Table 2.
Transplantation Outcomes*

Patient No.	Time to ANC >0.5 × 10^9/L, d	Time to Platelets >20 × 10^9/L, d	Acute GVHD						Chronic GVHD (Involved Organs)	Follow-up, d	Current Disease Status	Cause of Death
			Grade	Skin	Liver	Gut	mPSL, mg/kg	Response				
1	16	26	IV	3	4	4	2	PG	NE	121	Dead	Acute GVHD
2	35	30	0	0	0	0	—	—	NE	133	Dead	Relapse
3	17	—	0	0	0	0	—	—	NE	56	Dead	Relapse
4	14	15	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	439	Dead	BO
5	15	22	I	1	0	0	—	—	Ext (skin, mouth, liver)	286	Dead	IP
6	21	38	II	3	0	0	—	—	NE	260	Dead	Relapse
7	14	25	I	2	0	0	—	—	Ext (mouth, liver)	687+	CR, alive	
8	20	30	II	3	1	0	1	PR	Ext (skin)	667+	CR, alive	
9	22	—	II	3	0	0	—	—	Ext (skin, mouth, eyes)	336	Dead	Organizing pneumonia
10	18†	—	0	0	0	0	—	—	NE	94	Dead	Secondary graft failure
11	16	23	0	0	0	0	—	—	Ext (skin, mouth)	564+	CR, alive	
12	16†	—	IV	2	4	3	2	PG	NE	69	Dead	Acute GVHD
13	18	23	I	1	0	0	1	CR	Ext (mouth, eyes, liver)	525+	CR, alive	
14	18	—	IV	3	4	2	1	UE	NE	64	Dead	Relapse
15	14	16	0	0	0	0	—	—	Ext (mouth, eyes)	511+	CR, alive	
16	26	112	0	0	0	0	—	—	Lim (mouth)	463+	CR, alive	
17	22	38	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	276	Dead	BO + aspergillosis

*ANC indicates absolute neutrophil count; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PG, progressive response; NE, not evaluable; CR, complete response; Ext, extensive disease; BO, bronchiolitis obliterans; IP, interstitial pneumonitis; PR, partial response; UE, unevaluated; Lim, limited disease.

†Secondary graft failure occurred after neutrophil recovery.

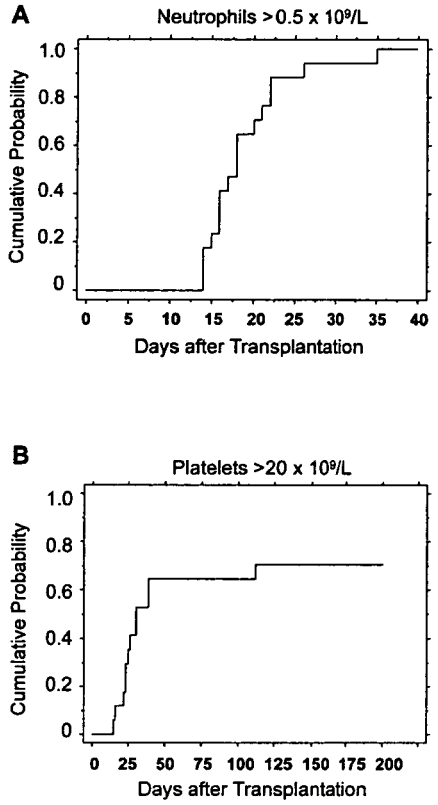

Figure 1. Engraftment after unrelated-donor bone marrow transplantation following reduced-intensity conditioning expressed as the cumulative probability of a neutrophil count >0.5 × 10^9/L (A) and a platelet count >20 × 10^9/L (B). All patients achieved neutrophil recovery, but 5 patients did not achieve platelet recovery. The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (15-112 days), respectively. Late graft failure was observed in 2 patients.

marrow from a donor with allele-level mismatches at 3 HLA loci. Two patients with grade IV acute GVHD involving only the skin were successfully treated with methylprednisolone. Grade II acute GVHD involving only the skin was treated solely with CsA in 2 patients (Table 2). In 7 patients without relapse or secondary graft failure, CsA was tapered from a median of day 120 (range, day 96-169). Only 2 of the 7 patients were able to discontinue CsA (at days 203 and 288). Chronic GVHD was documented in all patients who survived beyond day 100 (1 with limited GVHD, 9 with extensive disease). There was no significant correlation between HLA disparity at the allele level and the incidence of GVHD, although it was difficult to analyze the data statistically because of the small number of patients in this study.

3.5. Survival and Causes of Death

The median follow-up period was 286 days (range, 56-687 days). Overall, 11 patients died, but 6 patients are currently in remission (2 in remission and 4 not in remission at the time of transplantation). The estimated 100-day and 1-year nonrelapse mortality rates were 14% (95% CI, 12%-17%) and 46% (95% CI, 33%-57%), respectively (Figure 2B). Estimated 1-year overall survival and progression-free survival rates were both 41% (95% CI, 32%-51%; Figure 3). There were 4 deaths due to recurrent or progressive disease at a median time of 55 days (range, 32-93 days). The causes of the 7 treatment-related deaths included acute GVHD (n = 2), secondary graft failure with sepsis (n = 1), interstitial pneumonitis (n = 1), organizing pneumonia (n = 1), bronchiolitis obliterans (n = 1), and bronchiolitis obliterans with invasive aspergillosis (n = 1).

4. Discussion

In our previous study in an unrelated-donor BMT setting, 5 patients underwent conditioning with a combination of Flu (30 mg/m^2 for 6 days) or cladribine (0.11 mg/kg for 6 days), BU (4 mg/kg for 2 days), and antithymocyte globulin (2.5 mg/kg for 4 days) without TBI, but secondary graft failure in 2 of these patients alerted us to a possible higher risk of graft rejection when we used bone marrow instead of peripheral blood cells as the stem cell source. In this study, we demonstrated that the addition of 4 Gy of TBI to the widely applied combination of Flu (30 mg/m^2 for 6 days) and BU (4 mg/kg for 2 days) reduces the risk of graft failure and enables the rapid achievement of full donor chimerism without donor lymphocyte infusion (DLI) and that the regimen-related toxicity was acceptable. Nevertheless, a relatively high incidence of nonrelapse mortality was observed. We lost 4 patients who developed extensive chronic GVHD and subsequent pulmonary complications in the later phase, more than 6 months after transplantation. Because many patients develop extensive GVHD, we assume that the pulmonary complications were primarily due to GVHD and not the consequence of our reduced-intensity stem cell transplantation (RIST) regimen incorporating 4 Gy of TBI. However, Deeg et al reported that more pulmonary compli-

Table 3.
Maximum Toxicities (N = 17)*

Grade	Cardiac, n	Mucositis, n	GI, n	Hepatic, n	CNS, n	Hyponatremia, n	Pulmonary, n	Renal, n
0	12	0	9	1	16	6	11	15
I	4	0	3	2	0	7	2	0
II	0	2	4	7	0	0	0	2
III	1	15	1	5	1	4	4	0
IV	0	0	0	2	0	0	0	0

*GI indicates gastrointestinal tract; CNS, central nervous system.

