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Prospective Comparison of the Diagnostic Potential of Real-Time PCR, Double-Sandwich Enzyme-Linked Immunosorbent Assay for Galactomannan, and a (1→3)-β-D-Glucan Test in Weekly Screening for Invasive Aspergillosis in Patients with Hematological Disorders

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The establishment of an optimal noninvasive method for diagnosing invasive aspergillosis (IA) is needed to improve the management of this life-threatening infection in patients with hematological disorders, and a number of noninvasive tests for IA that target different fungal components, including galactomannan, (1→3)-β-D-glucan (BDG), and *Aspergillus* DNA, have been developed. In this study, we prospectively evaluated the diagnostic potential of three noninvasive tests for IA that were used in a weekly screening strategy: the double-sandwich enzyme-linked immunosorbent assay (ELISA) for galactomannan (Platelia Aspergillus), a real-time PCR assay for *Aspergillus* DNA (GeniQ-Asper), and an assay for BDG (β-glucan Wako). We analyzed 149 consecutive treatment episodes in 96 patients with hematological disorders who were at high risk for IA and diagnosed 9 proven IA cases, 2 probable IA cases, and 13 possible invasive fugal infections. In a receiver-operating characteristic (ROC) analysis, the area under the ROC curve was greatest for ELISA, using two consecutive positive results (0.97; $P = 0.036$ for ELISA versus PCR, $P = 0.055$ for ELISA versus BDG). Based on the ROC curve, the cutoff for the ELISA could be reduced to an optical density index (O.D.I.) of 0.6. With the use of this cutoff for ELISA and cutoffs for PCR and BDG that give a comparable level of specificity, the sensitivity/specificity/positive predictive value/negative predictive value of the ELISA and the PCR and BDG tests were 1.00/0.93/0.55/1.00, 0.55/0.93/0.40/0.96, and 0.55/0.93/0.40/0.96, respectively. In conclusion, among these weekly screening tests for IA, the double-sandwich ELISA test was the most sensitive at predicting the diagnosis of IA in high-risk patients with hematological disorders, using a reduced cutoff of 0.6 O.D.I.

Invasive aspergillosis (IA) is one of the most serious complications in patients with hematological malignancies. It has an extremely high mortality rate (11) and affects not only terminally ill patients with refractory leukemia or lymphoma but also patients who could otherwise be expected to experience a potential cure of the underlying leukemia or lymphoma. Among several factors that contribute to the high mortality rate, difficulties in establishing a reliable diagnosis early enough for successful intervention have been repeatedly discussed (10). A definitive diagnosis usually requires invasive tissue sampling, which is often hampered by the critical condition of the patients, while a delay in initiating antifungal therapy, or, conversely, a hasty use of empiric or prophylactic amphotericin B before making a definitive diagnosis may result in treatment failure for full-blown infection or excess toxicity, respectively.

To overcome this problem and to improve the treatment outcome, advances have been made over the past decade in the fields of both diagnostics and therapeutics, including improvements in diagnostic imaging (7, 8, 18) and histopathology (1), and the development of broad-spectrum antifungal agents with low toxicities (4, 24, 29, 33). In the field of diagnostics, much attention has recently been given to the development of several types of noninvasive laboratory tests for IA. These tests are designed to sensitively detect circulating *Aspergillus* components and include a double-sandwich enzyme-linked immunosorbent assay (ELISA) for galactomannan (GM) antigen (Platellia Aspergillus) (30), tests for (1→3)-β-D-glucan (BDG) (β-glucan Wako or FungiTec G test) (23, 25), and a number of PCR-based assay systems for *Aspergillus* DNA (5, 6, 12, 34).

The ELISA for GM uses a rat monoclonal antibody directed against the 1→5-β-galactofuranoside side chains of the GM molecule as both the capture and detection antibodies for ELISA and can detect as little as 1.0 ng of circulating GM per ml (30). The excellent sensitivity and specificity of this assay have been repeatedly demonstrated and validated in tests of patients with hematological disorders (22, 27, 32). BDG is a ubiquitous component of diverse fungal species and a possible target for the diagnostic detection of IA. Two assay systems are currently available for the sensitive detection of circulating

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BDG, and both are based on the *Limulus* reaction, in which a trace amount of BDG can trigger a horseshoe crab coagulation cascade through factor G (23, 25). The BDG test is a useful method for screening for invasive fungal infection (IFI) and is widely used in Japan. The other test that has long been under intensive investigation for the sensitive detection of IA is PCR amplification of *Aspergillus* DNA, mainly of the 18S ribosomal gene (5, 6, 12, 34). Moreover, recently introduced real-time PCR designs have made it possible to quantitatively evaluate a fungal load with high sensitivity (9, 17, 21).

With regard to an antifungal strategy, it would be interesting to determine which of these tests is the best for diagnosing IA in patients with hematological disorders. Although high sensitivity and specificity are reported for PCR-based assays, the question whether PCR-based assays are superior to GM ELISA is still controversial (3, 5, 19, 34). Previously, we developed a sensitive real-time PCR system for detecting *Aspergillus* 18S ribosomal DNA, with which as few as 40 copies of aspergillus DNA per ml of plasma could be stably detected. We reported that the sensitivity of our real-time PCR for IA in 33 IA patients was higher than those of the double-sandwich ELISA for GM and the BDG test, with only a slightly lower specificity than that of GM ELISA (17). However, this previous study may have been biased by its partially retrospective design, limited sampling points in each case or infectious episode, and use of an inappropriately high cutoff value for ELISA. In the present purely prospective analysis, we consecutively enrolled 96 patients with hematological disorders who were at high risk for IA, monitored the levels of *Aspergillus* DNA, GM, and BDG in plasma, as well as the development of IA, at weekly intervals, and evaluated their diagnostic potentials by using receiver-operating characteristic (ROC) analyses.

MATERIALS AND METHODS

Study population and design. From March 2001 through April 2002, a consecutive series of adult patients with hematological disorders who had been admitted to our hospital and were thought to be at high risk for IA were enrolled in the study, and their levels of *Aspergillus* DNA in plasma and GM in serum, and BDG in plasma were monitored weekly. Patients were considered to be at high risk for IA if (i) they underwent chemotherapy and were expected to be neutropenic (less than 500 neutrophils per μl) for at least 10 days, (ii) they had refractory disease or were neutropenic and presented for more than 96 h with persistent fever that was refractory to appropriate broad-spectrum antibacterial treatments, (iii) they had presented with acute graft-versus-host disease (GVHD) of grade 2 or greater or had extensive chronic GVHD, or (iv) they had received corticosteroids for more than 3 weeks within the previous 60 days. Plasma *Aspergillus* DNA levels, serum GM levels, and plasma BDG levels were to be measured once weekly whenever the patients were thought to be at high risk. Each period during which measurement was performed was defined as one treatment episode. Omission of sampling was permitted unless two consecutive samples were lacking. Treatment episodes with only one or two samples for each test were excluded from the analysis.

The level of *Aspergillus* DNA in plasma was measured using real-time PCR, as described previously (17). The ELISA for GM (Platelia Aspergillus; Sanofi Diagnostics Pasteur, Marnes-La-Cosuette, France) and the β-glucan Wako test (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) were performed as specified by the manufacturers. Each sample was tested twice for GM and BDG, and the average of the two measurements was taken.

Antifungal prophylaxis consisted of daily administration of 200 mg of fluconazole or itraconazole capsules with or without 15 mg of aerosolized amphotericin B or 10 mg of intravenous amphotericin B for patients with a suspected history of IA. Neutropenic fever was treated with broad-spectrum antibiotics in accordance with the published guidelines (16). Blood samples were used for bacterial, mycobacterial, and fungal cultures prior to the initiation of antibiotics. When IFI was suspected, treatment with 1 mg intravenous amphotericin B per kg was initiated. During the febrile period, patients were intensively surveyed for possible sites of infection and causative microorganisms. Diagnostic procedures included routine cultures of urine and stools, repeated cultures of blood and sputum, weekly chest X rays, high-resolution computed tomography (CT) scan of the chest, and, when possible, bronchoscopic examinations and open biopsies.

Case definitions. For each treatment episode, a diagnosis was made following the published case definition criteria for invasive fungal infections from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC-IFICG and NIAID-MSG) (2), with the necessary modification that the plasma GM level was not included in the microbiological criteria.

Statistical analysis. As described by Maertens et al. (22), we made a set of different estimates (A/B, C, and D) for the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test, where different definitions of disease status for an episode were used to calculate these statistical indexes, since there is an intrinsic uncertainty regarding the true disease status of IA so that the calculation of these values could be significantly affected by the definition of the disease status. Estimate A/B defines "proven IA" and "probable IA" as truly positive and only "no IA" as truly negative, whereas estimates C and D incorporate "possible IFI" into the truly positive and truly negative groups, respectively. In all of the estimates, "no-IA" episodes were considered truly negative. Since our objective was to validate and compare the potentials of different diagnostic tests in a setting where these tests are performed weekly to monitor the development of IA, the positivity or negativity of a test was defined for each episode, where an episode was considered positive if at least one sample (method I), or any two consecutive samples (method II) became positive. There is also a practical reason for this approach. The onset and resolution of an IA episode are not always clear and, indeed, are rather poorly defined in many cases. Even in proven cases, there might be several febrile episodes and the onset might be insidious. In this setting, the sample-based calculation of sensitivity and specificity might be severely biased. In addition, we determined a proper cutoff value for each test through a ROC analysis, in which sensitivity and specificity were calculated as a function of the cutoff value, (1 − specificity) was plotted against the sensitivity, and the areas under the ROC curves (AUCs) were calculated. The significance of the difference in the AUCs of any two diagnostic measures was statistically tested as described above, and *P* values were calculated by the paired method under the null hypothesis that the two ROC curves represent random samples from similar underlying data for sensitivities and specificities (13). Therefore, the *P* values can be used only to compare two ROC curves at a time. The calculated *P* values reflect the one-tailed significance of difference between two ROC curves.

