The cytogenetic response to CML treatment, ie, reduction in Ph-positive metaphases, can be usually obtained with IFN, imatinib, or allogeneic hematopoietic stem cell transplantation (allo-HSCT), and cytogenetic evaluation is used to evaluate the response to treatment [9,10]. Approximately 80% of the patients in the chronic phase who use imatinib will achieve the disappearance of Ph-positive metaphases (a complete cytogenetic response [CCR]) [5], making a molecularly analysis necessary to precisely evaluate the efficacy of imatinib, which has been used to follow minimal residual disease, mostly after allo-HSCT [11,12]. On the basis of the reports of many clinical trials, imatinib has become the first choice of treatment for patients with newly diagnosed CML [1].

Patients participating in clinical trials are usually selected according to strict eligibility criteria. These regulations are widely accepted as necessary in clinical trials to clearly answer the questions asked and to ensure the protection of the patients' safety and rights. In practical situations, however, the clinical features of patients are much more heterogeneous than those defined by the selection criteria in clinical trials [13]. For example, physicians need to treat patients who range from young, previously untreated patients to elderly patients who have had prior treatments and who may have many accompanying diseases and organ dysfunctions that sometimes may not allow use of the recommended drug doses.

At the time imatinib became widely available in Japan (December 2001), we wanted to know whether imatinib could reproduce in a practical setting the high efficacy that had been reported in clinical trials. To answer this question, we followed as many patients as possible over a wide variety of CML patients in Nagasaki prefecture, Japan, and we evaluated the efficacy of imatinib with molecular techniques. We also investigated how the treatment for CML changed with imatinib therapy in the same area.

By following almost 100 patients for more than 3.5 years, this study demonstrated the significantly higher efficacy and excellent clinical effects of imatinib treatment in a practical setting for both patients with newly diagnosed CML and those with previously treated CML.

2. Patients and Methods

2.1. Patients

The 99 CML patients who participated in this study were reported from 11 hospitals in Nagasaki prefecture and included (1) patients with CML newly diagnosed between December 2001 and July 2005 and (2) patients alive at the beginning of this study (December 2001). Informed consent was obtained from 78 of the 99 patients to measure the amount of bcr-abl fusion transcripts and to analyze gene mutation of the abl kinase domain in peripheral blood or bone marrow samples. In all, 554 samples were collected (250 peripheral blood and 304 bone marrow samples).

2.2. RNA Extraction, Complementary DNA Synthesis, and Polymerase Chain Reaction Conditions

Peripheral blood and bone marrow samples were sent to the Department of Hematology, Nagasaki University, where all measurements of bcr-abl fusion transcript amounts and sequence analyses of the kinase domain of the abl gene were performed. Mononuclear cells were separated from samples and disrupted to extract total RNA with the RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized from total RNA with random hexamer primers and a ProSTAR First Strand RT-PCR kit (Stratagene, La Jolla, CA, USA) for quantification of the bcr-abl fusion gene.

Quantitative real-time reverse transcriptase–polymerase chain reaction (RQ-PCR) analysis was performed with the LightCycler (Roche Diagnostics, Mannheim, Germany) and LightCycler Fast Start DNA Master SYBR Green 1 (Roche Diagnostics). PCR conditions and primer sequences are available on request. Each PCR reaction was independently performed at least twice, with monitoring of melting curves and gel electrophoresis of the products to ensure correct amplification. The amount of the fusion gene in the original sample was calculated by means of a standard curve (created with the bcr-abl fusion gene or the abl gene cloned in plasmids) and expressed as the bcr-abl/abl ratio. The lower limit of quantification was 1×10^{-4}. In several samples, the entire region of the abl kinase domain was sequenced in both forward and reverse directions by means of a BigDye Terminator v3.1 Cycle Sequencing Kit and the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

2.3. Clinical Parameters, Including Response to Therapy

Progression-free survival (PFS) was calculated from the first day of imatinib administration to the date of death, the date of development of the accelerated phase or blastic crisis of CML, or the date of the last follow-up. Overall survival (OS) was calculated from the day of diagnosis (for newly diagnosed patients, group I; see "Results") or the first day of imatinib administration (for patients with previously diagnosed CML, group II) to the date of any cause of death or the date of the last follow-up. The daily dose of imatinib was calculated as an average dose, the total amount of imatinib administered divided by the number of days of the administration period.

A CCR was defined as the absence of Ph-positive metaphases in the sample. In the event that no cytogenetic data were available but RQ-PCR or fluorescence in situ hybridization (FISH) results for bcr-abl fusion were available, we included CCR-equivalent responses (a bcr-abl/abl ratio <0.01 by RQ-PCR or below the limit of detection of bcr-abl signal by FISH) in determining the CCR rate. In terms of the molecular response, which was analyzed by RQ-PCR analysis, a 3-logarithm reduction in the data compared with those at diagnosis was categorized as a major molecular response (MMR), and the disappearance of the fusion product was defined as a complete molecular response.

2.4. Statistical Analysis

We evaluated the distribution of clinical characteristics for the 2 groups with the chi-square test or the Fisher exact test for categorical parameters and used the 2-sample

Table 1.
Clinical Characteristics of Patients in Groups I and II*

	Group I (n = 43)	Group II (n = 56)	P
M/F sex, n	27/16	30/26	.36
Median age at diagnosis (range), y	53 (21-71)	54 (15-74)	.56
CML phase at diagnosis, n			.36
CP	37	42	
AP	6	13	
BC	0	0	
Unknown	0	1	
Sokal score at diagnosis, n			.12
Low	19	15	
Intermediate	16	21	
High	7	13	
Unknown	1	7	
Median time after diagnosis (range), y	2.0 (0.2-3.6)	6.8 (3-22.3)	<.0001

*CML indicates chronic myelogenous leukemia; CP, chronic phase; AP, accelerated phase; BC, blastic crisis.

Student t test or the Wilcoxon rank sum test for continuous parameters. Imatinib doses for the 2 groups were compared with the 2-sample t test. The probabilities of CCR, PFS, and OS were estimated by the Kaplan-Meier methods. Comparisons of curves were performed with the log-rank test. All statistical analyses were performed with the JMP software package (SAS Institute, Cary, NC, USA). P values <.05 were regarded as statistically significant. All analyses were performed for data collected as of the end of July 2005.

3. Results

3.1. Number and Characteristics of Patients

During this study period (44 months, after the introduction of imatinib into clinical practice), there were 43 patients with newly diagnosed CML (group I). At the time this study started, 56 patients who had already received a CML diagnosis were alive (group II). There were no differences between the 2 groups with respect to the clinical phase of CML, Sokal score, or age at diagnosis (Table 1).

3.2. Treatments

For most patients in group I (40 of 43 patients), the initial treatment was imatinib (Figure 1). Two patients initially treated with imatinib were later changed to IFN therapy because of intolerance to imatinib. Ultimately, 42 of 43 patients in this group received imatinib at some point.

Patients in group II had received a variety of treatments until imatinib became available (Figure 1). Forty-seven patients had undergone treatment with an IFN-containing

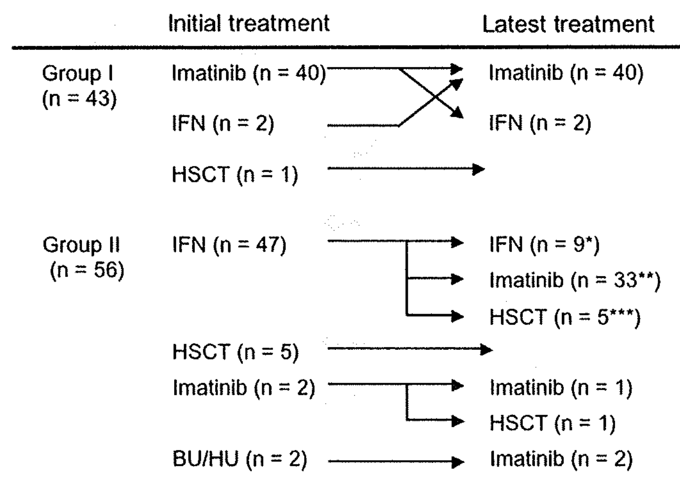

Figure 1. Initial and last treatments for patients in groups I and II. Only 1 of 43 patients in group I underwent allogeneic hematopoietic stem cell transplantation (HSCT), compared with 11 of 56 in group II (*, 1 patient discontinued interferon (IFN) treatment later; **, 2 patients discontinued imatinib treatment later; ***, 1 patient received imatinib before HSCT). Most patients were under imatinib treatment at the end of this study. BU indicates busulfan; HU, Hydrea (hydroxyurea).

