for the degree of dysplasia in each lineage was defined as 10%. Still, the impact of this threshold (10%) in each lineage on prognosis has not been fully assessed. Nosslinger *et al.*¹⁴ reported that WHO-RA patients did not show more favorable prognoses when compared to the RCMD patients. However, the threshold of dysplasia in their report was 50%. This demonstrates that the threshold of dysplasia for RCMD is still controversial. In the present study, all patients showed dys E $\geq 10\%$. Therefore, dys E $\geq 10\%$ did not have a prognostic effect. Dys M_{gk} $\geq 10\%$ was not an unfavorable prognostic factor for OS and LFS. On the other hand, dys G $\geq 10\%$ and dys M_{gk} $\geq 40\%$ were significant adverse prognostic factors correlated with OS and LFS. And, these threshold levels have similar prognostic effects between Japanese and German patients in uni- and multivariate analyses. We reported earlier that Pelger and mM_{gk} were correlated with OS and LFS in Japanese patients.^{10,11} However, when mature neutrophils had two lobes, the definition of 'Pelger' in this previous report was different from that in the present study. In this previous report, we defined mature neutrophils with the two lobes joined by a thin, hair-like bridge ('pince-nez type cells') as 'Pelger'. In contrast, we defined hypo-segmented mature neutrophils with

strikingly clumpy chromatin as 'Pelger' in the present study. Because of this difference of the definition, the frequency of Pelger in the present study was higher than that in this previous report. In the present study, Pelger+ and mMgk+ were significant adverse prognostic factors for OS and LFS. Again, these results were similar between the Japanese and German patients in uni- and multivariate analyses. The results of the present study support our previous results not only in Japanese patients, but also in German patients. Of note, the prognosis of dys G $\geq 10\%$ with Pelger+ was not different from that of dys G $\geq 10\%$ without Pelger+. In contrast, the prognosis of dys Mgk $\geq 40\%$ with mMgk+ was worse than that of dys Mgk $\geq 40\%$ without mMgk+.

We recently compared the clinical features of Japanese and German patients with FAB-RA and found some different prognostic factors, e.g. cytopenias according to IPSS publication were found to be useful for the assessment of prognosis in German FAB-RA patients, but not in Japanese FAB-RA patients.¹² In contrast, the prognostic relevance of the morphological features was similar in Japanese and German FAB-RA patients in the study presented here. However, in the multivariate analyses, there were slight differences between Japanese and German FAB-RA patients. For this reason, we speculate that the prognostic effects of the age category of Japanese patients may have an influence. In the present study, Japanese FAB-RA patients aged 60 years or less had a more favorable prognosis than German FAB-RA patients aged 60 years or less in OS ($P=0.001$) as in our previous study.¹² The degree of dysplasias was more severe in Japanese patients aged older than 60 years than those 60 years or younger. The frequency of RCMD according to our modified criteria was higher in Japanese patients aged older than 60 years than those 60 years or younger (48% vs 35%). Therefore, it seems that morphological features may not be significant independent prognostic factors due to the effects of age category in Japanese patients. In contrast, there were no differences in the frequency of RCMD according to our modified criteria between the German patients aged older than 60 years and those 60 years or younger (60% vs 62%). In addition, because the frequency of poor staining of the films was high in German patients, only a small number of cases could be judged in the morphological study ($n=58$) of German patients. It is expected that the significance as prognostic factors of morphological features becomes certain in multivariate analyses if we can examine more examples even in German patients. Concerning model C including cytogenetics, the number of patients with poor karyotype among 84 RCMD patients according to the modified definition was 17 (20%). In contrast, the number of patients with poor karyotype among 89 WHO-RA patients was only 4 (5%). We thought that the degree of dysplasias was related to the cytogenetic findings. Therefore, in model C, morphological features may not be significant independent prognostic factors.

