Decreased Risk of Acute Graft-versus-Host Disease Using Reduced Intensity Conditioning Compared to Myeloablative Conditioning is Independent of Donor-Recipient T-cell Chimerism

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Abstract

Background: It is not known if the reduced risk of graft-versus-host disease (GVHD) among patients receiving reduced intensity conditioning (RIC) as opposed to myeloablative conditioning (MAC) is due to differences in mixed donor-recipients chimerism, or to the intensity of the regimen.

Methods: We compared patients with acute myeloid leukemia (AML) selected for RIC (n=47) to 46 patients selected for MAC before allogeneic hematopoietic stem cell transplantation (HSCT).

Results: Time to neutrophils >0.5 x 109/L was median 15 days in the MAC group, which was faster than 17 days in the RIC group (p=0.001). MAC patients required more erythrocytes (p=0.001) and platelet transfusions (p=0.003). At four weeks, mixed donor-recipient T-cell chimerism was seen in 29% of the MAC patients and 46% of the RIC patients. Acute GVHD grades II-IV was 55% and 17% in the two groups, respectively (p<0.001). In multivariate analysis, acute GVHD was reduced using RIC (hazards ratio (HR) 0.23, p<0.001), for year of HSCT (HR 1.27, p=0.01), but not for mixed donor-recipient T-cell chimerism (HR 1.11, p=0.80). Transplant-related mortality (TRM) at three years was 15% versus 13%. Chronic GVHD and relapse were similar. Overall mortality was not affected by conditioning (HR 1.39, p=0.36).

Discussion: To conclude, patients treated with RIC had an increased risk of acute GVHD as opposed to recipients of MAC, which was due to less intense conditioning and not due to mixed donor T-cell chimerism.

Keywords: Acute myeloid leukemia; Reduced intensity conditioning; Myeloablative conditioning; Graft-versus-host disease; Donor-recipient chimerism

Introduction

Reduced intensity conditioning (RIC) has been used for one and a half decades to enable allogeneic hematopoietic stem cell transplantation (HSCT) in patients who are not fit for standard myeloablative conditioning (MAC) [1,2]. RIC takes advantage of the graft-versus-leukemia (GVL) effect induced by immunocompetent cells in the graft rather than the antitumor effect of high-dose chemoradiotherapy. The GVL effect may be potentiated by donor lymphocyte infusions (DLIs) [3]. Most patients with leukemia, for example acute myeloid leukemia (AML), are selected for either RIC or MAC depending on risk factors for toxicity or relapse. Retrospective registry analyses suggest that there is more toxicity and transplant-related mortality (TRM) using MAC, but more relapse using RIC, and that the two methods have similar overall survival and leukemia-free survival (LFS) [4-6]. To our knowledge, there have only been two randomized studies comparing RIC and MAC, one German multicenter study and one toxicity study from our unit [7,8]. The multicenter study involved 195 patients and found no difference in TRM, relapse, disease-free survival, or overall survival between patients randomized to RIC or MAC. The randomized toxicity study showed that the RIC patients had significantly less toxicity such as mucositis, less cytomegalovirus (CMV) reactivation, and less hemorrhagic cystitis than the MAC patients [8].

Patients receiving RIC have been reported to have less acute GVHD than patients treated with MAC [4,9-11]. T-cell mixed donor-recipient chimerism have been reported to be associated with a decreased risk of acute GVHD [12-14]. RIC patients more often are mixed donor T-cell chimeras than those receiving MAC [8,15]. We wanted to analyze if mixed donor T-cell chimerism in RIC patients was a reason for the reduced risk of acute GVHD. We also wanted to compare other outcome parameters in patients selected for RIC or MAC based on age and comorbidity.

Patients and Methods

Patient characteristics

Adult patients with AML in first or second complete remission (CR) were selected for RIC (n=47) if they were above 60 years of age or had comorbidity, such as organ impairment or infections, making them unsuitable for MAC. Patients ≤ 61 years of age with possible high risk of relapse as judged by the referring physician were selected for MAC (n=46). Patients ≤ 61 years of age with no comorbidity and judged not to have an excessive risk of relapse were included in a prospective randomized toxicity study comparing RIC with MAC (n=29) [8]. The randomized patients were excluded from the present study. All other AML patients submitted were included in this study. The characteristics regarding age, disease stage, type of donor, stem cell source, and cell dose in patients selected for MAC or RIC are given in (Table 1).