Table 2.
Hematologic and Nonhematologic Toxicities of Imatinib

Hematologic toxicity (grades 3 and 4)	58.6%*
Anemia	20%
Neutropenia	34%
Thrombocytopenia	24%
Nonhematologic toxicity (grades 2 to 4)	66%*
Fluid retention	24%
Skin rash	16%
Elevation of CPK	15%
Infection	8%
Elevation of liver enzymes	5%
Fever	5%
Nausea	5%
Phlegmon	4%
Others	25%

*Several patients experienced 2 or more adverse events.

regimen at least once, and 11 patients (including 5 who had a history of IFN treatment and 1 patient who had received imatinib treatment) had undergone allo-HSCT. After imatinib became available, only 9 patients remained on IFN treatment (1 patient later discontinued IFN because of associated toxicities). Imatinib was administrated to 38 patients in group II. Four patients in this group participated in the imatinib clinical trial so that they could use the drug before it became available. One patient who received imatinib after allo-HSCT for the treatment of CML relapse achieved an MMR. Eighty of the 99 patients, including the patient treated with imatinib after HSCT, took imatinib at least once during the study period.

3.3. Toxicity of Imatinib and Complications

Hematologic toxicity of grades 3 to 4 was observed for imatinib in 41 (58.6%) of 70 evaluable patients (Table 2); most recovered after the discontinuation of imatinib treatment. Nonhematologic toxicity of grades 2 to 4 observed in 53 patients (66.3%, Table 2) necessitated treatments in several of the patients. Fluid retention (24%), skin rash (16%), and the elevation of creatine phosphokinase (CPK) levels (15%) were frequent adverse events. Of note was 1 patient who had rhabdomyolysis during imatinib treatment that resulted in death of the patient. Malignancy other than CML was diagnosed in 15 of 99 patients before (6 patients), after (5 patients), or at the same time (4 patients) CML was noticed (Figure 2). In 4 patients, gastric cancer was found during the general medical checkup performed when CML was diagnosed.

3.4. Imatinib Dosage and Molecular and Cytogenetic Responses

The time course of the molecular response to imatinib treatment is shown in Figure 4. For group I, 16 (61.5%) of 26 patients achieved an MMR by 24 months, and 20 of 32 patients (62.5%) ultimately maintained an MMR (Figure 4A). In group II, 14 (58.3%) of 24 patients achieved an MMR with imatinib treatment (Figure 4B). The 2 groups did not differ in the time required to reach a CCR (Figure 3). Among the evaluable patients, 86.1% in group I (n = 37) and 77.9% in group II (n = 31) had reached a CCR at 30 months. Table 3 summarizes the CCR and MMR data for the 2 groups.

The imatinib dosage initially was 400 mg/day in almost all patients and was later modified for a variety of reasons (Table 4). Cytopenia was the most frequently observed reason for dosage reduction, especially in group II. The imatinib dosage was reduced without toxicity of grade 3 or 4 in 10 patients, including 4 patients with no apparent adverse events. Only 16 (21.1%) of 76 patients could take 400 mg/day or more of imatinib without requiring a dosage reduction. These changes produced a difference between groups I and II in the imatinib dosage: the daily dose was significantly higher in group I (as an initial therapy) than in group II (as a second-line therapy) during the first 12 months of imatinib treatment (Table 5).

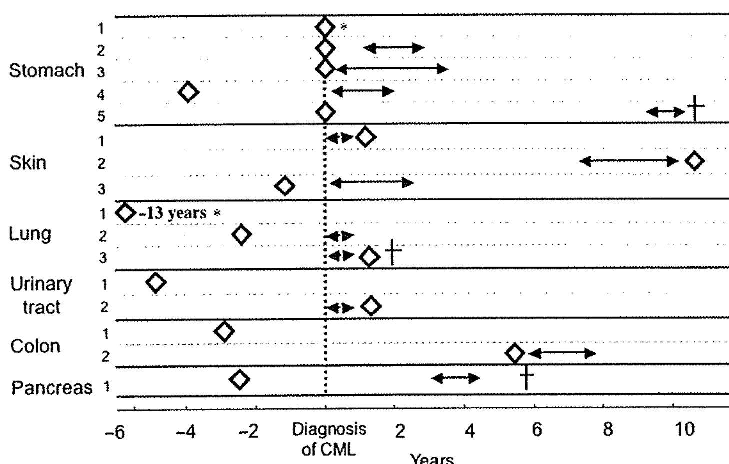

Figure 2. Malignancies other than chronic myelogenous leukemia (CML) among the patients in this study. Sixteen malignancies were found in 15 of 99 patients before, after, or at the time of CML diagnosis. Indicated are times of malignancy diagnosis (◊), the same patient (*), periods of imatinib treatment (↔), and patient death (†).

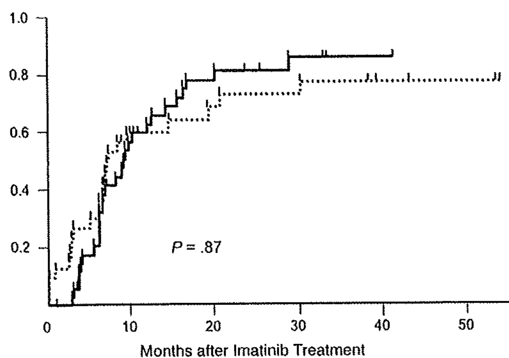

Figure 3. Accumulation of complete cytogenetic responses (CCR) in groups I and II. Time to reach CCR (and CCR equivalent as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis) is shown. The difference between group I (n = 37, solid line) and group II (n = 31, dotted line) in the time required to obtain CCR after the start of imatinib treatment was not statistically significant.

The daily imatinib dose was related to the cytogenetic response (Figure 5). Forty-one patients (54% of patients treated with imatinib) received more than 300 mg/day of imatinib. The CCR rate at 30 months was 91.9% for 40 of these 41 patients (for whom cytogenetic data were available), 86.1% for those who received 250 to 300 mg/day of imatinib (n = 12), and 50.6% for those who received less than 250 mg/day (n = 15). There was a significant difference in CCR rate among the 3 groups (P = .0052, Figure 5). The MMR rates for the patients who received more than 300 mg/day, 250 to 300 mg/day, and less than 250 mg/day were also different in both group I and group II: 68.2%, 71.4%, and 0%, respectively, in group I and 83.3%, 75.0%, and 37% in group II. Among the 15 patients whose average daily dose was less than 250 mg, cytopenia was also the major reason for the insufficient imatinib treatment (13 of 15 patients); a CCR was achieved in only 5 of these patients. Compared with these 5 patients who achieved a CCR, patients in this category without a CCR received far less imatinib (125 mg/day versus 173 mg/day), and 4 of the 10 patients who lacked a CCR died of CML progression.

3.5. Disease Progression and Survival of Imatinib-Treated Patients

In group I, disease progression was observed in 3 patients: transition from the accelerated phase to blastic crisis in 2 patients and the acquisition of an additional cytogenetic abnormality in 1 chronic-phase patient. Two patients in group I died during imatinib treatment (from rhabdomyolysis in 1 patient, as mentioned above, and from disease complicated by lung cancer in another). In group II, disease status progressed in 8 of 33 patients treated with imatinib. Forty patients in group II were alive at the end of this study.

The OS rates at 3.5 years for imatinib-treated patients were 88.7% in group I (n = 40) and 79.8% in group II (n = 36) and were not significantly different (P = .45, Figure 6A). There was no significant difference in PFS at 3.5 years for the same patient population (85.2% in group I and 76.6% in group II; P = .51, Figure 6B).

3.6. Mutation in the Kinase Domain of the bcr-abl Fusion Gene

Of the 49 patients who lacked a complete molecular response with imatinib treatment, 10 patients did not reach a CCR. Four of these 10 patients had a point mutation in the abl kinase domain (F311I, 1 patient; T315I, 2 patients; E459K, 1 patient); these data have already been reported [14,15]. At the time we noted the mutations, 3 patients were in the accelerated phase, and 1 patient was hematologically stable but with an additional cytogenetic change. Two of these 4 patients died when their disease progressed to blastic crisis.

4. Discussion

To follow CML patients in this study with no or minimal selection bias, we had to register as many CML patients as possible throughout an entire prefecture (Nagasaki prefecture, population approximately 1.5 million). This strategy allowed us to evaluate the clinical usefulness of imatinib in the context of a daily clinical setting. In 2002, 19 CML

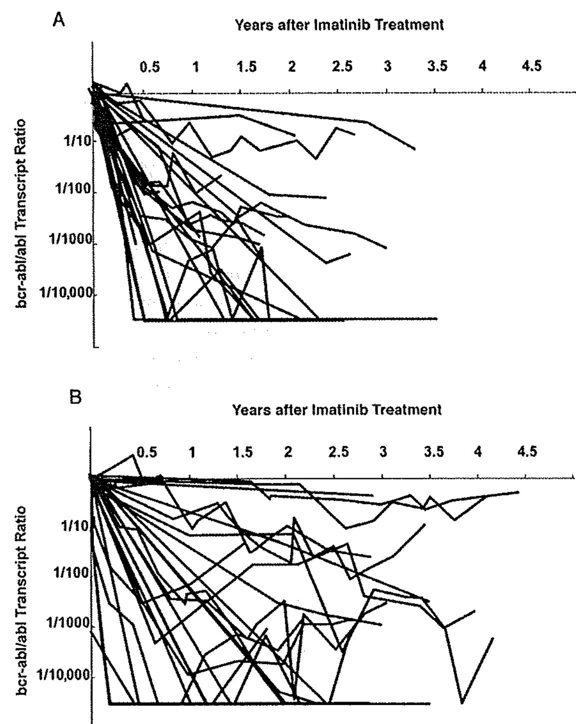

Figure 4. Reductions in bcr-abl fusion transcripts among imatinib-treated patients in group I (A) and group II (B). The changes in the amount of bcr-abl transcripts as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis and the times after imatinib treatment are shown. Each line represents 1 patient.