In univariate analyses, dys G $\geq 10\%$ was correlated with OS and LFS in all patients and in patients from each country. However, dys Mgk $\geq 10\%$ was not correlated with OS and LFS in all patients or in patients from either country. In the present patients, RCMD patients diagnosed by using a uniform threshold of 10% for dys G and dys Mgk according to the original WHO classification did not show a worse prognosis than WHO-RA patients. We think that it may be necessary to revise the morphological definition of RCMD to improve the WHO classification. Therefore, we propose modified morphological criteria for RCMD.

This morphological analysis in MDS patients has several limitations. We held two meetings on BM morphology and

made great efforts to achieve morphological consensus. However, the evaluation of dysplasias might be different among different observers. The number of evaluable cells in the megakaryocytic lineage is smaller than that in other lineages. Therefore, the concordance rate of frequency of dys Mgk among different observers might be lower than that of dys G or dys E. We think that the different morphological interpretation of megakaryocytes among different observers is one of the main causes of the disagreement in the diagnosis of WHO classification. For example, patients of FAB-RA in which three megakaryocytes among 25 megakaryocytes are judged to be dysplastic are classified as RCMD according to the original WHO classification. In contrast, patients of FAB-RA in which only two megakaryocytes among 25 megakaryocytes are judged to be dysplastic are classified as WHO-RA. Therefore, we think that this threshold (10%) of dys Mgk has problems not only for the assessing of the prognosis but also for the diagnosis of RCMD. It was reported that mMgk was specific dysplasias in MDS patients¹⁵ and the concordance rates concerning mMgk was sufficient.¹⁰ We think that the disagreement rate of morphological diagnosis might be decreased by using our modified criteria combining the frequency of dys Mgk and mMgk. The threshold (40%) of dys Mgk of our modified criteria is different from that (10%) of the original WHO criteria. The German group had already shown the usefulness of the WHO classification in several large-scale studies^{7,13} using this threshold (40%). Therefore, we believe that the usefulness of this threshold shown in the present study is certain.

In conclusion, the present results showed that the degree of dysplasia in FAB-RA patients was related to OS and LFS, and the prognostic effect of dysplasia was similar between the Japanese and German FAB-RA patients. However, the thresholds of dysplasia influencing prognosis were different from the original threshold of the WHO classification. We propose to raise the threshold of dys Mgk in the criteria for RCMD from 10 to 40% and add mMgk+.

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ORIGINAL ARTICLE

Total body irradiation and granulocyte colony-stimulating factor-combined high-dose cytarabine as a conditioning regimen in allogeneic hematopoietic stem cell transplantation for advanced myelodysplastic syndrome: a single-institute experience

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In this study, we retrospectively evaluated the efficacy and safety of total body irradiation (TBI) and granulocyte colony-stimulating factor (G-CSF)-combined high-dose cytarabine as a conditioning regimen for allogeneic hematopoietic stem cell transplantation (HSCT) in patients with advanced myelodysplastic syndrome (MDS). We evaluated 22 patients with advanced MDS, including refractory anemia with excess blasts (RAEB; $n = 10$), RAEB in transformation ($n = 2$), acute myelogenous leukemia transformed from MDS ($n = 6$) and chronic myelomonocytic leukemia ($n = 4$). The conditioning regimen consisted of 12 Gy of TBI and high-dose cytarabine (3 g/m^2) every 12 h for 4 days, and the cytarabine was combined with continuous administration of G-CSF. The stem cell sources were bone marrow or peripheral blood stem cells from human leukocyte antigen (HLA)-identical siblings ($n = 12$) and bone marrow from HLA serologically matched unrelated donors ($n = 10$). Three patients experienced disease relapse, two of whom died of disease progression. Of 22 patients, 16 are currently alive and disease-free. The 5-year estimated overall survival, disease-free survival, relapse and non-relapse mortality rates are 76.7, 72.2, 16.6 and 14.1%, respectively. These results suggest that G-CSF-combined high-dose cytarabine could be a promising component of the conditioning regimen of allogeneic HSCT for advanced MDS, providing a low incidence of both relapse and treatment-related mortality.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative therapy for myelodysplastic syndrome (MDS). However, the reported disease-free survival rates of patients undergoing allogeneic HSCT for MDS remains low, ranging between 24 and 56%.^{1–8} The most important cause of treatment failure is disease recurrence, which is reported to range between 23 and 48%.^{1–8} As granulocyte colony-stimulating factor (G-CSF) has been shown to increase the susceptibility of some myeloid leukemia cells to cytarabine *in vitro*,^{9–11} it has been used clinically in combination with cytarabine for refractory acute myelogenous leukemia (AML) or MDS.^{12–15} In our previous report, G-CSF was administered simultaneously with high-dose cytarabine as part of the conditioning regimen for advanced MDS based on these findings, and the 5-year overall survival rate of 14 patients was 75.5% with only one case of disease relapse.¹⁶ The present study, which is a single institute evaluation of more patients, including HSCT recipients from alternative donors, examined the efficacy and safety of a G-CSF-combined high-dose cytarabine regimen for advanced MDS.