Characteristics	MAC (n = 46)	RIC (n = 47)	p-value
Diagnosis:			
AML	46	47	
Favorable cytogenetics/intermed/adverse	5/28/2009	2/33/11	ns
Disease stage (CR1/ > CR2)	28/18	22/25	ns
Sex (M/F)	28/18	23/24	ns
Age	39 (21–59)	59 (31–69)	< 0.001
Donor:			0.06
Sibling	22	13	
MUD	21	30	
Allele MM MUD	3	4	
Donor sex (M/F)	26/19	29/18	ns
Donor age	37 (18–66)	34 (20–71)	ns
Female to male	9	3	0.07
Stem cell source (BM/PBSCs)	Apr-42	May-42	ns
NC dose (×10⁶/kg)	11.1 (1.5–28.8)	11.3 (2.3–32.7)	ns
CD34 dose (×10⁶/kg)	7.7 (0.2–25.5)	6.9 (0.6–18.1)	ns
GVHD prophylaxis:			
CsA + MTX	42	42	
CsA + MMF	0	1	
Tacrolimus + Sirolimus	4	4	
Conditioning:			
Busulfan-based	40	41	
TBI-based	4	0	
Chemo-based	0	6*	
+ATG	24 (52%)	41 (87%)	< 0.001

*Fu + Cy: n = 1; Flu + Treo: n = 5.

Abbreviations: AML = Acute Myeloid Leukemia; CR1 = Complete Remission; M = Male; F = Female; MUD=Matched Unrelated Donor; MM = Mismatch; BM= Bone Marrow; PBSCs = Peripheral Blood Stem Cells; NC dose = Nucleated Cell dose; CsA = Cyclosporine; MTX = Methotrexate; MMF = Mycophenolate mofetil; TBI = Total Body Irradiation; ATG = Antithymocyte Globulin.

Table 1: Characteristics of patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC).

Donors

Grafts from HLA-identical sibling donors were given to 35 patients. An HLA-A, HLA-B, and DRB1-identical matched unrelated donor (MUD) typed with high-resolution typing was found for each of 51 patients [16]. Seven patients got grafts from allele-mismatched MUDs. Peripheral blood stem cells from a related or an unrelated donor were favored and were given to 84 patients [17,18]. Bone marrow grafts were given to nine patients (Table 1).

Conditioning

MAC consisted of busulfan (4mg/kg/day) divided into four doses and given for four days, combined with cyclophosphamide (Cy, 120mg/kg) [19]. Busulfan doses were monitored depending on pharmacokinetics [20]. RIC included 30 mg/m²/day fludarabine for six days combined with 4mg/kg/day busulfan for two days.

Prophylaxis and Treatment of Graft-Versus-Host Disease

Cyclosporine (CsA) combined with four doses of methotrexate (MTX) was given to 84 patients [21]. CsA was given i.v. on day -1 and day 0, and thereafter orally with a dose ranging from 3 to 12mg/kg to achieve a trough level of 100ng/ml in recipients of grafts from HLA-identical siblings, and between 150 and 300ng/ml in patients with MUDs [22]. One patient received CsA combined with mycophenolate mofetil and eight patients participated in a randomized study and were treated with tacrolimus (0.1mg/kg/day) orally, aiming at trough levels between

5 and 15ng/ml in combination with sirolimus to achieve trough levels of 5-10ng/ml [23]. Antithymocyte globulin (Thymoglobulin, Genzyme, Cambridge, MA) was given at a dose of 6-8mg/kg to all recipients of grafts from MUDs and to seven patients with HLA-identical sibling grafts who were treated with RIC (Table 1) [24,25].

Supportive Care

Allopurinol was given to all patients during conditioning. To prevent hemorrhagic cystitis, forced diuresis, urinary alkalinization, and uromitexan 12mg/kg/dose for six doses were given after each dose of cyclophosphamide. Patients were treated in hospital in reversed isolation or at home during the pancytopenic phase after conditioning [26]. When the number of copies of cytomegalovirus (CMV) DNA in blood was 1,000-2,000 using PCR, pre-emptive therapy with ganciclovir (5mg/kg i.v. twice daily), foscarnet (90mg/kg i.v. twice daily) or oral valganciclovir (150mg/kg/day twice a day) was given for two weeks or longer if PCR results remained positive. Details of the supportive care have already been published [22,26].