Table 3.
Complete Cytogenetic Response (CCR) and Major Molecular Response (MMR) in Groups I and II

	Group I		Group II	
Time after imatinib treatment, mo	CCR, %	MMR, %	CCR, %	MMR, %
6	32.2	10.3	36.4	16.7
12	63.0	34.7	60.1	33.6
24	81.5	63.1	73.4	51.3

patients were reported to the Nagasaki Prefecture Cancer Registry (NPCR), whereas 11 patients were registered in this study. Patients who received their diagnoses outside of Nagasaki prefecture but who lived in Nagasaki prefecture and patients who received their diagnoses and were followed at home were very difficult to include. From 1985 to 1998, 187 patients with newly diagnosed CML were recorded in the NPCR (13.4 patients/year) [16], and the number of patients with newly diagnosed CML was 11.7/year in this study (43 patients during 44 months). These data indicate that our study did not include all CML patients but did cover a sufficient variety of CML patients to evaluate the practical usefulness of imatinib. Compared with the IRIS study, more patients in this study were older than 60 years (37.4% versus 21.9% in the IRIS study), and fewer patients had a low Sokal score (37.4% in this study and 50.4% in the IRIS study).

This study clearly reproduced the imatinib efficacy results described in the IRIS study, not only for patients with newly diagnosed CML but also for those with prior therapy who might include more patients in the late chronic phase. The rates of OS (88.7% at 3.5 years, n = 43) and CCR (86.1% at 30 months) for imatinib-treated patients in group I were comparable with those reported in the IRIS study (OS, 97.2% at 18 months) [4,5] and in subsequent reports [1,2,17,18].

The profiles of adverse events in this study were also similar to those in the IRIS study, and most patients could tolerate these events without the discontinuation of imatinib treatment. However, we did observe the elevation of CPK levels (grades 1 to 4) in 42% of the patients. Although not clearly mentioned in previous reports, monitoring CPK levels and muscular symptoms during imatinib treatment seemed necessary, given that one of the patients experienced rhabdomyolysis. We observed complications of additional malignancies in 15 patients (15%) in this study, including 4 patients with gastric cancer identified at the time of CML diagnosis. Although no report has described an increase in secondary malignancies with imatinib treatment, we need to pay attention to secondary and even tertiary malignancies, because imatinib treatment will prolong the survival of CML patients and we still do not know the long-term effects of imatinib on neoplasm development.

The administered imatinib dose seemed lower than that reported in the IRIS study (median dose, 400 mg/day); in our study, only 21.1% of patients could take 400 mg/day of imatinib without a reduction in dosage. In 10 patients (13.1%), the physicians reduced the imatinib dosage without a toxicity of grade 3 or 4, probably reflecting complicated situations in practice. Cytopenia was the major reason for dosage reduction and was more frequently observed in group II (Table 4), making the average imatinib dose lower in group II (259 mg/day) than in group I (334 mg/day, Table 5). This reduction might have been caused by the slow recovery of non-Ph hematopoiesis in group II after imatinib suppression of Ph-positive cells, because group II patients had a longer history of CML than group I. Fortunately, however, we observed no difference between these 2 groups in OS and PFS, which were achieved with a lower dose than recommended. This result could be explained, at least in part, by the increment of imatinib dose with time in group II. The dose gradually increased from 234 mg/day for the first 3 months to 302 mg/day after 2 years, making the average dose more than

Table 4.
Events Related to Imatinib Dosage Reduction

Events	Group I, n	Group II, n	Total, n
Patients with adverse events of grade 3 or 4			
Cytopenia alone	8	8	16
Cytopenia plus skin rash/fluid retention	1	13	14
Cytopenia plus other events	4	4	8
Skin rash alone	1	0	1
Skin rash plus events other than cytopenia	4	3	7
Patients without adverse events of grade 3 or 4			
With minor adverse events	5	1	6
Without apparent adverse events	3	1	4
Others	1	2	3
Unknown	0	1	1
Total	27	33	60

Table 5.

Comparison of Imatinib Dosages for Groups I and II

Time after Imatinib	Group	No. of Patients	Imatinib Dosage, mg/d			*P*
			Average*	Range	SD	
0-3 mo	I	40	328	142-400	80.8	<.0001
	II	36	234	48-400	100.5	
4-6 mo	I	34	344	122-648	100.4	.0001
	II	33	216	0-400	149.5	
7-9 mo	I	32	324	0-400	113.7	.024
	II	33	252	0-425	137.7	
10-12 mo	I	29	336	100-600	110.2	.026
	II	31	262	0-400	141.6	
1-2 y	I	27	338	89-600	111.9	.17
	II	28	297	93-400	103.1	
>2 y	I	16	310	50-400	119.3	.85
	II	23	302	106-582	121.1	

*Average imatinib dose administered to the patients in each group during the respective periods.

250 mg/day (259 mg/day). We also need to keep in mind that group II patients were selected for having maintained their CML status in a relatively stable clinical course for a certain period (3-22.3 years) before this study began. This fact would also be a reason for the fair response to imatinib in group II, even with an average dose lower than that in group I. Patients who did not (or could not) receive 250 imatinib mg/day showed insufficient clinical results. In our experience, when cytopenia makes it difficult to maintain an average imatinib dose of more than 250 mg/day, other treatment options, including allo-HSCT, should be considered. We do not recommend reducing the imatinib dosage (to less than 400 mg/day) on the basis of these observations because many groups have reported the importance of the imatinib dosage for obtaining clinical effects [19-22]. Our data rather have demonstrated that imatinib provided benefit, even for those who could not receive a sufficient dose for a period of at least 3.5 years. From our observations, the daily dose could be

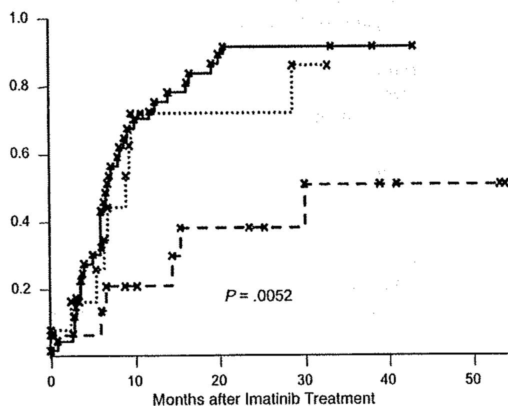

Figure 5. Time to reach a complete cytogenetic response (CCR) according to the daily imatinib dose. The differences in the times to reach a CCR (including CCR equivalent as measured by real-time quantitative reverse transcriptase–polymerase chain reaction analysis) among the 3 dosage groups were statistically significant (P = .0052). Solid line, ≥300 mg/day of imatinib (n = 40); dotted line, 250-300 mg/day (n = 12); broken line, <250 mg/day (n = 15).

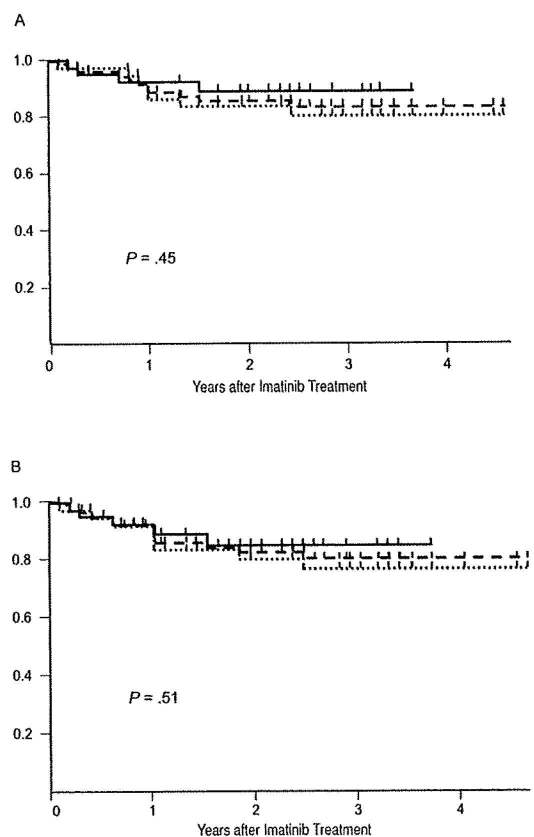

Figure 6. Overall survival (OS) (A) and progression-free survival (PFS) (B) for imatinib-treated patients in groups I and II. There were no significant differences between the 2 groups in OS and PFS. Solid line, group I; dotted line, group II; broken line, all cases.

gradually increased along with treatment for patients who could take 250 to 300 mg/day of imatinib. Imatinib could be continued at the same or an increased dosage as long as sufficient efficacy was maintained.