RESULTS

Study episodes. There were 149 treatment episodes in 96 consecutive patients, including 9 proven IA, 2 probable IA, 13 possible IFI, and 125 no-IA episodes. Of these, 56 episodes (38%) were associated with stem cell transplantation. The patient characteristics and sample distributions are summarized in Table 1. Nineteen treatment episodes had no host factors. Overall, 1,251 samples were analyzed by the real-time PCR assay, 1,233 were analyzed by double-sandwich ELISA for GM, and 1,243 were analyzed by the BDG test. On average, approximately eight samples were examined for each treatment episode. The characteristics of the 24 episodes of proven IA, probable IA, and possible IFI are shown in Table 2. There were 24 fatal episodes, of which 8 were proven IA, 1 was probable IA, 4 were possible IFI, and 11 were no IA. Autopsies were performed in 14 episodes (58%), including 6 proven IA and 8 no-IA cases. In the remaining 10 fatal episodes, autopsy was not permitted by the patients' families. The 3 proven IA episodes were diagnosed based on histopathology of a pharyngeal biopsy specimen, a surgical specimen of the brain, and a skin biopsy specimen, respectively. Although postmortem examinations disclosed superinfections of disseminated *Trichosporon* infection and atypical mycobacteriosis in episode 1 and

TABLE 1. Patient characteristics

Characteristic	Patients with:				Total[b]
	Proven IA	Probable IA	Possible IFI	No IA	
No. of episodes	9	2	13	125	149 (96)
No. of deaths	8	1	4	11	24
No. of autopsies	6	0	0	8	14
Age (yr)					
Mean	46	47	43	45	45
Median	42	47	40	47	46
Range	19–69	40–53	18–68	17–74	17–74
Sex (no. male/no. female)	6/3	2/0	12/1	82/43	102/47 (67/29)
No. with disease[a]					
AML	3	1	5	48	57 (29)
ALL	1	0	4	26	31 (19)
CML	0	1	2	8	11 (9)
MDS	3	0	2	11	16 (14)
NHL	2	0	0	28	30 (21)
AA	0	0	0	2	2 (2)
Other	0	0	0	2	2 (2)
No. with allografts	4	2	6	44	56
Duration of episode (days)					
Mean	126	92	78	50	57
Median	135	92	57	37	43
Range	36–234	50–134	35–172	11–181	11–234
No. with host factor:					
Neutropenia	7	1	8	86	102
Fever	6	1	7	37	51
GVHD	2	2	5	17	26
Steroid	2	1	4	28	35
None	1	0	0	18	19
Duration of neutropenia (days)					
Mean	63	10	42	16	21
Median	37	10	18	14	15
Range	0–205	0–20	0–162	0–120	0–205
No. of samples tested					
PCR	154	25	146	926	1,251
Mean (per episode)	17.1	12.5	11.2	7.4	8.4
Median (per episode)	17	13	9	6	6
Range (per episode)	7–32	6–19	4–24	3–26	3–32
GM	155	24	140	914	1,233
Mean (per episode)	17.2	12.0	10.8	7.3	8.3
Median (per episode)	18	12	9	5	6
Range (per episode)	7–30	5–19	5–24	2–26	2–30
BDG	158	24	147	914	1,243
Mean (per episode)	17.6	12.0	11.3	7.3	8.3
Median (per episode)	19	12	9	6	6
Range (per episode)	7–31	5–19	6–24	3–23	3–31

[a] AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CLL, chronic nyelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA, aplastic anemia.

[b] Values in parentheses are numbers of patients. Other values refer to numbers of episodes.

episode 9, respectively, no invasive candidiasis was documented during the study period.

Among the 125 no-IA episodes, 11 deaths occurred, and the diagnosis of no IA was confirmed by autopsy in 8. The other three fatal episodes were not confirmed by autopsy and included two respiratory failures following chemotherapy and one case of severe stomatitis following a second bone marrow transplantation. One respiratory failure was due to bacterial pneumonia, in which *Pseudomonas aeruginosa* was cultured from the sputum and the blood. In the other episode, respiratory failure developed in association with rapid tumor growth. Although no pathogen was identified despite repeated cultures, we could not completely exclude a possible infectious origin of this episode. The episode of severe stomatitis became suddenly fatal after the patient aspirated the clot and was asphyxiated.

ROC analysis. Figure 1 shows ROC curves for each test, using different definitions of the disease status. First, we examined the behaviors of the ROC curves for different diagnostic tests by using an "ideal" estimate (estimate A/B), in which episodes were expected to be most accurately defined. ELISA has a larger AUC in both method I (ELISA, 0.93; PCR, 0.81; BDG, 0.85) and method II (ELISA, 0.97; PCR, 0.76; BDG, 0.79). To increase the sensitivity for GM, we could more easily decrease its cutoff value with a small decrease in specificity. In contrast, a higher sensitivity could be obtained for the PCR and BDG tests by decreasing their cutoff values, but this would be at a significant cost in terms of specificity. When we shifted the diagnostic algorithm from method I (one positive sample) to method II (two consecutive positive samples), the AUC for the GM test was further increased while those for the PCR and BDG tests decreased, indicating that the GM test has higher

TABLE 2. Diagnosis of IA and its documentation

Episode no.	Patient characteristics[a]: Age (yr)	Sex	IA	Primary disease	Status of primary disease	Outcome	Host factors	Clinical evidence	Culture and its source	Histological evidence	Maximum value (method I/method II): PCR (copies/ml)	GM (O.D.I.)	BDG (ng/ml)
1	41	F	P	AML M1	Post-allo, RD	Dead	NF	Erosion of sinus walls	*A. flavus* and *A. fumigatus* from pharyngeal mucosa	Biopsy	2,000/200	3.8/3.6	19.7/4.7
2	32	M	P	MDS (RAEB-t)	Post-allo, CR	Dead	GS	Dyspnea, pleural effusion		Autopsy	32/0	1.3/1.0	60.5/36.5
3	58	M	P	AML M1	RD	Dead	NF	Halo sign		Autopsy	90/42.5	7.7/6.4	25/1.5
4	38	F	P	AML M2	Post-allo, CR	Alive	NS	Cavity within area of consolidation	*A. fumigatus* from broncheal lavage fluid	Biopsy	33.5/0	1.9/1.7	2.8/0
5	51	M	P	Macroglobulinemia	Stable disease	Dead	None	Extensive skull base destruction	*A. fumigatus* from epidural abscess	Biopsy	0/0	1.2/0.8	37.4/7.1
6	19	M	P	MDS RA	RD	Dead	NF	Multiple nodular lesions in the lung field, pleural effusion		Autopsy	3,500/1,000	2.5/1.5	155.5/59.2
7	42	M	P	MDS/AML	Post-allo, RD	Dead	NFG	Dyspnea, pleural effusion		Autopsy	24/9	2.4/0.6	0/0
8	63	F	P	ATL acute type	RD	Dead	NF	Dyspnea, pleural effusion		Autopsy	50/12.5	1.9/0.7	2.4/0
9	69	M	P	ALL PreB	RD	Dead	NF	No specific clinical evidence		Autopsy	100,000/5,000	4.2/1.1	171.7/12.6
10	53	M	PP	AML M2	Post-allo, CR	Dead	FG	Dyspnea, pleural effusion	*A. spergillus* spp. from bronchoalveolar lavage fluid	NA[b]	5/0	5.3/0.7	4.5/2.2
11	40	M	PP	CML CP1	Post-allo, CR	Alive	NGS	Halo sign	*A. fumigatus* from sputum	NA	11.5/7.5	2.3/2.0	0/0
12	68	M	PPP	MDS/AML	RD	Dead	NF	Multiple nodular lesions in the lung field, intraparenchymal brain mass lesion, seizure, hemiparesis		NA	155/100	2.2/1.5	18.3/16.6
13	24	M	PPP	AML M4E	CR, HDAraC	Alive	NF	Nodular skin lesion without any other explanation, multiple nodular lesions in the lung field		NA	20.5/0	4.5/0.3	0/0
14	61	M	PPP	AML M4E	CR, HDAraC	Alive	N	Halo sign		NA	1,000/9	0.2/0.1	3.5/2.9
15	30	M	PPP	ALL precursor B	Post-allo, CR	Alive	NFGS	Nonspecific abnormal shadow in lung field, pleural effusion		NA	60/60	0.6/0.4	0/0
16	61	M	PPP	AML M2	RD	Dead	NF	Multiple nodular lesions in the lung field, halo sign, cavity within area of consolidation		NA	84.5/0	1.1/0.7	2/0
17	68	M	PPP	CML BC	RD	Dead	NS	Dyspnea, pleural effusion		NA	165/0	0.3/0.2	0/0
18	25	M	PPP	ALL precursor B	RD	Alive	NG	Cavity within area of consolidation		NA	400/0	0.7/0.6	3.2/0
19	32	M	PPP	ALL PreB	Post-allo, CR	Dead	FGS	Dyspnea, pleural effusion		NA	27/1	0.7/0.5	3.7/2.4
20	18	F	PPP	AML M2	CR, HDAraC	Alive	N	Halo sign		NA	0/0	0.6/0.1	0/0
21	55	M	PPP	MDS RA	Stable disease	Alive	F	Cough, dyspnea, pleural effusion		NA	19/4	0.8/0.3	0/0
22	28	M	PPP	CML CP1	Post-allo, CR	Alive	G	Cough, dyspnea, pleural effusion		NA	0/0	0.4/0.3	0/0
23	40	M	PPP	CML CP1	Post-allo, CR	Alive	GS	Cough, dyspnea, new infiltrate not fulfilling the major radiological criteria without an alternative diagnosis		NA	6/0	0.5/0.4	0/0
24	54	M	PPP	ALL precursor B	Post-allo, CR	Alive	F	Dyspnea, new infiltrate not fulfilling the major radiological criteria without an alternative diagnosis		NA	10.5/0	0.5/0.3	0/0

[a] F, female; M, male; P, proven; PP, probable; PPP, possible; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; RA, refractory anemia; RAEB-t, RA with excess of blasts in transformation; ALL, acute lymphoblastic leukemia/lymphoma; CML, chronic myelogenous leukemia; CP, chronic phase; BC, blastic crisis; allo, allogeneic hematopoietic stem cell transplantation; CR, complete remission; RD, refractory disease; HDAraC, high-dose cytrabine; N, neutropenia; F, persistent fever; G, GVHD, S, prolonged use of corticosteroid.

[b] NA, not available.

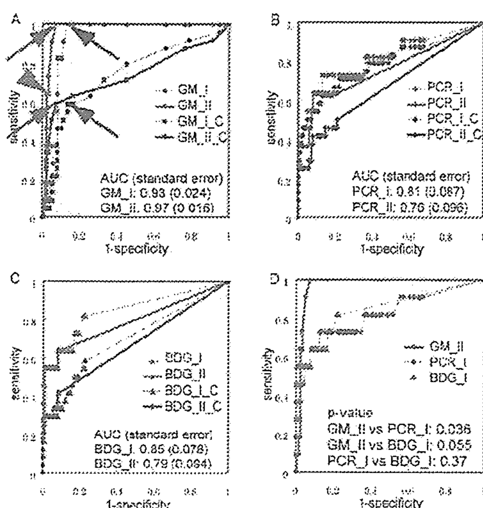

FIG. 1. (A to C) ROC curves of the GM (A), PCR (B), and BDG (C) tests for screening for IA. Both methods I and II were used. The ROC curves obtained by estimate A/B are shown in red, and those obtained by estimate C are shown in blue. The ROC curves obtained by method II are indicated by solid lines, and those obtained by method I are indicated by dotted lines. (D) Combination of ROC curves of the GM test (method II) and those of the PCR and BDG tests (method I).

reproducibility than the other two tests. The comparison of ROC curves of ELISA (method II), PCR (method I), and BDG (method I) is presented in Fig. 1D. When estimate C was applied for ROC analyses, these characteristics of the ROC curve for GM were partially obscured. In estimate C, a large decrease in sensitivity shifted the ROC curve downward and caused a significant reduction in AUC for the ELISA and BDG test, as expected. On the other hand, the ROC curve for the PCR test did not significantly change, since an expected decrease in sensitivity due to false-positive episodes in the possible IFI group is thought to be counterbalanced by a gain due to false-positive PCR results in these episodes. The ROC curves for the GM test in estimates A/B, C, and D, which is not presented but is similar to that for A/B, represent extreme cases, and the unknown "real" ROC curve might be mapped between these extremes.