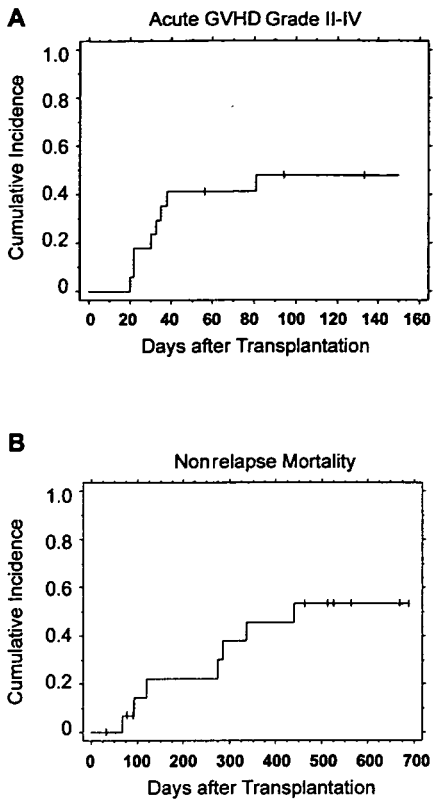

Figure 2. Cumulative incidence of acute GVHD (grades II-IV) (A) and nonrelapse mortality (B) after unrelated bone marrow transplantation following reduced-intensity conditioning. Acute GVHD (grades II-IV) was diagnosed in 8 patients (48%) (grade II in 3 patients and grade IV in 5) at a median of day 32 (range, day 20-81). The estimated 100-day and 1-year nonrelapse mortality rates were 14% and 46%, respectively.

cations developed in patients with aplastic anemia who received 4 to 6 Gy of TBI in combination with cyclophosphamide/antithymocyte globulin for unrelated-donor BMT than in patients who received 2 Gy TBI [20]. These investigators recommended that a 2-Gy TBI dose is sufficient to allow stable engraftment without increased toxicities, and this proposal should be evaluated in future studies. On the other hand, Maris et al described a nonmyeloablative conditioning regimen consisting of 2 Gy TBI and Flu (90 mg/m^2) for unrelated-donor HSCT [8]. In their study, the use of bone marrow rather than G-CSF–mobilized peripheral blood cells as the source of hematopoietic stem cells led to a lower engraftment rate (56% versus 85%), as well as lower rates of overall survival (33% versus 57%) and progression-free survival (17% versus 44%). Because bone marrow is currently the only stem cell source available from volunteer donors in Japan, we may need a more intensified regimen than the combination of 2 Gy TBI and 90 mg/m^2 Flu.

In this study, the rates of acute GVHD of grades II to IV and extensive chronic GVHD in patients who survived for more than 100 days were 48% and 90%, respectively. Grade IV acute GVHD was the primary cause of death in 2 patients. Moreover, the quality of life of patients who develop extensive chronic GVHD rapidly deteriorates, particularly in elderly patients. Although CsA was tapered from a median of day 120 in this series, it might be better to delay the start of CsA tapering in elderly patients, who are associated with higher GVHD rates. Studies have incorporated in vivo T-cell depletion through the addition of antithymocyte globulin or alemtuzumab in order to reduce the risk of GVHD [21-26]. In the study reported by Chakraverty et al, severe GVHD following RIST from an unrelated donor was decreased with in vivo use of alemtuzumab in the preparative regimen [23]. In their study, the rates of acute GVHD (grades II to IV) and chronic GVHD were 21% and 8%, respectively. The long half-life of alemtuzumab (15-21 days) may disturb the induction of full donor chimerism, however. If patients cannot achieve full donor chimerism, the usual option is DLI, which carries a risk of GVHD [26]. Moreover, lymphocytes for DLI are not always available for every patient, particularly in unrelated-donor transplantation settings. In this regard, we think that a regimen that routinely involves DLI after transplantation cannot be considered a universal strategy. In the present study, 2 patients who had

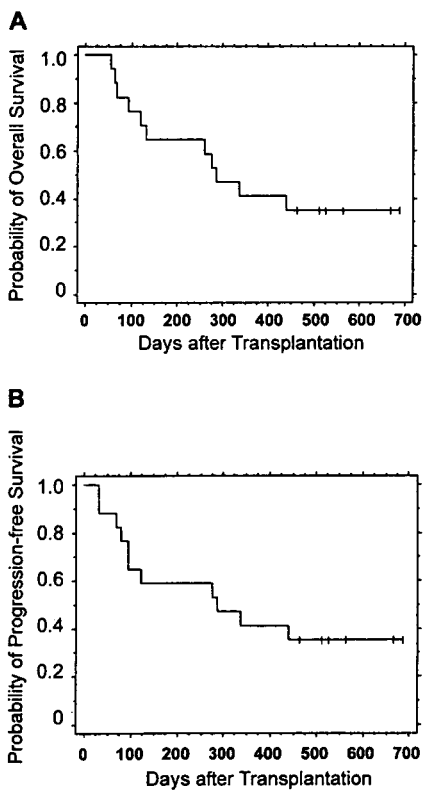

Figure 3. Kaplan-Meier actuarial probability of overall survival (OS) (A) and progression-free survival (PFS) (B) after unrelated-donor bone marrow transplantation following reduced-intensity conditioning. The median follow-up was 286 days (range, 56-687 days). The 1-year OS and PFS rates were both 41%. All 6 of the surviving patients (2 in remission and 4 not in remission at transplantation) remain in remission.

secondary graft failure did not receive DLI, because of grade IV acute GVHD in 1 patient and a reduced performance status in the other. Another approach to preventing severe GVHD is the use of novel immunosuppressive regimens. Several combinations of agents for GVHD prophylaxis, including CsA/mycophenolate mofetil [8,14,16] and tacrolimus/methotrexate [10,15,27], have been reported previously, and their value should be tested in prospective trials.