In summary, we reconfirmed the efficacy of imatinib in patients with CML, even in the practical setting. Imatinib will prolong the survival of CML patients in situations broader than those defined in clinical trials.

Appendix

The members of the Nagasaki CML Study Group are as follows: S. Atogami (Nagasaki Municipal Medical Center); M. Yamamura (Nagasaki Municipal Hospital); S. Momita, T. Joh, Y. Takasaki (The Japanese Red Cross Nagasaki Atomic Bomb Hospital); Y. Yoshida (St. Francis Hospital); Y. Moriuchi, J. Taguchi, T. Tsuchiya, Y. Onimaru (Sasebo Municipal General Hospital); S. Yoshida, M. Honda, M. Tawara (National Hospital Organization, Nagasaki Medical Center); Y. Matsuo (Nagasaki Prefectural Shimabara Hospital); H. Soda (Health Insurance, Isahaya General Hospital); H. Nonaka (Japan Labour Health and Welfare Organization, Nagasaki Rosai Hospital); S. Ikeda (Hirado Municipal Hospital); C. Kawasaki (Sasebo Kyosai Hospital); I. Jinnai (Saitama Medical School); K. Kuriyama (University of the Ryukyus); M. Kusano (Senju Hospital); Y. Moriwaki (Gotoh Central Hospital); and other authors at Nagasaki University.

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References

1. Goldman JM, Melo JV. Chronic myeloid leukemia: advances in biology and new approaches to treatment. *N Engl J Med.* 2003;349:1451-1464.
2. Borthakur G, Cortes JE. Imatinib mesylate in the treatment of chronic myelogenous leukemia. *Int J Hematol.* 2004;79:411-419.
3. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood.* 2005;105:2640-2653.
4. O'Brien SG, Guilhot F, Larson RA, et al, for the IRIS Investigators. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N Engl J Med.* 2003;348:994-1004.
5. Hughes TM, Kaeda J, Branford S, et al, for the International Randomised Study of Interferon versus STI571 (IRIS) Study Group. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med.* 2003;349:1423-1432.
6. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed *BCR-ABL*-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol.* 2006;24:460-466.
7. Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood.* 2004;103:4396-4407.
8. Champagne MA, Capdeville R, Krailo M, et al. Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. *Blood.* 2004;104:2655-2660.
9. Bonifazi F, de Vivo A, Rosti G, et al, for the European Study Group on Interferon in Chronic Myeloid Leukemia. Chronic myeloid leukemia and interferon-α: a study of complete cytogenetic responders. *Blood.* 2001;98:3074-3081.
10. Kim YJ, Kim DW, Lee S, et al. Comprehensive comparison of FISH, RT-PCR, and RQ-PCR for monitoring the BCR-ABL gene after hematopoietic stem cell transplantation in CML. *Eur J Haematol.* 2002;68:272-280.
11. Olavarria E, Kanfer E, Szydlo R, et al. Early detection of *BCR-ABL* transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. *Blood.* 2001;97:1560-1565.
12. Branford S, Rudzki Z, Harper A, et al. Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. *Leukemia.* 2003;17:2401-2409.
13. Wright JR, Bouma S, Dayes I, et al. The importance of reporting patient recruitment details in phase III trials. *J Clin Oncol.* 2006;24:843-845.
14. Branford S, Rudzki Z, Walsh S, et al. Detection of *BCR-ABL* mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. *Blood.* 2003;102:276-283.
15. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, et al. Several types of mutations of the *Abl* gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. *Blood.* 2002;100:1014-1018.
16. Soda M, Ikeda T, Matsuo T, Suyama A. Cancer incidence in Nagasaki prefecture 1993-1997. In: Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, eds. *Cancer Incidence in Five Continents Vol. VIII.* Lyon, France: International Agency for Research on Cancer/International Association of Cancer Registry; 2003:390-393.
17. Cortes J, Talpaz M, O'Brien S, et al. Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. *Clin Cancer Res.* 2005;11:3425-3432.
18. Morishima Y, Ogura M, Nishimura M, et al. Efficacy and safety of imatinib mesylate for patients in the first chronic phase of chronic myeloid leukemia: results of a Japanese phase II clinical study. *Int J Hematol.* 2004;80:261-266.
19. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.* 2001;344:1031-1037.
20. Talpaz M, Silver RT, Druker BJ, et al. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. *Blood.* 2002;99:1928-1937.
21. Kantarjian HM, Talpaz M, O'Brien S, et al. Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. *Blood.* 2003;101:473-475.
22. Kantarjian H, Talpaz M, O'Brien S, et al. High-dose imatinib mesylate therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. *Blood.* 2004;103:2873-2878.

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ORIGINAL ARTICLE

Severe hemorrhagic complications during remission induction therapy for acute promyelocytic leukemia: incidence, risk factors, and influence on outcome

Masamitsu Yanada[1], Tadashi Matsushita[1], Norio Asou[2], Yuji Kishimoto[3], Motohiro Tsuzuki[4], Yasuhiro Maeda[5], Kentaro Horikawa[2], Masaya Okada[6], Shigeki Ohtake[7], Fumiharu Yagasaki[8], Tadashi Matsumoto[9], Yukihiko Kimura[10], Katsuji Shinagawa[11], Masako Iwanaga[12], Yasushi Miyazaki[12], Ryuzo Ohno[13], Tomoki Naoe[1]

[1]Nagoya University Graduate School of Medicine, Nagoya; [2]Kumamoto University School of Medicine, Kumamoto; [3]Kansai Medical University, Moriguchi; [4]Fujita Health University School of Medicine, Toyoake; [5]Kinki University School of Medicine, Osaka-Sayama; [6]Hyogo College of Medicine, Nishinomiya; [7]Kanazawa University Hospital, Kanazawa; [8]Saitama Medical University, Saitama; [9]Jiaikai Imamura Hospital, Kagoshima; [10]Tokyo Medical University, Tokyo; [11]Okayama University Graduate School of Medicine, Okayama; [12]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; [13]Aichi Cancer Center, Nagoya, Japan

Abstract

Background: Even after the introduction of all-*trans* retinoic acid (ATRA), early hemorrhagic death remains a major cause of remission induction failure for acute promyelocytic leukemia (APL). *Methods:* To investigate severe hemorrhagic complications during remission induction therapy with respect to incidence, risk factors, and influence on outcome. Results were analyzed for 279 patients enrolled in the APL97 study conducted by the Japan Adult Leukemia Study Group (JALSG). *Results:* Severe hemorrhage occurred in 18 patients (6.5%). Although most of them were receiving frequent transfusions, the targeted levels of platelet counts (30×10^9/L) and plasma fibrinogen (1.5 g/L) for this study were reached at the day of bleeding in only 71% and 40%, respectively. Nine of them succumbed to an early death, while the remaining nine patients eventually achieved complete remission (CR). The 5-yr event-free survival rate was 68.1% for those who did not suffer severe hemorrhage, and 31.1% for those who did ($P < 0.0001$). For patients who achieved CR, on the other hand, there was no difference in disease-free survival between patients with and without severe hemorrhage ($P = 0.6043$). Risk factor analysis identified three pretreatment variables associated with severe hemorrhage: initial fibrinogen level, white blood cell count, and performance status. Additionally, patients with severe hemorrhage were more easily prone to develop retinoic acid syndrome or pneumonia than patients without hemorrhage. *Conclusions:* These results indicate that fatal hemorrhage represents a major obstacle in curing APL, and that patients with such high-risk features may benefit from more aggressive supportive care.

Key words acute promyelocytic leukemia; coagulopathy; hemorrhage; early hemorrhagic death; all-*trans* retinoic acid

Correspondence Masamitsu Yanada, MD, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya, Aichi 466-8550, Japan. Tel.: +81 52 744 2955, Fax: +81 52 744 2801; e-mail: myanada@med.nagoya-u.ac.jp

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Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML), characterized by specific biologic and clinical features including the presence of the t(15,17) chromosomal translocation, frequent observation of significant coagulopathy at presentation, and high susceptibility to all-*trans* retinoic acid (ATRA) (1–3). The introduction of ATRA has dramatically improved the outcome for APL, and by using current

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induction therapy, complete remission (CR) rates have been increased up to 90% or higher. As a result, early hemorrhagic death is now the primary cause of remission induction failure. The frequency of fatal hemorrhage reportedly ranges from 2.4% to 11.6% (4–13), which appears to be lower than those in the pre-ATRA era. Nevertheless, severe hemorrhage, particularly in central nervous system (CNS) and lung, represents a major obstacle in curing APL. In this study, we investigated incidence, risk factors, and influence on outcome of severe hemorrhagic complications during remission induction therapy for APL by analyzing data of the patients entered into the prospective trial (APL97) conducted by the Japan Adult Leukemia Study Group (JALSG).

