凍結

Pro	Con
●日程調整しやすい ●Poor mobilizer対策 ●ドナー・採取医・患者・主治医のストレス回避 ●採取と並行して前処置が実施されないので，無理な採取が防止できる	●骨髄と同じであるべき ●廃棄が増え，ドナーの善意がむだになる ●凍結によるリスク

⇩

骨髄も同じ

凍結の可否は，骨髄も含めて決めるべき，との結論に至った

それまでは，原則禁止だが
凍結を希望する場合は，個別審査で対応？

凍結を希望する場合とは

ドナー・採取施設と，移植時期の調整がつきにくい
ドナー・患者に大きな体重差？（これらの状況はBMでもある）

凍結を認める条件

●凍結は移植施設で実施
●施設認定の際に，凍結の質が担保されるかどうか厳格に審査する
　"院内における血液細胞処理の指針"をクリアするか否か
●凍結がドナー・患者双方にメリットとなる
●凍結後の移植計画が妥当（使用することが明確）
●凍結する場合は，1日目，2日目ともに当日運搬し，凍結する
●後日，適切に使用されたか，検証する

廃棄

シチュエーション	廃棄場所	費用負担
		G-CSF，入院，アフェレーシス，輸血費
アフェレーシス後	採取施設	パンク？
運搬後	移植施設	パンク？
凍結後	移植施設	パンク？

安易な廃棄に歯止めをかけるため，患者負担という意見もあった
廃棄するよりは研究利用を考えてはという意見もあった

採取されたPBSCが過剰な場合

●血縁PBSCTで，CD34が多いと（$8x10^6$/kg）慢性GVHDが多いといういくつかの施設からの報告あるが，否定的な論文もある

●非血縁PBSCTでは，NMDP932例の検討（$0.3-29x10^6$/kg）で，CD34多いほうが予後がよい，慢性GVHDの増加なし

●従って，現時点では，慢性GVHDを危惧して輸注量を"間引く"べきではないと考えられる

Blood 114: 2606,2009

DLI用として一部を凍結可能
使用の有無を報告する

ただし，凍結するPBSCは採取されて24時間以内のものの一部

院内における血液細胞処理のための指針

日本輸血・細胞治療学会　日本造血細胞移植学会

●末梢血幹細胞の処理・凍結保存・解凍
●骨髄　赤血球／血漿除去

非閉鎖系での処理はクリーンベンチで行う
取り違え防止：バーコード管理，コンピューター管理
　　ダブルチェック
フリーザーの温度管理，保守

輸血や薬剤と同じように扱えということ！

Ⅳ. 平成 22 年度
研究成果の刊行に関する一覧表

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Ⅴ. 平成 22 年度研究成果の刊行物

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Increased risk of bacterial infection after engraftment in patients treated with allogeneic bone marrow transplantation following reduced-intensity conditioning regimen

A. Shigematsu, S. Yamamoto, J. Sugita, T. Kondo, M. Onozawa, K. Kahata, T. Endo, S. Shiratori, S. Ota, K. Yamaguchi, K. Wakasa, M. Takahata, H. Goto, S. Ito, R. Takemura, J. Tanaka, S. Hashino, M. Nishio, T. Koike, M. Asaka, M. Imamura. Increased risk of bacterial infection after engraftment in patients treated with allogeneic bone marrow transplantation following reduced-intensity conditioning regimen. Transpl Infect Dis 2010: **12**: 412–420. All rights reserved

Abstract: Although bacterial infection is a major cause of death even after reduced-intensity conditioning (RIC) for allogeneic stem cell transplantation (SCT), little is known about the epidemiology and risk factors. The incidence of bacterial infection in 43 patients who received allogeneic bone marrow transplantation (BMT) using a RIC regimen was compared with that in 68 patients who received BMT using a myeloablative conditioning regimen, and risk factors for bacterial infection were identified. Before engraftment, incidences of febrile neutropenia (FN) and documented infections (DI) were significantly decreased in RIC patients (FN: 59.5% vs. 89.6%, $P<0.01$, DI: 4.8% vs. 17.9%, $P<0.01$). However, incidence of bacterial infection was significantly increased in RIC patients in the post-engraftment phase (53.8% vs. 11.1%, log-rank, $P<0.01$). Blood stream was the most frequent focus of infection in both groups. In multivariate analysis, RIC and acute graft-versus-host disease were revealed to be significant risk factors for bacterial infection in this phase. In summary, risk of bacterial infection after engraftment was significantly higher in RIC patients, although infection was decreased before engraftment, and we need to develop a RIC-specific strategy against bacterial infection after RIC SCT.

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Key words: reduced-intensity conditioning; bone marrow transplantation; bacterial infection; febrile neutropenia; graft-versus-host disease

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Infection is a major cause of morbidity and mortality in patients after hematopoietic stem cell transplantation (SCT) (1). Recently, reduced-intensity conditioning (RIC) regimens have been developed for patients who are considered ineligible for SCT using a myeloablative conditioning (MAC) regimen because of advanced age or medical contraindications (2, 3). Although many studies have shown that infection before engraftment was reduced in patients who underwent RIC owing to a shorter neutropenic period and lower rate of severe mucositis (4–8), infection is still a significant complication even after RIC (4, 5) and some studies have indicated that risk of infection after engraft-

ment was not reduced in patients using RIC regimens (4, 6, 9 15). Although many studies have revealed risk factors for infections, including severe mucositis, long-lasting neutropenia, use of a central venous catheter (CVC), graft-versus-host disease (GVHD), and use of immunosuppressants (4, 16 19), current knowledge of risk factors for infection after SCT is still primarily based on results of analyses performed using MAC regimens. We need to consider the effect of the difference between RIC protocols on infection, because various RIC protocols have been developed and the toxicity profile might vary from one protocol to another, because of the variability in degree of immunosuppression or myeloablation (2 5, 10, 11, 13). We should also consider the difference between stem cell sources (11, 20).