The induction of adequate antileukemic activity is another primary concern with a RIST procedure, particularly for patients with refractory diseases. de Lima et al reported a promising regimen that consisted of once-daily intravenous BU (130 mg/m^2 for 4 days) and Flu (40 mg/m^2 for 4 days) for patients with AML or MDS [27]. Replacement of oral BU with an intravenous preparation may result in an improved toxicity/survival profile. In our series, 4 patients achieved remission after RIST, although they were not in remission at the time of transplantation. Hence, it is likely that the antileukemic effect exerted by 4 Gy TBI in combination with Flu and BU is valuable even for the immediate control of leukemic blasts, although this possibility needs to be confirmed in further studies. The use of DLI has allowed the rescue of relapsed patients after allogeneic HSCT. In this study, however, we did not give DLI to 4 patients with progressive or relapsed diseases after transplantation because the relevance of the graft-versus-leukemia effect in rapidly proliferating diseases was not fully established and 2 of the patients had developed acute GVHD.

In conclusion, our regimen of 4 Gy TBI, Flu (180 mg/m^2), and BU (8 mg/kg) was effective in reducing the risk of graft failure following unrelated-donor transplantation. We confirmed, however, that a high incidence of nonrelapse mortality, primarily due to GVHD and/or pulmonary complications, still remains a major obstacle for the wider application of this procedure to elderly or medically infirm patients. Further studies to identify ways to ameliorate transplantation-related toxicities are urgently required.

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bjh research paper

Chronic graft-*versus*-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program

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Summary

Chronic graft-*versus*-host disease (GVHD) remains the major cause of late morbidity and mortality after allogeneic stem cell transplantation. We retrospectively analysed 2937 patients who underwent bone marrow transplantation from an unrelated donor (UR-BMT) facilitated by the Japan Marrow Donor Program (JMDP) and survived beyond day 100 after transplantation. The cumulative incidence of chronic GVHD (limited + extensive) or extensive chronic GVHD at 5 years post-transplant was 45·8% and 28·2%, respectively. On multivariate analysis, seven variables predicting chronic GVHD were identified: recipient age over 20 years, donor age over 30 years, primary diagnosis of chronic myeloid leukaemia, human leucocyte antigen (HLA)-A or -B mismatch, total body irradiation-containing regimen, platelet count not having reached 50×10^9/l by day 100, and prior acute GVHD. Among 2609 patients with haematological malignancy, overall survival was significantly higher in patients with limited chronic GVHD but lower in patients with extensive chronic GVHD compared with those without chronic GVHD. The cumulative incidence of relapse among patients with limited or extensive chronic GVHD was significantly lower than that among patients without chronic GVHD. Our results suggest that limited chronic GVHD provides a survival benefit to patients with haematological malignancies by reducing the risk of relapse without increasing the risk of death from chronic GVHD.

Keywords: chronic graft-*versus*-host disease, unrelated bone marrow transplantation, Japan Marrow Donor Program, relapse, graft-*versus*-leukaemia effect.

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†A complete list of the centres that participated in the bone marrow transplantations facilitated by the Japan Marrow Donor Program (JMDP) appears in Appendix 1.

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Haematopoietic stem cell transplantation (HSCT) has become established as one of the curative therapies for haematological malignancies and other haematological or immunologic disorders (Armitage, 1994). However, various late complications of HSCT rather than relapse decrease the quality of life of HSCT recipients (Socie *et al*, 1999; Kiss *et al*, 2002). Among late complications that may occur beyond 100 d post-transplant, chronic graft-*versus*-host disease (GVHD) affects approximately 30–70% of long-term survivors depending on the degree of human leucocyte antigen (HLA)-mismatch with the donor and the source of the stem cells, and remains a major cause of late morbidity and mortality post-transplantation (Atkinson *et al*, 1990; Sullivan *et al*, 1991; Vogelsang, 2001; Lee *et al*, 2002; Farag, 2004). Despite improvements in other areas of supportive care, little significant progress has been made in the management of chronic GVHD (Vogelsang, 2001). Patients with chronic GVHD have decreased performance status, impaired quality of life, and increased risk of mortality (Duell *et al*, 1997; Socie *et al*, 1999). In spite of its adverse effects, chronic GVHD is associated with a lower incidence of leukaemia relapse by a graft-*versus*-leukaemia (GVL) effect that is comparable or greater than that ascribed to acute GVHD (Weiden *et al*, 1981; Sullivan *et al*, 1989; Kataoka *et al*, 2004).

Bone marrow transplantation (BMT) from an unrelated volunteer donor (UR-BMT) has become established as an accepted treatment for patients in need of HSCT who do not have a HLA-matched sibling donor (Kernan *et al*, 1993; Hansen *et al*, 1998; Kodera *et al*, 1999; Davies *et al*, 2000). The incidence of chronic GVHD is assumed to be higher after UR-BMT than after transplants from an HLA-matched sibling donor. Previous studies have identified the incidence and risk factors for chronic GVHD after sibling transplant (Storb *et al*, 1983; Ringden *et al*, 1985; Atkinson *et al*, 1990; Remberger *et al*, 2002); however, there are no definite data available on the incidence and risk factors for chronic GVHD among patients who have undergone UR-BMT. The Japan Marrow Donor Program (JMDP) was established in December 1991. We previously analysed the data of 1298 patients who underwent UR-BMT facilitated by the JMDP between 1993 and 1998 to identify the effect of HLA matching on acute GVHD, chronic GVHD, engraftment, survival and relapse (Morishima *et al*, 2002). In that study, HLA-A and/or HLA-B allele mismatch and patient age were found to be significant risk factors for the occurrence of chronic GVHD. The current study extended the analysis to include the data of 2937 patients who underwent UR-BMT facilitated by the JMDP between January 1993 and June 2004 and survived for at least 100 d post-transplant to clarify the incidence and risk factors for chronic GVHD, and the effect of chronic GVHD on survival and relapse in UR-BMT recipients.