Patients and methods

Patients

The JALSG APL97 study enrolled patients aged between 15 and 70 yr with newly diagnosed APL. Eligibility criteria included adequate functioning of the liver [serum bilirubin level < 34.2 μM (2.0 mg/dL)], kidneys [serum creatinine level < 152.50 μM (2.0 mg/dL)], lungs ($PaO_2 \geq 60$ mmHg), and heart (no severe abnormalities detected on electrocardiograms) and an Eastern Cooperative Oncology Group performance status between 0 and 3. The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients prior to registration.

Study design and treatments

For remission induction therapy, ATRA was administered to all patients at a daily dose of 45 mg/m^2 until CR or for 60 d, whichever was shorter. The chemotherapy protocol depended on the initial white blood cell (WBC) count and blast cell count in peripheral blood (PB). If the initial WBC count did not exceed 3.0×10^9/L and the PB blast count was less than 1.0×10^9/L (Group A), simultaneous chemotherapy was withheld. If the initial WBC count was between 3.0×10^9/L and 10.0×10^9/L and/or the PB blast count exceeded 1.0×10^9/L (Group B), patients received 12 mg/m^2 of idarubicin (IDR) on days 1 and 2, and 80 mg/m^2 of cytarabine (Ara-C) on days 1 to 5. If the initial WBC count was 10.0×10^9/L or higher (Group C), 12 mg/m^2 of IDR was administered on days 1 to 3, and 100 mg/m^2 of Ara-C on days 1 to 5. Patients whose PB blast counts exceeded 1.0×10^9/L during the induction course were given an additional 12 mg/m^2 of IDR for 2 d, and 80 mg/m^2 of Ara-C for 5 d (Group D). If patients in Group A, B, and C were treated with additional chemotherapy because of an increase in the PB blast count, they were designated as Groups AD, BD, and CD, respectively.

For the treatment of coagulopathy, platelet transfusions were administered to maintain the platelet count above 30×10^9/L, and fresh frozen plasma was transfused to maintain the plasma fibrinogen level above 1.5 g/L. Anticoagulants were used according to the discretion of the participating institutions. Retinoic acid (RA) syndrome was treated with methylprednisolone at 20 mg/kg/d for 3 d while ATRA was immediately discontinued.

Consolidation therapy consisted of three courses of intensive chemotherapy: using mitoxantrone and standard-dose Ara-C for the first course, daunorubicin, etoposide and standard-dose Ara-C for the second course, and IDR and standard-dose Ara-C for the third course. Methotrexate, Ara-C, and prednisolone were administered by intrathecal injection before the third course.

Patients who were positive for the promyelocytic-retinoic acid receptor-alpha (PML-RARα) fusion transcript in bone marrow (BM) after the completion of the consolidation therapy were treated with ATRA for 28 d followed by six courses of intensification therapy. If they were 50 yr old or younger and a suitable donor was available, allogeneic hematopoietic stem cell transplantation (HSCT) was recommended. Patients who were negative for PML-RARα after the consolidation courses were randomly assigned to either six courses of intensification therapy or no further therapy.

Evaluation of patients

CR was defined as the presence of all of the following: less than 5% of blasts in BM, no leukemic blasts in PB, recovery of PB values to neutrophil counts of at least 1.5×10^9/L and platelet counts of at least 100×10^9/L, and no evidence of extramedullary leukemia. Relapse was defined as the presence of at least one of the following: recurrence of more than 10% leukemic cells in BM or any leukemic cells in PB or extramedullary sites. Toxicity evaluation was based on the National Cancer Institute Common Toxicity Criteria Version 2.0. Severe hemorrhagic complication was defined as intracranial or pulmonary hemorrhage of grade 3 or higher. Early hemorrhagic death was defined as death during the remission induction course because of severe hemorrhagic complications.

Statistical analysis

Comparisons of baseline characteristics between patients with and without severe hemorrhage were made with the

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Fisher's exact test for categorical variables, and with the Wilcoxon rank-sum test for continuous variables. Kaplan-Meier analysis was used to estimate the probability of event-free survival (EFS) and disease-free survival (DFS). EFS was defined as the time from the first day of therapy to relapse, death, or last visit, and patients who failed to achieve CR were categorized as failure cases at time zero. DFS was defined as the time from the day of achievement of CR to relapse, death, or last visit. Patients undergoing HSCT were censored at the time of transplantation. Differences between curves were compared by using a log-rank test. Cumulative incidence of severe hemorrhage was calculated with death because of other causes considered as a competing risk. To determine risk factors for the development of severe hemorrhage, variables with *P*-values of less than 0.10 in univariate logistic analysis were included in the multivariate logistic model. Cut-off points were determined according to statistical and clinical perspectives. The odds ratio (OR) was calculated in conjunction with the 95% confidence interval (CI). Stata ver. 8 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses.

Results

Incidence of severe hemorrhage and patient characteristics

Of the 283 patients registered and evaluable in the JALSG APL97 study, four were excluded because of insufficient data for the purpose of the present analysis. Thus, a total of 279 patients were analyzed. They comprised 76 in Group A, 67 in Group B, 52 in Group C, 78 in Group AD, and 6 in Group BD. Severe hemorrhage during remission induction therapy occurred in 18 patients (6.5%), comprising intracranial hemorrhage in 12, pulmonary hemorrhage in 4, and both in 2 cases. Cumulative incidence at 60 d was 5.8% (95% CI: 4.8–9.0%) (Fig. 1). Baseline characteristics of patients with and without severe hemorrhage are summarized in Table 1. Patients who developed severe hemorrhage were likely to present with worse performance status, lower levels of plasma fibrinogen, and higher fibrin degradation product (FDP) ratios, which were calculated by dividing the serum FDP value by its upper normal limit. No differences in age, morphological subtype (M3 or M3v), WBC counts, or platelet counts were observed between the two groups. Table 2 shows details of those who developed severe hemorrhage, indicating that none of the cases had been treated with ATRA alone (Group A). The median duration from the start of chemotherapy to the onset of bleeding was 5 d (range, 0–17 d).

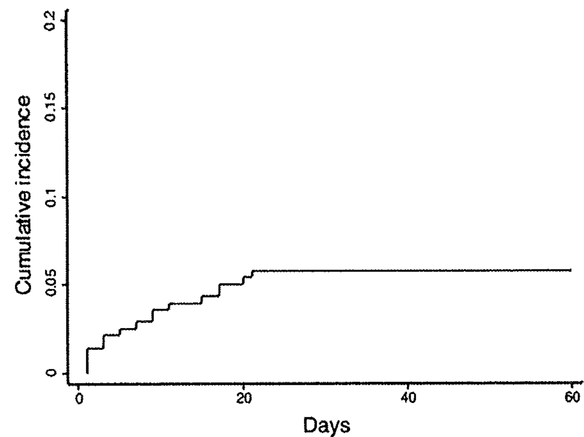

Figure 1 Cumulative incidence of severe hemorrhage. The incidence of grade 3 or higher intracranial and/or pulmonary hemorrhage during remission induction therapy was 5.8%.

At the day of onset, 71% of the patients reached the targeted level of platelet count (30×10^9/L), whereas only 40% reached the targeted level of fibrinogen (1.5 g/L).

Risk factors

Next, risk factors for severe hemorrhage were investigated. Univariate analysis disclosed that three pretreatment factors, i.e., fibrinogen level ($\geq$ or <1.0 g/dL), WBC count ($\geq$ or $<20 \times 10^9$/L), and performance status (0–1 or 2–3) were associated with severe hemorrhagic complications, all of which maintained their predictive value by multivariate analysis (Table 3). When the cut-off point of WBC count was set at 10×10^9/L, statistically significant association was not observed (P = 0.106). Also, no significant correlation was detected for age, platelet count, or FDP ratio ($P = 0.896$, 0.741, and 0.440, respectively). The ratios for patients who developed severe hemorrhage were 12.5% for those with fibrinogen levels below 1.0 g/dL, 15.6% for those with WBC counts exceeding 20×10^9/L, 13.7% for those with performance status of 2 or 3. Additionally, patients with sever hemorrhage were more easily prone to develop RA syndrome (OR, 3.97; 95% CI, 1.38–11.4) or pneumonia (OR, 3.27; 95% CI, 0.99–10.8) than patients without hemorrhage.