The present study was a retrospective analysis to compare bacterial infection in 43 consecutive patients who received bone marrow transplantation (BMT) using a RIC regimen, which mainly consisted of fludarabine (FLU), busulfan (BU), and total body irradiation (TBI), at our institution with bacterial infection in 68 patients who received MAC BMT during the same period. Bacterial infections were separately analyzed according to 3 phases of infection after SCT, pre-engraftment phase (phase 1), post-engraftment phase (phase 2, engraftment to day 100), and late phase (phase 3, > day 100) (21), and risk factors for bacterial infections in each phase were investigated.

Patients and methods

Patients

A total of 111 consecutive adult patients who received allogeneic BMT using RIC regimens ($n = 43$) or MAC regimens ($n = 68$) between September 2000 and March 2007 at Hokkaido University Hospital in Japan were analyzed for bacterial infection and febrile neutropenia (FN). A difference in the risk of infection depending on stem cell source has been reported, and we cannot use peripheral blood stem cell (PBSC) from an unrelated donor (PBSC can be used only from related donors) in Japan. Moreover, it has been reported that cord blood showed differences in the incidences of engraftment and infections from other stem cell sources. Therefore, we analyzed only patients who received BMT. Twenty-eight patients received an RIC regimen because of advanced age (> 50 years old) and 10 patients received RIC because of prior autologous transplantation (5 patients overlapped with patients of advanced age). Four patients received RIC because of organ dysfunction and 6 patients received RIC because of indolent disease (myelodysplastic syndrome, refractory anemia, $n = 4$; idiopathic myelofibrosis, $n = 2$). Patients who had already received allogeneic SCT were excluded from this study.

Conditioning regimens

In the RIC group, 38 (88.4%) of the patients received a conditioning regimen of FLU/BU/TBI, which consisted of FLU at a dose of 30 mg/m^2 once daily administered intravenously (IV) on days − 7 to − 2 (total dose: 180 mg/m^2) and BU at 1 mg/kg 4 times daily administered orally (p.o.) on days − 3 and − 2 (total dose: 8 mg/kg) combined with fractionated TBI at 2 Gy twice daily on day − 1 (total dose: 4 Gy) (22), and the other 5 patients received FLU plus melphalan ($n = 4$) or FLU plus cyclophosphamide (CY) ($n = 1$). In the MAC group, 10 patients received a conditioning regimen of CY and TBI, which consisted of CY at a dose of 60 mg/kg once daily administered IV on days − 5 and − 4 combined with fractionated TBI at 2 Gy twice daily on days − 3 to − 1 (total dose: 12 Gy) and 44 patients received CY/TBI plus VP-16 (VP/CY/TBI), in which VP-16 was added to CY/TBI at a dose of 15 mg/kg once daily administered IV on days − 7 and − 6 (total dose: 30 mg/kg) (23). The other patients received other regimens of BU/CY or CY/TBI plus cytarabine. GVHD prophylaxis consisted of cyclosporin A and a short course of methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6) for human leukocyte antigen (HLA)-matched related donor recipients, and tacrolimus plus a short course of methotrexate was given for HLA-matched unrelated donor or HLA-mismatched donor recipients. The patients received GVHD prophylaxis from day − 1 for 3 months and drug doses were tapered in patients with no active GVHD, with the dose of cyclosporin A or tacrolimus being adjusted by plasma level.

Supportive care and infection prophylaxis

A tunneled double-lumen CVC was inserted in all patients before transplantation via the subclavian vein, and all patients were treated in a HEPA filter-equipped room until the time of neutrophil engraftment. The CVC was removed when the patient was able to take food by mouth, or when the patient developed CVC-related infections. Levofloxacin (300 mg daily p.o.) was administered for prevention of bacterial infections until engraftment, and antifungals (fluconazole at 400 mg daily p.o., itraconazole capsules at 200 mg daily p.o., or micafungin at 100 mg daily IV) were administered for prevention of fungal infections. Oral acyclovir was given on days − 7 to 35 for prevention of herpes simplex virus infection. Oral trimethoprim sulfamethoxazole or pentamidine inhalation was started after engraftment for prevention of *Pneumocystis jirovecii* infec-

tion. Granulocyte colony-stimulating factor was administered from day 1 until engraftment. For patients with persistent fever, physical examination, chest x-rays, and multiple blood cultures were performed to identify the source of infection. Treatment of neutropenic fever was performed in accordance with standard practice guidelines (24). In brief, patients who developed FN were treated empirically with broad-spectrum antibiotics, usually cefepime, tazobactam/piperacillin, or carbapenem, and antibiotics were adjusted on the basis of results of microbial sensitivity tests for patients with positive cultures. The supportive care was the same for RIC patients and MAC patients.

Definitions

Day of neutrophil engraftment was defined as the first of 2 days with absolute neutrophil count $> 0.5 \times 10^9$/L. Toxicity after SCT was graded by the National Cancer Institute common toxicity criteria version 3 (NCI, Bethesda, Maryland, USA). FN was defined as a single axillary body temperature (BT) > 37.5°C in patients with peripheral neutrophil counts $< 0.5 \times 10^9$/L, as reported previously (24). Infection after BMT was divided into 3 phases, as described above (21). According to the standard guidelines, bacteremia or fungemia was defined by the isolation of bacteria or fungi from any blood culture in the context of fever or other clinical signs consistent with infection (25). Bacterial pneumonia was diagnosed by chest x-ray examination or computed tomography, and a bacterial pathogen was identified by culture of sputum, bronchoalveolar lavage fluid, pleural fluid, or blood specimen. A diagnosis of invasive fungal infection was made as described previously (14). For coagulase-negative *Staphylococci* (CNS), at least 2 blood cultures with the same antimicrobial susceptibilities were required to be positive. Patients censored for an infection in one period were not considered at risk for the same period. Infection-related mortality (IRM) was defined as death mainly due to infection in patients without relapse. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded by standard criteria (26, 27).

