Authorship and Disclosures

MY designed and co-ordinated the study, analyzed the data, and wrote the paper; JT, NU, FY, SM, and IJ designed the study, and provided patient sample and clinical data; IS, HA, KN, YU, MT, and AM provided patient sample and clinical data; HN co-ordinated the study, and revised the paper. YM provided patient sample and clinical data, and engaged in data management. SO designed the study, provided patient sample and clinical data, and engaged in data management; KM designed the study, and analyzed the data; TN chaired the study group, co-ordinated the study, and revised the paper; RO served as the principal investigator, chaired the study group, and revised the paper. All authors reviewed the paper, interpreted the results, and approved the final version. The authors reported no potential conflicts of interest.

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CASE REPORT

Successful cord blood transplantation for mycosis fungoides

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Abstract A 26-year-old female diagnosed as mycosis fungoides (MF, clinical stage IV) was treated with single-agent chemotherapy, multi-drug chemotherapy and unrelated bone marrow transplantation with reduced-intensity conditioning (engraftment failure), resulting in failure. Unrelated cord blood transplantation (CBT) as second transplantation following myeloablative conditioning brought complete remission (CR), but relapse of MF occurred 3 months after transplantation. However, discontinuation of immune suppressant led to the regression of MF regions and to second CR that continued for more than 23 months. This is the first report of successful CBT for MF, suggesting the graft-versus-MF effect in a setting of CBT.

Keywords Mycosis fungoides · Cord blood transplantation · Graft-versus-lymphoma effect

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Mycosis fungoides (MF) is a cutaneous T-cell lymphoma associated with the invasion of transformed mature T-cells into the skin demonstrating polymorphic atrophic patches, plaques, to generalized erythrodermia. In general, the prognosis for advanced MF patients with metastasis to other sites has been reported to be poor even when treated with systemic therapies [1, 2]. Several reports of allogeneic hematopoietic stem cell transplantation (allo-HSCT) with both myeloablative and reduced-intensity conditioning regimens [3–5] suggest the efficacy of allo-HSCT for MF through a graft-versus-lymphoma (GVL) effect [6].

In June 2004, a 26-year-old woman was admitted to our hospital because of generalized erythrodermia, a skin tumor of the head, and multiple lymphadenopathy. Her medical history started from 1996 with itchy erythema diagnosed as parapsoriasis in January 2001. Skin tumors developed 3 years later on the head, diagnosed as MF on biopsy. In June 2004, her disease status advanced with generalized multiple skin tumors and lymphadenopathy, eosinophilia (20% of WBC), and the elevation of LDH (367 IU/L, normal range 119–229). Lymph node biopsy and bone marrow analysis revealed the invasion of abnormal T cells, leading to a diagnosis of stage IV MF. Since systemic combination (biweekly CHOP, 8 cycles) or low-dose chemotherapy did not elicit any clinical response, allo-HSCT was considered appropriate for the treatment of this patient. In April 2005, allogeneic bone marrow transplantation from an unrelated donor was performed after reduced-intensity conditioning (fludarabine at 25 mg/m^2 day^{-1} for 5 days and melphalan at 70 mg/m^2 day^{-1} for 2 days) infusing 2.9×10^8 cells/kg of bone marrow cells, which resulted in the rejection of donor cells. MF lesions that showed temporal regression after conditioning recurred within 5 weeks after transplantation. Another chemotherapy regimen with cladribine and etoposide did not lead to any

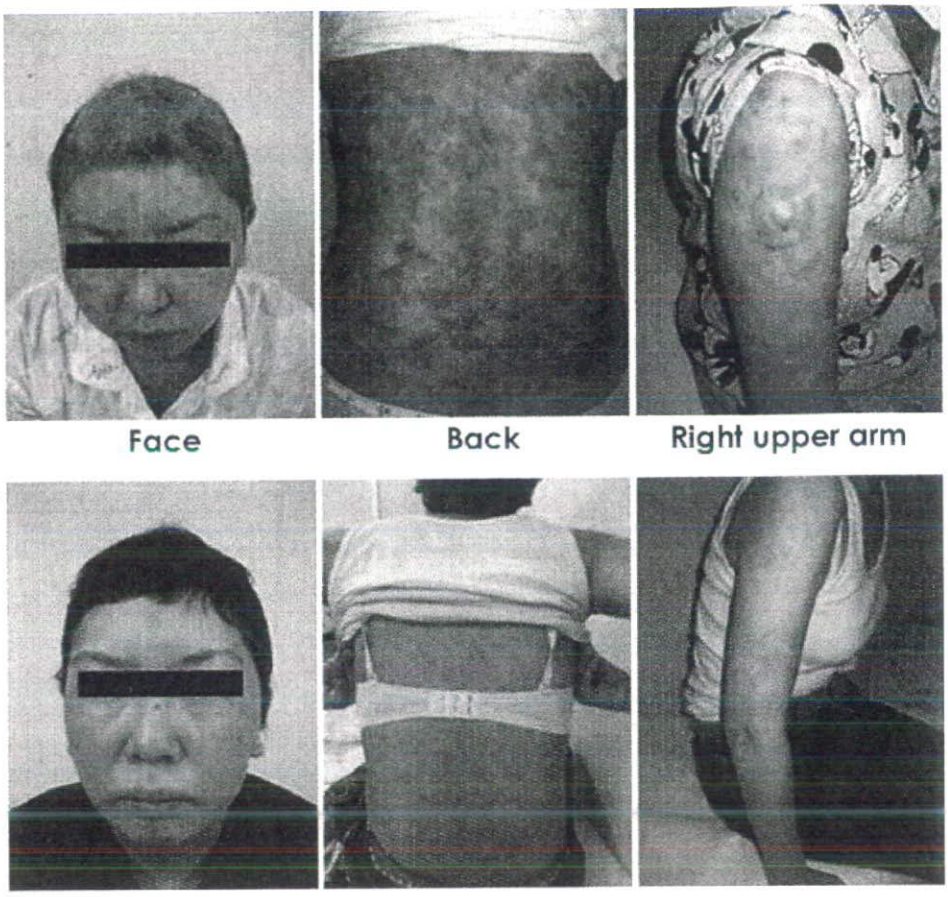

Fig. 1 Skin lesions of MF before CBT (**a**), and those after the discontinuation of tacrolimus (**b**). Skin tumors (*on the head, right eyelid, back,* and *upper arm*) and erythroderma markedly improved (**b**)

improvement of MF after the first transplantation. She had multiple skin tumors with generalized erythrodermia and lymphadenopathy.

Considering the refractory nature of MF in this patient, we decided to perform a second allo-HSCT. In August 2005, after total body irradiation (12 Gy, 6 fractions) and cyclophosphamide (60 mg/kg day^{-1}, 2 days), cord blood (2.2×10^7 cells/kg, HLA 2 loci mismatched, from a male donor) from the Japanese Cord Blood Bank Network was transplanted. For prophylaxis for graft-versus-host disease (GVHD), tacrolimus (0.03 mg/kg, continuous infusion) was used as a single agent. Neutrophils recovered on day 14, and engraftment was confirmed in bone marrow by FISH analysis of sex chromosomes. Platelet recovery (>50,000 per mm^3 without transfusion) was observed on day 41. In terms of MF regions, skin tumors, erythrodermia, and lymphadenopathy began to diminish during conditioning, and disappeared by the time of engraftment, achieving clinical complete remission. Around day 85 after transplantation, skin tumors appeared again on both her legs with itchy skin regions, along with multiple duodenal ulcers (by endoscopic examination) and multiple areas of lymph node swelling (neck, axilla, mediastinum, and para-aorta by CT scan). Skin tumor biopsy confirmed the relapse of MF, and histological analysis of the duodenal ulcer strongly suggested EB virus-associated lymphoproliferative disease. Tacrolimus was reduced and discontinued within 2 weeks; then, skin tumors and skin lesions showed a gradual decrease in size and completely diminished by day 140 (Fig. 1). No chemotherapy was added. There was no clear sign of acute or chronic GVHD even after the discontinuation of tacrolimus. There was no sign of MF on her skin and no lymphadenopathy on CT scan at more than 23 months after the second CR, with a Karnofsky score of 90%.