Optimal cutoff value. Determination of an optimal cutoff value may be somewhat arbitrary depending on the purpose of the diagnostic test. A loss of specificity may be allowed to obtain a higher sensitivity. Based on the conventional or manufacturer-recommended cutoff values, an optical density index (O.D.I.) of 1.0, in two serial samples for GM (2, 22), i.e., 40 copies/ml for PCR and 11 pg/ml for the BDG test, all tests showed excellent specificity (0.98) in estimate A/B whereas their sensitivity was generally low (0.64, for GM, 0.45 for the PCR test, and 0.55 for the BDG test) even in estimate A/B, with further decreases as low as 0.33 for the GM test and 0.29 for the BDG test in estimate C. The current standard for ELISA (red arrowhead in Fig. 1A) seems to be inadequate. It could be reduced to 0.6 O.D.I. in method II (red arrows in Fig. 1A), or the criteria for positivity could be relaxed to those in method I while retaining the same cutoff (1.0 O.D.I.) (blue arrows), without great loss of specificity. With regard to specificity, the former may be recommended (P = 0.0334 by Fisher's direct test), which reflects a more leftward displacement of the ROC curve for method II. Both cutoff values represent the inflexion point of each ROC curve, around which the diagnostic efficacy is maximum for both cutoffs. The sensitivity/specificity and PPV/NPV of the GM test are 1.0/0.93 and 0.55/1.0 for a cutoff value of 0.6 O.D.I. in method II and 1.0/0.86 and 0.38/1.0 for a cutoff value of 1.0 O.D.I. in method I. Various diagnostic statistical parameters in different calculations are presented in Table 3. We may improve the diagnostic efficiency by using two or three tests in combination. In our analyses, however, we could not obtain better sensitivity by combination use of multiple tests employing much reduced cutoff values while maintaining high specificity (data not shown). This is also accompanied by significant delay of diagnosis.

Time interval between the first positive result and the antemortem diagnosis. Chronological relationships between the first positive results of different screening tests, histopathology, and diagnostic imaging are summarized in Fig. 2 and 3. For the PCR and BDG tests, the conventional cutoff was used, while the second of the first two consecutive results equal to or greater than 0.6 or 1.0 O.D.I. was plotted for ELISA. When the new reduced cutoff was used, the first positive date for GM was brought forward by a median of 10 (0 to 70, n = 9, mean = 24) days compared to the conventional cutoff value. Using the conventional cutoff, only one episode was identified to have a positive ELISA result before definitive treatment was started. In contrast, with the new reduced cutoff, the first positive ELISA result preceded the initiation of broad-spectrum antifungal treatment in seven IA-positive episodes (median, 31 days; range, 2 to 127 days; mean, 28 days). It became positive 51 days before a positive histopathology result (10 to 127 days; mean, 31 days).

Unfortunately, chronological comparisons between the three different assays were possible for only six episodes, in which patients had refractory leukemia and their IA tended to have a rapidly progressive course as a terminal infection (Fig. 3). In these episodes, ELISA gave positive findings earlier than (five episodes) or at the same time as (one episode) the BDG test (median, 16.5 days; range, 0 to 76 days). The PCR test was positive in 11 of 24 IA patients in estimate C. A comparison was possible in 5 of the 11 episodes, which were also positive for ELISA, but there was no significant difference in the date of the first positive result between ELISA and the PCR tests.

DISCUSSION

In this study, we compared the diagnostic potential of three different laboratory tests used to screen for IA in a prospective setting, where GM, DNA, and BDG levels in a cohort of patients at high risk for IA were measured weekly. The statistical parameters of a diagnostic test can be dramatically affected by the predetermined cutoff value, and when there is some uncertainty regarding the disease status, as in this case, they can also be influenced by the definition of the disease status. Therefore, to meaningfully compare the diagnostic potentials of these different tests, we performed an ROC analysis for each test by using the same cohort of patients with different positive result criteria (methods I and II) and various definitions of the disease status (estimates A/B, C, and D). As a

TABLE 3. Statistics for some selected thresholds

Method and threshold	Sensitivity A/B (C)	Specificity A/B (D)	PPV A/B (D)	NDV A/B (C)	Efficacy A/B (C)
Method I					
GM (O.D.I.)					
0.5	1.00 (0.88)	0.34 (0.33)	0.12 (0.11)	1.00 (0.93)	0.40 (0.43)
0.6	1.00 (0.79)	0.55 (0.54)	0.16 (0.15)	1.00 (0.93)	0.59 (0.59)
1.0	1.00 (0.58)	0.86 (0.85)	0.38 (0.34)	1.00 (0.91)	0.87 (0.81)
1.5	0.82 (0.46)	0.90 (0.89)	0.41 (0.38)	0.98 (0.90)	0.89 (0.83)
PCR (copies/ml)					
5	0.91 (0.88)	0.43 (0.41)	0.12 (0.11)	0.98 (0.95)	0.47 (0.30)
10	0.82 (0.79)	0.60 (0.55)	0.15 (0.13)	0.97 (0.94)	0.62 (0.63)
20	0.73 (0.67)	0.78 (0.75)	0.23 (0.19)	0.97 (0.92)	0.78 (0.77)
40	0.45 (0.46)	0.98 (0.93)	0.63 (0.36)	0.95 (0.90)	0.93 (0.89)
BDG (ng/ml)					
2	0.82 (0.58)	0.77 (0.76)	0.24 (0.21)	0.98 (0.91)	0.78 (0.74)
3	0.64 (0.46)	0.84 (0.82)	0.26 (0.23)	0.96 (0.89)	0.82 (0.78)
5	0.55 (0.29)	0.92 (0.92)	0.38 (0.35)	0.96 (0.87)	0.89 (0.82)
11	0.55 (0.29)	0.98 (0.97)	0.67 (0.60)	0.96 (0.88)	0.94 (0.87)
Method II					
GM (O.D.I.)					
0.5	1.00 (0.63)	0.84 (0.83)	0.35 (0.31)	1.00 (0.92)	0.85 (0.81)
0.6	1.00 (0.58)	0.93 (0.91)	0.55 (0.48)	1.00 (0.92)	0.93 (0.87)
1.0	0.64 (0.33)	0.98 (0.97)	0.70 (0.64)	0.97 (0.88)	0.95 (0.87)
1.5	0.45 (0.25)	0.98 (0.97)	0.63 (0.56)	0.95 (0.87)	0.93 (0.86)
PCR (copies/ml)					
5	0.64 (0.43)	0.87 (0.86)	0.30 (0.27)	0.96 (0.89)	0.85 (0.80)
10	0.45 (0.30)	0.94 (0.93)	0.38 (0.33)	0.95 (0.88)	0.90 (0.84)
20	0.36 (0.26)	0.98 (0.97)	0.67 (0.50)	0.95 (0.88)	0.93 (0.87)
40	0.36 (0.26)	1.00 (0.99)	1.00 (0.67)	0.95 (0.88)	0.95 (0.89)
BDG (ng/ml)					
2	0.64 (0.42)	0.91 (0.90)	0.39 (0.33)	0.97 (0.89)	0.89 (0.83)
3	0.55 (0.29)	0.95 (0.95)	0.50 (0.66)	0.96 (0.88)	0.92 (0.85)
5	0.55 (0.29)	0.98 (0.97)	0.67 (0.60)	0.96 (0.88)	0.94 (0.87)
11	0.45 (0.25)	0.99 (0.99)	0.83 (0.71)	0.95 (0.87)	0.95 (0.87)

result, the ROC curve for the GM test seemed to be better than those for the other two tests.

We previously reported that this real-time PCR for *Aspergillus* DNA was highly sensitive in vitro and with clinical samples (17): it could stably detect as few as 40 copies/ml in vitro and showed a higher sensitivity (79%) than those of the GM (58%) and BDG (67%) tests. In the present prospective analysis with consecutive patients, however, these results were not reproduced. This may be partly explained by the fact that our previous study included many retrospective samples. Furthermore, we intentionally selected IA patients and used a higher cutoff value for the GM test. Although several authors have also reported excellent sensitivity in PCR assays for IA (5, 6, 14, 34), we cannot directly compare those results with ours since there were differences in the target genes, methods of DNA extraction, starting materials, and designs of the PCR amplifications. Some form of standardization is required to make an international comparison possible. We used our real-time PCR system (GeniQ-Asper) (17) because it is most widely used in Japan. Several authors, including Loeffler et al. and Costa et al., also published excellent real-time PCR detection systems for *Aspergillus* DNA (9, 21, 26, 28), and their systems might produce superior results in the diagnosis of IA, which should be addressed in future studies.

As a diagnostic test, PCR requires more time and more complicated processing and thus costs more than the BDG and GM tests. It costs six times (15,700 yen/test) as much as the BDG and GM assays (2,700 yen/test) in Japan. A specialized laboratory as well as an expensive assay system and reagents are also required. These problems should be addressed before PCR is widely accepted as a standard screening test for IA, although it still seems to have value in making a diagnosis when a variety of clinical samples are used (20, 26, 28, 31).

The BDG test has also been widely used in Japan as a noninvasive diagnostic test for IFI. While it covers wide ranges of fungal species and may be potentially more useful as a screening test for IFI, it can cause frequent nonspecific reactions to various medical materials. Three kinds of assay systems for BDG have been developed in Japan: a chromogenic assay (FungiTec G test), β-glucan test Maruha) and a kinetic assay (β-glucan test Wako), but there is still some debate regarding their diagnostic potential. According to a sample-based analysis by Yoshida et al. (35), the chromogenic assay seems to be more sensitive (87.9 and 72.7%, respectively) than the kinetic assay but much less specific (43.3 and 75.2%, respectively) when the cutoff values recommended by the manufacturers are used. In the present study, where we used a kinetic assay, we could not obtain sufficient sensitivity even with the cutoff being maximally reduced. Furthermore, even if positive results were obtained, the positive results with the BDG test tended to occur later in the clinical course. The present result (55% sensitivity and 98% specificity) is consistent with our previous results (67% sensitivity and 84% specificity) using the chromogenic assay and also with other reports. This seems to be an inherent limitation of BDG assays