End points and statistical analysis

The aims of this study were to compare the incidences of FN and bacterial infection in phase 1 and the incidence of bacterial infection in phase 2 in patients who received RIC and MAC regimens, and to identify risk factors for infections at each phase. Patients who relapsed or died were censored for analyses of infection at the time of relapse or death. Univariate analyses were performed using the χ^2 test and Fisher's exact test, as appropriate. Overall survival was calculated from the day of SCT until death or last follow-up. The probability of survival was estimated using the Kaplan Meier method. The effects of conditioning regimens on survival and infections were studied using the log-rank test. Multivariate logistic regression models were used to analyze the influence of selected variables on the risk of FN (phase 1) and bacterial infection in phase 2. The variables included in these models are described in 'Results'. All *P*-values were 2-sided and a *P*-value of 0.05 was used as the cutoff for statistical significance.

Results

Patients and transplantation characteristics

Patients and transplantation characteristics are summarized in Table 1. Median age, GVHD prophylaxis, underlying disease, prior autologous transplantation, and months from diagnosis to transplantation were significantly different between RIC patients and MAC patients. Disease status at transplant was not different between RIC patients and MAC patients. Parameters for infections, including positive culture (including colonization) for methicillin-resistant *Staphylococcus aureus* (MRSA) and cytomegalovirus serostatus, were not different between RIC patients and MAC patients. In 11 patients who were positive for MRSA before SCT, 10 patients had colonization and only 1 patient who received RIC had active infection (pneumonia).

Engraftment

Clinical outcomes after alloSCT are summarized in Table 2.

All patients were assessed for engraftment and infection during phase 1; 93% of RIC patients and 95% of MAC patients achieved engraftment with median neutrophil recovery at days 16 and 15, respectively ($P = 0.81$). Mean duration of neutropenia was significantly shorter in RIC patients than in MAC patients (13.8 days vs. 16.5 days, $P < 0.01$). Stomatitis and diarrhea were assessed as regimen-related mucositis, and grade ≥ 3 stomatitis and grade ≥ 3 diarrhea were significantly less in RIC patients (stomatitis: $n = 8$ [19.0%] vs. $n = 27$ [41.5%], $P = 0.02$; diarrhea: $n = 5$ [11.9%] vs. $n = 22$ [34.4%], $P < 0.01$).

Infections in phase 1 (pre-engraftment)

Forty-two patients who received RIC and 67 patients who received MAC were assessed for FN and documented infections (DI) (Table 3). FN occurred in 25 (59.5%) of the RIC

Patients and transplantation characteristics

	RIC ($n = 43$)	MAC ($n = 68$)	*P*-value
Age (years), median (range)	52 (17–66)	34.5 (15–58)	< 0.01
Patient sex			
Male	48.8%	63.2%	0.13
Donor			
HLA-matched related donor	23.3%	30.9%	0.48
HLA-matched unrelated donor	67.4%	55.9%	
HLA-mismatched donor	9.3%	13.2%	
Conditioning regimen			
Fludarabine/busulfan	88.4%	0.0%	–
Fludarabine/melphalan	9.3%	0.0%	
CY/VP-16/TBI	0.0%	64.7%	
CY/TBI	0.0%	14.7%	
Others	2.3%	20.6%	
TBI	88.4%	91.2%	0.63
GVHD prophylaxis			
Cyclosporin A + methotrexate	44.2%	67.6%	0.02
Tacrolimus + methotrexate	53.5%	30.9%	
Cell dose (ANC, $\times 10^8$/kg), median (range)	2.4 (0.7–5.5)	2.7 (0.4–9.4)	0.10
Underlying disease			
ALL	2.3%	27.9%	< 0.01
AML	14.0%	26.5%	
MDS	27.9%	10.3%	
CML	9.3%	20.6%	
NHL	18.6%	7.4%	
ATL	9.3%	2.9%	
MM	11.6%	0.0%	
Others[1]	7.0%	4.4%	
Prior autologous SCT	23.3%	0.0%	< 0.01
Diagnosis to SCT (months), median (range)	22.5 (1.7–240)	8.3 (5.0–276.1)	< 0.01
Complete remission at SCT[2]	53.5%	68.7%	0.11
MRSA culture[3]			
Positive	14.3%	9.3%	0.44
CMV serostatus			
High risk (R +)	93.0%	88.2%	0.71
Intermediate risk (R – /D +)	4.7%	8.8%	
Low risk (R – /D –)	2.3	2.9%	

[1]Other diseases included aplastic anemia (RIC: $n = 1$, MAC: $n = 1$), idiopathic myelofibrosis (RIC: $n = 1$), essential thrombocytemia (RIC: $n = 1$), desmoplastic small round cell tumor (MAC: $n = 1$), and rhabdomyosarcoma (MAC: $n = 1$).
[2]Complete remission at SCT included MDS RA and CML CP at SCT.
[3]Positive for MRSA culture included MRSA colonization.
RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; HLA, human leukocyte antigen; CY, cyclophosphamide; TBI, total body irradiation; GVHD, graft-versus-host disease; ANC, absolute neutrophil count; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; RA, refractory anemia; CML, chronic myelogenous leukemia; CP, chronic phase; NHL, non-Hodgkin's lymphoma; ATL, advanced T-cell leukemia; MM, multiple myeloma; SCT, stem cell transplantation; R, recipient; D, donor; MRSA, methicillin-resistant *Staphylococcus aureus*; CMV, cytomegalovirus.