Patients and methods

Patients and transplant procedure

Between January 1993 and June 2004, 2937 Japanese patients who underwent UR-BMT through the JMDP, engrafted and survived for at least 100 d after UR-BMT were included in this analysis. We excluded patients who survived <100 d after UR-BMT to exclude the effect of early mortality. Because peripheral blood stem cell harvest has not been performed through the JMDP, all transplants were BMTs. Baseline characteristics and follow-up data were obtained using standard report forms designed by the JMDP. Follow-up reports were submitted at 100 d, 1 year, and annually thereafter post-transplantation. A final clinical survey of these patients was performed on 1 November 2004. The median follow-up time was 822 d (range, 100–4129 d). Informed consent was obtained from the patients and donors according to the Declaration of Helsinki.

The characteristics of the patients and donors are summarised in Table I. The median age of the patients was 27 years and the median age of the donors was 33 years. As much as 59·7% of the patients and 59·5% of the donors were male. The number of patients with a haematological malignancy was 2667 (90·8%). Transplantation was performed according to the protocol of each centre, and therefore the conditioning regimen and GVHD prophylaxis varied among patients. A conditioning regimen containing anti-thymocyte globulin (ATG) was used in 203 patients (6·9%), and a conditioning regimen containing total body irradiation (TBI) was used in 2329 patients (79·3%). Only 14 patients (0·5%) received T cell-depleted marrow.

HLA matching and typing

According to the donor selection criteria of the JMDP, patients received marrow transplants from serologically HLA-A, -B and -DR antigen completely matched or serologically 1 antigen mismatched donors. Genomic typing of HLA-A, -B and -DR antigens was also performed. 68·5% of the donors were fully HLA-matched by both serological and genomic typing.

Statistical analysis

The incidence of chronic GVHD was the primary endpoint of our study. Diagnosis of chronic GVHD and its clinical grading were performed according to the standard criteria at each institution (Atkinson, 1990). Chronic GVHD was graded as limited (localised skin or single organ involvement) or extensive (generalised skin or multiple organ involvement). The cumulative incidence of chronic GVHD was calculated from the time of transplantation. To evaluate potential risk factors for developing chronic GVHD, the time-dependent

Table I. Characteristics of the patients who underwent UR-BMT and donors.

Number of patients	2937
Median age of patients, years (range)	27 (0–67)
Patient sex (male/female), *n*	1753/1184
Diagnosis, *n*	
Haematological malignancy	
AML	793
ALL	768
CML	604
MDS	285
NHL	168
Others	49
Non-malignant disease	
AA	191
Hereditary disorders	68
Conditioning, *n*	
ATG	203
TBI	2329
GVHD prophylaxis, *n*	
CsA + MTX	1545
FK506 + MTX	1118
Others	274
Median age of donors, years (range)	33 (20–52)
Donor sex (male/female), *n*	1748/1189
Sex (recipient/donor), *n*	
Male/male	1151
Male/female	602
Female/female	587
Female/male	597
HLA disparity, *n*	
Full match	2012
Class I one locus or one allele mismatch	286
Class II one locus or one allele mismatch	473
Others	166
Blood-type disparity, *n*	
Match	1535
Major mismatch	677
Minor mismatch	616
Major–minor mismatch	72
Bone marrow treatment, *n*	
No	1529
Yes	
Removal of red blood cells	764
Removal of plasma	750
T cell depletion	14
Time from diagnosis to BMT, months	
<13	1180
13–24	865
≥25	865
Median time from BMT to WBC = $1{\cdot}0 \times 10^9$/l, d (range)	17 (1–99)
Platelet count = 50×10^9/l by day 100 from BMT, *n*	
Yes	2714
No	223
Prior acute GVHD, *n*	
No	884
Grade I	915
Grade II	793
Grade III	281
Grade IV	64

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia, CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-*versus*-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC, white blood cell count.

Cox proportional hazard regression model was used for univariate and multivariate analyses (Cox, 1972). Factors with a *P*-value of 0·2 or less in the univariate analysis were included in the multivariable analysis. Factors that remained significant were retained in the final model.

Patients were also analysed for overall survival (OS) and relapse. To illustrate the effect of chronic GVHD on relapse and survival, semi-landmark plots were constructed (Baron *et al*, 2005). In patients who developed chronic GVHD, the post-transplant day of development of chronic GVHD was defined as the landmark day; in patients who did not develop chronic GVHD, post-transplant day 112, which was the median day of occurrence of chronic GVHD, was defined as the landmark day. OS was calculated from the landmark day to death from any cause or date of last contact. Relapse was defined on the basis of evidence of the respective malignancy and its cumulative incidence was plotted as a function of time since the landmark day.

Survival analyses were performed by the Kaplan–Meier method (Kaplan & Meier, 1958) and the log-rank test was used for univariate comparisons. The cumulative incidences of chronic GVHD and relapse were calculated using the Gray method, considering death without chronic GVHD or death without relapse, respectively, as the competing risk (Gray, 1988). For most of the statistical analyses, the Statistical Package for the Social Sciences (SPSS) software version 11 (SPSS Inc., Chicago, IL, USA) was used. Analyses of cumulative incidences were carried out with package 'cmprsk' of the R statistical software 2.1.0 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org). All *P*-values were two-sided and differences were considered to be statistically significant when $P < 0{\cdot}05$. Differences with *P*-values > 0·10 are reported as not significant (NS), whereas differences with *P*-values between 0·05 and 0·1 are reported in detail.

Results

Incidence and severity of chronic GVHD

Among the 2937 patients, 1267 (43·1%) developed chronic GVHD, of whom 268 patients (21·2%) had *de novo* onset of

chronic GVHD. The median time to onset of chronic GVHD was 112 d following transplant. The 5-year cumulative incidence of chronic GVHD was 45·8%, and that of extensive chronic GVHD was 28·2% (Fig 1A). Fig 1B shows the cumulative incidences of chronic GVHD according to the primary diagnosis.

Risk factors for developing chronic GVHD

Multivariate analysis for risk factors for the development of chronic GVHD included the 2909 patients in whom data on the variables with $P \leq 0{\cdot}2$ in the univariate analysis were available (Table II). Recipient age ≥20 years, donor age ≥30 years, primary diagnosis of chronic myeloid leukaemia (CML), HLA-A or -B mismatch by serological or genomic typing, total body irradiation (TBI)-containing regimen, platelet count $<50 \times 10^9$/l by day 100, and prior acute GVHD remained in the optimal model on multivariate analysis and increased the risk of chronic GVHD significantly. Aplastic anaemia (AA) and hereditary disorders were significantly associated with a low incidence of chronic GVHD.