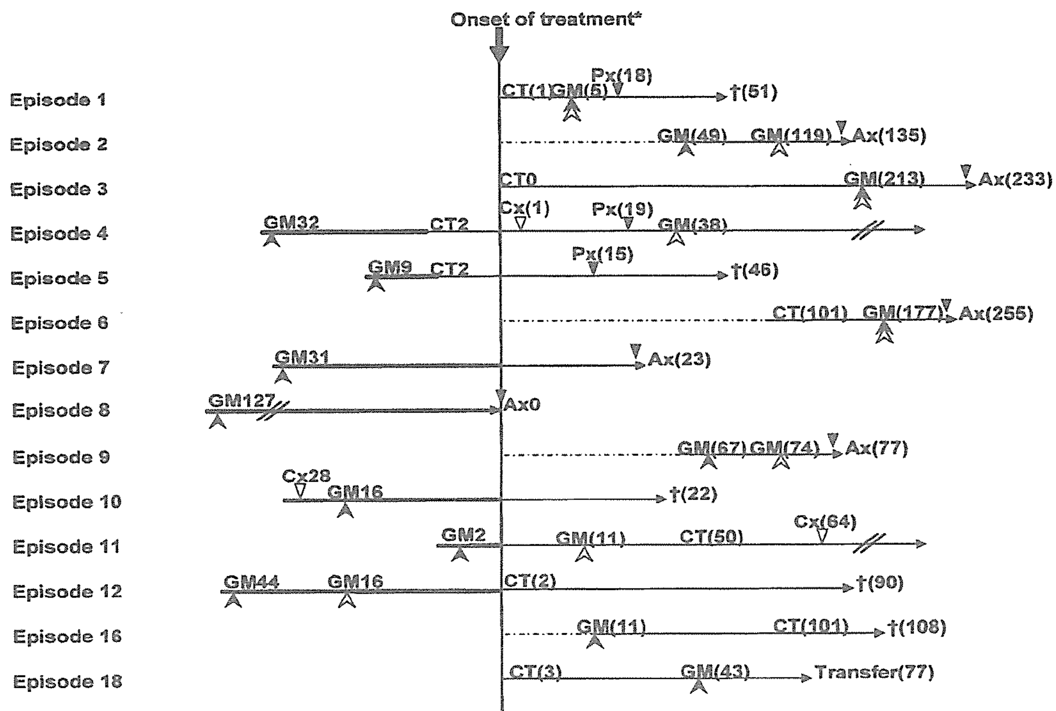

FIG. 2. Number of days from when GM assays become positive to the onset of treatment, using a threshold of 0.6 O.D.I. by method II (solid arrowheads) or 1.0 O.D.I. by method II (open arrowheads), or positive findings on CT. Open triangles indicate the date of positive culture, and solid triangles indicate when the histopathological diagnosis was made (Px, biopsy; Ax, autopsy). The values in parentheses indicate the number of days after the onset of treatment. For example, for episode 11, CT showed specific findings 50 days after the onset of treatment and the GM assay become positive 2 days before treatment. Episode numbers correspond to those in Table 2. Episodes whose GM assays did not reach the threshold are not shown. For episodes 2 and 9, a CT scan was not performed, and for episodes 7, 8, 10, 17, 19, 21, and 22, the CT findings were nonspecific and could not be used for decision-making. Each treatment was started at the discretion of the physician, taking into account various prices of clinical information, including CT findings and the results of GM assays. For Episode 8, IA was not suspected and no antifungal agent was administered. Therefore, the date of death was used instead of the date of treatment onset.

for the diagnosis of IA, although they show a very high sensitivity and specificity for candidiasis (25).

The diagnostic potential of double-sandwich ELISA for GM has been repeatedly validated in recent large-scale studies (15, 22). However, a direct comparison of the results of different studies, including ours, is not always easy and in fact can be quite difficult or impractical. Many factors can influence the apparent sensitivity and specificity and of course the PPV and NPV. Therefore, the important point is the way in which these results should be interpreted, and this depends on the objective and design of each study. From this perspective, our results are comparable to those of Maertens et al. (22) but in contrast to those of Herbrecht et al. (15). The latter addressed principally the diagnostic potential of the GM test in the presence of an unknown neutropenic fever or some respiratory signs and symptoms in cancer patients. On the other hand, in our study as well as in that of Maertens et al., the principal concern was the potential of the test in serial screenings with multiple measurements throughout the entire period of hematology care. For example, the mean numbers of measurements per episode in our study and that of Maertens et al. (8.3 and 11.2 per episode, respectively, with GM measured weekly) are significantly different from that in the study of Herbrecht et al. (5.5 per episode, with GM measured daily or weekly), consistent with the study designs. The difference becomes more prominent for proven IA episodes (17.3 and 19 versus 6.8). The differences in the mean number and timing of measurements clearly affect the apparent sensitivity and specificity of the studies. Hence, the apparent statistical values obtained by Herbrecht et al. are expected to be lower than ours and those of

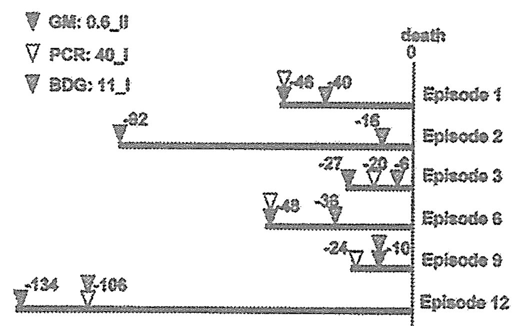

FIG. 3. Number of days before death that each test gave positive results. Solid triangles indicate the date when GM became positive, using a threshold of 0.6 O.D.I. by method II; open triangles indicate the date when PCR exceeded a cutoff value of 40 copies/ml; and shaded triangles indicate the date when the BDG test exceeded a cutoff value of 11 ng/ml, by method I. In episode 2, PCR never exceeded the cutoff value. Episode numbers correspond to those in Table 2.

Maertens et al., but they should provide a better approximation of the corresponding sample-based statistics, even though the patient population was more heterogeneous.

According to the ROC analysis of double-sandwich ELISA, the conventionally used cutoff seems to be too high: our recommendation is 0.6 O.D.I., and two consecutive positive results should be taken into consideration. With these new criteria, the GM test showed an excellent chronological profile. It gave the first positive diagnostic result in 9 of 14 GM-positive IA episodes and in 5 of 9 IA or possible IFI episodes where both CT and GM were positive. It preceded the initiation of empiric or definitive antifungal therapy in seven episodes. Using the novel criteria, positivity was ascertained a median of 10 days before conventional positivity was noted, and in six cases the GM test gave positive results only with the novel criteria. These chronological advantages were not observed with a threshold of 1.0 O.D.I. by method II: for episodes 5, 7, 8, and 10, the GM assay did not become positive; for episode 4, the GM assay exceeded the criteria 38 days after the onset of treatment; for episode 12, the GM assay gave positive results 16 days before the onset of treatment. According to the high PPV with the novel cutoff criteria (0.55 for proven or probable IA and 0.48 for proven, probable, or possible IFI) and the early timing of its positivity, we could have initiated antifungal therapy in a preemptive manner for episodes 4, 5, 7, 8, 10, 11, and 12.

Our result does not justify a discontinuation or moratorium of empiric antifungal treatment based only on a single negative result in the face of an impending threat of IA. It should be stressed that the extremely high NPVs provided here are episode-based calculations. Sample-based NPVs should be much lower, especially when patients are at high risk. We could not exclude a possibility of other IFI. Similarly, PPV does not always represent the probability of currently having IA but, rather, predicts the probability that the subject has or will have IA. In addition, while there was a sufficient number of no-IA episodes in this study to permit reliable estimations of specificity and NPV, there is much uncertainty regarding the estimations of the absolute values of sensitivity and PPV because of the small number of IA patients.

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EXTENDED REPORT

A phase I-II trial of autologous peripheral blood stem cell transplantation in the treatment of refractory autoimmune disease

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Objectives: To carry out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood stem cell transplantation (PBSCT) in the treatment of severe and refractory autoimmune disease (AD).
Methods: Peripheral blood stem cells (PBSCs) were mobilised during haematological recovery after relatively high dose CY (2 g/m^2) for 2 days, followed by administration of granulocyte colony stimulating factor. After collecting PBSCs—more than 2×10^6 CD34+ cells/kg—by apheresis, CD34+ cells were immunologically selected and cryopreserved. Eight patients were enrolled—five had systemic sclerosis (SSc) alone, one had SSc with systemic lupus erythematosus, one amyopathic dermatomyositis (ADM), and one Wegener's granulomatosis (WG). All of the patients were treated with high dose CY (50 mg/kg) for 4 days and autologous PBSCT.
Results: Haematopoietic reconstitution was rapid and sustained. Toxicity due to the regimen included various infections such as pneumonia, sepsis, cystitis, herpes zoster, and acute heart failure. However, there was no treatment related mortality. Encouraging results were obtained after autologous PBSCT. Sclerosis of the skin was markedly improved in all of the patients with SSc. Interstitial pneumonia (IP), evaluated by PaO_2, serum KL-6 levels, and pulmonary high resolution computed tomography, improved significantly. In a patient with ADM, severe and progressive IP also improved markedly. In a patient with WG, the size of the left orbital granuloma decreased substantially, resulting in reduction of the exophthalmos.
Conclusions: These observations suggest that high dose CY with autologous PBSCT is feasible and may be effective in the treatment of severe and refractory AD.

Although most patients with autoimmune disease (AD) have a relapsing, remitting or smouldering disease, some of them are damaged severely or fatally from the uncontrolled disease progression, and conventional treatments are not effective. The concept of high dose immunosuppressive treatment and autologous haematopoietic stem cell transplantation (HSCT) for AD is based on the finding that HSCT is effective for animal models of AD,[1–3] and that patients receiving autologous HSCT for treatment of malignant diseases can achieve long term remission of coincidental AD.[4–6]

Autologous HSCT as a treatment for AD was initiated in 1996, and more than 800 patients with AD have been treated.[7] Clinically significant responses were found in two thirds of the patients who received HSCT, and treatment related mortality (TRM) was reported to be relatively high (9%) in the early period until 2000.[8] The mechanism for inducing remission in AD is based not only on the eradication of autoreactive lymphocytes by an immunoablative pretransplant conditioning regimen but also on the correction of a dysregulated immune balance by newly developed lymphocytes derived from the haematopoietic stem cells transplanted.[9]

Many reports have examined the clinical results of autologous HSCT for AD. However, few studies provided detailed information about the effect of autologous HSCT on interstitial pneumonia (IP), which is often associated with AD. In the study by McSweeney *et al*, 19 patients with systemic sclerosis (SSc) were treated with high dose immunosuppression followed by autologous HSCT, resulting in no significant changes in carbon monoxide transfer factor (TLCO) or vital capacity (VC) at 12 months after autologous HSCT.[10] We carried out a phase I-II trial to elucidate the feasibility and efficacy of high dose cyclophosphamide (CY) supported by autologous peripheral blood CD34 selected stem cell transplantation (PBSCT) in patients with severe and refractory AD. We report encouraging results obtained in eight patients, suggesting that high dose CY with autologous PBSCT may be effective for treatment of AD complicated by IP.

Abbreviations: A-aDO_2, alveolar-arterial oxygen tension difference; AD, autoimmune disease; ADM, amyopathic dermatomyositis; ATG, antithymocyte globulin; CY, cyclophosphamide; HRCT, high resolution computed tomography; G-CSF, granulocyte-colony stimulating factor; HSCT, haematopoietic stem cell transplantation; IP, interstitial pneumonia; mRSS, modified Rodnan skin score; NHL, non-Hodgkin's lymphoma; NIH-CTC, National Cancer Institute-Common Toxicity Criteria; PaO_2, arterial oxygen pressure; PBSCs, peripheral blood stem cells; PBSCT, peripheral blood stem cell transplantation; SSc, systemic sclerosis; TBI, total body irradiation; TLCO, carbon monoxide transfer factor; TRM, treatment related mortality; VC, vital capacity; WG, Wegener's granulomatosis

Table 1 Patient profile

Patient No	Diagnosis	Sex	Age (years)	PS	mRSS	Major disorders associated with AD	VC/TLCO (%)	Auto-antibody	Prior treatment	Follow up (months)
1	SSc+SLE	F	54	2	16	IP, digital ulcer	58/51	Anti-Scl-70	St, CY	33
2	SSc	M	55	2	15	IP	65/47	Anti-Scl-70	St	25
3	SSc	M	58	2	31	IP	63/44	–	St	22
4	SSc	F	54	1	26	IP	73/60	Anti-Scl-70	St	20
5	SSc	F	53	1	28	IP	74/29	Anti-Scl-70	St	17
6	SSc	F	48	2	32	IP	77/25	Anti-Scl-70	St, CY, CsA	13
7	ADM	F	54	2		IP	50/50	–	St, CY, CsA	31
8	WG	M	21	1		Exophthalmos		Anti-PR-3	St, CY, CsA	16

PS, performance status; mRSS, modified Rodnan skin score for systemic sclerosis; AD, autoimmune disease; F, female; M, male; SSc, systemic sclerosis; IP, interstitial pneumonia; ADM, amyopathic dermatomyositis; WG, Wegener's granulomatosis; PR3, proteinase 3; St, corticosteroids; CY, cyclophosphamide; CsA, ciclosporin A.