Several groups described that neither conventional chemotherapy nor high-dose chemotherapy with autologous stem cell support was sufficient for the long-term remission of MF [7, 8]. Based on the successful reports of allo-HSCT for MF and the efficacy of the withdrawal of immunosuppressants for some relapsed MF cases, the important role of the GVL effect for the control of MF is suggested [9, 10]. This is the first report of successful CBT for advanced MF with the graft-versus-MF effect. Since cord blood is available for many patients through cord blood banks and the waiting period is relatively short, CBT could be a therapeutic option for MF patients who are candidates for allo-HSCT but lack suitable related or unrelated donors.

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Prospective monitoring of *BCR-ABL1* transcript levels in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia undergoing imatinib-combined chemotherapy

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Summary

The clinical significance of minimal residual disease (MRD) is uncertain in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) treated with imatinib-combined chemotherapy. Here we report the results of prospective MRD monitoring in 100 adult patients. Three hundred and sixty-seven follow-up bone marrow samples, collected at predefined time points during a uniform treatment protocol, were analysed for *BCR-ABL1* transcripts by quantitative reverse transcription polymerase chain reaction. Ninety-seven patients (97%) achieved complete remission (CR), and the relapse-free survival (RFS) rate was 46% at 3 years. Negative MRD at the end of induction therapy was not associated with longer RFS or a lower relapse rate ($P = 0{\cdot}800$ and $P = 0{\cdot}964$ respectively). Twenty-nine patients showed MRD elevation during haematological CR. Of these, 10 of the 16 who had undergone allogeneic haematopoietic stem cell transplantation (HSCT) in first CR were alive without relapse at a median of 2·9 years after transplantation, whereas 12 of the 13 who had not undergone allogeneic HSCT experienced a relapse. These results demonstrate that, in Ph+ ALL patients treated with imatinib-combined chemotherapy, rapid molecular response is not associated with a favourable prognosis, and that a single observation of elevated MRD is predictive of subsequent relapse, but allogeneic HSCT can override its adverse effect.

Keywords: acute lymphoblastic leukaemia, Philadelphia chromosome, *BCR-ABL1*, imatinib, minimal residual disease.

The recent development of imatinib-combined chemotherapy has drastically improved overall treatment results in Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) (Ottmann & Wassmann, 2005; Yanada & Naoe, 2006; Thomas, 2007). Nearly 95% of newly diagnosed patients now achieve complete remission (CR) (Thomas *et al*, 2004;

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Lee *et al*, 2005; Wassmann *et al*, 2006; Yanada *et al*, 2006). However, outcome after CR depends on the individual patient and is not predictable. Young patients generally undergo allogeneic haematopoietic stem cell transplantation (HSCT) after achieving CR if a suitable donor is available, based on the concept that it is the established treatment with curative potential for this disease (Cornelissen *et al*, 2001; Dombret *et al*, 2002; Stirewalt *et al*, 2003; Yanada *et al*, 2005). Nevertheless, a fraction of patients experience a relapse even prior to transplantation, whereas some remain alive in remission for years without undergoing HSCT.

Minimal residual disease (MRD), as measured by reverse transcription-polymerase chain reaction (RT-PCR) or flow cytometry, has been shown to be useful for predicting prognosis in paediatric (Brisco *et al*, 1994; Cave *et al*, 1998; Coustan-Smith *et al*, 1998; van Dongen *et al*, 1998; Dworzak *et al*, 2002; Nyvold *et al*, 2002; Zhou *et al*, 2007) and adult ALL patients (Brisco *et al*, 1996; Mortuza *et al*, 2002; Vidriales *et al*, 2003; Bruggemann *et al*, 2006; Raff *et al*, 2007). However, the utility of MRD as a prognostic indicator has been established on the basis of data from patients treated with chemotherapy alone, and it remains to be determined whether it is useful in patients treated with chemotherapy in combination with imatinib. The Japan Adult Leukemia Study Group (JALSG) recently conducted a phase II trial of imatinib-combined chemotherapy in newly diagnosed Ph+ ALL patients (Towatari *et al*, 2004; Yanada *et al*, 2006, 2008). In that trial, *BCR-ABL1* transcript levels in bone marrow were prospectively monitored at predetermined time points using quantitative real-time RT-PCR (RQ-PCR). The results are presented here, with particular emphasis on the prognostic significance of rapid MRD clearance and MRD kinetics.

Patients and methods

Patients

The patient eligibility requirements of the phase II trial were as follows: newly diagnosed with Ph+ ALL, aged 15–64 years, an Eastern Cooperative Oncology Group performance status of 0–3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration. The protocol was reviewed and approved by the institutional review boards of all of participating centres and was conducted in accordance with the Declaration of Helsinki. This trial was registered at http://www.clinicaltrials.gov as #NCT00130195.

The treatment schedule is summarized in Table I. Allogenic HSCT was allowed after achieving CR if the patient had a suitable donor. The original target sample size was 77 patients (Yanada *et al*, 2006), with the CR rate defined as the primary endpoint. Eighty patients had been enrolled by January 2005, when enrolment was extended to 100 patients to attain a more precise point estimate of the overall survival (OS) rate. This sample size enabled the lower limit of the 95% confidence interval (CI) of OS rate (expected to be 70% at 1 year) to be higher than 60%.

MRD evaluation

Molecular monitoring was performed with use of the RQ-PCR assay in a single independent laboratory. Bone marrow samples were collected at diagnosis; at days 28 and 63 of the induction course; after the first, second, fifth and sixth consolidation courses; after 1 year of treatment; and at the end of therapy (2 years from the date of CR).

Total RNA was extracted from mononuclear cells using the QIAamp RNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of RNA were measured by spectrophotometric determination of the A260/A280 ratio. Total RNA (1·5 μg) was transcribed to cDNA in a 22·5-μl reaction mixture containing 500 ng of random hexamer (Invitrogen, Carlsbad, CA, USA), 50 units of reverse transcriptase (Invitrogen), 40 units of RNase inhibitor (Invitrogen) and 500 μmol/l dNTP. The reaction mixture (total volume: 50 μl) contained 7·5 μl of a 22·5-μl RNA mixture (corresponding to 500 ng of RNA), 15 pmol of forward and reverse primers, 10 pmol of TaqMan probe, and 25 μl of 2× TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA, USA). The primer and probe sequences have been described elsewhere (Towatari *et al*, 2004). Amplification was carried out with an initial activation of the polymerase at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles consisting of two steps: 95°C for 15 s and 60°C for 1 min. Fluorescent emission spectra were monitored every 7 s and analysed using the PRISM 7700 system with SEQUENCE DETECTION SYSTEM software (version 1.7; Applied Biosystems). Amplified cDNA fragments were cloned into the pCRII vector (Invitrogen) and used as the reference standard. The copy number of each plasmid was calculated from the DNA concentration (determined by measuring A260) and the molecular weight of the plasmid. The copy number of the *BCR-ABL1* transcripts was calculated by comparing the C_t values of samples with those of the standard and converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The threshold for quantification was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}. Detectable MRD levels below this threshold were referred to as '<50 copies/μg' to distinguish from undetectable MRD. Nested PCR was not performed in this study. Samples with *GAPDH* levels below $5{\cdot}7 \times 10^5$ copies/μg RNA were not eligible for MRD evaluation.