When the patients were divided by age decade, the incidence of chronic GVHD was significantly lower in recipient groups aged <10 years and 10–19 years; however, among recipients aged ≥20 years, there were no differences in the incidence of chronic GVHD (Fig 2). When the donors were divided by age decade, the cumulative incidence of chronic GVHD was significantly lower among patients transplanted from donors aged 20–29 years than among patients transplanted from donors aged ≥30 years ($P = 0{\cdot}005$, method of Gray). No differences in the incidence of chronic GVHD were found among patients transplanted from donors aged ≥30 years.

Prior acute GVHD was the strongest risk factor for chronic GVHD (Table II and Fig 1C). Among patients with no history of acute GVHD ($n = 870$), risk factors for chronic GVHD on multivariate analysis were recipient age ≥20 years {hazard ratio (HR) = 1·45 [95%confidence interval (95% CI), 1·06–1·98], $P = 0{\cdot}019$}, donor age ≥30 years [HR = 1·54 (95%CI, 1·19–2·00), $P = 0{\cdot}001$] and one locus mismatch or one allele mismatch at HLA-A/-B loci [*versus* full match, HR = 1·50 (95% CI, 1·02–2·20) $P = 0{\cdot}039$]. Among patients with a history of grade II–IV acute GVHD ($n = 1107$), platelet count $<50 \times 10^9$/l by day 100 [HR = 1·30 (95%CI, 1·00–1·67), $P = 0{\cdot}048$] was the only risk factor on multivariate analysis.

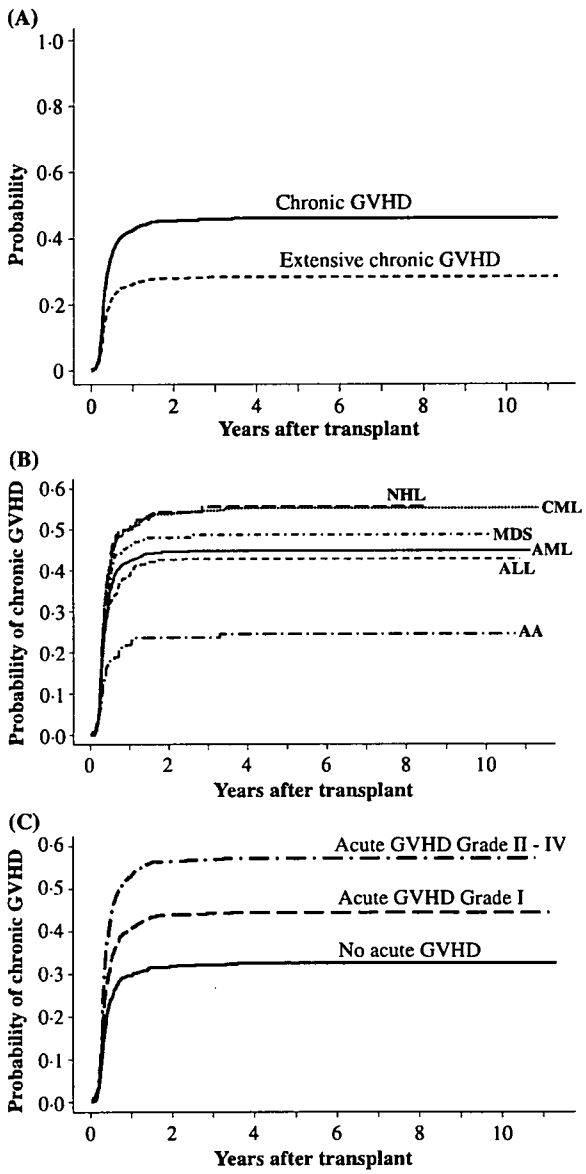

Fig 1. Cumulative incidence of chronic GVHD after UR-BMT. (A) Cumulative incidences of chronic GVHD (limited + extensive) and extensive chronic GVHD. The 5-year cumulative incidence of chronic GVHD was 45·8% (95% CI, 43·9–47·7%) and that of extensive chronic GVHD was 28·2% (95% CI, 26·5–29·9). Competing risks were death without chronic GVHD and death without chronic extensive GVHD (19·3% and 24·4%, respectively). (B) Cumulative incidences of chronic GVHD according to the primary diagnosis. The 5-year cumulative incidence and competing risk were 44·7% and 25·4% among patients with acute myeloid leukaemia (AML, solid line), 42·9% and 25·7% among patients with acute lymphoblastic leukaemia (ALL, dashed line), 49·0% and 16·1% among patients with myelodysplastic syndrome (MDS, dot-dash line), 55·3% and 12·8% among patients with chronic myeloid leukaemia (CML, dotted line), 55·7% and 11·5% among patients with non-Hodgkin lymphoma (NHL, long-dash line), and 24·4% and 6·1% among patients with aplastic anaemia (AA, dot-long dash line), respectively. (C) Cumulative incidences of chronic GVHD according to the severity of prior acute GVHD. The 5-year cumulative incidence was 32·4% among patients without a history of acute GVHD (solid line), 44·4% among patients with a history of grade I acute GVHD (dashed line), and 57·3% among patients with a history of grades II–IV acute GVHD (dot-dashed line). Competing risks were 20·0% without prior acute GVHD, 20·2% for grade I, and 17·9% for grades II–IV.

Table II. Univariate and multivariate analyses of risk factors for the development of chronic GVHD.