PATIENTS AND METHODS

Protocol

The protocol of this phase I-II clinical trial was approved by the ethics committee of Kyushu University Hospital. Written informed consent was obtained from all patients.

Patients and eligibility

Patients aged between 16 and 65 years were eligible at the time of pretransplant evaluation. Patient eligibility depended on a diagnosis of AD. All of the patients were followed up for at least 12 months after transplantation for the evaluation of treatment outcomes.

Patients with SSc were eligible when they had severe diffuse SSc that had rapidly developed over the previous 4 years. They also had to have at least one of the following: (*a*) pulmonary involvement including VC or TLCO <70% predicted or arterial oxygen pressure (Pa_{O_2}) at room temperature below 70 mm Hg and evidence of interstitial lung disease defined by pulmonary high resolution computed tomography (HRCT); (*b*) cardiac disease, which was reversible congestive heart failure or significant arrhythmia; and (*c*) renal involvement such as hypertension, persistent urine analysis abnormalities, microangiopathic haemolytic anaemia, and renal insufficiency.

Patients with limited scleroderma were considered eligible when progressive and life threatening IP was present.

Patients with amyopathic dermatomyositis (ADM) were eligible when they had the following criteria: (*a*) clinical diagnosis of ADM by the criteria reported[11]; (*b*) progressive and life threatening IP that was refractory to conventional immunosuppressive treatment.

Table 2 Apheresis and CD34+ selection in eight patients

Characteristic	Median (range)
Apheresis	
Number of apheresis/patient	2 (1–4)
Total cells $\times 10^9$	22.3 (8.7–90.4)
CD34+ (%)	2.06 (0.11–4.72)
CD34+ $\times 10^7$	36.8 (10.1–161.1)
CD34+ $\times 10^6$/kg	7.61 (2.06–35.80)
CD34+ selection	
Total cells $\times 10^7$	33.4 (11.6–95.0)
Total cells $\times 10^6$/kg	5.11 (2.34–21.11)
CD34+ $\times 10^7$	28.0 (10.1–94.3)
CD34+ $\times 10^6$/kg	4.90 (2.10–21.10)
CD3+ $\times 10^5$	5.78 (0–15.00)
CD3+ $\times 10^4$/kg	0.91 (0–2.95)
Purity (%)	96.4 (87.0–99.3)
Yield (%)	75.7 (58.6–100.0)

Patients with Wegener's granulomatosis (WG) were eligible when they had the following criteria: (*a*) clinical diagnosis of WG by the criteria reported[12]; (*b*) vasculitis or granuloma causing severe organ damage that was refractory to conventional immunosuppressive treatment.

Exclusion criteria

Patients were excluded from the study when they had uncontrolled arrhythmia, heart failure with left ventricular ejection fraction (LVEF) <50%, mean pulmonary artery pressure >50 mm Hg, TLCO <20% predicted, and creatinine clearance below 40 ml/min/m^2.

Peripheral blood stem cell (PBSC) mobilisation, CD34 cell selection, and autologous PBSCT

PBSCs were mobilised during haematological recovery after relatively high dose CY (2 g/m^2) for 2 days followed by administration of recombinant human granulocyte-colony stimulating factor (G-CSF, filgrastim; Kirin Brewery, Tokyo, Japan) at a dose of 10 µg/kg as previously described.[13] After collecting PBSCs to obtain 2×10^6 CD34+ cells/kg or more by apheresis, CD34+ cells were positively selected using immunomagnetic beads with an anti-CD34 monoclonal antibody (CliniMACS, Miltenyi Biotec, Germany). Mobilisation of PBSCs was repeated when 2×10^6 CD34+ cells/kg were not obtained. For pretransplant conditioning, high dose CY (50 mg/kg) was given for 4 days from day −5 to −2. After transplantation of frozen-thawed CD34+ cells on day 0, G-CSF was administered from day 1. Acyclovir (250 mg/day, from day 1 to 18), ciprofloxacin (600 mg/day from day −7 to −1), fluconazole (400 mg/day from day −7 to 14, 200 mg/day from day 15 to 100), trimethoprim-sulfamethoxazole (1920 mg/day, from day −14 to −2 and 1920 mg/day, twice a week from day 30 to 100) were prophylactically given as previously described.[13]

Treatment outcome

The modified Rodnan skin score (mRSS) was used to evaluate the improvement of skin sclerosis in patients with SSc.[14] Arterial blood gas at room temperature, a pulmonary function test, pulmonary HRCT, and serological examinations were used to evaluate the effect of high dose CY on IP. HRCT scans were graded and scored blinded according to the relative amount of ground glass opacity and reticular infiltrates as follows: 1 = pure ground glass; 2 = ground glass more than reticular; 3 = ground glass equals reticular; 4 = reticular more than ground glass; 5 = pure reticular.[15] The lower grade indicates more active inflammation in this system. Regimen related toxicity was determined and graded according to the National Cancer Institute-Common Toxicity Criteria (NIH-CTC) version 2. Cytomegalovirus antigenaemia was determined as previously described.[16]

Table 3 Number of reinfused cells and haematological recovery

Patient No	Number of reinfused CD34+ cells ($\times 10^6$/kg)	Number of reinfused CD3+ cells ($\times 10^4$/kg)	ANC $>0.5\times10^9$/l (days)	Platelet $>50\times10^9$/l (days)	Interval between PBSC harvest and PBSCT (days)
1	8.4	0.33	9	20	27
2	4.9	0.27	9	10	64
3	2.2	2.95	10	12	39
4	2.1	1.71	11	16	87
5	7.2	13.00	13	9	51
6	4.0	2.35	11	11	355
7	4.9	0.50	8	10	31
8	5.0	0.52	13	11	50

ANC, absolute neutrophil count; PBSC, peripheral blood stem cell; PBSCT, peripheral blood stem cell transplantation.

Statistical analysis

Wilcoxon's signed rank test was used for statistical analysis of the data.

RESULTS

Patients

Eight patients (three male, five female) with a median age of 54 years (range 21–58) were studied (table 1). Patients 1–6 were diagnosed as diffuse SSc. Patient 1 had had systemic lupus erythematosus (SLE) for 22 years and SSc for 2 years. She had progressive IP and severe digital ulcers due to SSc while the SLE was inactive. Patients 2, 3, 5, 6 (SSc), and 7 (ADM) also developed severe and progressive IP. Patient 4 had mild IP. Patients 3, 4, 5, and 6 showed severe skin sclerosis. Patient 3 had been in complete remission of non-Hodgkin's lymphoma (NHL) for 1 year and he was considered to be eligible. Patient 8 (WG) presented with severe exophthalmos due to a granuloma, which was 18 mm in diameter and located in the upper lateral region of the left orbit affecting the superior rectus muscle. He needed monthly steroid pulse therapy to prevent further growth of the granuloma. Eastern Cooperative Oncology Group performance status[12] was <3 in all patients. Anti-Scl-70 antibody was positive in 5/6 patients with SSc. CY and ciclosporin A were used in four and three of the patients, respectively. All patients were treated with corticosteroids, and the median duration of follow up was 21 months (range 13–33). Results are reported as of February 2005.

PBSC mobilisation and CD34+ cell selection

PBSCs were collected by apheresis after CY plus G-CSF-induced mobilisation in all patients as previously described.[13] The median of the total number of CD34+ cells collected was 7.61×10^6/kg (range 2.06–35.80) after apheresis (table 2). CD34+ cell selection was performed using CliniMACS. Purity and yield of the CD34+ cells selected were 96.4% (range 87.0–99.3) and 75.7% (range 58.6–100), respectively. Mobilisation was repeated in patient 4 because an insufficient number of CD34+ cells (2×10^6/kg) were collected after the initial mobilisation.

Autologous PBSCT

All the patients received autologous transplantation of frozen-thawed CD34+ cells after pretransplant conditioning with high dose CY. The median numbers of CD34+ and CD3+ cells infused were 4.92×10^6/kg (range 2.1–8.4) and 1.17×10^4/kg (range 0.27–13.0), respectively (table 3). All the patients achieved rapid haematopoietic engraftment. Median days to an absolute neutrophil count $>0.5\times10^9$/l and a platelet count $>50\times10^9$/l were 10.5 (range 8–13) and 11.5 (range 9–20), respectively. The interval between PBSC harvest and PBSCT was a median of 50.5 days (range 27–355).

Toxicity

Patients 1, 6, and 7 developed post-transplant infections and showed grade 3–4 toxicity according to NCI-CTC (table 4). Patient 1 also developed pneumonia of unknown cause and adenoviral cystitis, and patient 6 showed positive blood cultures for *Streptococcus mitis* in addition to adenoviral cystitis. Adenoviral cystitis was successfully treated with cidofovir. Patient 7 had positive blood cultures for *Listeria monocytogenes*. Four patients developed herpes zoster with grade 2–3 toxicity around 12 months after transplantation. Five patients showed cytomegaloviral antigenaemia. Epstein-Barr titres were not checked in this study. Patient 1 had ventricular arrhythmia, and patient 4 showed ST depression in ECG during intravenous administration of CY. Patient 6 developed acute heart failure, requiring temporary intubation

Table 4 Toxicity (NCI)

Patient No	Infection	CMV antigen-aemia	Cardiovascular	Haemorrhage	Pulmonary	Gastro-intestinal	Hepatic
1	Pneumonia (3) cystitis, adenovirus (3)	+ (2)	VPCs (3)	–	–	Nausea (2)	Raised transaminase (1)
2	–	–	–	GI bleeding (3)	–	(1)	(1)
3	HZ (3)	+ (1)	–	–	–	(2)	(1)
4	HZ (3)	+ (1)	Cardiac ischaemia (2)	–	–	(2)	(2)
5	HZ (3)	–	–	–	Hypoxia (3)	(1)	–
6	Sepsis, *Strep. mitis* (3), cystitis, adenovirus (3)	+ (1)	CHF (4)	–	–	(2)	(3)
7	Sepsis, *Listeria monocytogenes* (3), HZ (2)	+ (2)	–	–	–	(2)	(2)
8	–	–	–	–	–	(1)	(2)

NCI, National Cancer Institute; CMV, cytomegalovirus; HZ, herpes zoster; *Strep*, *Streptococcus*; VPC, ventricular premature capture; GI, gastrointestinal; CHF, congestive heart failure; (number), grade of toxicity.