Statistical analysis

Relapse-free survival (RFS) was defined as the time from CR to relapse, death, or last follow-up, and OS was defined as the time from registration to death or last follow-up. A Kaplan–Meier survival analysis was performed to estimate the probabilities of RFS and OS, with differences between the curves

Table I. Treatment schedule.

Drug	Dose	Route	Days
Induction			
Cyclophosphamide	1200 mg/m^2 (800 mg/m^2)*	IV (3 h)	1
Daunorubicin	60 mg/m^2 (30 mg/m^2)*	IV (1 h)	1–3
Vincristine	1·3 mg/m^2†	IV (bolus)	1, 8, 15, 22
Prednisolone	60 mg/m^2	PO	1–21 (1–7)*
Imatinib	600 mg	PO	8–63
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	29
Consolidation #1			
Methotrexate	1 g/m^2	IV (24 h)	1
Cytarabine	2 g/m^2 (1 g/m^2)* twice a day	IV (3 h)	2, 3
Methylprednisolone	50 mg twice a day	IV (bolus)	1–3
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #2			
Imatinib	600 mg	PO	1–28
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #3	Repeat #1		
Consolidation #4	Repeat #2		
Consolidation #5	Repeat #1		
Consolidation #6	Repeat #2		
Consolidation #7	Repeat #1		
Consolidation #8	Repeat #2		
Maintenance‡			
Vincristine	1·3 mg/m^2†	IV (bolus)	1
Prednisolone	60 mg/m^2	PO	1–5
Imatinib	600 mg	PO	1–28

IV, intravenously; PO, orally; IT, intrathecally.
*For patients aged 60 and older.
†Maximum 2·0 mg.
‡Repeated every 4 weeks up to 2 years from the date of complete remission.

qualified with the log-rank test. The cumulative incidence of relapse was calculated with death during CR considered as a competing risk, and differences between the curves were qualified with Gray's test. STATA version 8 software (StataCorp, College Station, TX, USA) and R software version 2.4.0 (The R Foundation for Statistical Computing, http://www.r-project.org) were used for statistical analyses. P values ≤0·05 were considered to be statistically significant.

Results

Patients and treatment results

The median patient age was 45 years (range 15–64 years); 55 were male and 45 were female. Twenty-five patients were positive for major *BCR-ABL1*, and 75 for minor *BCR-ABL1*. Ninety-seven patients (97%) achieved CR. The median and maximum follow-up periods were 3·2 and 5·1 years respectively. The outcomes of 100 patients are detailed in Fig 1. Relapse occurred in 38 patients after a median CR duration of 7·3 months (range 2·1–37·4). Allogeneic HSCT was performed in 60 patients during first CR, and in 19 patients beyond first CR. For patients allografted in first CR, the median time to HSCT was 5·3 months (range 2·2–17·1). No patient underwent autologous HSCT. The probability of OS for the entire cohort was 55% at 3 years. The 1-year OS rate, the endpoint for the study extension, was 83% (95% CI 74–89%). Among the 97 patients who achieved CR, the probability of RFS was 46% at 3 years. Neither transcript types nor copy numbers at diagnosis were associated with RFS (P = 0·709 and P = 0·851 respectively).

MRD kinetics

The number of patients who underwent MRD monitoring decreased with time because of prior relapse, death, or transfer to allogeneic HSCT. Thus, the total number of follow-up samples was 367 (77% of all possible samples at all time points): 86 of 98 (88%) at day 28, 85 of 97 (88%) at day 63, 75 of 90 (83%) after the first consolidation (C#1), 55 of 73 (75%) after C#2, 31 of 38 (82%) after C#5, 22 of 32 (69%) after C#6, 11 of 15 (73%) at 1 year, and 2 of 9 (22%) at 2 years.

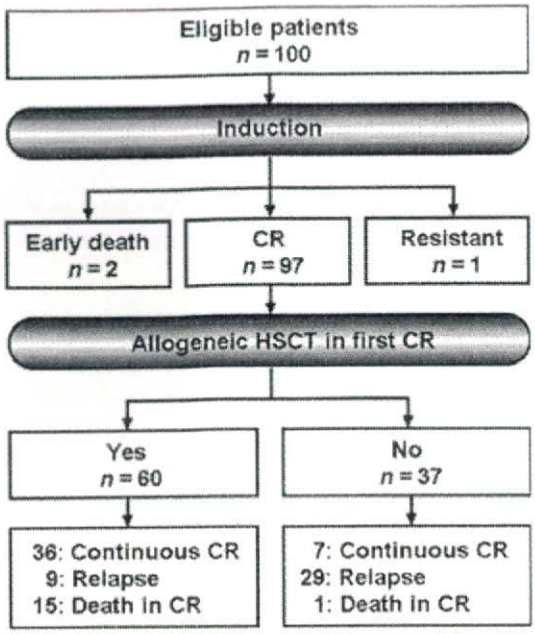

Fig 1. Flow diagram showing patient outcomes. CR, complete remission; HSCT, haematopoietic stem cell transplantation.

Figure 2 shows the percentages of patients with negative and low (<50 copies/μg) MRD levels at each time point. There was a progressive increase in the percentage of patients with negative MRD during the early treatment courses, with 24% at day 28, 48% at day 63, 68% after C#1, and 67% after C#2. Nearly all samples measured at 1 year and at 2 years were negative for MRD, although only a small number of samples were analysed at these time points. The only patient whose MRD was positive (87 copies/μg) at 1 year experienced a relapse 8 months later. All of the three patients who experienced a relapse during maintenance therapy had showed MRD elevation prior to haematological relapse.

Rapid MRD clearance and outcome

RQ-PCR results at the end of induction therapy (day 63) were available for 85 patients. One patient with a *BCR-ABL1* level of 160 000 copies/μg failed to achieve CR. Figure 3 shows the RFS rates and cumulative incidences of relapse in 84 CR patients according to MRD detection at day 63. PCR negativity was not associated with a higher RFS rate (46% vs. 42% at 3 years, $P = 0{\cdot}800$; Fig 3A) or a lower relapse rate (40% vs. 41% at 3 years, $P = 0{\cdot}964$; Fig 3B). A relatively small number of patients ($n = 11$) whose MRD levels exceeded 1000 copies/μg at day 63 had trends toward lower RFS ($P = 0{\cdot}092$, Fig 4A) and higher relapse rate ($P = 0{\cdot}070$, Fig 4B). Neither PCR negativity at day 28 nor after C#1 was associated with higher RFS ($P = 0{\cdot}867$ and $P = 0{\cdot}549$) or lower relapse rates ($P = 0{\cdot}796$ and $P = 0{\cdot}667$).