Outcome

Of the 18 patients with severe hemorrhage, nine suffered an early death at a median of 15 d (range, 1–22 d) after start of treatment. Death within 7 d occurred in three patients. Their causes of deaths comprised intracranial

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Table 1 Patient characteristics

Severe hemorrhage	Absent	Present	*P*-value
No. of patients	261	18	
Age (yr)	48 (15–70)	44 (16–63)	0.837
Sex (male/female)	147/114	9/9	0.631
FAB type (M3/M3v)	243/18	18/0	0.616
Performance status (0–1/2–3)	215/44	11/7	0.003
WBC count ($\times 10^9$/L)	1.7 (0.1–151.6)	3.3 (0.6–256.5)	0.257
PB blast count ($\times 10^9$/L)	0.5 (0.0–145.6)	2.5 (0.0–252.7)	0.079
Platelet count ($\times 10^9$/L)	15 (2–238)	40 (4–128)	0.834
Fibrinogen (g/L)	1.37 (0.20–5.80)	0.96 (0.42–2.28)	0.020
FDP ratio[1]	11.1 (0.29–524)	16.6 (6.56–190)	0.014

Continuous variables are presented as median (range).
[1] Calculated by dividing the FDP value by its upper normal limit.
FAB, French-American-British; WBC, white blood cell; PB, peripheral blood; FDP, fibrin degradation product.

Table 2 Details of patients who developed severe hemorrhage during remission induction course

UPN	Induction therapy[1]	Site of bleeding	Onset of hemorrhage	Platelet count at onset ($\times 10^9$/L)	Fibrinogen level at onset (g/L)	Pneumonia	RA syndrome	Outcome	Survival (yr)
26	C	CNS	Day 3	NA	NA	–	–	Alive in CR1	6.1+
37	AD	CNS	Day 7	35	1.11	–	–	Death in CR1	2.9
46	B	CNS	Day 5	47	1.63	–	–	Death in CR1	1.0
75	C	CNS	Day 11	2	NA	–	–	Early death	
85	AD	Lung	Day 21	9	0.69	–	–	Early death	
103	C	CNS	Day 1	46	1.05	+	–	Alive in CR1	2.0+
112	B	CNS	Day 3	32	1.65	–	+	Early death[2]	
116	AD	Both	Day 9	60	2.54	+	+	Death in CR1	0.2
124	AD	Lung	Day 17	7	4.49	+	+	Early death	
125	C	Both	Day 1	42	1.27	–	–	Early death	
147	C	CNS	Day 1	108	2.70	–	–	Early death	
164	AD	Lung	Day 20	36	2.24	+	+	Alive in CR1	5.1+
167	AD	Lung	Day 17	9	0.60	–	+	Alive in CR1	5.0+
206	B	CNS	Day 0	46	0.56	–	–	Alive in CR1	4.2+
233	AD	CNS	Day 15	13	0.90	–	–	Early death	
239	C	CNS	Day 1	51	0.67	–	+	Early death	
256	B	CNS	Day 9	51	0.67	–	–	Early death	
310	AD	CNS	Day 0	66	NA	–	–	Alive in CR1	2.6+

[1] Types of induction therapies are detailed in the text.
[2] The main cause of death for this patient was RA syndrome, but in association with CNS bleeding.
RA, retinoic acid; CNS, central nervous system; NA, not assessed; CR1, first remission; UPN, unique patient number.

Table 3 Factors associated with development of severe hemorrhage

	Univariate analysis	Multivariate analysis		
	P-value	*P*-value	OR (95% CI)	Factor
Fibrinogen level	0.022	0.024	3.28 (1.17–9.19)	Lower than 1.0 g/L
			1.00	1.0 g/dL or higher
WBC count	0.033	0.029	3.61 (1.14–11.4)	20 × 10^9/L or higher
			1.00	Lower than 20 × 10^9/L
Performance status	0.026	0.045	3.04 (1.02–9.02)	2–3
			1.00	0–1

OR, odds ratio; 95% CI, 95% confidence interval; WBC, white blood cell.

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hemorrhage ($n = 6$), pulmonary hemorrhage ($n = 2$), and RA syndrome ($n = 1$). All of the remaining nine patients eventually achieved CR, but two remitters died because of intracranial hemorrhage which had developed during the induction course. None of the patients died of hemorrhagic complications occurring at other sites. Figure 2 shows Kaplan-Meier curves of EFS for patients with and without severe hemorrhagic complications during the remission induction course. The 5-yr EFS rate was 68.1 ± 3.2% for those who did not suffer severe hemorrhage and 31.1 ± 11.5% for those who did ($P < 0.0001$). For patients who achieved CR, on the other hand, there was no difference in DFS between patients with and without severe hemorrhage ($P = 0.6043$, Fig. 3). Six of the seven patients who survived severe hemorrhagic complications during induction therapy were alive and disease-free after a median follow-up duration of 4.6 yr (range, 2.0–6.1 yr).

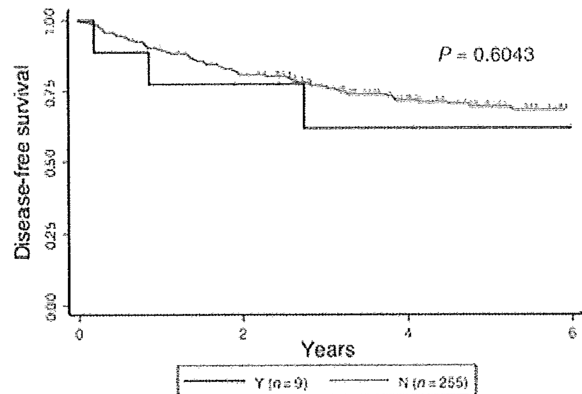

Figure 3 Probability of disease-free survival. Patients who did (Y) and did not (N) develop severe hemorrhage during remission induction therapy are compared.

Discussion

APL presents significant coagulopathy, which is occasionally exacerbated by the initiation of cytotoxic chemotherapy. In the pre-ATRA era, early hemorrhagic death reportedly occurred in up to 20% of the patients (14, 15). Recent laboratory and clinical studies have shown that ATRA produces rapid resolution of coagulopathy (16, 17), and reduces the incidence of fatal hemorrhage to a range of 2.4–11.6% (4–13). However, early hemorrhagic death remains a matter of vital concern because current treatment combining ATRA and chemotherapy induces CR in nearly all APL patients if early hemorrhagic death can be avoided, and a majority of them are to be potentially cured after the completion of standard postremission therapy (4–13). In this study, we analyzed the findings for 279 patients registered in the JALSG APL97 study, to describe severe hemorrhagic complications during induction therapy with respect to incidence, risk factors, and influence on outcome.

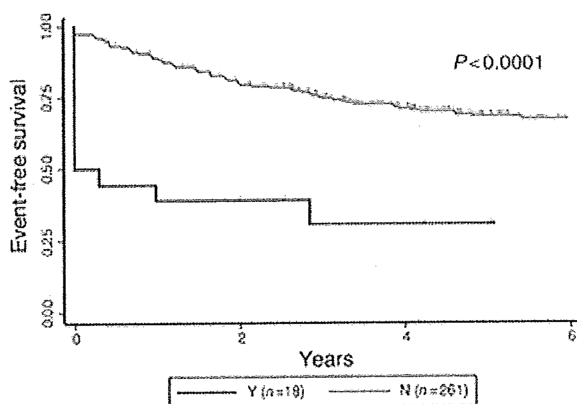

Figure 2 Probability of event-free survival. Patients who did (Y) and did not (N) develop severe hemorrhage during remission induction therapy are compared.

Severe hemorrhage developed in 6.5%, half of whom succumbed to an early death. Although direct comparisons with other studies would have some limitations particularly because of different induction regimens, the early hemorrhagic death rate of 3.2% for our patients was in the lower range of previously reported studies (Table 4). The rate in our previous study was also as low as 3.3% (11). Considering that no patient treated with ATRA alone suffered a severe hemorrhage in this study, one of the specific reasons for these findings might be the withholding of chemotherapy in a subset of patients (Group A). However, this should be interpreted with caution because eight patients in Group AD developed severe hemorrhage. Also, it should be noted that 5.8% of early hemorrhagic deaths were reported in one study in which all patients were treated with ATRA alone (6).

Although most of the patients who developed severe hemorrhage were receiving frequent transfusions, the targeted levels of platelet counts and plasma fibrinogen were reached at the day of bleeding in only 71% and 40%, respectively (Table 2). This finding indicates that for patients at high risk of hemorrhage, more intensive transfusion policy may be beneficial. The risk factors identified by our analysis: initial fibrinogen level, WBC count, and performance status, should be helpful for the assessment of high-risk patients.