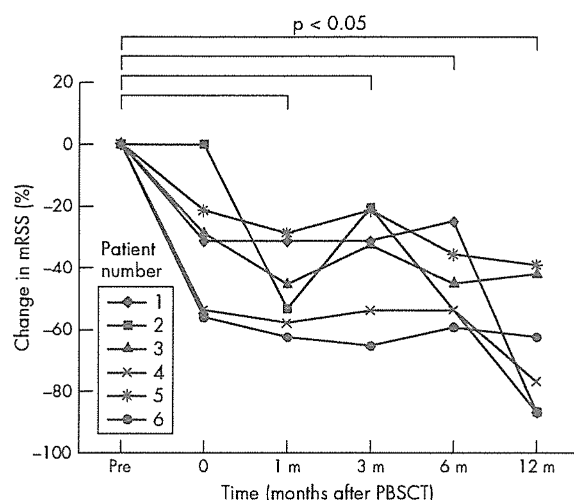

Figure 1 Evaluation of the modified Rodnan skin score (mRSS). The serial skin score data are presented for six patients with SSc. The proportional change from baseline measurement was calculated for each patient at each available time point. The x axis is not drawn to scale. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively.

after mobilisation. Patient 2 was complicated by grade 3 bleeding from an intestinal ulcer due to a non-steroidal anti-inflammatory drug during mobilisation. Patient 5 showed hypoxia due to transient worsening of IP shortly after administration of G-CSF. All the patients had grade 1–2 nausea and seven patients showed grade 1–3 hepatic toxicity. Twelve months after autologous PBSCT, patient 3 experienced a relapse of the NHL, which was successfully treated to reinduce complete remission by chemotherapy including rituximab. All the patients are alive with performance status 1 or 2.

CLINICAL OUTCOME

SSc

Figure 1 shows post-transplant changes in the mRSS for patients with SSc. A decline in skin score is considered significant if it is >25% of the baseline or >10% of the maximum skin score. When this definition is used, 6/6 (100%) patients showed significant improvement. The mean skin scores at 1, 3, 6, and 12 months post-transplant were significantly less than those before mobilisation ($p<0.05$). Five out of six patients showed an improvement in the skin score after mobilisation before pretransplant conditioning, although it was not statistically significant. Reincreases in skin score were not seen in any of the three patients who were followed up for 18 months or more after autologous PBSCT (data not shown).

To investigate the effect of autologous PBSCT on IP, blood gas analysis, a pulmonary function test, and pulmonary HRCT were performed at 3 and 12 months after transplantation. Figure 2A shows that Pao_2 was significantly increased from the median value of 66.5 mm Hg (range 51–88.7) before transplantation to 78.3 mm Hg (69.7–102) and 83.2 mm Hg (72.6–93.2) at 3 and 12 months after transplantation, respectively. Improvement of alveolar-arterial oxygen tension difference (A-aDo_2) was also seen in four patients at 12 months (fig 2B). The VC was improved in four and five patients at 3 and 12 months, respectively (fig 2C). Improvement of TLCO was seen in only one patient (fig 2D). Serum levels of KL-6, a marker for IP,[18] significantly decreased from the median value, 1823 U/ml (range 1080–2988) before transplantation to 890 U/ml (740–1962) and 989 U/ml (532–1273) at 3 and 12 months after transplantation, respectively (fig 2E). The ground glass opacity markedly regressed in all of the patients, although reticular infiltrates remained essentially unaffected after transplantation (fig 3), resulting in significant improvement of pulmonary HRCT grading from the median value of 2.5 (range 2–3) before transplantation to 4 (range 3–4) at 12 months after transplantation (fig 2F).

ADM

Skin lesions had resolved by conventional immunosuppressive treatment before mobilisation. Pao_2 was increased from 65.6 mm Hg before transplantation to 87.8 and 83.9 mm Hg at 3 and 12 months after transplantation, respectively. VC increased from 52.6% to 59.3% and 74.1% of the predicted value at 3 and 12 months, respectively. KL-6 decreased from 3280 IU/ml before transplantation to 1020 and 425 IU/ml at 3 and 12 months after transplantation, respectively. Both the ground glass opacity and the reticular infiltrates were markedly improved in pulmonary HRCT at 12 months post-transplant. The clinical course of this case has been described in detail elsewhere.[19]

WG

The size of the left orbital granuloma markedly decreased, resulting in an improvement of the exophthalmos, and regrowth of the granuloma has not been seen. Monthly steroid pulse therapy was not necessary to maintain this remission state. A serum level of proteinase 3 (PR3)-anti neutrophil cytoplasmic antibodies (ANCA) decreased from 72 IU/ml before transplantation to 39 IU/ml at 3 months after transplantation. However, it increased again to 157 IU/ml 12 months after transplantation.

DISCUSSION

In this study, we demonstrated that high dose CY with autologous PBSCT was feasible and effective in the treatment of refractory AD. For patients with SSc, we first showed that high dose CY and autologous PBSCT had favourable effects not only on skin sclerosis but also on IP. We thought our patient with WG was probably the first to have been treated with high dose CY and autologous PBSCT. However, during the revision of this manuscript a similar case was reported in the *Annals*.[19a]

We used a combination of high dose CY and G-CSF to mobilise a sufficient number of PBSC without the disease flare, although G-CSF alone was able to mobilise PBSC.[10] Flares of AD when G-CSF is used have been reported in rheumatoid arthritis,[20] multiple sclerosis,[21] and SSc.[22] In the European trial for SSc, the use of CY+G-CSF (84% of the cases) was preferred rather than G-CSF alone (10.7%).[23] In our trial, one patient had to repeat the mobilisation because an insufficient number of PBSC were obtained by the initial mobilisation. In another study, one of 12 PBSC mobilisations failed with the same protocol and autologous bone marrow transplantation was subsequently performed instead.[24]

We used immunological selection of CD34+ cells from PBSC harvests to minimise the risk of reinfusing autoreactive lymphocytes.[25] The selection device (CliniMACS) permitted good yield and purity of CD34+ cells with few contaminated T cells. In a study of patients with malignancy and concomitant AD, a high rate of recurrent AD was seen when unmanipulated autografts were used.[26] In the European phase I-II trial for SSc, 47/55 (85%) patients received CD34+ selection.[23] On the other hand, a randomised trial of 31 patients with rheumatoid arthritis comparing T cell depleted *v* unmanipulated autologous PBSCT after high dose CY (200 mg/kg) without additional T cell purging agents failed to

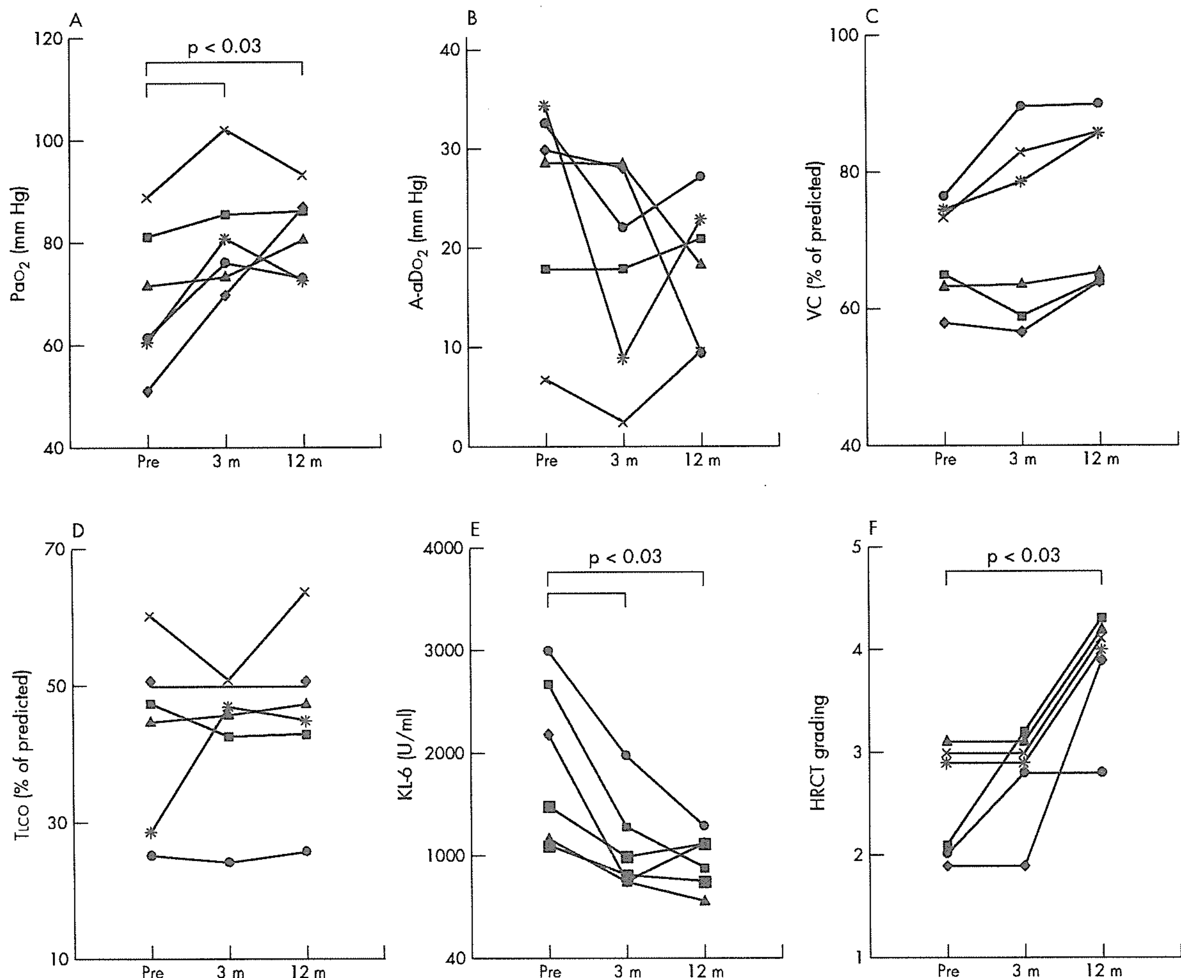

Figure 2 Evaluation of variables associated with IP in six patients with SSc before mobilisation, and at 3 and 12 months after autologous PBSCT. (A) Pao_2 at room temperature; (B) A-aDo_2; (C) VC; (D) TLCO; (E) KL-6; (F) pulmonary HRCT grading. The x axis shows time. Data obtained before mobilisation and just before conditioning are shown as "Pre" and "0" respectively. m, month.

demonstrate significant differences between the two groups.[27] The usefulness of T cell depletion should also be investigated carefully in patients with other AD.

Six of the eight patients had infectious episodes. Viral infections were more common than bacterial infections. Other toxicity included cardiac toxicity of CY and temporary exacerbation of IP by G-CSF. Patient 6 developed acute heart failure, requiring temporary intubation after mobilisation, which might be due to the combination of viral myocarditis and cardiac toxicity of CY. She recovered from heart failure and received autologous PBSCT 1 year later. Patient 3 had a relapse of NHL. The relation between autologous PBSCT and the relapse of NHL was not clear because autologous PBSCT was also used for treatment of NHL. There was no TRM in our study, whereas an early European study and the study by McSweeney *et al* reported that the overall TRM was 9% and 15.8%, respectively.[5 10] One of the most important toxicities was cardiotoxicity, possibly related to direct CY toxicity and hyperhydration. The patient selection was reported to be important to reduce TRM[23] and a full cardiological assessment before transplantation was recommended by the European group.[28] Careful patient selection, especially in the light of cardiac function evaluation, which is often underestimated in patients with severe rheumatic diseases, may have allowed us to avoid TRM.