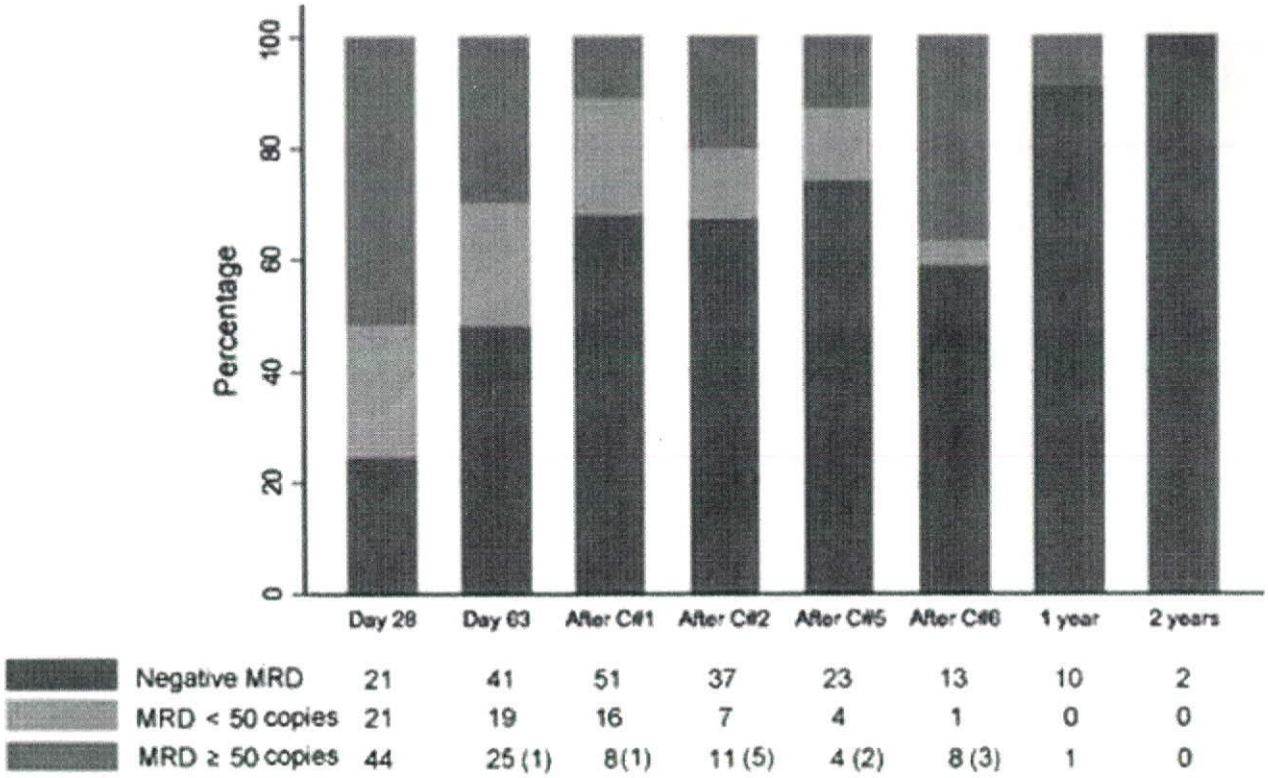

Fig 2. Frequencies of negative and low (<50 copies/μg RNA) *BCR-ABL1* transcript levels at each time point. Figures in parentheses represent the number of patients who developed haematological relapse at that time point.

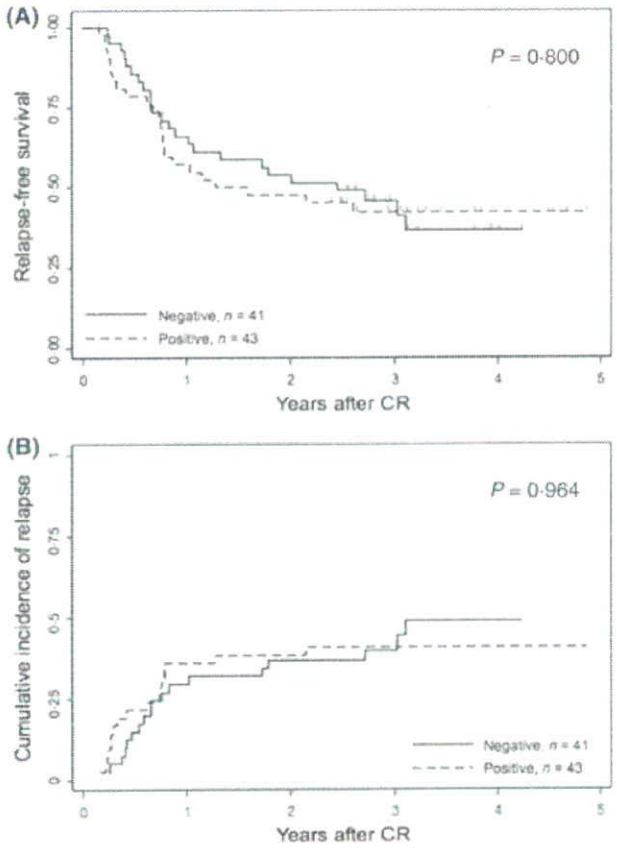

Fig 3. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with negative and positive *BCR-ABL1* transcript levels at the end of induction therapy.

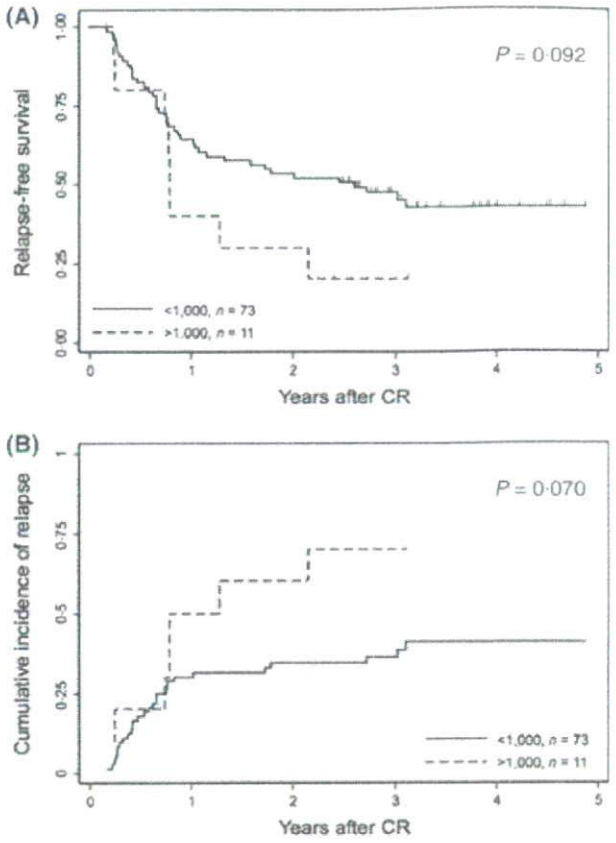

Fig 4. Relapse-free survival (A) and cumulative incidence of relapse (B) in patients with *BCR-ABL1* transcript levels below or above 1000 copies/μg RNA at the end of induction therapy.

MRD elevation during haematological remission

Elevated MRD levels during CR were documented in 29 patients. Of these, six patients experienced MRD elevation twice, and the second elevation was accompanied by simultaneous haematological relapse in five patients. The outcome and duration from the first observation of MRD elevation to relapse or allogeneic HSCT, whichever came first, in each patient are presented in Table II. Sixteen underwent allogeneic HSCT in first CR. The median duration from the first documentation of elevated MRD to allogeneic HSCT was 2·3 months (range 0·4–5·6). Death during first CR and relapse after transplantation occurred in three patients each, and 10 remained in first CR at a median of 2·9 years (range 2·0–4·6 months) after transplantation. In contrast, among the 13 non-transplantation patients, 12 had a relapse at a median of 2·0 months (range 0·5–35·0) after the first MRD elevation. Another patient once achieved PCR negativity after C#2, but showed detectable MRD below the threshold (<50 copies/μg) after C#5. However, MRD became negative after C#6, and the patient remained alive without relapse at 2·8 years after MRD elevation. The conversion from negative MRD to '<50 copies/μg' was observed in another six patients. Four remained in first CR after undergoing allogeneic HSCT, and the remaining two who had not undergone HSCT experienced a relapse.