Definitions

The cytogenetic abnormalities in AML were classified as good: included t(18;21), t(15;17), inv or del (16;), poor included: 11q23 abnormalities, complex caryotype ≥ 3 and abnormalities of chromosomes 5 and 7 [27]. All other abnormalities including trisomies were regarded as intermediate-risk cytogenetic abnormalities. Bacterial septicemia was defined as a positive blood culture during a febrile episode with ≥ 38.5°C. Acute GVHD was graded according to the Seattle criteria [28]. Chronic graft-versus-host disease was defined as limited or extensive, or as mild, moderate or severe, based on the judgment of the treating physician [29,30]. CMV infection was defined as CMV DNA positivity by PCR and CMV disease was defined as symptomatic organ involvement. Hemorrhagic cystitis was defined as painful hematuria with a negative urine culture without any other explanation. It was graded from 1 to 5 according to NCI criteria [31]. AML relapse was defined as > 20% blasts in the bone marrow, or leukemia in extra medullary organs.

Chimerism

Chimerism was analyzed 1, 3, 6, 9, and 12 months after HSCT. PCR amplification of variable numbers of tandem repeats was used to evaluate the degree of donor and recipient chimerism in CD19+, CD3+, CD33+ cells enriched using magnetic beads (Dynal, Oslo, Norway) as previously described in detail [32]. Donor chimerism (DC) was defined as < 5% recipient cells. Until April 2005, chimerism analysis was based on mini-satellites. After that date, a real-time PCR method based on single nucleotide polymorphism (SNP) was used, which has also been described previously [33]. In this study, we used CD3+ chimerism at one month for evaluation, because this was the average date of appearance of GVHD.

Statistics

Comparisons between groups were performed using Fisher's exact test, the chi-square test, or the Mann-Whitney U-test. The probabilities of survival and LFS were estimated using the Kaplan-Meier method and compared with a log-rank test (Mantel-Haenszel) [34]. TRM, GVHD, and relapse were estimated using cumulative incidence curves, taking competing events into consideration [35]. Factors with a p-value of p<0.1 or known risk-factors were included in the multivariate analysis. Analysis was performed using the CMPRSK package (developed by

Gray, June 2001), Splus 6.2 software (Insightful, Seattle, WA) and Statistica software (StatSoft, Tulsa, OK).

Results

Engraftment, transfusions, and discharge

All patients but one in each group had engraftment. Median time to neutrophil counts > 0.5×10^9/L was 15 (range 11-35) in the MAC group and 17 (0-34) in the RIC group (Figure 1, p = 0.001). Time to reach platelet counts > 30×10^9/L was the same in both groups: 13 (9-31) and 13 (0-40), respectively. RIC patients received fewer erythrocyte transfusions, median 2 (0-12), than MAC patients (2 (0-14), p = 0.003). RIC patients also required fewer platelet transfusions: 1 (0-6) as opposed to 2 (0-18) in the MAC group (p = 0.001). Granulocyte transfusions due to infection and/or severe mucositis were given to seven MAC patients and two RIC patients (p = 0.36). Median time to discharge from the hospital was 20 (12-69) in the MAC patients and 20 (15-42) in the RIC patients (ns).

Infections

Herpes simplex virus infection was seen in 8 of the MAC patients (17%) and 3 of the RIC patients (6%) (p = 0.12). Bacteremia was diagnosed in 15 patients in the MAC group (33%) and in 8 patients in the RIC group (17%) (p = 0.1). CMV reactivation was diagnosed by PCR in 26 patients in the MAC group (56%) and in 27 patients in the RIC group (57%). The corresponding Figure s for CMV disease in the two groups were 4 (9%) and 3 (6%).