Significant improvement in the skin score of >25% after autologous PBSCT occurred in all of the patients with SSc. In the European study and the study by McSweeney *et al*, it occurred in 69% and 100% of the patients transplanted, respectively.[10 29] The mechanism for the effect of autologous PBSCT on skin sclerosis may be due to intensive immunosuppression and immune reconstitution. In the European study and the study by McSweeney *et al*, 35% and 0% of the patients with an initial response relapsed during 20 and 14.7 months of median follow up after transplantation, respectively.[10 23] A longer follow up is necessary to assess the response duration of skin sclerosis in our trial.

Improvement of IP has been demonstrated in patients with SSc and ADM, whereas pulmonary function remained unaffected in other studies.[10 23 29] In this study, Pao_2, KL-6, and pulmonary HRCT grading were significantly improved, while TLCO values showed no significant change. KL-6 is a high molecular weight glycoprotein recently identified in humans as MUC1 mucin. It is a useful marker in the evaluation of disease activity not only of idiopathic pulmonary fibrosis but also of IP associated with collagen vascular

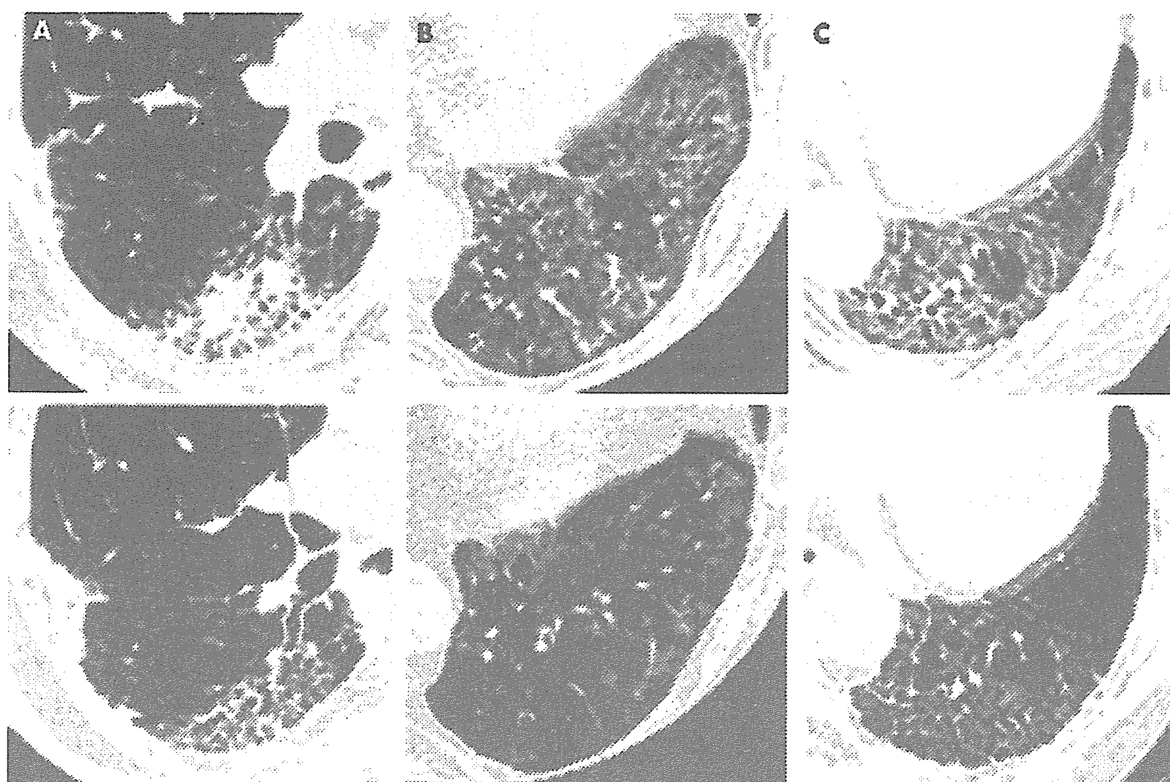

Figure 3 Pulmonary HRCT in patients with SSc. Upper panel, before mobilisation; lower panel, 12 months after autologous PBSCT. (A) Patient 1; (B) patient 2, (C) patient 4.

disease.[30] VC was increased in 5/6 patients with SSc, although it was not statistically significant. McSweeney *et al* treated 19 patients with SSc with high dose CY, total body irradiation (TBI), and antithymocyte globulin (ATG) followed by autologous PBSCT. They reported a significant decrease in the TLco values at 3 months but not at 12 months and no significant change in the VC at 3 or 12 months after autologous PBSCT.[10]

Because IP was not evaluated with respect to Pa_{O_2}, KL-6, or pulmonary HRCT grading in previous studies, the improvement of IP might have been undetectable. Selection biases of patients may be another reason; we might have selected patients with more active IP without honeycombing, whereas more patients with inactive and stable IP might have been selected in other studies. A different treatment regimen, especially the use of TBI, might be responsible for the different results. It is reasonable to suppose that high dose CY and autologous PBSCT could provide favourable effects on the IP of patients with SSc because intravenous pulse CY was reported to be effective for IP in patients with collagen vascular diseases including SSc.[15 31] In this study, improvement of TLco was not seen, as it was in previous studies, despite the improvement of KL-6 and pulmonary HRCT grading. Because TLco reflects not only interstitial lesions but also microvascular lesions of the lung,[32] periphery distributed microvascular impairment of the lung due to SSc may not have improved after autologous PBSCT compared with the improvement of interstitial lesions, resulting in the absence of an improved TLco.

In this study, a patient with WG receiving autologous PBSCT was described. In the European study, three patients with WG receiving autologous PBSCT were listed, but the treatment outcome was not described.[8 33] In our case, G-CSF in combination with CY did not cause a disease flare, and high dose CY with autologous PBSCT produced long term remission for more than 16 months.

We did not incorporate ATG into the conditioning regimen. Although ATG is believed to be useful for deleting the residual T cells and is often used in the other settings,[10 23 29] its usefulness has not been fully proved. Because we obtained significant clinical responses and considerable susceptibility to infections when treating with CY alone, ATG did not seem to be necessary. We did not incorporate TBI for a similar reason. Although our conditioning regimen was less intense than high dose CY with ATG and/or TBI, initial clinical responses were comparable, at least at the 12 month follow up. It is important to look at the response duration of our regimen in comparison with those of more immunosuppressive regimens. A randomised controlled trial will be necessary to assess the usefulness of ATG and/or TBI.

Most of the patients showed an improvement in disease activity after high dose CY and G-CSF for PBSC mobilisation before pretransplant conditioning, as shown in a previous study.[34] Haematopoietic stem cells express high levels of aldehyde dehydrogenase, an enzyme responsible for cellular resistance to CY. Hence, high dose CY should have strong effects on fully differentiated and aggressive autoreactive lymphocytes and allow immune reconstitution by newly developed lymphocytes from CY resistant haematopoietic stem cells.[35]

In conclusion, the present phase I-II study demonstrated that high dose CY with autologous PBSCT is feasible and effective in the treatment of refractory AD. We first demonstrated the clinical effects of high dose CY with autologous PBSCT on IP of SSc and on granuloma of WG. A prospective study with longer follow up time and more

patients will be necessary to assess the efficacy of this treatment modality in AD.

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Myeloablative allogeneic hematopoietic stem cell transplantation for non-Hodgkin lymphoma: a nationwide survey in Japan

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We retrospectively surveyed the data of 233 patients who underwent myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) for non-Hodgkin lymphoma (NHL). Donors were HLA-matched relatives in 154 patients (66%) or unrelated volunteers in 60 (26%). Ninety patients (39%) were in complete remission. One hundred ninety-three (83%) received a total body irradiation (TBI)–based regimen, and 40 (17%) received a non–TBI-based regimen. Acute graft-versus-host disease (GVHD) occurred in 155 (67%) of the 233 evaluable patients; grade II to IV in 90 (39%), and grade III to IV in 37 (16%). Treatment-related mortality (TRM) was observed in 98 patients (42%), and 68% of them were related to GVHD. In a multivariate analysis, chemoresistance, prior autograft, and chronic GVHD were identified as adverse prognostic factors for TRM. Relapse or progression of lymphoma was observed in 21%. The 2-year overall survival rates of the patients with indolent (n = 38), aggressive (n = 111), and lymphoblastic lymphoma (n = 84) were 57%, 42%, and 41%, respectively. In a multivariate analysis, chemoresistance, prior autograft, and prior radiotherapy were identified as adverse prognostic factors for overall survival. Although myeloablative allo-HSCT represents an effective therapeutic option for patients with NHL, more work is still needed to decrease TRM and relapse. (Blood. 2006;108:382-389)

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Introduction

Hematopoietic stem cell transplantation (HSCT) for patients with non-Hodgkin lymphoma (NHL) has been mainly focused on an autograft strategy. High-dose therapy with autologous HSCT (auto-HSCT) can increase remission rates and possibly prolong disease-free survival and overall survival (OS) in patients with chemotherapy-sensitive NHL at relapse.[1] This is also effective as first-line therapy for those with advanced aggressive lymphoma.[2] Nevertheless, relapse is a frequent cause of treatment failure after auto-HSCT.[1,3]

Allogeneic HSCT (allo-HSCT) has several advantages over auto-HSCT, because the former can avoid the reinfusion of malignant cells and can also be associated with a graft-versus-lymphoma (GVL) effect, which might reduce the risk of relapse. Most physicians believe that a small fraction of patients with end-stage aggressive lymphoma can still achieve prolonged lymphoma-free survival with the application of allo-HSCT. However, the high incidence of treatment-related mortality (TRM) (up to 55%) associated with allogeneic HSCT with a myeloablative regimen has prevented the wider application of this strategy.[4-8] Several reports on allo-HSCT for refractory or advanced lymphoma, as well as studies comparing auto- versus allo-HSCT for NHL, have been published over the past decade.[8-10] However, most of these studies were small and nonrandomized, and incorporated patients who had heterogeneous backgrounds. Thus, the role of allo-HSCT in the treatment of NHL remains controversial. Moreover, the outcome of allo-HSCT in each histologic subtype has not been fully determined. Previous studies have suggested that allo-HSCT improves the prognosis of patients with advanced follicular lymphoma (FL),[7,10,11] whereas few reports have been published on its benefit in aggressive lymphoma.[12,13] In particular, there has been very little information available on subtypes, including mantle-cell lymphoma[11,14]; peripheral T-cell lymphoma, unspecified (PTCL)[15]; natural killer (NK) cell lymphoma[16]; and anaplastic large cell lymphoma.

The application of reduced-intensity stem cell transplantation (RIST) or "nonmyeloablative" HSCT has been reported to decrease

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TRM.[17-19] Additionally, the recent development of supportive treatments may have decreased the risk of TRM and facilitated the application of allo-HSCT to NHL.[20] Therefore, we conducted a retrospective nationwide survey on Japanese patients with NHL who had undergone conventional allo-HSCT to establish a benchmark of myeloablative allo-HSCT in the treatment of NHL.