Discussion

Minimal residual disease levels at various time points in CR, especially at the end of induction therapy, are considered an important prognostic factor in ALL (Pui *et al*, 2008). Although there were few studies that focused on Ph+ ALL with a relatively large number of patients (Dombret *et al*, 2002; Pane *et al*, 2005), Pane *et al* (2005) reported that significant reductions in *BCR-ABL1* levels after induction and consolidation therapy were associated with better outcomes. Most published studies on imatinib-combined chemotherapy include MRD findings (Thomas *et al*, 2004; Towatari *et al*, 2004; Lee *et al*, 2005; Rea *et al*, 2006; Wassmann *et al*, 2006; Yanada *et al*, 2006; de Labarthe *et al*, 2007; Ottmann *et al*, 2007a), but the prognostic significance of early treatment response remains to be determined. Our data remarkably demonstrated that the RFS rate for the patients with negative MRD at the end of induction therapy was similar to that for patients with positive MRD. We considered the possibility that this lack of difference was influenced by the confounding effect of allogeneic HSCT.

Table II. Outcome of patients who experienced an MRD elevation during haematological CR.

UPN	Outcome	Months from MRD elevation to relapse	UPN	Outcome	Months from MRD elevation to HSCT	Outcome after HSCT
63	CCR without HSCT	–	36	HSCT	1·0	CCR
17	Relapse	0·5	72	HSCT	1·0	CCR
43	Relapse	0·9	12	HSCT	2·1	CCR
50	Relapse	1·5	77	HSCT	2·2	CCR
58	Relapse	1·5	10	HSCT	2·6	CCR
82	Relapse	1·6	1	HSCT	2·9	CCR
62	Relapse	1·9	81	HSCT	3·3	CCR
14	Relapse	2·0	94	HSCT	3·6	CCR
85	Relapse	3·6	55	HSCT	4·8	CCR
56	Relapse	4·3	8	HSCT	5·1	CCR
51	Relapse	7·9	34	HSCT	0·4	Relapse
60	Relapse	8·4	87	HSCT	2·0	Relapse
18	Relapse	35·0	47	HSCT	2·4	Relapse
			48	HSCT	1·9	NRM
			16	HSCT	2·0	NRM
			49	HSCT	5·6	NRM

UPN, unique patient number; MRD, minimal residual disease; HSCT, haematopoietic stem cell transplantation; CCR, continuous complete remission; NRM, non-relapse mortality.

However, MRD negativity was not beneficial in terms of relapse rate ($P = 0{\cdot}964$) or even in terms of RFS after we censored patients who underwent allogeneic HSCT at the time of transplantation ($P = 0{\cdot}470$). A trend toward a higher relapse rate in the 11 patients (13%) with MRD levels of ≥1000 copies/μg suggests that MRD levels at the end of induction therapy may be helpful in identifying a small subgroup of patients at high risk for relapse. However, the finding that negative MRD was not associated with a favourable outcome precludes prognostication of the remaining majority of patients, and indicates that relapse risk in these patients depends on factors unrelated to initial treatment response. Acquisition of resistance during treatment may explain why rapid molecular response is not prognostically relevant.

Another important finding of this study was the significant relationship between MRD elevation and relapse. This finding is in accordance with those of several studies published in the 1990s in which the conversion from negative to positive RT-PCR results was associated with subsequent relapse in Ph+ ALL patients (Miyamura *et al*, 1992; Preudhomme *et al*, 1997; Radich *et al*, 1997; Mitterbauer *et al*, 1999). Our results suggest that an increase in the MRD level at a single time point is predictive of subsequent relapse, but such patients can be successfully treated with allogeneic HSCT. Given a median duration of only 2 months from MRD elevation to haematological relapse, an alternative therapeutic intervention should be considered immediately after MRD elevation. Because of its rapid availability, cord blood transplantation may be a practical treatment option for patients without a related donor, if they are fit for the procedure. Switching from imatinib to other novel tyrosine kinase inhibitors, such as dasatinib (Talpaz *et al*, 2006; Ottmann *et al*, 2007b) and nilotinib (Kantarjian *et al*, 2006), may also be a reasonable option for patients without a mutation resistant to these agents. Additionally, frequent MRD monitoring increases the chances of detecting MRD elevation during CR, prolonging the duration prior to haematological relapse, and enabling the use of alternative therapies in patients who would otherwise experience an overt relapse.

When MRD data are analysed in relation to outcome, differences in conditions such as treatment and sampling time points can affect results. In this regard, strength of this study is that all samples were collected at scheduled time points during a uniform treatment protocol. On the other hand, one limitation of our study is that samples were not obtained from all patients at all time points. Nevertheless, the percentage of available samples collected at the end of induction therapy was 86% (84 of the 97 CR patients). Furthermore, sample availability did not seem to be a significant source of selection bias: we found no difference in RFS between patients whose samples were available or not at the end of induction therapy ($P = 0{\cdot}345$). Also the utility of MRD elevation in predicting subsequent relapse would have been strengthened if the proportion of missing samples had been smaller. Finally, it may be disputed that our detection method was partly different from those used in other countries, specifically in that results were reported as a copy number normalized by the control gene and in that PCR negativity was not confirmed by nested PCR. Nevertheless, we believe this point would not impair our main results.

In summary, our prospective MRD monitoring of Ph+ ALL patients treated with imatinib-combined chemotherapy revealed that rapid molecular response is not associated with a superior prognosis and that a single observation of elevated

MRD is strongly predictive of subsequent relapse but allogeneic HSCT can override its adverse effect. Such patients may also benefit from novel tyrosine kinase inhibitors. We conclude that frequent MRD monitoring is beneficial in clinical decision making for Ph+ ALL patients treated with imatinib-combined chemotherapy. Incorporating MRD data into a treatment protocol will be necessary in future clinical trials of Ph+ ALL.

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Japanese epidemiological survey with consensus statement on Japanese guidelines for treatment of iron overload in bone marrow failure syndromes

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Abstract Many patients with bone marrow failure syndromes need frequent transfusions of red blood cells, and most of them eventually suffer from organ dysfunction induced by excessively accumulated iron. The only way to treat transfusion-induced iron overload is iron chelating therapy. However, most patients have not been treated effectively because daily/continuous administration of deferoxamine is difficult for outpatients. Recently, a novel oral iron chelator, deferasirox, has been developed, and introduction of the drug may help many patients benefit from iron chelation therapy. In this review, we will discuss the current status of iron overload in transfusion-dependent patients, and the development of Japanese guidelines for the treatment of iron overload in Japan, which were established by the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan.

Keywords Bone marrow failure syndrome · Iron overload · Iron chelation · Guidelines

1 Introduction

Many patients with aplastic anemia (AA) or myelodysplastic syndromes (MDS) need frequent transfusions of red blood cells (RBCs). One unit (derived from 200 mL of whole blood) of RBC transfusion in Japan contains about 100 mg of iron. Because there is no physiological mechanism for iron excretion in humans, and daily iron excretion is no more than 1 mg in a healthy man, repeated RBC transfusions will soon result in iron overload. Excess iron is mainly deposited in the liver, heart and pancreas, and causes organ dysfunction [1, 2].

As phlebotomy is not an option because of the underlying bone marrow failure, the only way to treat iron overload is by iron chelation therapy. However, difficulty in optimal administration of deferoxamine (DFO, Desferal®) in Japan has hampered effective chelation, and currently most patients are not treated effectively [3].

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Recently, a novel oral iron chelator, deferasirox (Exjade®), has been introduced in more than 60 countries, including Japan. The introduction of deferasirox may improve compliance with iron chelation therapy [4]. Under these circumstances, the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan drew up Japanese guidelines for the treatment of transfusion-induced iron overload. Herein, we describe the current status of iron overload in transfusion-dependent patients in Japan, and development of the proposed guidelines for the treatment of transfusion-induced iron overload.