Graft-versus-host disease and chimerism

Of the MAC patients, 14 did not experience GVHD, as opposed to 29 in the RIC group. The incidence of acute GVHD of grade I, grade II, and grades III-IV was 7, 20, and 5 in the MAC group and 9, 8, and 1 in the RIC group (p < 0.001). The cumulative incidence of grade II-

IV acute GVHD was 55% in the MAC patients and 17% in the RIC patients (p < 0.001) (Figure 2a). At one month, 46% (13/28) of the RIC patients were mixed T-cell chimeras as compared to 29% (10/35) among the MAC patients. T-cell chimerism did not differ between the RIC patients and the MAC patients (Figure 3a). Among these patients, there was no correlation between donor T-cell chimerism and acute GVHD grades 0-I versus II-IV (Figure 3b). In multivariate analysis for factors associated with acute GVHD, RIC (p < 0.001) and year of transplantation (p = 0.01) were associated with reduced risk of acute GVHD, but chimerism was not (p = 0.80) (Table 2). Chronic GVHD was diagnosed in 13 of the MAC patients, 5 with limited disease and 8 with extensive disease. In the RIC patients, 10 had chronic GVHD; of these, 6 had limited disease and 4 had extensive disease. Mild, moderate, and severe chronic GVHD was seen in 9, 2, and 2 of the MAC patients and in 9, 1, and none of the RIC patients, respectively. The cumulative incidence of chronic GVHD at four years was 32% in the MAC group, as compared to 22% in the RIC group (p = 0.45) (Figure 2b).

Transplant-related mortality, relapse, overall survival, and leukemia-free survival

TRM at three years was 15% in the MAC group and 13% in the RIC group (Figure 2c). The cumulative probability of relapse was significantly higher in the RIC group in univariate analysis, being 44% at five years, as opposed to 24% in the MAC patients (p = 0.01) (Figure 2d). In multivariate analysis of factors associated with relapse, RIC was not associated with an increased risk of relapse compared to MAC (hazards ratio (HR) 1.62, p = 0.34) (Table 2). Patients treated with CsA and MTX had a reduced risk of relapse (p < 0.01) and those treated with ATG had an increased risk of relapse (p = 0.01) (Table 2).