As shown in Fig. 2, patients who did not experience hemorrhagic complications had an excellent long-term outcome, suggesting that early death represents the major obstacle for a cure of this disease. Although introduction of ATRA has resulted in a decrease in fatal

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Table 4 Incidence of early hemorrhagic death in various acute promyelocytic leukemia (APL) studies

Authors	Induction therapy	No. of patients	No. of CR (%)	No. of EHD (%)
Fenaux *et al.* (4)	ATRA ± DNR/Ara-C	54	49 (91)	3 (5.6)
Estey *et al.* (5)	ATRA + IDR	43	33 (77)	5 (11.6)
Tallman *et al.* (6)	ATRA	172	124 (72)	10 (5.8)
Mandelli *et al.* (7)	ATRA + IDR	240	229 (95)	8 (3.3)
Fenaux *et al.* (8)	ATRA + DNR/Ara-C	413	229 (95)	10 (2.4)
Sanz *et al.* (9)	ATRA + IDR	123	381 (92)	8 (6.5)
Lengfelder *et al.* (10)	ATRA + TAD/HAM	51	47 (92)	3 (5.9)
Asou *et al.* (11)	ATRA ± DNR/BHAC	369	333 (90)	12 (3.3)
Sanz *et al.* (12)	ATRA + IDR	426	384 (90)	25 (5.9)
Schlenk *et al.* (13)	ATRA + (IDR or ICE)	82	72 (88)	6 (7.3)
Current study	ATRA ± IDR/Ara-C	279	264 (95)	9 (3.2)

CR, complete remission; EHD, early hemorrhagic death; ATRA, all-*trans* retinoic acid; DNR, daunorubicin; Ara-C, cytarabine; IDR, idarubicin; TAD, 6-thioguanine, Ara-C, DNR; HAM, high-dose Ara-C and mitoxantrone; BHAC, behenoyl Ara-C; ICE, IDR, Ara-C, and etoposide.

hemorrhage, such complications remain of major importance and efforts to prevent them should be pursued. Our findings suggest that patients who have the high-risk features may benefit from aggressive supportive care. In the ongoing JALSG APL204 study, we are aiming to further reduce the incidence of severe hemorrhagic complications by stratifying patients into three groups on the basis of risk factors identified in the present study and by prospectively applying different criteria for transfusion threshold.

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References

1. Avvisati G, Lo Coco F, Mandelli F. Acute promyelocytic leukemia: clinical and morphologic features and prognostic factors. *Semin Hematol* 2001;**38**:4–12.
2. Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: evolving therapeutic strategies. *Blood* 2002;**99**:759–67.
3. Grimwade D, Lo Coco F. Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. *Leukemia* 2002;**16**:1959–73.
4. Fenaux P, Le Deley MC, Castaigne S, *et al.* Effect of all transretinoic acid in newly diagnosed acute promyelocytic leukemia. Results of a multicenter randomized trial. European APL 91 Group. *Blood* 1993;**82**:3241–9.
5. Estey E, Thall PF, Pierce S, Kantarjian H, Keating M. Treatment of newly diagnosed acute promyelocytic leukemia without cytarabine. *J Clin Oncol* 1997;**15**:483–90.
6. Tallman MS, Andersen JW, Schiffer CA, *et al.* All-*trans*-retinoic acid in acute promyelocytic leukemia. *N Engl J Med* 1997;**337**:1021–8.
7. Mandelli F, Diverio D, Avvisati G, *et al.* Molecular remission in PML/RAR alpha-positive acute promyelo cytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood* 1997;**90**:1014–21.
8. Fenaux P, Chastang C, Chevret S, *et al.* A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. The European APL Group. *Blood* 1999;**94**:1192–200.
9. Sanz MA, Martin G, Rayon C, *et al.* A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML/RARalpha-positive acute promyelocytic leukemia. PETHEMA group. *Blood* 1999;**94**:3015–21.
10. Lengfelder E, Reichert A, Schoch C, *et al.* Double induction strategy including high dose cytarabine in combination with all-*trans* retinoic acid: effects in patients with newly diagnosed acute promyelocytic leukemia. German AML Cooperative Group. *Leukemia* 2000;**14**:1362–70.
11. Asou N, Adachi K, Tamura U, *et al.* Analysis of prognostic factors in newly diagnosed patients with acute promyelocytic leukemia: the APL92 study of the Japan Adult Leukemia Study Group (JALSG). *Cancer Chemother Pharmacol* 2001;**48**(Suppl 1):S65–71.
12. Sanz MA, Martin G, Gonzalez M, *et al.* Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. *Blood* 2004;**103**:1237–43.
13. Schlenk RF, Germing U, Hartmann F, *et al.* High-dose cytarabine and mitoxantrone in consolidation therapy for acute promyelocytic leukemia. *Leukemia* 2005;**19**:978–83.

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14. Rodeghiero F, Avvisati G, Castaman G, Barbui T, Mandelli F. Early deaths and anti-hemorrhagic treatments in acute promyelocytic leukemia. A GIMEMA retrospective study in 268 consecutive patients. *Blood* 1990;**75**:2112–17.
15. Barbui T, Finazzi G, Falanga A. The impact of all-*trans*-retinoic acid on the coagulopathy of acute promyelocytic leukemia. *Blood* 1998;**91**:3093–102.
16. Watanabe R, Murata M, Takayama N, *et al.* Long-term follow-up of hemostatic molecular markers during remission induction therapy with all-*trans* retinoic acid for acute promyelocytic leukemia. Keio Hematology-Oncology Cooperative Study Group (KHOCS). *Thromb Haemost* 1997;**77**:641–5.
17. Tallman MS, Lefebvre P, Baine RM, Shoji M, Cohen I, Green D, Kwaan HC, Paietta E, Rickles FR. Effects of all-*trans* retinoic acid or chemotherapy on the molecular regulation of systemic blood coagulation and fibrinolysis in patients with acute promyelocytic leukemia. *J Thromb Haemost* 2004;**2**:1341–50.

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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

A Matsuda[1], U Germing[2], I Jinnai[1], M Iwanaga[3], M Misumi[1], A Kuendgen[2], C Strupp[2], Y Miyazaki[3], H Tsushima[3], M Sakai[3], M Bessho[1], N Gattermann[2], C Aul[4] and M Tomonaga[3]

[1]Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Saitama Medical University, Saitama, Japan; [2]Department of Hematology, Oncology and Clinical Immunology, Heinrich-Heine University, Düsseldorf, Germany; [3]Department of Hematology, Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan and [4]Department of Hematology, Oncology and Clinical Immunology, St Johannes Hospital, Duisburg, Germany

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 ⩾10% (Pelger+), micromegakaryocytes ⩾10% (mMgk+), dysgranulopoiesis (dys G) ⩾10% and dysmegakaryopoiesis (dys Mgk) ⩾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 ⩾10% was not correlated with OS and LFS. In the multivariate analysis, mMgk+ and dys Mgk⩾40% were adverse prognostic factors for OS for all patients, and dys G ⩾10% and dys Mgk⩾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 ⩾10%, dys Mgk⩾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.

Correspondence: Dr A Matsuda, Division of Hematology, Department of Internal Medicine, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama, Iruma-gun, Saitama, 350-0495, Japan.
E-mail: amatsu@saitama-med.ac.jp
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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