2 Current status of transfusion-induced iron overload in Japan

In 2005, the first nationwide survey on iron overload in transfusion-dependent patients in Japan was carried out [3]. This retrospective survey investigated the outcomes of iron overload-related morbidity and mortality from August 2001 to December 2005. A questionnaire was sent to hematology departments in hospitals all over Japan, and 43 hospitals responded by returning data on 292 patients.

Demographic data showed that MDS and AA accounted for about 80% of the underlying diseases: MDS, 52.1%; AA, 30.8%; pure red cell aplasia (PRCA), 5.1%; and myelofibrosis (MF), 4.5%. Serum ferritin levels were significantly correlated with the lifetime total number of RBC transfusion units received. Figure 1 shows the relationship between the number of RBC units and mean ferritin level, indicating the percentage of patients with an abnormal ferritin level (≥1,000 ng/mL) for any total number of RBC units received as analyzed by a logistics model. The goodness-of-fit of this model between theoretical and actual values was assessed by Pearson chi-squared test, and the estimated number of RBC units required to raise ferritin to ≥1,000 ng/mL in 50 and 75% of patients was calculated as 21.5 and 43.4 units, respectively.

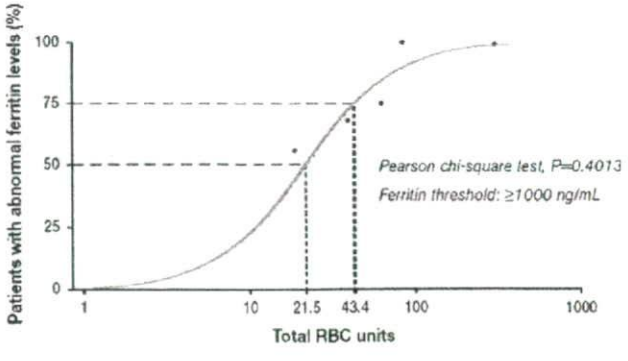

Fig. 1 Relationship between serum ferritin and total number of red blood cell units. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) abnormalities were significantly correlated with transfusion frequency and increased ferritin levels; there was a significantly ($P < 0.0001$) higher prevalence of SGOT and SGPT abnormality in patients with high serum ferritin than in those whose serum ferritin was <1,000 ng/mL (Fig. 2). Moreover, among patients in whom cardiac function was evaluated, abnormalities were found in 21.9%, and cardiac abnormality was weakly correlated with serum ferritin levels. These data indicate that ferritin levels can be a useful predictor of hepatic and cardiac dysfunction. Fasting blood sugar (FBS) abnormality was also correlated with transfusion frequency.

In the survey, 75 deaths were reported, most of which were caused by infection and leukemia. However, cardiac and hepatic failure was noted in 24% and 6.7% of cases, respectively. Patients who died from cardiac or hepatic failure had received more transfusions than those who died from other causes, and among 38 patients in whom serum ferritin levels were available, 37 patients died with serum ferritin levels ≥1,000 ng/mL; the majority of patients (24 patients) had serum ferritin levels >5,000 ng/mL. These data indicate that multiple transfusion therapy is associated with a high risk of fatal complications caused by iron overload. Recently, similar analyses have been reported describing that transfusion-dependent MDS patients show significantly shorter survival than those who do not require transfusions and that transfusion-induced iron overload significantly affects survival [5].

3 Iron chelation therapy

As phlebotomy is not an option because of underlying bone marrow failure, the only way to treat iron overload is with iron chelation therapy. Until recently, the only available iron chelating agent in Japan was DFO. Because of the limited absorption from the gastrointestinal tract and short biological half-life of the agent, the drug must be administered by parenteral injections at least 5–7 times a week, or continuously for optimal effectiveness [6]. In the survey, 43.2% of patients received DFO, but only 8.6% received DFO daily or continuously; most of the patients were administered the drug intermittently (average once per 1.9 weeks) or concurrently with transfusion [3]. While improvements in serum ferritin, SGOT, SGPT and FBS were noted in the patients who received DFO daily or continuously, these data did not improve, and rather worsened, in those without optimal administration (Table 1). This indicates that appropriate administration of the chelating agent is needed for sufficient therapeutic results.

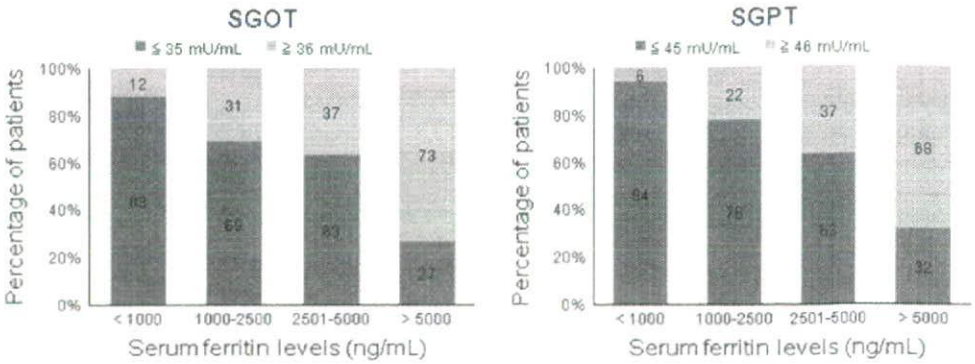

Fig. 2 Relationship between serum transaminase abnormality and serum ferritin levels. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Table 1 Average changes in laboratory values during the period of transfusion dependence in patients receiving deferoxamine treatment

Parameter	Intermittent (once/1.9 week)	Concurrent with transfusion	Daily/continuous
Serum ferritin[a,b] (ng/mL)	+2222.8 ($n = 36$)	+2204.8 ($n = 19$)	−1135.2 ($n = 9$)
SGOT[a,c] (mU/mL)	+28.0 ($n = 53$)	+40.0 ($n = 30$)	−9.2 ($n = 10$)
SGPT (mU/mL)	+28.6 ($n = 53$)	+10.3 ($n = 30$)	−28.8 ($n = 10$)
FBS (mg/dL)	+31.2 ($n = 31$)	+8.2 ($n = 12$)	−4.8 ($n = 5$)

[3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

[a] Intermittent versus continuous, $P < 0.05$

[b] Continuous versus concurrent, $P < 0.01$

[c] Continuous versus concurrent, $P < 0.05$

Moreover, it has also been reported that iron chelation not only reduced iron burden and improved organ dysfunction, but also ameliorated the hemoglobin levels of iron-overloaded patients [7, 8]. Although the biological mechanism of the hematopoietic recovery remains to be elucidated, this fact indicates that iron itself negatively impacts on hematopoiesis, and in some conditions removal of iron burden from the hematopoietic environment can restore normal hematopoiesis.

Deferasirox is easily absorbed in the gastrointestinal tract and has an elimination half-life of 8–16 h, which means that deferasirox is continuously present in the plasma with once-daily dosing [9]. In a large Phase III trial, deferasirox was comparable with DFO at decreasing iron burden in β-thalassemic patients [10]. Deferasirox also reduced iron burden in patients with various anemias including MDS [11]. These findings indicate that oral iron chelators can improve patients' quality of life by ameliorating organ dysfunction and preventing iron damage, even improving hematopoiesis itself. Oral iron chelators are expected to prolong survival of transfusion-dependent patients.

4 Japanese guidelines for the treatment of iron overload in transfusion-dependent patients

The clinical significance of iron chelation is undeniable and requires attention. With the availability of deferasirox in Japan, the frequency of continuous treatment may be strengthened and many more patients can benefit from chelation therapy. To help optimal iron chelation therapy, the National Research Group on Idiopathic Bone Marrow Failure Syndromes drew up the Japanese guidelines for the treatment of transfusion-induced iron overload. To date, guidelines for iron overload have been developed in several countries [6, 12–14], and the Japanese guidelines were designed to align with the international guidelines (see the paper by Dr. Gattermann in this issue). The essential features of the Japanese guidelines are depicted in Fig. 3 and Table 2.