Survival at five years was 67% in the MAC group and 48% in the RIC group (p = 0.08) (Figure 4a). In multivariate analysis, there was no difference in survival between the RIC patients and the MAC patients. Factors associated with reduced survival were adverse cytogenetics (p
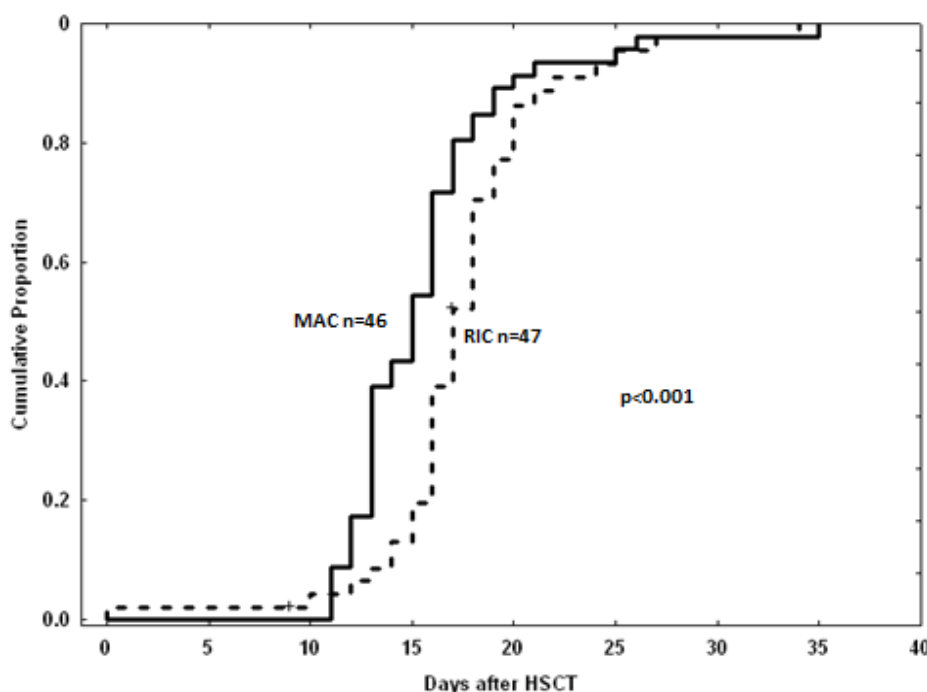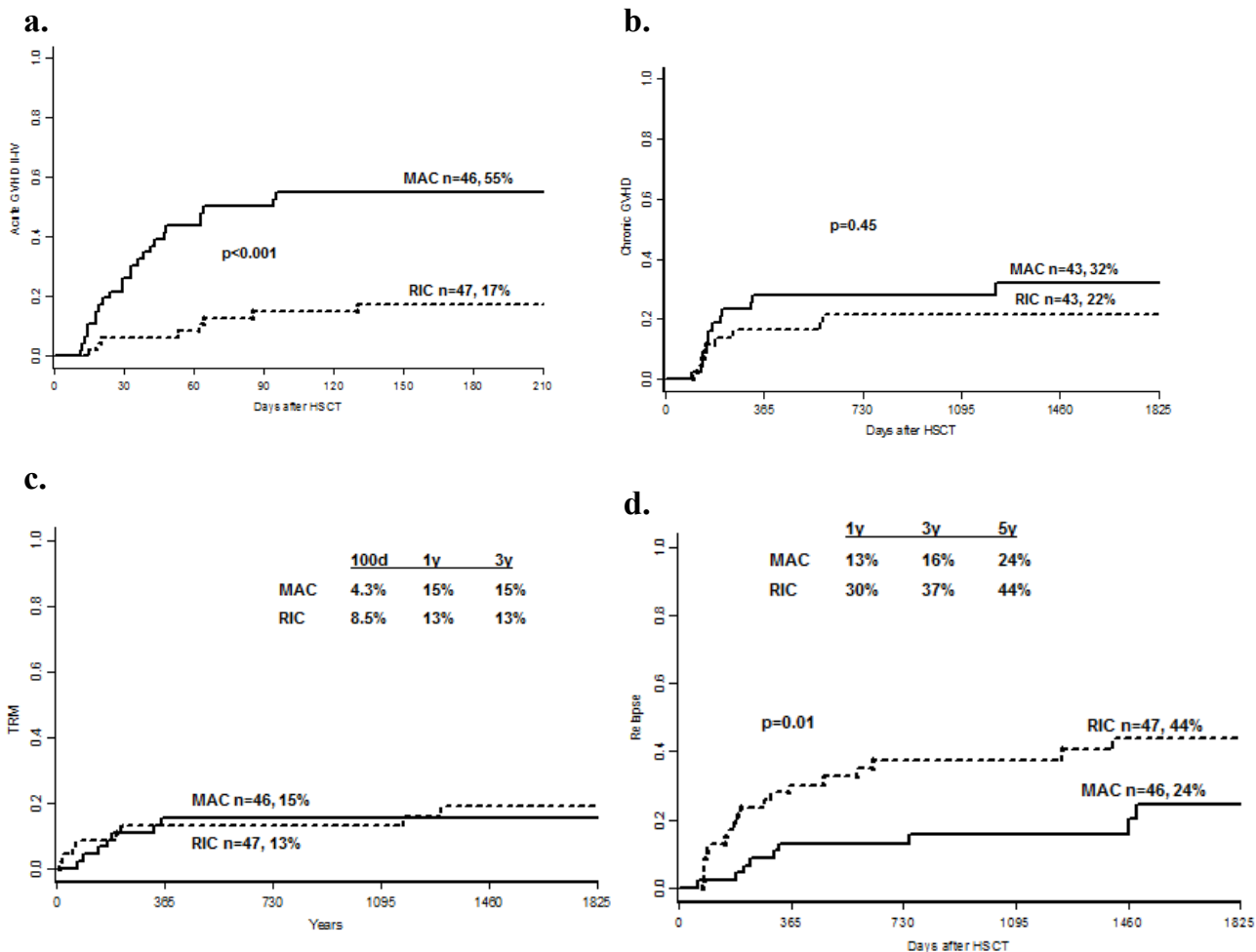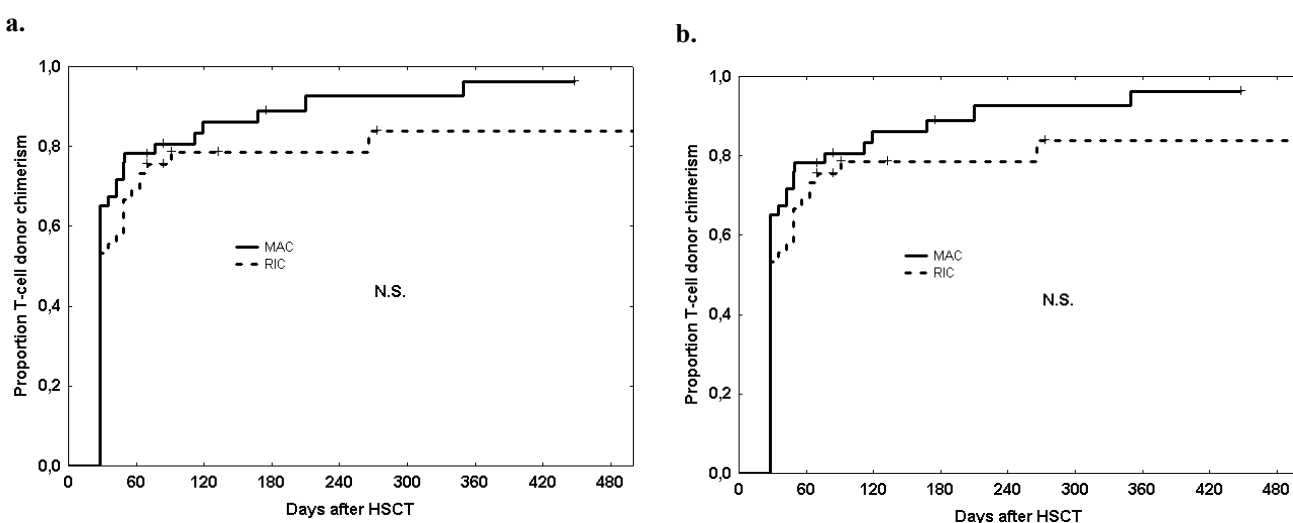