Patients and methods

Patients and diagnoses

The present study was a retrospective evaluation of patients with advanced MDS who underwent allogeneic HSCT at Keio University Hospital between May 1995 and

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September 2005 after being conditioned with total body irradiation (TBI) and G-CSF-combined high-dose cytarabine as described below. During this study period, all patients who underwent allogeneic HSCT for advanced MDS were conditioned with this regimen except for patients who underwent allogeneic HSCT from unrelated donors before June 2000. This cohort included four patients discussed in our previous report.¹⁶ The diagnosis of MDS subtypes was based on the French-American-British classification. Advanced MDS was defined as refractory anemia with excess blasts (RAEB), RAEB in transformation (RAEB-t), AML (more than 30% blasts in the bone marrow or peripheral blood) that had evolved from a preceding phase of MDS (AML-MDS) or chronic myelomonocytic leukemia. Patients with therapy-related MDS were excluded.

Conditioning regimen

TBI (12 Gy) was administered in six doses of 2 Gy each, followed by cytarabine at a dose of 3 g/m², which was administered intravenously over 2 h every 12 h for four consecutive days, as previously reported.^{11,16,17} This conditioning regimen was originally used in a pilot study for patients with AML.¹¹ Recombinant human G-CSF (lenograstim) was given by continuous infusion at a daily dose of 5 µg/kg, starting 12 h before the first dose of cytarabine and continuing until the last dose of cytarabine. All patients received steroid eye drops for the prophylaxis of keratoconjunctivitis owing to the cytarabine. No patients received antithymocyte globulin as part of the conditioning regimen.

HSCT procedure and supportive care

Two days after the completion of cytarabine and G-CSF administration, the patients received bone marrow transplantation or peripheral blood stem cell transplantation. The types of donor and human leukocyte antigen (HLA) compatibility were HLA-A, -B or -DR identical sibling, or HLA-A, -B or -DR serologically matched unrelated donor. T-cell depletion of the graft was not performed in any of the patients. For the prophylaxis of graft-versus-host disease (GVHD), the patients received cyclosporine A (CSA: 3 mg/kg i.v.) or tacrolimus (0.03 mg/kg iv) with short-term methotrexate (15 mg/m² on day 1, and 10 mg/m² on days 3 and 6). Recipients of bone marrow from an unrelated donor received additional methotrexate (10 mg/m²) on day 11. Both acute and chronic GVHD were diagnosed and graded on the basis of the published criteria.^{18,19} Each patient was isolated in a laminar air flow room and received antibiotics and antifungal agents orally. The administration of lenograstim at a dose of 5 µg/kg was initiated 1 day after HSCT and continued until neutrophil recovery was achieved. Regimen-related toxicities were assessed and graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Statistical analysis

Overall survival, disease-free survival, relapse and non-relapse mortality curves were calculated by the Kaplan-Meier method.