	Univariate analysis			Multivariate analysis (n = 2909)		
Factor	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Recipient age						
0–19 years	972	1·0		961	1·0	
≥20 years	1965	1·41 (1·24–1·59)	<0·0001	1948	1·19 (1·04–1·36)	0·013
Recipient sex						
Female	1184	1·0				
Male	1753	1·11 (0·99–1·25)	0·07			NS
Donor age						
20–29 years	1007	1·0		994	1·0	
≥30	1930	1·28 (1·14–1·45)	<0·0001	1915	1·20 (1·07–1·36)	0·003
Sex matching						
Match	1738	1·0		1721	1·0	
Female to male	602	1·01 (0·87–1·16)	0·94	595	1·05 (0·91–1·22)	NS
Male to female	597	0·89 (0·77–1·03)	0·11	593	0·85 (0·74–0·99)	0·03
Diagnosis						
AML	793	1·0		787	1·0	
ALL	768	0·92 (0·79–1·08)	0·31	764	0·89 (0·76–1·04)	NS
MDS	285	1·13 (0·92–1·38)	0·25	283	1·11 (0·90–1·36)	NS
CML	604	1·27 (1·09–1·48)	0·002	602	1·19 (1·02–1·39)	0·03
NHL	168	1·32 (1·04–1·67)	0·02	166	1·18 (0·93–1·50)	NS
AA	191	0·43 (0·32–0·60)	<0·0001	190	0·51 (0·37–0·71)	0·0001
Other haematological malignancies	49	1·05 (0·67–1·65)	0·83	49	0·94 (0·60–1·48)	NS
Hereditary disorders	68	0·47 (0·29–0·77)	0·003	68	0·56 (0·34–0·93)	0·02
Time from diagnosis to BMT						
<13 months	1180	1·0				
13–24 months	865	1·05 (0·92–1·20)	0·45			
≥25 months	865	0·98 (0·85–1·12)	0·71			
Blood type disparity						
Match	1535	1·0				
Major mismatch	677	1·03 (0·89–1·18)	0·73			
Minor mismatch	616	1·08 (0·94–1·24)	0·30			
Major minor mismatch	72	1·12 (0·79–1·58)	0·54			
HLA disparity						
Full match	2012	1·0		1991	1·0	
Class I one mismatch	286	1·26 (1·05–1·51)	0·01	285	1·26 (1·05–1·52)	0·01
Class II one mismatch	473	1·03 (0·88–1·20)	0·73	468	0·90 (0·77–1·05)	NS
≥2 mismatches	166	1·31 (1·05–1·64)	0·02	165	1·14 (0·91–1·43)	NS
Preparative regimen TBI for conditioning						
Non-TBI regimen	608	1·0		601	1·0	
TBI-based regimen	2329	1·23 (1·06–1·42)	0·005	2308	1·16 (1·00–1·35)	0·04
ATG for conditioning						
No	2718	1·0				
Yes	203	0·58 (0·44–0·75)	0·0001			NS
GVHD prophylaxis						
CsA + MTX	1545	1·0				
FK506 + MTX	1118	1·00 (0·88–1·19)	0·93			
Treatment of bone marrow						
No	1529	1·0				
Yes	1384	1·06 (0·95–1·19)	0·29			
Platelet recovery (50×10^9/l or more by 100 d from BMT)						
Yes	2714	1·0		2688	1·0	
No	223	1·33 (1·10–1·61)	0·003	221	1·34 (1·10–1·63)	0·004

Table II. *Continued*

Factor	Univariate analysis			Multivariate analysis (n = 2909)		
	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Days from BMT to WBC recovery						
<Day 18	1622	1·0				
≥Day18	1314	0·90 (0·80–1·00)	0·049			NS
Prior acute GVHD						
No	884	1·0		869	1·0	
Grade I	915	1·54 (1·31–1·80)	<0·0001	911	1·47 (1·25–1·72)	<0·0001
Grade II–IV	1138	2·28 (1·98–2·64)	<0·0001	1129	2·08 (1·80–2·42)	<0·0001

CI, confidence interval; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, antithymocyte globulin; TBI, total body irradiation; GVHD, graft-*versus*-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC recovery, the first of three consecutive days with a persistent white blood cell count >1·0 × 10^9/l; HR, hazard ratio.

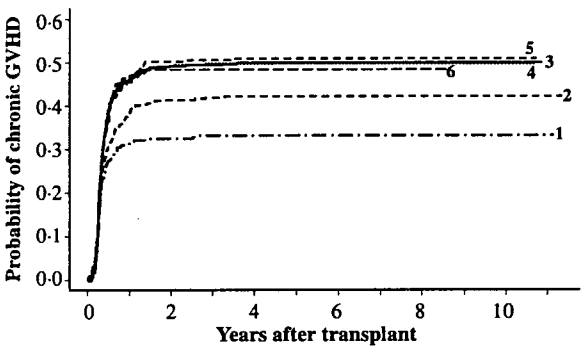

Fig 2. Cumulative incidence of chronic GVHD according to recipient's age decade. The competing risk was death without chronic GVHD. The 5-year cumulative incidence and competing risk were: 32·9% and 14·1% among patients aged 0–9 years (line 1; dot-dash line), 42·1% and 18·8% among those aged 10–19 years (line 2; dash line), 49·1% and 15·1% among those aged 20–29 years (line 3; solid line), 49·4% and 23·1% among those aged 30–39 years (line 4; dotted line), 51·0% and 23·2% among those aged 40–49 years (line 5; dash line), and 48·3% and 25·6% among those aged >50 years (line 6; long-dash line), respectively.

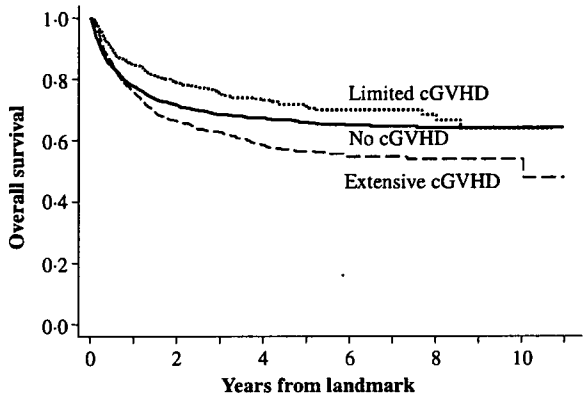

Fig 3. Overall survival according to chronic GVHD grading. The OS of all patients who survived beyond 100 d post-transplant according to chronic GVHD grade (n = 2877), is shown. The 5-year OS rate was 71·1% (95% CI, 66·4–75·8) among those with limited chronic GVHD (n = 489), 56·4% (95% CI, 52·3–60·5) among those with extensive chronic GVHD (n = 771), and 65·9% (95% CI, 63·2–68·5) among patients who did not develop chronic GVHD (n = 1617). The landmark day was the day of onset of chronic GVHD for patients with chronic GVHD, and it was day 112 from transplant, which was the median day of the onset of chronic GVHD, for patients without chronic GVHD. No chronic GVHD, solid line; limited chronic GVHD, dotted line; extensive chronic GVHD, dashed line.