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 (months)*			*Percentile of LFS (months)*			
		75%	*50%*	*P-value*	*90%*	*75%*	*50%*	*P-value*
All patients (n = 187)								
Age (years)								
Older than 60	96	21	59	<0.001	23	104	NR	0.053
60 or younger	91	68	202		53	NR	NR	
Sex								
Male	103	23	102	0.230	22	NR	NR	0.311
Female	84	44	NR		51	NR	NR	
Neutrophils								
Dys G								
⩾10%	41	20	33	<0.001	10	25	52	<0.001
<10%	122	54	175		104	NR	NR	
Pelger								
Positive*	26	16	29	<0.001	13	25	36	<0.001
Negative	153	31	158		52	NR	NR	
Dys G/Pelger								
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	
Megakaryocytes								
Number of Mgk								
Normo/increased	154	23	109	0.083	25	NR	NR	0.046
Decreased	29	88	NR		NR	NR	NR	
Dys Mgk								
⩾10%	129	23	98	0.254	23	104	NR	0.101
<10%	25	23	176		NR	NR	NR	
Dys Mgk								
⩾40%	75	20	42	<0.001	14	36	NR	<0.001
<40%	79	88	217		NR	NR	NR	
mMgk								
Positive**	25	12	23	<0.001	13	25	51	<0.001
Negative	158	44	158		38	NR	NR	
Dys Mgk/mMgk								
Dys Mgk ⩾40% with mMgk positive**	24	12	23	<0.001	13	22	51	0.034
Dys Mgk ⩾40% without mMgk positive**	51	26	76		20	38	NR	
Chomosome (IPSS)								
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)								
Age (years)								
Older than 60	42	20	44	<0.001	14	51	NR	0.005
60 or younger	58	56	157		NR	NR	NR	
Sex								
Male	53	29	176	0.802	25	NR	NR	0.702
Female	47	52	175		51	NR	NR	
Neutrophils								
Dys G								
⩾10%	17	22	29	<0.001	11	25	38	<0.001
<10%	79	88	176		104	NR	NR	
Pelger								
Positive*	12	27	31	0.003	22	25	38	0.010
Negative	87	52	176		74	NR	NR	
Dys G/Pelger								
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 (months)*			*Percentile of LFS (months)*			
		75%	*50%*	*P-value*	*90%*	*75%*	*50%*	*P-value*
Megakaryocytes								
Number of Mgk								
Normo/increased	81	31	175	0.411	37	NR	NR	0.234
Decreased	19	88	NR		NR	NR	NR	
Dys Mgk								
⩾10%	69	29	157	0.439	25	NR	NR	Not available
<10%	12	59	176		NR	NR	NR	
Dys Mgk								
⩾40%	38	23	52	0.001	22	38	NR	0.003
<40%	43	176	217		NR	NR	NR	
mMgk								
Positive**	12	22	23	<0.001	14	25	51	<0.001
Negative	88	62	176		74	NR	NR	
Dys Mgk/mMgk								
Dys Mgk ⩾40% with mMgk positive**	12	22	23	<0.001	14	25	51	0.019
Dys Mgk ⩾40% without mMgk positive**	26	42	106		37	NR	NR	
Chomosome (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								
⩾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 ⩾10% with Pelger positive*	14	12	20	0.072	5	13	31	0.177
Dys G ⩾10% without Pelger positive*	10	43	65		10	52	NR	
Megakaryocytes								
Number of Mgk								
Normal/increased	73	16	54	0.159	23	53	NR	Not available
Decreased	10	44	108		NR	NR	NR	
Dys Mgk								
⩾10%	60	16	52	0.412	20	52	NR	0.419
<10%	13	22	NR		NR	NR	NR	
Dys Mgk								
⩾40%	37	12	29	0.001	13	28	53	0.001
<40%	36	65	NR		NR	NR	NR	
mMgk								
Positive**	13	10	16	<0.001	13	31	31	0.044
Negative	70	26	136		28	NR	NR	
Dys Mgk/mMgk								
Dys Mgk ⩾40% with mMgk positive**	12	9	16	0.019	5	13	31	0.489
Dys Mgk ⩾40% without mMgk 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.
mMgk positive**: the presence of 10% or more mMgk 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 classification[6] as follows. Dysplasias of the nucleus in erythroid lineage cells were defined as budding, bridging, internuclear, karyorrhexis, multinuclearity or megaloblastoid 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 micromegagakaryocytes, 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 Mgk) 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 Mgk. Two distinct dysplastic changes, Pelger and mMgk, 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 'mMgk'. Positivity for Pelger (Pelger+) was defined as the presence of 10% or more Pelger among 200 mature neutrophils. Positivity for mMgk (mMgk+) was defined as the presence of 10% or more mMgk among 25 or more megakaryocytes. Patients with decreased megakaryocytes were judged to be negative for mMgk (mMgk−). 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$10%. Some marrow preparations could not be examined in detail. Especially concerning the frequency of dys G, 24 cases could not be evaluated, because the observation of granules of neutrophils was difficult owing to the poor staining condition of the films. Most of the patients with mMgk+ had dys Mgk$\geq$40%. Of the patients with dys Mgk<40%, only one patient had mMgk+. However, even with this patient it was judged that the frequency of dys Mgk 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+, mMgk+, dys G $\geq$10% and dys Mgk$\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+, mMgk+, dys G $\geq$10% or dys Mgk $\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 Mgk $\geq$40% were divided into dys Mgk $\geq$40% with or without mMgk+, the effect on the prognosis of dys Mgk $\geq$40% with mMgk+ was greater than that of dys Mgk $\geq$40% without mMgk+ on OS and LFS in all patients. In a separate analysis for each country, the prognosis of patients showing dys Mgk $\geq$40% with mMgk+ was worse than that of patients with dys Mgk $\geq$40% without mMgk+ 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)*
All patients	$n=136$	$n=136$	$n=136$	$n=136$	$n=136$	$n=136$
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 Mgk⩾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)
Chomosome (IPSS), low			1			1
Chomosome (IPSS), intermediate			2.4 (1.2–4.9)*			3.1 (0.9–11)
Chomosome (IPSS), poor			2.5 (1.1–5.7)*			10.5 (2.7–40)*
Japanese patients	$n=78$	$n=78$	$n=78$	$n=78$	$n=78$	$n=78$
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 Mgk⩾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)
Chomosome (IPSS), low			1			1
Chomosome (IPSS), intermediate			2.8 (0.9–8.6)			2.7 (0.2–31)
Chomosome (IPSS), poor			1.4 (0.4–4.8)			6.7 (1.2–37)*
German patients	$n=58$	$n=58$	$n=58$	$n=58$	$n=58$	$n=58$
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 Mgk⩾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)
Chomosome (IPSS), low			1			1
Chomosome (IPSS), intermediate			2.9 (1.0–8.8)*			4.7 (0.7–34)
Chomosome (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 Mgk category. Model B included the country, age category, sex, dys G category, Pelger, dys Mgk category and mMgk. Model C included parameters of model B and cytogenetic findings. In model A, dys G ⩾10% and dys Mgk⩾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 Mgk ⩾40% was a significant adverse prognostic factor for LFS. In German patients, dys G ⩾10% was a significant adverse prognostic factor for LFS, and Mgk ⩾40% was a significant adverse prognostic factor for OS. In model B, dys Mgk ⩾40% and mMgk+ were significantly associated with an adverse prognosis regarding OS, and dys G ⩾10% and dys Mgk ⩾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 Mgk ⩾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 Mgk ⩾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 ⩾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 Mgk 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 Mgk ⩾10% was not correlated with OS and LFS. However, the frequency of dys Mgk was correlated with prognosis in FAB-RA patients, excluding 5q-syndrome. Patients with dys Mgk ⩾70% or dys Mgk of 40–70% showed a more unfavorable prognosis than patients with dys Mgk of 10–40% or dys Mgk<10% (OS, $P<0.001$; LFS, $P<0.001$). Patients with dys Mgk ⩾70% had a more unfavorable prognosis than patients with dys Mgk of 40–70% (OS, $P=0.003$; LFS, $P=0.114$). However, there was no prognostic difference between the patients with dys Mgk of 10–40% and patients with dys Mgk<10% (OS, $P=0.277$; LFS, $P=0.881$). (Figure 2) Most of the patients with mMgk+ had dys Mgk⩾40%. Of the patients with dys Mgk<40%, only one patient had mMgk+. The prognostic effect of mMgk+ might relate to the prognostic difference between patients with dys Mgk ⩾40% and patients with dys Mgk<40%. To clarify this point, we compared the OS and LFS between patients showing dys Mgk⩾40% without mMgk+ (dys Mgk⩾40%/mMgk–) and patients with dys Mgk<40%. However, patients with dys Mgk⩾40%/mMgk– had a more unfavorable prognosis than patients with dys Mgk<40% (median survival: dys Mgk⩾40%/mMgk–, 76 months; dys Mgk<40%, 217 months; $P=0.001$, 10%; LFS: dys Mgk⩾40%/mMgk–, 20 months; dys Mgk<40%, not reached; 25% LFS: dys Mgk⩾40%/mMgk–, 38 months; $P<0.001$). In addition, dys Mgk⩾40% and mMgk+ 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⩾10%. Categories B1, B2 and B3 are defined as dys G⩾10%, dys Mgk⩾40% and mMgk+, 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

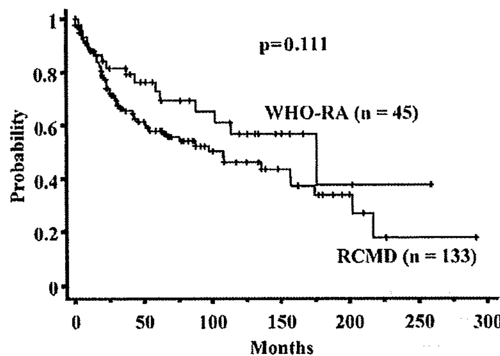

Figure 1 Cumulative overall survival and leukemia-free survival of WHO-RA and RCMD patients according to the original WHO classification. In overall survival, the RCMD patients, according to original WHO classification, did not show a more unfavorable prognosis than the original WHO-RA patients ($P=0.111$).

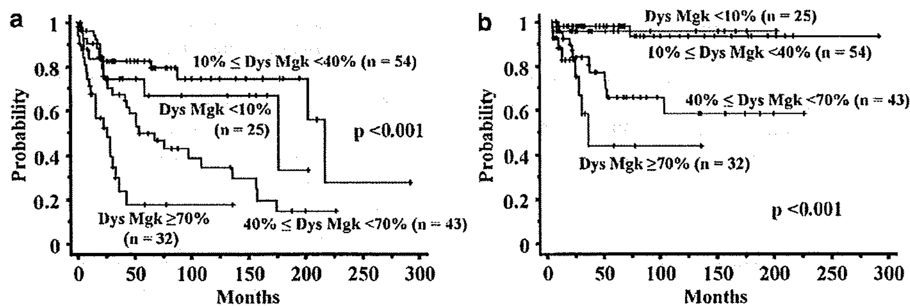

Figure 2 Cumulative overall and leukemia-free survival and frequency of dysmegakaryopoiesis in patients with FAB-RA, excluding 5q-syndrome. **(a)** Overall survival (OS). **(b)** Leukemia-free survival (LFS). **(a)** Patients with dys Mgk ⩾70% or dys Mgk of 40–70% showed a more unfavorable OS than patients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