Results

Patients

The medical records of a total of 22 patients were evaluated. The patient characteristics are shown in Table 1. Of our 22 patients, 10 were diagnosed with RAEB, two with RAEB-t, six with AML-MDS and four with CMML. The number of blasts immediately before transplantation exceeded 5% of bone marrow cells in all but three patients, and exceeded 30% in six patients. Twelve patients received stem cells from an HLA-identical sibling donor (bone marrow in nine cases, peripheral blood cells in three) and 10 patients received bone marrow from a serologically HLA-matched unrelated donor.

Table 1 Patient characteristics (n = 22)

Median age, years (range)	44.5 (18–54)
Gender, male/female	10/12
<i>Disease morphology at diagnosis</i>	
RAEB	10
RAEB-t	2
AML from MDS	6
CMML	4
<i>Number of blasts in the bone marrow or peripheral blood at the time of transplant</i>	
< 5%	3 ^a
5 ≤ < 30%	13
30 ≤ < 50%	2
≥ 50%	4
<i>Marrow fibrosis</i>	
Present	6
Absent	11
Not evaluated	5
<i>Risk of cytogenetic analysis^b</i>	
Good	11
Intermediate	6
Poor	4
Not evaluable	1
<i>IPSS risk category at diagnosis</i>	
Low	0
Intermediate-1	8
Intermediate-2	6
High	7
Not evaluable	1
<i>Stem cell source</i>	
Bone marrow/PBSC from related donor	9/3
Bone marrow from unrelated donor	10
<i>GVHD prophylaxis</i>	
Cyclosporine with methotrexate	10
Tacrolimus with methotrexate	12

Abbreviations: AML = acute myelogenous leukemia; CMML = chronic myelomonocytic leukemia; GVHD = graft-versus-host disease; IPSS = International Prognostic Scoring System; MDS = myelodysplastic syndrome; RAEB = refractory anemia with excess blasts; PBSC = peripheral blood stem cells.

^aTwo patients with RAEB (n = 1) and AML from MDS (n = 1) treated with chemotherapy and a patient with CMML.

^bGood, normal, 5q-, -Y, 20q-; poor, chromosome seven abnormalities and complex abnormalities (>3); intermediate, all other abnormalities.

Regimen-related toxicities and engraftment

Regimen-related toxicities are summarized in Table 2. Toxicities were generally well tolerated. We experienced toxicities of grades 3–4: hepatotoxicity ($n=3$) including one case of fatal hepatic veno-occlusive disease (VOD), mucositis ($n=3$) and conjunctivitis/keratitis ($n=6$).

Of the present 22 patients, 21 achieved neutrophil engraftment (an absolute neutrophil count exceeding $0.5 \times 10^9/l$) between days 14 and 28 after transplantation (median, day 20). The remaining patient died of hepatic VOD and could not be evaluated for engraftment.

Table 2 Regimen-related toxicities

	Grades ^a				
	0	1	2	3	4
Stomatitis	2	3	14	3	0
Diarrhea	3	13	6	0	0
Hepatotoxicity	1	16	2	2	1 ^b
Conjunctivitis/keratitis	10	5	1	6	0
Dermatitis	16	2	4	0	0

^aGrades were evaluated according to the National Cancer Institute Common Toxicity Criteria (ver. 2.0).

^bFatal hepatic veno-occlusive disease.

GVHD

Grades II to IV acute GVHD developed in 14 patients (eight with HLA-identical sibling donors, six with HLA-matched unrelated donors) and chronic GVHD developed in nine of 20 evaluable patients. Acute and chronic GVHD were successfully treated with the addition of glucocorticoids to cyclosporine or tacrolimus in all patients but one, who died of extensive-type chronic GVHD involving the lungs on day 314 after transplantation.