Table 1

Clinical outcomes after allogeneic transplantation

	RIC	MAC	*P*-value
Engraftment	93.0%	95.6%	0.56
Day, median (range)	16 (7 21)	15 (9 39)	0.81
Acute GVHD			
Overall	71.8%	78.5%	0.44
Onset day, median (range)	33 (18 127)	20 (8 59)	<0.01
Grades II IV	41.0%	52.3%	0.26
Grades III IV	17.9%	15.4%	0.73
Corticosteroid	46.2%	52.3%	0.54
Chronic GVHD			
Overall	77.1%	68.7%	0.24
Onset day, median (range)	121 (59 302)	100 (34 365)	0.51
Extensive	48.6%	50.9%	0.83
Corticosteroid	37.5%	39.6%	0.85
Death due to disease progression	11.6%	19.1%	0.30
NRM	18.6%	14.7%	0.59
IRM	11.6%	11.8%	0.88

RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; GVHD, graft-versus-host disease; NRM, non-relapse mortality; IRM, infection-related mortality.

Table 2

patients and in 60 (89.6%) of the MAC patients (log-rank, $P<0.01$; HR, 0.45; 95% confidence interval [95%CI], 0.26 0.65), and the median onset day was day 9 and 7, respectively (Fig. 1). Documented bacterial infection was also decreased in RIC patients compared with that in MAC patients ($n-2$ [4.8%] vs. $n-12$ [17.9%], $P<0.01$). In

Infections according to the phase after stem cell transplant

	RIC	MAC	*P*-value
Phase 1 (preengraftment)	*n* 42	*n* 67	
Febrile neutropenia	59.5%	89.6%	<0.01
Documented bacterial infection	4.8%	17.9%	<0.01
Phase 2 (engraftment to day 100)	*n* 39	*n* 64	
Documented infection	66.7%	45.3%	0.09
Documented bacterial infection	53.8%	11.1%	<0.01
Phase 3 (late infection, day 100)	*n* 35	*n* 55	
Documented infection	45.7%	25.5%	0.12
Documented bacterial infection	5.7%	9.0%	0.70

RIC, reduced intensity conditioning; MAC, myeloablative conditioning.

Table 3

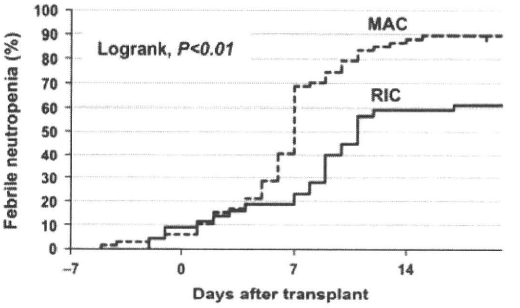

Fig. 1. Cumulative incidence of febrile neutropenia in phase 1. MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

RIC patients, MRSA bacteremia and CNS bacteremia occurred in 1 patient each and no patient developed gram-negative bacteremia or pneumonia. In the MAC group, bacteremia occurred in 12 patients, including 6 patients with gram-positive bacteria (MRSA $n-3$, CNS $n-3$), 5 patients with gram-negative bacteria, and 1 patient with *Nocardia*, and only 1 patient developed pneumonia. Probable fungal infection did not occur in any patients in either group. The highest BT and maximal levels of C-reactive protein (CRP), which are usually assessed as markers of severity of inflammation, were also lower in RIC patients (median highest BT: 37.6°C vs. 38.5°C, $P<0.01$; median maximal CRP: 3.3 mg/dL vs. 8.7 mg/dL, $P<0.01$). Three patients died from bacterial infection in phase 1; 1 patient who received an MAC regimen died of pneumonia and 2 patients who received an RIC regimen and developed bacteremia died. Univariate and multivariate analyses for FN were performed, and results are summarized in Table 4. The results of univariate analysis showed that MAC regimen, longer duration of neutropenia, younger age, and severe stomatitis were significantly associated with high incidence of FN. These 4 variables were evaluated in the multivariate logistic regression model, and only MAC regimen remained significant in multivariate analysis (odds ratio [OR], 5.6; 95%CI, 1.9 16.5; $P<0.01$).

aGVHD and infections in phase 2 (engraftment to day 100)

Except for 1 patient who died early after engraftment, all patients who achieved engraftment were assessed for aGVHD and infection in phase 2 (RIC: $n-39$, MAC: $n-64$) (Tables 2 and 3). Although incidences of overall aGVHD and grades II IVaGVHD and frequency of corticosteroid administration as treatment for aGVHD were not different between RIC patients and MAC patients, median

Univariate and multivariate analyses for febrile neutropenia (FN)

Variables	FN (%)	Univariate P	Multivariate Odds ratio (95% CI)	Multivariate P
Age				
49	84.2	0.03	1.1 (0.29 4.39)	0.67
50	64.7			
Conditioning regimen				
RIC	59.5	<0.01	5.6 (1.9 16.5)	<0.01
MAC	89.6			
Duration of neutropenia (days)				
15	69.2	0.04	0.4 (0.12 1.25)	0.10
16	87.2			
Stomatitis (grade)				
2	70.4	0.04	2.0 (0.48 7.98	0.29
3	91.4			
Diarrhea (grade)				
2	73.4	0.1	NA	
3	88.9			

CI, confidence interval; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; NA, not assessed.

Table 4

onset day of aGVHD was significantly delayed in RIC patients (day 33 vs. day 20, $P<0.01$).

DI occurred in 26 (66.7%) of the RIC patients and in 29 (45.3%) of the MAC patients (log-rank, $P=0.09$; HR, 1.57; 95%CI, 0.9 2.8), and the median onset day was day 48 (range: 17 84) and day 42 (range: 17 94), respectively ($P=0.51$). Incidence of bacterial infection was significantly increased in RIC patients (RIC: $n=21$ [53.8%] vs. MAC: $n=7$ [11.1%], log-rank, $P<0.01$; HR, 6.0; 95%CI, 3.1 14.9) (Fig. 2). Although median onset day of bacterial infection was not different between RIC patients and MAC patients (day 44 vs. day 37, $P=0.34$), delayed bacterial infection (day 50) seemed to have occurred more in RIC patients (RIC: $n=9$, MAC: $n=1$).