Figure 1: Time to and cumulative incidence of absolute neutrophil count > 0.5×10^9 in patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC).

Figure 2: a. Time to and cumulative incidence of acute GVHD, grades II–IV, in patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) (p < 0.001). Multivariate analysis (HR 0.27, p < 0.01). b. Time to and cumulative incidence of chronic GVHD in patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) (p = 0.45). c. Time to and cumulative incidence of transplant-related mortality (TRM) in patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC). d. Time to and cumulative incidence of leukemic relapse in patients selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) (p = 0.01). Multivariate analysis (HR 1.62, p = 0.34).

Figure 3: a. Time to and cumulative incidence of T-cell donor chimerism in patients treated with reduced intensity conditioning (RIC) or myeloablative conditioning (MAC). b. Time to and cumulative incidence of T-cell chimerism in patients who developed grades 0-I acute GVHD and those who developed grades II-IV acute GVHD.

Factor	Relative hazards	95% confidence interval	p-value
Acute GVHD II–IV			
RIC vs. MAC	0.23	0.10–0.51	< 0.001
Year of HSCT	1.27	1.07–1.52	0.01
Mixed chimerism	0.9	0.40–2.00	0.8
Relapse			
RIC vs. MAC	1.62	0.61–4.31	0.34
CsA + MTX	0.35	0.15–0.81	0.014
ATG	4.48	1.36–14.8	0.014
Acute GVHD I–IV	0.45	0.21–0.99	< 0.05
Mortality			
RIC vs. MAC	1.39	0.69–2.81	0.36
Adverse cytogenetics	2.31	1.14–4.68	0.02
ATG	2.64	1.00–7.06	0.05
Bone marrow	2.33	1.04–2.39	0.04
Transplant failure (opposite of leukemia-free survival)			
RIC vs. MAC	1.61	0.85–3.06	0.14
ATG	3.95	1.51–10.3	0.005
Adverse cytogenetics	2.13	1.10–4.12	0.025

Abbreviations: RIC = Reduced Intensity Conditioning; MAC = Myeloablative Conditioning; ATG = Antithymocyte Globulin; CsA = Cyclosporine; MTX = Methotrexate; GVHD = Graft-Versus-Host Disease.