The contents of the guidelines are as follows:

Patients who may benefit from chelation therapy: The guidelines are applicable to transfusion-dependent patients with primary (MDS, AA, PRCA, MF, etc.) and secondary (chemotherapy-induced, etc.) bone marrow failure. Transfusion-dependent patients are defined as those receiving >2 RBC units/month for ≥6 months. Because organ dysfunction becomes symptomatic after a certain period of time, it is suggested that iron chelation therapy is offered to patients with an expected survival of more than 1 year. The international guidelines for MDS patients also recommend that they should have a life expectancy of ≥1 year.

Diagnosis of iron overload: After patients become transfusion dependent, regular examination of serum ferritin is required to monitor iron burden at least once every 3 months. For early diagnosis of organ dysfunction,

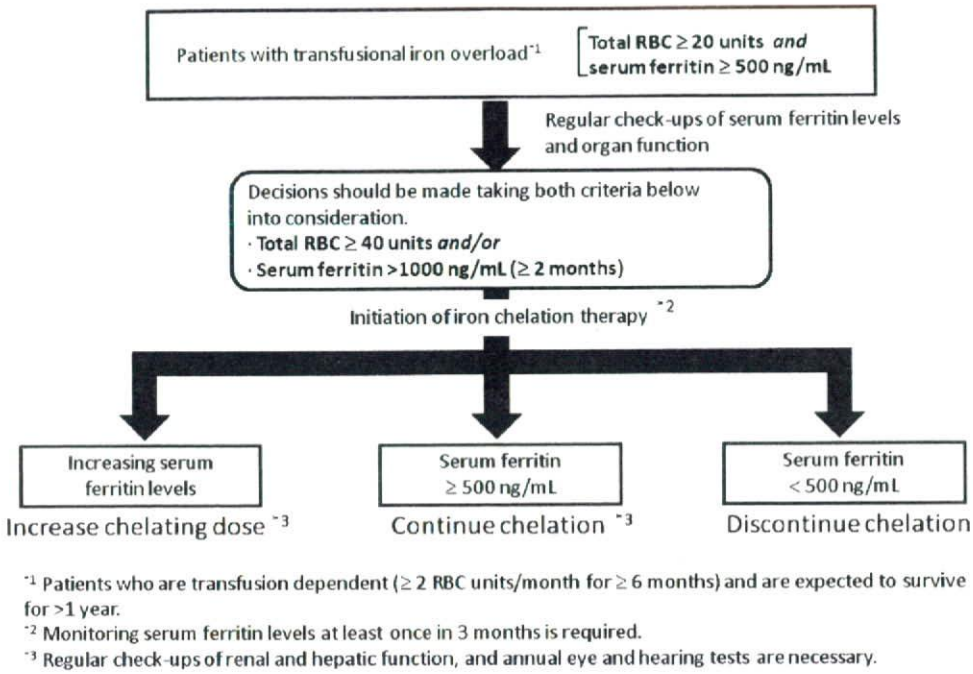

Fig. 3 A flow chart for the treatment of transfusion-dependent iron overload

*1 Patients who are transfusion dependent (≥ 2 RBC units/month for ≥ 6 months) and are expected to survive for >1 year.
*2 Monitoring serum ferritin levels at least once in 3 months is required.
*3 Regular check-ups of renal and hepatic function, and annual eye and hearing tests are necessary.

periodic check-ups of cardiac, hepatic and pancreatic endocrine functions are recommended.

Patients can be said to be iron overloaded when their serum ferritin levels reach >500 ng/mL and when they have received >20 Japanese RBC units (in pediatric patients, >50 mL/kg body weight). Severity of iron overload is determined by serum ferritin levels and organ dysfunction (Table 2, lower part).

Initiating iron chelation therapy: Administration of an iron chelator is the only recommended treatment for iron overload in patients with bone marrow failure. To initiate iron chelation therapy, confirmation of serum ferritin levels >1,000 ng/mL for more than 2 months, at least in two successive examinations, is recommended. The nationwide survey reported that more than 90% of patients who suffered from organ dysfunction had serum ferritin levels >1,000 ng/mL, and prevalence of hepatic dysfunction increases in parallel with ferritin levels [3] (Fig. 2). Therefore, a serum ferritin level >1,000 ng/mL is considered the appropriate point to initiate iron chelation. However, serum ferritin levels are not reliable in patients with inflammatory conditions such as Still's disease and hemophagocytic syndrome, or in those with malignancies. In these cases, transfusion history should be taken into account. Therefore, receiving a total of more than 40 Japanese RBC transfusion units (in pediatric patients, >100 mL/kg body weight) was included as another recommended criterion. As mentioned previously, about 75% of patients who received >40 RBC units have serum ferritin levels >1,000 ng/mL, indicating that 40 units of RBC transfusion can be a good indicator of transfusion-induced hyperferritinemia. However, transfusion history alone is also not reliable, because serum ferritin levels may not increase in patients with chronic bleeding and hemolysis. Furthermore, patients who have already discontinued transfusion therapy with successful treatment may not require iron chelation therapy. If neither of these two criteria is applicable, chelation therapy should not be started.

Target ferritin maintenance levels and adverse effects of iron chelators: During chelation therapy, monitoring of iron burden and organ functions should be continued. After initiating chelation therapy, serum ferritin levels should decrease, but if they continue to increase, even 3–6 months after starting treatment, an increase in dose is necessary. When patients are minimally transfusion dependent (<2 RBC units/month) or already free of transfusions, dose adjustment must be determined carefully.

It is recommended that serum ferritin levels are maintained at 500–1,000 ng/mL, and when ferritin levels are below 500 ng/mL at two successive examinations, chelators should be discontinued. As an excessive reduction in iron burden is harmful, the guidelines have determined this target value (500–1,000 ng/mL) with a safety margin.

As iron chelating agents can induce adverse effects on the kidney, liver and sensory organs [10], regular examination of renal and hepatic functions, and periodical (prior to treatment and annually after initiation) ophthalmologic examinations and hearing tests, are recommended. If an abnormal increase in serum creatinine level is noticed, the drug should be decreased or discontinued. In patients with a high risk of renal dysfunction, weekly monitoring of creatinine level is recommended, at least during the first

Table 2 Japanese guidelines for transfusional iron overload (main points)

Patients	Transfusion-dependent patients with bone marrow failure syndromes who are likely to survive for >1 year
Diagnosis of iron overload	1. Total RBC >20 units[a] (in pediatric patients, RBCs >50 mL/kg body weight) *and* 2. Serum ferritin >500 ng/mL
Criteria for initiating chelation therapy	1. Total RBC >40 units[a] (in pediatric patients, RBCs >100 mL/kg body weight) *and/or* 2. Serum ferritin >1,000 ng/mL Decisions should be made taking both criteria into consideration, especially for patients: –with chronic bleeding or hemolysis; –who no longer need RBC transfusions; –with complications that chronically raise serum ferritin levels independently of transfusion; e.g., Still's disease, hemophagocytic syndrome and malignancies
Target serum ferritin maintenance level	Serum ferritin 500–1,000 ng/mL

Classified severity of iron overload

Serum ferritin (ng/mL)	With normal organ function	With organ dysfunction
>500	Stage 1A	Stage 1B
>1,000	Stage 2A	Stage 2B
>2,500	Stage 3A	Stage 3B
>5,000	Stage 4A	Stage 4B

The severity of iron overload is defined by serum ferritin level and organ dysfunction (cardiac, liver and pancreatic endocrine dysfunction). The dysfunction must be considered to be related to iron overload; i.e., the organ dysfunction progresses as serum ferritin or transfusion burden increase

The criteria for specific organ dysfunction are as follows

–Cardiac dysfunction: LVEF <50%

–Hepatic dysfunction: abnormal transaminase levels, fibrosis and cirrhosis of the liver

–Pancreatic endocrine dysfunction: impaired glucose tolerance

[a] 20 and 40 units of the Japanese RBC transfusion correspond to 10 and 20 Western RBC units, respectively

month. Furthermore, if drug-induced hepatic injury is suspected, withdrawal of the drug with appropriate treatments is needed. It has been reported that iron chelators can cause hearing loss and cataracts. Therefore, if any signs of dysfunction are noticed a dose reduction or discontinuation of the drug is necessary and prompt consultation by an ophthalmologist or otorhinolaryngologist is required. In pediatric patients, annual monitoring of height, weight and state of secondary sex characteristics are needed for an early diagnosis of abnormal development.