Survival, disease-free survival and relapse

At the time of writing, 17 of 22 patients are alive, and 16 patients remain disease-free between 12 and 138 months after transplantation (median, 60.6 months). Three patients suffered disease relapse on days 53 (RAEB), 518 (AML-MDS) and 373 (RAEB-t). Two of these three patients then underwent a second transplantation, but both again experienced disease relapse after the second transplantation. The causes of death included disease progression ($n=2$), hepatic VOD ($n=1$), bacterial infection ($n=1$) and extensive-type chronic GVHD ($n=1$). The 5-year estimated overall survival, disease-free survival, relapse and non-relapse mortality rates were 76.7, 72.2, 16.0 and 14.1%, respectively (Figure 1).

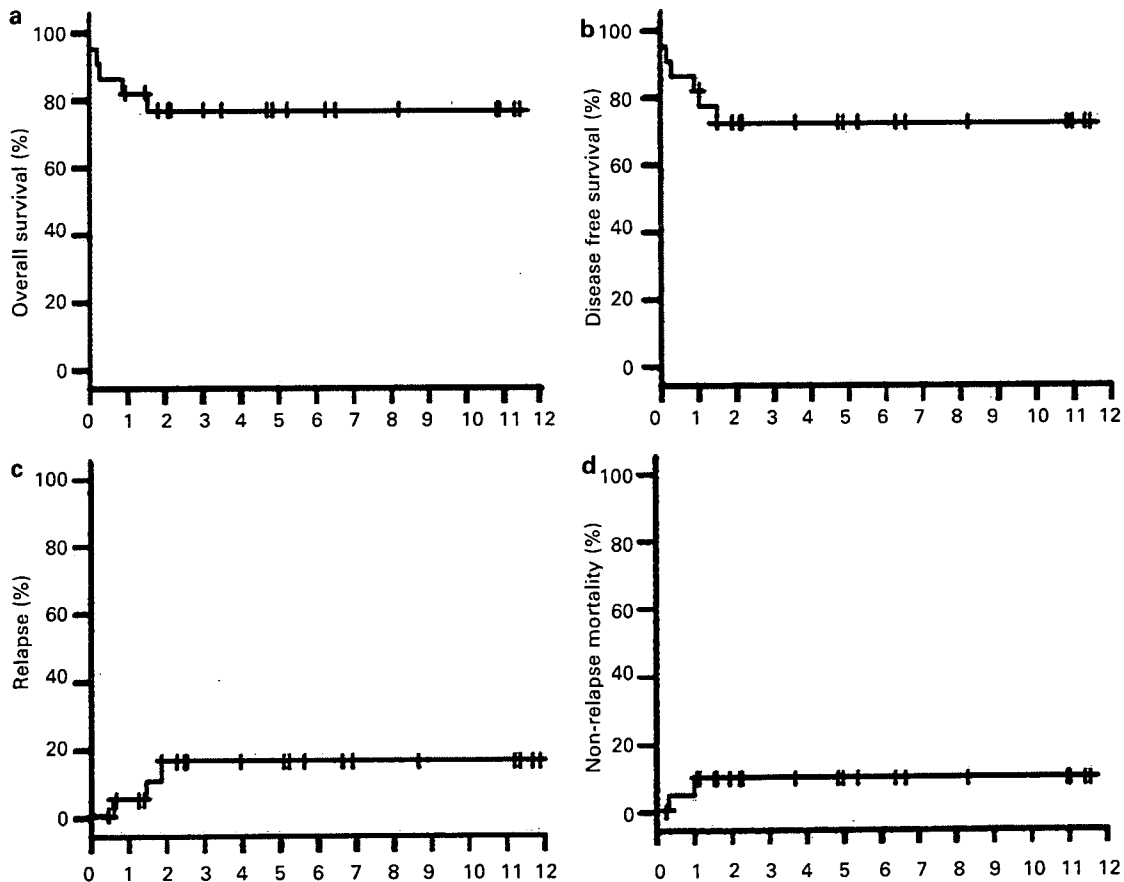


Figure 1 Kaplan-Meier estimates of (a) overall survival rate, (b) disease-free survival rate, (c) relapse rate and (d) non-relapse mortality rate. The + indicates a censored patient.