Influence of chronic GVHD on OS and relapse

We analysed how chronic GVHD affects the prognosis after UR-BMT among 2877 patients (Fig 3). Patients with limited chronic GVHD had significantly better prognosis than patients with extensive chronic GVHD (log-rank test, P < 0·0001) or patients without GVHD (P = 0·009), whereas patients with extensive chronic GVHD had significantly poorer prognosis (*versus* without chronic GVHD, P = 0·003). The same tendencies were observed among 2609 patients with a haematological malignancy. On multivariate analysis using the Cox proportional hazard model with chronic GVHD as a time-dependent covariate, patients with extensive chronic GVHD had significantly increased mortality and patients with limited chronic GVHD had a survival advantage compared with those without chronic GVHD (Table III). However, patients with chronic GVHD had a lower cumulative incidence of relapse than patients without chronic GVHD (*versus* limited chronic GVHD, P = 0·049; *versus* extensive chronic GVHD, P = 0·009). There was no difference in relapse rate between patients with limited chronic GVHD and those with extensive chronic GVHD. The 5-year probability of relapse was 15·8% (95% CI, 12·1–19·5) among patients with limited chronic GVHD, 15·3% (95% CI, 12·3–18·4) among patients with extensive chronic GVHD, and 21·0% (95% CI, 18·5–23·6) among patients without chronic GVHD.

Table III. Multivariate analysis of prognostic factors in patients with haematological malignancies.

Factor	HR (95% CI)	*P*-value
Recipient age		
≥20 years	1·54 (1·30–1·83)	<0·0001
Donor age		
≥40 years	1·18 (1·18–1·38)	0·04
Diagnosis		
CML (*versus* AML)	0·67 (0·55–0·82)	0·0001
HLA disparity		
Class I one mismatch (*versus* full-match)	1·58 (1·27–1·97)	0·0001
≥2 mismatches (*versus* full-match)	1·52 (1·14–2·01)	0·0038
Platelet recovery (≥50 × 10^9/l by day 100 from BMT)		
No	1·58 (1·24–2·01)	0·0002
Prior acute GVHD		
Grade II–IV (*versus* No prior acute GVHD)	1·60 (1·31–1·95)	<0·0001
Relapse		
Yes	11·62 (10·06–13·41)	<0·0001
Secondary malignancies		
Yes	6·23 (3·28–11·83)	<0·0001
Chronic GVHD		
Limited (*versus* No)	0·67 (0·54–0·83)	0·0003
Extensive (*versus* No)	1·21 (1·03–1·43)	0·02

CI, confidence interval; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; HR, hazard ratio.

Discussion

In the present study, the 5-year cumulative incidence of chronic GVHD was 45·8% and that of extensive chronic GVHD was 28·2%. These cumulative incidences, especially the cumulative incidence of extensive chronic GVHD, are slightly lower than those of the data of the National Marrow Donor Program (NMDP) (Kollman *et al*, 2001) and other previous reports (Sullivan, 1999). Notably, nearly 100% of the recipient and donor pairs in the present study were composed of a single ethnic population of Japanese people. Recently, Oh *et al* (2005) reported that Japanese and Scandinavian people had significantly lower incidences of acute GVHD than American and Irish people in HLA-identical sibling BMT. Because Japanese people have been geographically isolated for a long period of time historically, Japanese people are genetically more similar than people of the USA. or Western countries and it is unclear whether our results apply to other more diverse genetic groups.

Our previous study revealed two significant risk factors for chronic GVHD by multivariate analysis: HLA-A/-B allele mismatch and patient age (Morishima *et al*, 2002). In the current extended analysis, seven risk factors were found to be significant for the development of chronic GVHD on multivariate analysis.

Zecca *et al* (2002) reported that the incidence of chronic GVHD in children after HSCT was 27%, which was assumed to be lower than that in adult recipients. In the current analysis, the incidence of chronic GVHD among patients <20 years of age was significantly lower than that among patients over 20 years of age. However, there was no significant difference in the incidence of chronic GVHD when adult patients over 20 years of age were grouped by age decade, although the OS rate was significantly lower in older adults than in younger adults, probably because of an increased incidence of death from other causes rather than chronic GVHD.

Donor age ≥30 years was a significant risk factor for the development of chronic GVHD and it also tended to decrease the survival rate. Kollman *et al* (2001) also reported that younger donor was a significant predictor of lack of development of chronic GVHD. Although the reason for this is not well understood, our findings suggest that donors of younger age may be preferable when selecting from comparably HLA-matched volunteer donors.

In our previous study (Morishima *et al*, 2002), HLA-C allele mismatch also tended to increase the incidence of chronic GVHD, while HLA-DR/-DQ mismatch showed no effect. Petersdorf *et al* (2004) showed that a single HLA-C mismatch conferred increased risk of mortality compared with matches. Greinix *et al* (2005) also showed that HLA class I mismatch, as detected by high-resolution typing, had a significant impact on the development of chronic GVHD and survival of UR-BMT recipients. The present study returned the same result as that in the previous report, although the effect of HLA-C was not analysed.

Previous analysis of risk factors for chronic GVHD after HLA-identical sibling BMT (Atkinson *et al*, 1990) revealed that the strongest risk factor for chronic GVHD was the existence of prior acute GVHD. In that report, several risk factors including recipient age >20 years predicted a higher risk of chronic GVHD in patients with a history of grade I acute GVHD or without a history of acute GVHD; however, among patients with a history of moderate to severe acute GVHD, no other risk factor predicted the development of chronic GVHD. In our study, recipient age and donor age were important risk factors for *de novo* onset of chronic GVHD, whereas in patients with a history of moderate to severe acute GVHD, patient age and donor age were not risk factors for chronic GVHD. These results are similar to the results of the other report (Atkinson *et al*, 1990). Remberger *et al* (2002) revealed that CML was a risk factor for chronic GVHD. We also identified that the incidence of chronic GVHD among patients with CML was significantly higher than that among patients with acute myeloid leukaemia (AML).

Whether the primary disease was a haematological malignancy or not significantly affected the development of chronic GVHD. In our previous study, among patients with AA who underwent UR-BMT, the incidence of chronic GVHD was

30% (Kojima *et al*, 2002), and it was 24·4% in the present extended analysis. Moreover, we found that the incidence of chronic GVHD among patients with hereditary disorders was significantly low in multivariate analysis. This finding might be due to the difference in treatment strategies for patients with haematological malignancy and those with AA. Immunosuppressive agents might be stopped or decreased earlier in patients with haematological malignancy than in those with non-malignant disease in order to induce the GVL effect.