Patients, materials, and methods

Data sources

This survey collected the data of 233 consecutive patients who received myeloablative allo-HSCT for NHL between 1990 and 2001 in 56 participating hospitals. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of graft-versus-host disease (GVHD). The indications for allo-HSCT were left to the discretion of each institution. The patients included in this study received a conditioning regimen with an intensity that was equivalent to that of total body irradiation (TBI) plus cyclophosphamide or busulfan plus cyclophosphamide. Patients who had previously received monoclonal antibody therapy or T-cell–depleted transplantation, those younger than 14 years, and those who received RIST were not included. Additionally, those with adult T-cell leukemia/lymphoma were excluded because their clinical course differed from that of other types of lymphoma. The minimum data required for the inclusion of a patient in this study were age, sex, histologic diagnosis, prior treatment details, status at transplantation, donor information, conditioning regimen, date of transplantation, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression/death, and cause of death. Approval was obtained from the institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Definitions

The initial institutional histologic diagnosis was further reviewed by a pathologist (K. Takeuchi) using the WHO classification.[21] Briefly, NHL was divided into 3 clinical subtypes: indolent, aggressive, and lymphoblastic lymphoma. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma. Transformed indolent lymphoma and Burkitt lymphoma were classified as aggressive lymphoma. Furthermore, because most of the patients were evaluated before publication of the WHO classification, this analysis only included those who had tumors that formed lesions, such as T-cell lymphoblastic lymphoma (T-LBL), and all other patients who had features of leukemia were excluded. Those with chemosensitive disease included all patients who had shown a response to the last chemotherapy prior to transplantation (partial remission [PR], complete remission [CR] unconfirmed, and CR), whereas chemoresistant disease included those with primary refractory disease or refractory relapse prior to transplantation. Acute and chronic GVHD was graded according to the consensus criteria.[22,23] Patients who survived 100 days were evaluable for the assessment of chronic GVHD. OS was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until disease progression (PD)/relapse or death from any cause. Patients who died from transplantation-related causes were classified as TRM regardless of their disease status.

Statistical analysis

OS and PFS were calculated using the Kaplan-Meier method.[24] Surviving patients were censored on the last day of follow-up, in July 2002. The associations among patient-, disease-, and transplantation-related factors and OS were assessed by using univariate and multivariate Cox proportional hazards models. The associations between these factors and TRM were assessed by using univariate and multivariate logistic models. The variables analyzed included age, clinical subtype, histologic diagnosis, chemosensitivity, history of autograft or radiotherapy, years of transplantation, donor, source of stem cells, TBI-containing regimen, GVHD prophylaxis, and acute and chronic GVHD. Acute GVHD was treated as a time-dependent covariate in the Cox model. Stepwise variable selection at a significance level of .05 was used to identify covariates associated with outcomes. TRM and disease progression/relapse were calculated by using cumulative incidence. The statistical analysis was performed with the SAS 8.2 program package (SAS Institute, Cary, NC).

Results

Patients' characteristics

The patients' characteristics are listed in Table 1. All patients were younger than 60 years at the time of transplantation, with a median age of 31 years. Thirty-eight patients (16%) had indolent lymphoma, 111 (48%) had aggressive lymphoma (diffuse large B-cell, n = 44; PTCL, n = 22; extranodal NK/T-cell, n = 19; anaplastic large cell, n = 7; mantle cell, n = 5; Burkitt, n = 4; angioimmunoblastic T cell, n = 2; blastic NK cell, n = 2; hepatosplenic T-cell, n = 2; subcutaneous panniculitis like T cell, n = 2; mycosis fungoides with visceral dissemination, n = 2), and 84 (36%) had lymphoblastic lymphoma. Ninety patients (39%) were in CR, 38 (16%) were in PR, 42 (18%) were in primary refractory, and 63 (27%) had refractory relapse at the time of allo-HSCT. Ninety patients (39%) had received 4 or more chemotherapy regimens before allo-HSCT. Forty patients (17%) had received prior autograft, and 81 (35%) had received prior radiotherapy. One hundred fifty-four patients (66%) received a transplant from a human leukocyte antigen (HLA)–matched related donor, 19 (8%) from a 1-antigen–mismatched related donor, 43 (19%) from a matched unrelated donor, and 17 (7%) from a 1-antigen–mismatched unrelated donor. One hundred fifty-nine (68%) patients received bone marrow (60 from an unrelated donor) and 70 (30%) received granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood. One hundred ninety-three patients (83%) received TBI-based myeloablative regimens, including TBI 12 Gy plus cyclophosphamide (n = 60); a combination of TBI, cyclophosphamide, and etoposide (n = 47); or TBI, cyclophosphamide, and cytarabine (n = 40). Forty patients (17%) received a non–TBI-based myeloablative regimen, including a combination of busulfan and cyclophosphamide with or without other agents (n = 27); melphalan, thiotepa, and busulfan (n = 3); cytarabine, ranimustine, carboplatin, cyclophosphamide, and total lymphoid irradiation (n = 2); or cytarabine, etoposide, and busulfan (n = 2). The remaining 6 patients received individualized regimens. GVHD prophylaxis included a combination of cyclosporin and methotrexate in 204 (88%) or tacrolimus and methotrexate in 22 (9%). Two hundred twenty-six patients (97%) were treated with G-CSF, starting at days +1 to +6 after graft infusion until engraftment.

GVHD

Acute GVHD occurred in 155 (67%) of the 233 patients: grade I in 65 (28%), grade II to IV in 90 (39%), and grade III to IV in 37 (16%) patients. Of the 165 patients who survived the initial 100 days after allo-HSCT, chronic GVHD occurred in 79 (48%), with extensive type in 48 (29%). In allo-HSCT from related (n = 173) and unrelated (n = 60) donors, grade II to IV acute GVHD occurred, respectively, in 61 (35%) and 30 (50%), grade III to acute GVHD occurred in 25 (15%) and 12 (20%), chronic GVHD occurred in 54 (31%) and 25 (42%) patients, and chronic extensive

Table 1. Patient-, disease-, and transplantation-related characteristics

Variable	No. (%)*
Patient characteristics	
Younger than 40 y	158 (68)
40 y or older	75 (32)
Male sex	150 (64)
Disease characteristics at diagnosis	
Histology	
Indolent	38 (16)
Follicular	37 (16)
MALT	1 (0)
Aggressive	111 (48)
Diffuse large B cell	44 (19)
Peripheral T cell, unspecified	22 (9)
Extranodal NK/T cell, nasal type	19 (8)
Anaplastic large cell	7 (3)
Mantle cell	5 (2)
Others	14 (6)
Lymphoblastic	84 (36)
Precursor B cell	7 (3)
Precursor T cell	77 (33)
Stage I	9 (4)
Stage II	25 (11)
Stage III	30 (13)
Stage IV	150 (64)
No data	19 (8)
Disease characteristics at transplantation	
Response to chemotherapy†	
Sensitive	128 (55)
Complete remission‡	90 (39)
Partial remission	38 (16)
Resistant	104 (45)
Primary refractory disease	41 (18)
Refractory relapse	63 (27)
No. of prior chemotherapy regimens†	3 (0-11)
Fewer than 4 regimens	143 (61)
At least 4 regimens	90 (39)
Prior autograft	40 (17)
Prior radiotherapy	81 (35)
Transplantation characteristics	
Year of transplantation	
1990-1995	46 (20)
1996-2001	187 (80)
No. of patients receiving a transplant per hospital	
Fewer than 9 patients	146 (63)
At least 9 patients	87 (37)
Donor	
HLA-matched related	154 (66)
HLA-1 antigen-mismatched related	19 (8)
HLA-matched unrelated	43 (19)
HLA-1 antigen-mismatched unrelated	17 (7)
Donor-recipient sex match	
Male-male	80 (34)
Male-female	66 (28)
Female-male	33 (14)
Female-female	46 (20)
Donor-recipient CMV status§	
+/+	131 (57)
−/+	14 (6)
+/−	14 (6)
−/−	11 (5)
Source of stem cells	
Bone marrow	159 (68)
Peripheral blood cells	70 (30)
Bone marrow + peripheral blood cells	2 (1)
Cord blood	2 (1)
Conditioning regimen	
TBI-containing	193 (83)
Non-TBI	40 (17)
GVHD prophylaxis	
Cyclosporin + methotrexate	204 (88)
Tacrolimus + methotrexate	22 (9)
Others	7 (3)

The study included 233 patients. The median age was 31 years (range, 15-59 years). Age was a continuous variable.

MALT indicates extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; NK, natural killer; HLA, human leukocyte antigen; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Categoric variable.

†One patient with mediastinal B-LBL did not receive prior chemotherapy for an unknown reason but did receive prior radiotherapy.

‡Includes 2 patients in complete remission, unconfirmed.

§Sixty-three pairs were not evaluated for CMV status.

GVHD occurred in 33 (19%) and 16 (27%). In allo-HSCT from HLA-matched (n = 197) and mismatched (n = 36) donors, grade II to IV acute GVHD occurred, respectively, in 76 (39%) and 15 (42%), grade III to IV acute GVHD occurred in 30 (15%) and 7 (19%), chronic GVHD occurred in 65 (33%) and 14 (39%), and chronic extensive GVHD occurred in 41 (21%) and 7 (19%). The distribution pattern of the incidences of acute and chronic GVHD by background factors was analyzed by using a chi-square test. Although none of the factors correlated with acute GVHD, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate ($P = .015$, chi-square test; $P = 0.023$, Fisher exact test).

Disease response

Of the 143 patients who had measurable disease at allo-HSCT, 89 (62%) achieved CR, 7 (5%) PR, 6 (4%) stable disease (SD), and 12 (8%) PD, whereas 29 (20%) were not evaluable because of early death. Of the 90 patients who were in CR at transplantation, 80 (89%) maintained CR, 4 (4%) showed PD, and 6 (7%) were not evaluable because of early death. Thirty-five patients died before the first response evaluation, with a median survival of 29 days (range, 0-72 days) after allo-HSCT. In the 27 patients with indolent lymphoma who had measurable disease at allo-HSCT, 22 (81%) achieved CR or PR. In the 72 patients with aggressive lymphoma who had measurable disease at allo-HSCT, 49 (68%) achieved CR or PR. In the 41 patients with lymphoblastic lymphoma who had measurable disease at allo-HSCT, 26 (63%) achieved CR.

TRM, disease relapse, and progression

Ninety-eight patients (42%) died of TRM, and its cumulative incidence is shown in Figure 1. Of the 98 patients who died of therapy-related complications, 60 (61%) died within day 100 of transplantation and 38 (39%) died thereafter. The major causes of TRM included GVHD (n = 11), infection (n = 29), interstitial pneumonitis (n = 16), venoocclusive disease of the liver (n = 11), thrombotic microangiopathy (n = 8), heart failure (n = 7), hemorrhage (n = 4), renal failure (n = 3), and others (n = 9), as shown in Table 2. The causes of infection-related mortality (n = 29) were bacterial (n = 13), fungal (n = 11), or viral (n = 5). Seventeen (59%) of 29 patients died of infections within 100 days of allo-HSCT, 7 (24%) from 101 days to 1 year and 5 (17%) thereafter. Fourteen patients died of TRM before engraftment. Of the 98 patients who died of TRM, 67 (68%) had GVHD, and 11 of