In phase 2, CNS was the main organism that caused bacterial infection both in RIC patients (61.5%) and in MAC patients (57.1%). Other pathogens in RIC patients were MRSA (15.3%) and *Pseudomonas aeruginosa* (23.0%), and those in MAC patients were MRSA (28.6%) and *Klebsiella pneumoniae* (14.3%). Blood stream was the most frequent focus of infection in both groups (RIC: 61.9%, MAC: 71.4%) and some patients developed respiratory infections (RIC: 21.1%, MAC: 14.3%). Univariate and multivariate analyses for bacterial infection in phase 2 were performed,

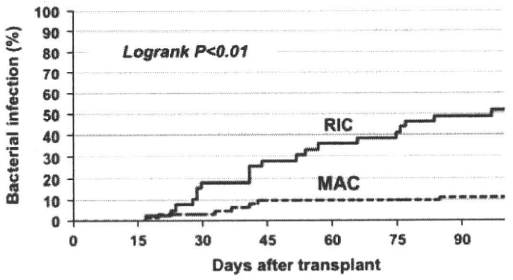

Fig. 2. Cumulative incidence of bacterial infection in phase 2. MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

and the risk factors are summarized in Table 5. In univariate analysis, older age, RIC regimen, underlying disease, prior autologous SCT, and later engraftment were significantly associated with bacterial infection during phase 2, and aGVHD and longer duration between diagnosis and transplantation showed marginal significance. Age, conditioning regimen, underlying disease, prior autologous SCT, months from diagnosis to SCT, day of engraftment, and aGVHD were evaluated in the multivariate logistic regression model. Only RIC regimen and aGVHD were significant in multivariate analysis (RIC: OR, 12.1; 95%CI, 4.1 35.6; $P<0.001$, aGVHD: OR, 6.0; 95%CI, 1.4 25.3; $P=0.01$). Incidence of fungal infection was not different between RIC patients and MAC patients (RIC: $n=6$ [15.4%] vs. MAC: $n=5$ [7.9%], $P=0.23$).

cGVHD and infections in phase 3 (late infection)

All patients who were alive at day 100 were assessed for cGVHD and late infection (RIC: $n=35$, MAC: $n=55$). In the RIC group, overall cGVHD and extensive cGVHD occurred in 27 (77.1%) of the patients and in 17 (48.6%) of the patients, respectively, at median onset day of 121 (range: 59 302) (Table 2). In the MAC group, overall cGVHD occurred in 36 (66.7%) of the patients and extensive cGVHD occurred in 28 (50.9%) of the patients at median day of 100 (range: 34 365). There were no differences between RIC patients and MAC patients (overall cGVHD, $P=0.24$; extensive cGVHD, $P=0.83$; onset day, $P=0.51$). Corticosteroid administration as treatment for cGVHD was not different between the 2 groups (RIC: $n=12$ [37.5%] vs. MAC: $n=21$ [39.6%], $P=0.85$).

Late infection occurred in 16 (45.7%) of the RIC patients and in 14 (25.5%) of the MAC patients ($P=0.12$), and late bacterial infection occurred in 2 (5.7%) of the RIC patients and 5 (9.0%) of the MAC patients (Table 3). In univariate analysis, extensive cGVHD and corticosteroid

Univariate and multivariate analyses for bacterial infection in phase 2

Variables	Infection (%)	Univariate P	Multivariate Odds ratio (95% CI)	Multivariate P
Age				
–49	17.8	<0.01	0.82 (0.2–3.8)	0.8
50–	48.3			
Conditioning regimen				
RIC	51.3	<0.01	12.1 (4.1–35.6)	<0.01
MAC	25.3			
Underlying disease				
ALL	10.0	0.02		1.00
AML	23.8			
MDS	27.8			
CML	23.1			
NHL	38.5			
ATL	50.0			
MM	80.0			
Others	0.0			
Prior autologous SCT				
Yes	80.0	<0.01	3.8 (0.6–23.9)	0.16
No	20.7			
Diagnosis to SCT (months)				
–11	19.3	0.06	1.0 (0.3–3.7)	0.98
12–	35.6			
Engraftment (day)				
–15	16.3	0.03	0.4 (0.1–1.3)	0.13
16–	35.8			
Acute GVHD				
Yes	31.2	0.07	6.0 (1.4–25.3)	0.01
No	12.0			
Corticosteroid				
Yes	32.7	0.15	NA	
No	20.0			

CI, confidence interval; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; GVHD, graft-versus-host disease; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; ATL, advanced T-cell leukemia; MM, multiple myeloma; SCT, stem cell transplantation; NA, not assessed.

Table 5

administration were significantly associated with occurrence of late infections (extensive cGVHD, $P = 0.05$; corticosteroid, $P = 0.03$).

Survival and IRM

The median follow-up period was 18.1 months (range: 0.1 83.4 months). Thirteen of the RIC patients and 23 of the MAC patients died. Among the RIC patients, 5 patients (11.6%) died of disease progression and 8 patients (18.6%) died of transplantation-related complications (nonrelapse mortality, NRM). There was no difference in cause of death between RIC patients and MAC patients (MAC: disease progression, $n = 13$ [19.1%], NRM, $n = 10$ [14.7%]). Five (11.6%) of the RIC patients and 8 (11.8%) of the MAC patients died of IRM (log-rank, $P = 0.88$). IRM occurred at median day 123 (range: 8 867) and 3 patients, all of whom had active infection at transplantation, died of bacterial infections before engraftment (Table 2). All of the patients who died of infection after engraftment had GVHD (aGVHD: $n = 4$, cGVHD: $n = 6$), and corticosteroid was administered for all of them except 1. Two patients died of bacterial infection in both the RIC and MAC groups after engraftment. Univariate analysis revealed active infection at transplantation ($P = 0.03$) as a significant adverse prognostic factor for IRM, and corticosteroid administration for cGVHD tended to have an adverse impact on IRM ($P = 0.05$). None of the other parameters, including age, conditioning regimen, FN, day of neutrophil engraftment, aGVHD and cGVHD, were predictive factors for IRM.