Limited chronic GVHD had a significant impact on increasing patient survival, whereas patients with extensive chronic GVHD had a poor prognosis. In patients with a haematological malignancy, we found no significant difference in relapse rates between patients with limited chronic GVHD and those with extensive chronic GVHD, indicating that extensive chronic GVHD does not provide a strong GVL effect compared with limited chronic GVHD.

We used the grading system of limited and extensive chronic GVHD, which was originally proposed in 1980 based on the clinicopathological findings in 20 patients (Shulman *et al*, 1980). However, this grading system has several limitations. Akpek *et al* (2001, 2003) proposed a new prognostic model by analysing GVHD-specific survival and suggested that three factors, i.e. skin involvement, platelet count and progressive-type onset, significantly influence the survival of patients who developed chronic GVHD. However, a recent Japanese report showed that Japanese patients could not be accurately classified when these proposed prognostic models were used because the manifestation of chronic GVHD differed between Japanese and Western ethnic populations (Atsuta *et al*, 2006). We have started to collect more detailed information on Japanese patients with chronic GVHD, such as organ involvement, treatment strategy, and treatment outcome, to establish prognostic models.

In conclusion, this large-scale study demonstrated the incidence of chronic GVHD after UR-BMT in a single Japanese ethnic population and provides strong evidence for seven risk factors for chronic GVHD after UR-BMT. This study also suggests that limited chronic GVHD provides a survival benefit to patients with a haematological malignancy by reducing the risk of relapse without increasing the risk of death from chronic GVHD. Extended intervention and clinical trials are necessary to overcome extensive chronic GVHD.

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Appendix 1

The following centres participated in the bone marrow transplantations facilitated by the JMDP: Asahikawa Medical College Hospital, Asahikawa Red Cross Hospital, Sapporo Medical University Hospital, Sapporo Hokuyu Hospital, Hokkaido University Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hirosaki University Hospital, Aomori Prefectural Central Hospital, Akita University Hospital, Iwate Medical University Hospital, Miyagi Cancer Centre, Tohoku University Hospital, Yamagata University Hospital, Fukushima Medical University Hospital, Ibaraki Children's Hospital, Tsukuba University Hospital, Tsuchiura Kyodo General Hospital, Jichi Medical School Hospital, Dokkyo Medical University Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Saitama Medical University Hospital, Saitama Cancer Centre Hospital, Saitama Children's Medical Centre, Fukaya Red Cross Hospital, National Defense Medical College Hospital, Kameda General Hospital, Matsudo Municipal Hospital, Chiba Children's Hospital, Chiba Aoba Municipal Hospital, Chiba University Hospital, Jikei University Kashiwa Hospital, Keio University Hospital, Toranomon Hospital, National Cancer Centre Central Hospital, International Medical Centre of Japan, National Centre for Child Health and Development, Juntendo University Hospital, Showa University Hospital, Teikyo University Hospital, Tokyo Medical and Dental University Hospital, Tokyo Medical College Hospital, Jikei University Hospital, Tokyo Women's Medical University Hospital, Research Hospital of the Institute of Medical Science-the University of Tokyo, The University of Tokyo Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo Metropolitan Hospital of Fuchu, Toho University Omori Medical Centre, National Hospital Organisation Tokyo Medical Centre, Nippon Medical School Hospital, Japanese Red Cross Medical Centre, Nihon University Itabashi Hospital, Yokohama City University Medical Centre, Yokohama City University Hospital, Kanagawa Cancer Centre, Kanagawa Children's Medical Centre, St. Marianna University School of Medicine Hospital, Tokai University Hospital, Niigata University Medical & Dental Hospital, Nagaoka Red Cross Hospital, Niigata Cancer Centre Hospital, University of Yamanashi Hospital, Saku Central Hospital, Shinshu University Hospital, Nagano Children's Hospital, Nagano Red Cross Hospital, Toyama Prefectural Central Hospital, Kanazawa Medical University Hospital, Kanazawa University Hospital, Ishikawa Prefectual Central Hospital, University of Fukui Hospital, Hamamatsu Medical Centre, Seirei Hamamatsu General Hospital, Shizuoka Children's Hospital, Shizuoka General Hospital, Shizuoka Red Cross Hospital, Hamamatsu University School of Medicine Hospital, Aichi Medical School Hospital, Aichi Cancer Centre Hospital, Anjo Kousei Hospital, Showa Hospital, National Hospital Organisation Nagoya Medical Centre, Fujita Health University Hospital, Nagoya City University Hospital, Nagoya University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Nagoya Ekisaikai Hospital, Meitetsu Hospital, Mie University Hospital, Yamada Red Cross Hospital, Suzuka Kaisei Hospital, Suzuka General Hospital, Shiga University of Medical Science Hospital, Kyoto Katsura Hospital, Kyoto City Hospital, Kyoto University Hospital, Kyoto First Red Cross Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Rinku General Medical Centre, Kansai Medical University Hospital, Kinki University Hospital, Matsushita Memorial Hospital, Osaka Medical College Hospital, Osaka City University Hospital, Osaka Red Cross Hospital, Osaka University Hospital, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka Medical Centre and Research Institute for Maternal and Child Health, Kobe City General Hospital, Kobe University Hospital, Hyogo College of Medicine Hospital, Hyogo Children's Hospital, Hyogo Medical Centre for Adults, Tenri Hospital, Nara Medical University Hospital, Wakayama Medical University Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Shimane Prefectural Central Hospital, Okayama University Hospital, National Hospital Organisation Okayama Medical Centre, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima University Hospital, National Hospital Organisation Kure Medical Centre, Kurashiki Central Hospital, Yamaguchi University Hospital, Tokushima University Hospital, Kagawa University Hospital, Ehime Prefectural Central Hospital, Ehime University Hospital, Matsuyama Red Cross Hospital, Kochi Medical School Hospital, Kurume University Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Centre, University of Occupational and Environmental Health Hospital, Kokura Memorial Hospital, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, National Hospital Organisation Kumamoto Medical Centre, Oita Prefectural Hospital, Oita University Hospital, Miyazaki Prefectural Hospital, Imamura Hospital, and Kagoshima University Hospital.