5 Conclusions

The retrospective survey of transfusion-dependent patients revealed that the mortality rate is raised in heavily iron-overloaded patients, with liver and cardiac dysfunction being the primary cause of death [3]. Daily or continuous chelation therapy is effective in reducing iron burden and improving organ function, but practically, daily or continuous administration through parenteral injection is difficult. In Japan, a novel oral chelator, deferasirox, has recently been approved. Oral iron chelators can improve compliance of treatment and many more patients who need iron chelation may benefit from a reduction in iron burden and improvement of organ function, which ultimately may lead to the improvement of patients' prognosis and quality of life.

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ORIGINAL ARTICLE

Diagnosis of acute myeloid leukemia according to the WHO classification in the Japan Adult Leukemia Study Group AML-97 protocol

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Abstract We reviewed and categorized 638 of 809 patients who were registered in the Japan Adult Leukemia Study Group acute myeloid leukemia (AML)-97 protocol using morphological means. Patients with the M3 subtype were excluded from the study group. According to the WHO classification, 171 patients (26.8%) had AML with recurrent genetic abnormalities, 133 (20.8%) had AML with multilineage dysplasia (MLD), 331 (51.9%) had AML not otherwise categorized, and 3 (0.5%) had acute leukemia of ambiguous lineage. The platelet count was higher and the rate of myeloperoxidase (MPO)-positive blasts was lower in AML with MLD than in the other WHO categories. The outcome was significantly better in patients with high (≥50%) than with low (<50%) ratios of MPO-positive blasts ($P < 0.01$). The 5-year survival rates for patients with favorable, intermediate, and adverse karyotypes were 63.4, 39.1, and 0.0%, respectively, and 35.5% for those with 11q23 abnormalities ($P < 0.0001$). Overall survival (OS) did not significantly differ between nine patients with $t(9;11)$ and 23 with other 11q23 abnormalities ($P = 0.22$). Our results confirmed that the cytogenetic profile, MLD phenotype, and MPO-positivity of blasts are associated with survival in patients with AML, and showed that each category had the characteristics of the WHO classification such as incidence, clinical features, and OS.

Keywords AML · WHO classification · Myeloperoxidase · Multilineage dyplasia · 11q23 abnormalities

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1 Introduction

The French-American-British (FAB) classification of acute myeloid leukemia (AML), based on morphological and cytochemical findings, was established in 1976 and has since become the standard classification [1, 2]. However, specific chromosomal and genetic abnormalities that have been extracted from analyses of prognostic factors for AML are recognized as important in selecting treatment strategies and are reflected in the AML classification as

factors that are required to establish the disease entity [3]. The 1999 World Health Organization (WHO) classification includes morphological, immunological, cytogenetic, genetic, and clinical features [4–6]. The WHO and FAB classifications differ in several aspects. The blast threshold required for a diagnosis of AML was reduced from 30 to 20%, and new AML categories have been added for cytogenetic abnormalities, the presence of multilineage dysplasia (MLD), as well as a history of chemotherapy and subtypes for acute basophilic leukemia, acute panmyelosis with myelofibrosis, and myeloid sarcoma. The WHO classification comprises more subtypes and is more comprehensive than the FAB classification.

Cytogenetic features are important prognostic factors in AML [3, 7–12]. However, 11q23 abnormalities have not yet been established as a cytogenetic risk classification. Over 30 partner genes with 11q23 abnormalities have been described, and some reports indicate that patients with $t(9;11)$ have a relatively more favorable prognosis than those with other partner chromosomes/partner genes [13–16].

In the present study, we reviewed stained smears of blood and bone marrow from patients who were registered in the Japan Adult Leukemia Study Group (JALSG) AML-97 trial, and classified them into FAB subtypes and WHO categories. We also evaluated their survival on the basis of the WHO classification, the myeloperoxidase (MPO)-positivity of blasts, and cytogenetic findings including 11q23 abnormalities.

2 Patients and methods

2.1 Patients

Between December 1997 and July 2001, 809 patients aged from 15 to 66 years with untreated AML (excluding M3) were registered from 103 institutions in the AML-97 trial of the JALSG. The patients were diagnosed with AML according to the FAB criteria at each institution. Patients with a history of MDS, hematological abnormalities before the diagnosis of AML, or a history of chemotherapy were not eligible for the AML-97 trial.

2.2 Treatment strategies

Details of the JALSG AML-97 treatment protocol are described elsewhere [17]. In brief, all patients underwent induction therapy consisting of idarubicin (3 days) and Ara-C (7 days). Patients who achieved complete remission were randomized into one of two arms of consolidation chemotherapy alone or in combination with maintenance chemotherapy. Patients who were placed into intermediate/poor risk groups according to the JALSG scoring system [17] and who had an HLA-identical sibling ($\leq$50 years old) were simultaneously assigned to receive allogeneic hematopoietic stem cell transplantation during their first remission.

2.3 Morphologic and cytochemical analyses

Peripheral blood and bone marrow smears from registered patients were sent to Nagasaki University for staining with May-Giemsa, MPO, and esterase, and the diagnosis was then reevaluated by the Central Review Committee for Morphological Diagnosis. Patients were subsequently categorized according to the FAB and WHO classifications. Dyserythropoietic features were defined as >50% dysplastic features in at least 25 erythroblasts and dysgranulopoietic features including $\geq$3 neutrophils with hyposegmented nuclei (pseudo-Pelger–Heut anomaly), and hypogranular or agranular neutrophils (>50% of $\geq$10 neutrophils). Dysmegakaryopoietic features were defined as $\geq$3 megakaryocytes that were micronuclear, multiseparate nuclear, or large mononuclear [18].

We assessed the ratios (%) of MPO-positive blasts on MPO-stained bone marrow smears using the diaminobenzidine method [19].

2.4 Cytogenetic analysis

Cytogenetic analysis was performed at either laboratories in participating hospitals or authorized commercial laboratories. The karyotypes of leukemic cells were collected through the JALSG AML-97 case report forms and reviewed by the Central Review Committee for Karyotyping. The patients were classified into favorable, intermediate, or adverse risk groups based on karyotypes according to results of the Medical Research Council (MRC) AML 10 trial [3]. The favorable risk group included patients with $t(8;21)$ and inv(16), whether alone or in combination with other abnormalities. The intermediate risk group included those with a normal karyotype and other abnormalities that were not classified as either favorable or adverse. The adverse risk group included patients with a complex karyotype with four or more numerical or structural aberrations, −5, deletion (5q), and −7, whether alone or in combination with intermediate risk or other adverse risk abnormalities.

2.5 Statistical analysis

The overall survival (OS) for all patients was defined as the interval from the date of diagnosis to that of death. We applied the Kaplan–Meier method to estimate OS and