Discussion

RIC is less myeloablative and is associated with less mucosal injury, and patients receiving an RIC regimen therefore tended to have fewer infectious complications than the MAC patients (3, 4, 7, 8). Many RIC regimens with various intensities of myeloablation and immunosuppression have been developed, and we therefore need to assess infectious complications after RIC according to conditioning regimens (2 5, 9 11, 13). We should also consider the difference between stem cell sources (11, 20).

In this study, we analyzed the incidence and characteristics of bacterial infections in patients who received allogeneic BMT after an RIC regimen of FLU/BU/TBI.

Many studies have shown that infections before engraftment were fewer in RIC patients than in MAC patients (4 8). The present study confirmed these results by analysis of incidences of FN and DI, although patients receiving a RIC regimen were older than MAC patients and more RIC patients had prior autologous transplantation. Although we defined FN as a single axillary BT >37.5°C in patients with neutropenia, in accordance with the guideline in Japan (24), the same result was obtained when FN was defined as

a single axillary BT > 38.3°C, the criterion used in western countries (RIC: 28.6% vs. MAC: 58.2%, $P < 0.01$) (28).

Severe and long-lasting neutropenia, neutropenia at the time of conditioning, mucositis, and CVC have been reported as risk factors for infection in phase 1 (4, 5, 17–19). In the current study, multivariate analysis revealed that the RIC regimen was strongly associated with fewer infections in phase 1 and this seemed to be mainly a result of a shorter neutropenic period and less mucosal injury, although patients who received our RIC regimen including TBI tended to have a longer neutropenic period and more mucosal injury than those patients who received other RIC regimens (4, 5), and all of our patients received a CVC and IV hyperalimentation. None of our patients developed gram-negative bacteremia in phase 1, suggesting that less mucosal injury by the RIC regimen resulted in less bacterial translocation from the gut into the blood stream (4, 9). All of our patients received bone marrow as a source of stem cells and no patients showed delayed engraftment or rejection, which may also account for fewer infections in RIC patients. Again, smaller degree of highest BT and smaller degree of maximal levels of CRP in RIC patients suggested that infection in RIC patients was milder than that in MAC patients, although there was no difference of IRM in phase 1.

One of the important findings in the present study was an increasing risk of infection in phase 2 among RIC patients, especially bacterial infection after day 50. Some studies have shown the same tendency (4, 10, 11). Junghanss et al. (4) reported on bacterial infection in patients who received a nonmyeloablative regimen (TBI 2 Gy ± FLU) compared with that in patients who received a myeloablative regimen. Although they found a lower incidence of bacterial infection during the first 30 days in nonmyeloablative patients (9% vs. 27%, $P = 0.01$) with statistical significance, bacterial infection from days 0 to 100 was not decreased in nonmyeloablative patients (27% vs. 41%, $P = 0.07$). This fact suggests that incidences of bacterial infection from days 30 to 100 were almost the same in the 2 groups. They also reported that the incidence of CVC-related infection was not decreased in nonmyeloablative patients, although mucositis-associated infection was significantly decreased. Mohty et al. (10, 11) reported an increased incidence of bacterial infection in phase 2 following a regimen of FLU/BU plus anti-thymocyte globulin; 25% of the patients developed bacteremia, which occurred after engraftment in most cases (23%) (10, 11). In the current study, many RIC patients developed blood stream infection due to CNS and the bacterial infection improved in most of patients after removal of the CVC, suggesting CVC-related infection (4, 6, 9). The fact that most of our patients had a CVC after engraftment might explain the unexpectedly high rate of bacterial infection in RIC patients. However, this cannot explain the difference in incidence of bacterial infection between RIC patients and MAC patients, because there was no change in removal policy of the CVC between RIC patients and MAC patients, as described in 'Patients and methods,' and actual day of CVC removal was not different between RIC patients and MAC patients (RIC: median day 31 [0–83], MAC: median day 35 [14–143], $P = 0.64$). aGVHD and corticosteroid administration have been reported as risk factors for infection in phase 2 (4, 11), and our study showed that grades II–IV aGVHD was significantly associated with bacterial infection in phase 2, but that corticosteroid administration was not. This might be explained by the fact that we usually started corticosteroid treatment for aGVHD at a lower dose (prednisolone at 1 mg/kg/day) than the dose used in other countries (prednisolone at 2 mg/kg) because of the lower incidence of critical aGVHD in Japan because of racial homogeneity (29). Delayed-onset aGVHD in RIC patients might reflect delayed-onset bacterial infection in RIC patients. However, aGVHD cannot explain the difference in incidence of bacterial infection between RIC patients and MAC patients because incidence of grades II–IV aGVHD was not different between the 2 groups. Again, the difference between bacterial infection in RIC patients and that in MAC patients was not due to the RIC regimen itself, because our RIC regimen did not include long-lasting immunosuppressants, such as antithymocyte globulin or Campath-1H, which have been reported as risk factors of infection after engraftment (9, 11, 13). We think that the difference is mainly a result of the patients' background (age, prior autologous transplantation, and underlying disease), and univariate analysis showed these factors as significant risk factors for bacterial infection in phase 2 (6). These factors were more frequent in RIC patients and might reflect lower immune function at SCT, and a combination of these risk factors reflected higher incidence of bacterial infection in RIC patients in phase 2.

IRM was not different between RIC patients and MAC patients, and univariate analysis showed that only presence of active infection at transplantation was a significant risk factor for IRM, especially mortality in phase 1. All patients who died of infection after engraftment had GVHD and all patients but 1 received corticosteroid treatment. Therefore, patients with active infection may need to be managed with a new strategy, and management of GVHD remains important even in RIC patients.

It should be noted that it was difficult to directly compare infectious complications in RIC patients and MAC patients because RIC patients' backgrounds and MAC patients' backgrounds were significantly different. However, in a clinical setting, we need to consider how to prevent or manage infectious complications in patients receiving an RIC regimen who have different characteristics from those in MAC patients. The present study suggests that there is an increasing risk for bacterial infection in phase 2, especially CVC-related gram-positive bacteremia, and we need to

establish a new strategy in this phase (i.e., earlier removal of the CVC or prophylactic antibiotics for CNS or MRSA in high-risk patients). MRSA infection developed more frequently in patients who had positive culture for MRSA before SCT (27.3% in positive patients vs. 5.9% in negative patients, $P = 0.046$); therefore, we also need a new strategy for patients who have MRSA before SCT.

Although our analysis has limitations owing to its retrospective nature and the small sample size, our results showed that patients who received an RIC regimen had lower risk of infection before engraftment but had an increased risk of bacterial infection after engraftment. We need to develop an RIC-specific strategy against infection after SCT.

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ORIGINAL ARTICLE

Clinical significance of minimal residual disease in adult acute lymphoblastic leukemia

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Abstract Monitoring minimal residual disease (MRD) in patients with acute lymphoblastic leukemia (ALL) is a useful way for assessing treatment response and relapse. We studied the value of MRD and showed a correlation with relapse for 34 adult patients with ALL. MRD was evaluated by real-time quantitative polymerase chain reaction (RQ-PCR) with probes derived from fusion chimeric genes (BCR/ABL) ($n = 12$) or PCR-based detection of clonal immunoglobulin and T cell receptor gene rearrangements ($n = 16$), or both ($n = 6$). We analyzed 27 of the 34 patients who could be examined for MRD on day 100 after induction therapy. The overall survival (OS) rate (45.0%) and relapse-free survival (RFS) rate (40.0%) at 2 years in complete remission (CR) patients with MRD level $\geq 10^{-3}$ ($n = 12$) were significantly lower than those in CR patients with MRD level $<10^{-3}$ ($n = 15$) (OS rate 79.0%, RFS rate 79.4%) (log-rank test, $P = 0.017$ and 0.0007). We also applied multicolor flow cytometry for comparison with MRD results analyzed by PCR methods. The comparison of results obtained in 27 follow-up samples showed consistency in 17 samples (63.0%) ($P = 0.057$). MRD analysis on day 100 is important for treatment decision in adult ALL.

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Keywords MRD · Adult ALL · Allogeneic transplantation · Polymerase chain reaction · Flow cytometry

1 Introduction

The prognosis of adult patients with acute lymphoblastic leukemia (ALL) is dismal. Although most adult patients with ALL enter complete remission (CR), only 30–40% of patients survive for 5 or more years [1]. The major cause of treatment failure is relapse, affecting approximately half of the patients who have achieved CR [2]. Survival depends on risk factors such as age, white blood cell (WBC) count, time to CR, disease immunophenotype, cytogenetics, and molecular abnormalities [1], and several studies have shown that detection of minimal residual disease (MRD) in childhood and adult ALL is an independent risk parameter of high clinical relevance [3]. Early indicators of disease outcome would be particularly useful for the design of new treatments.

The rationale of MRD analysis is to improve estimation of treatment response, to provide independent prognostic information, and to optimize therapeutic strategies. Established methods for detecting MRD are polymerase chain reaction (PCR) amplification of antigen receptor genes and of fusion transcripts, and flow cytometric detection of ectopic or aberrant immunophenotypes [4].

Flow cytometric detection of MRD is based on the identification of immunophenotypic combinations expressed on leukemic cells, but not on normal hematopoietic cells [4]. Abnormal antigen expression or leukemia-specific gene rearrangements or fusion transcripts are suitable for MRD detection; however, they cannot be identified in all patients. Therefore, the complementary use of both methods might allow monitoring of virtually all patients for MRD.

We previously reported that molecular MRD status by PCR amplification of antigen receptor genes is a strong predictor of outcome in adult ALL [5]. In this study, we accumulated more patients and reevaluated the significance of MRD. We also compared the two methods of MRD detection: PCR amplification and flow cytometry.

2 Patients and methods

2.1 Patient characteristics

A total of 46 adult ALL patients were included in the study during the period from May 2001 to December 2007 at Hokkaido University Hospital and hospitals associated with the Hokkaido Leukemia Study Group. They were registered with the study when the diagnoses of ALL were made. Twelve of the 46 patients who entered the study were excluded [patients with no IGH, TCRδ, or TCRγ clonal marker or fusion transcripts at diagnosis ($n = 5$), patients who died before or during induction chemotherapy ($n = 5$), and patients who did not achieve CR ($n = 2$)]. In total, MRD could be monitored in 34 of the 46 initial patients.

The characteristics of the 34 patients are summarized in Table 1. Seventeen (50.0%) of the patients were male and 17 (50.0%) were female. Their median age was 48.5 years (range 15–79 years) and median duration of follow-up was 567.5 days (range 49–2040 days). The median WBC count was 10.9×10^9/L (range $1.7–3272.5 \times 10^9$/L). Thirty patients had B-precursor ALL and 4 had T-ALL. Eighteen patients were Philadelphia (Ph) chromosome positive: 13 patients (55.6%) had $p190^{BCR/ABL}$ transcripts, 3 patients (16.7%) had $p210^{BCR/ABL}$ transcripts, and the other 2 (11.1%) had both transcripts. One patient was positive for SIL/TAL1 and another patient was positive for E2A/PBX1, but these fusion transcripts were not used to detect MRD. Eighteen (52.9%) of the 34 patients received chemotherapy only, and the remaining 16 (47.1%) received allogeneic stem cell transplantation after chemotherapy. Eight patients had related donors and eight patients had unrelated donors. Clinical characteristics of the patients are shown in Table 2.

Twenty-six patients were treated with intravenous cyclophosphamide, vincristine, daunorubicin or doxorubicin, etoposide, cytosine arabinoside, steroid, L-asparaginase, with or without imatinib, and intrathecal methotrexate for induction therapy. One patient received only imatinib, another patient received etoposide and imatinib, and another patient was treated with idarubicin and cytosine arabinoside because the diagnosis of acute myelogenous leukemia was made at the first time. The induction therapies for other five patients were not known. The conditioning regimen for allogeneic transplantation consisted of total body irradiation in combination with cyclophosphamide and etoposide for 13 patients or fludarabine for 1 patient. The conditioning regimen was unknown for the other three patients.

Clinical decisions concerning treatment of the patients were made by their physicians regardless of MRD results. Enrollment in the study was contingent upon informed consent of the patient. The study was approved by the Institutional Review Board of Hokkaido University Graduate School of Medicine.

2.2 Remission and relapse

Morphologic CR was defined as less than 5% blast cells in a regenerated bone marrow (BM) aspirate, absence of extramedullary leukemia, and peripheral blood (PB) neutrophil

Table 1 Characteristics of the patients

Characteristics	*N*
Number of patients	34
Gender (%)	
Male	17 (50.0%)
Female	17 (50.0%)
Median age, years (range)	48.5 (15–79)
Age (years)	
<35	14 (41.2%)
≥35	20 (58.8%)
<55	21 (61.8%)
≥55	13 (38.2%)
Median follow-up period, days (range)	567.5 (49–2040)
Immunophenotype	
B-ALL	30 (88.2%)
T-ALL	4 (11.8%)
Median WBC, $\times 10^9$/L (range)	10.9 (1.7–3272.5)
WBC <30 × 10^9/L (B), <100 × 10^9/L (T)	25 (73.5%)
WBC ≥30 × 10^9/L (B), ≥100 × 10^9/L (T)	5 (14.7%)
Unknown	4 (11.8%)
Median hemoglobin, g/dL (range)	10.3 (4.1–14.9)
Median platelet, $\times 10^9$/L (range)	50.5 (3–302)
Median LDH, IU/L (range)	730 (206–11730)
Philadelphia chromosome	
Negative	16 (47.1%)
Positive	18 (52.9%)
$p190^{BCR/ABL}$	13 (38.2%)
$p210^{BCR/ABL}$	3 (8.8%)
$p190^{BCR/ABL}$ and $p210^{BCR/ABL}$	2 (5.9%)
Treatment	
Chemotherapy	18 (52.9%)
Chemotherapy → Transplantation	16 (47.1%)
Conditioning	
Myeloablative	12 (75.0%)
Reduced intensity	2 (12.5%)
Unknown	2 (12.5%)
Stem cell source	
Bone marrow	6 (37.5%)
Peripheral blood	7 (43.8%)
Cord blood	3 (18.8%)
Median time to CR, days (range)	35 (15–105)
Outcome at 2 years	
Overall survival	54.6%
Relapse-free survival	51.8%

and platelet counts of >1.5 × 10^9 and >100 × 10^9/L, respectively. Clinical relapse was defined as detection of at least 5% blast cells in BM or detection of leukemic cells extramedullary.

2.3 Sample processing

BM or PB was collected at the time of initial diagnosis and at several clinical points (day 30 and 100 after diagnosis, before the conditioning regimen, after transplantation, and any points at the end of each chemotherapy). Some samples were collected after completion of the treatment. The samples were used for PCR study of IGH/TCR rearrangements and BCR/ABL fusion gene transcripts. Some of the samples were also used for flow cytometry study. A total of 231 samples (220 BM and 11 PB samples) were analyzed; the median number of samples analyzed per patient was 6 (range 2–22) (Table 3). For a total of 27 BM samples from five patients, MRD was analyzed by both PCR amplification and flow cytometry. We did not evaluate the difference in values of MRD between BM and PB samples.

2.4 Quantitative real-time PCR analysis of BCR/ABL

Samples were analyzed for BCR/ABL, TEL/AML1, MLL/AF4, MLL/AF9, MLL/AF6, MLL/ENL, E2A/PBX1, and SIL/TAL1 chimeric genes. Samples were amplified by real-time quantitative polymerase chain reaction (RQ-PCR) and quantified by parallel amplification of serial dilutions of transcript-containing plasmids. A 10^{-5} sensitivity for MRD detection could be obtained.

2.5 PCR analysis of IGH/TCR gene rearrangements

When a BCR/ABL chimeric fusion gene was not present, leukemia-specific probes were generated by genomic amplification and sequencing of VDJ regions of the immunoglobulin heavy chain (IGH) and T cell receptor δ and γ (TCRδ and TCRγ) genes. The method of PCR analysis of IGH/TCR gene rearrangements has been described previously [5]. The sensitivity was 10^{-3}. When two MRD probes gave different results in the same patient, the higher MRD level was considered valid for the purpose of the study.

2.6 Flow cytometry

Leukemia-associated immunophenotypes were identified by flow cytometry with four color combinations of monoclonal antibodies. The flow cytometric method used has been described previously [6]. For each case, one or more marker combinations that allowed the identification of one leukemic cell in 10^3–10^4 normal nucleated cells were selected at diagnosis and used to study MRD during therapy. This approach reached sensitivities up to 10^{-4}.