Table 2: Multivariate analysis for acute GVHD of grades II–IV, relapse, mortality, and transplant failure (leukemia-free survival).
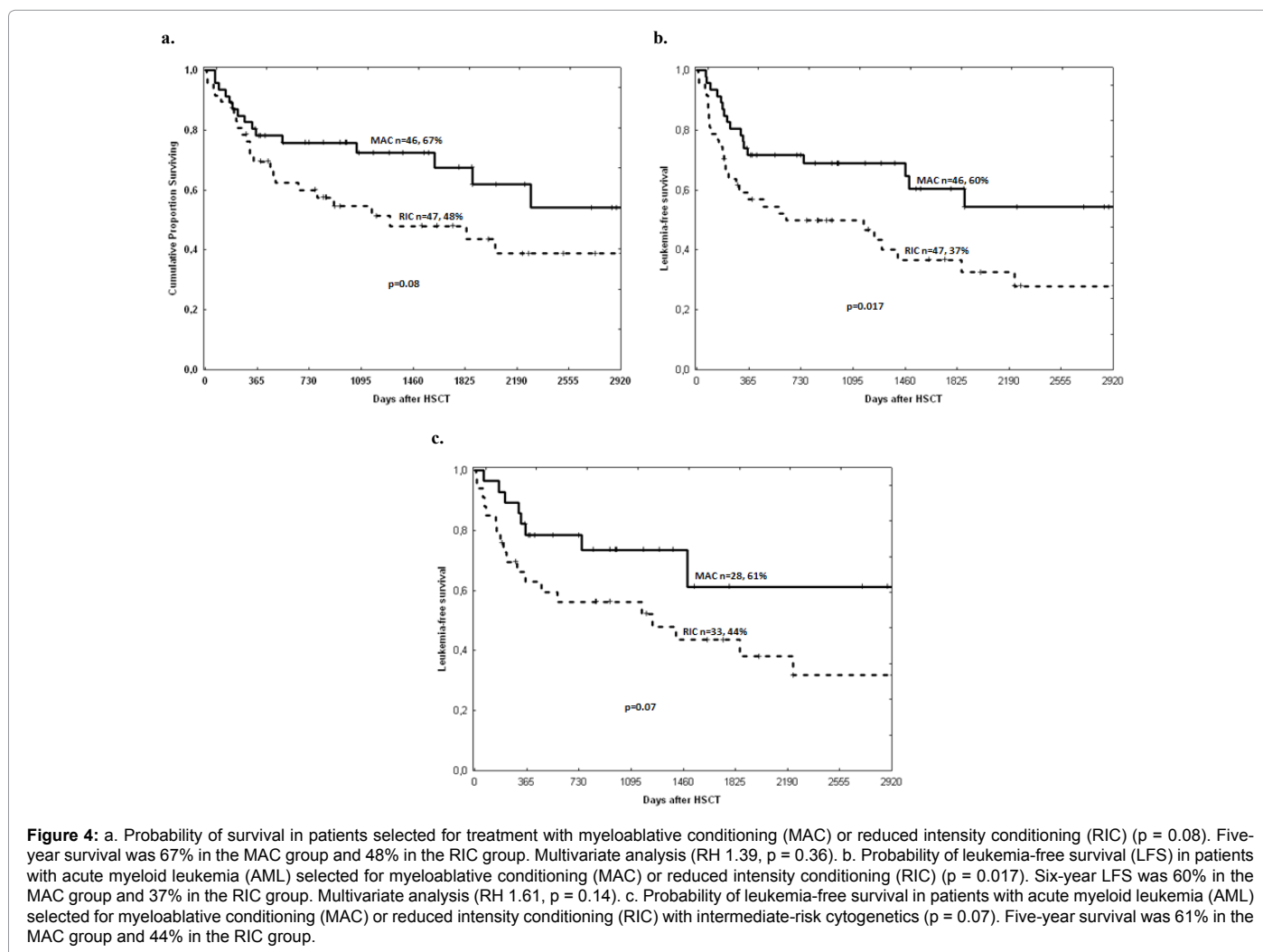
= 0.02), treatment with ATG (p = 0.05), and bone marrow graft rather than PBSCs (p = 0.04) (Table 2).

LFS at five years was 60% in the MAC patients, as opposed to 37% in the RIC patients (p = 0.017) (Figure 4b). LSF for patients with intermediate-risk cytogenetics was 61% and 44% in the two groups, respectively (p = 0.07) (Figure 4c). For patients with adverse-risk cytogenetics, three-year survival was 44% in the MAC patients and 45% in the RIC patients. In multivariate analysis, there was no difference in LFS between patients receiving MAC or RIC (Table 2). Factors associated with reduced LFS were adverse cytogenetics and ATG.

Causes of death for the MAC and RIC patients were: GVHD (4 and 1, respectively), relapse (5 and 14), infection (5 and 6), and others (1 and 3).

Discussion

Like most studies published so far that have compared MAC and RIC in AML patients undergoing HSCT, this study included patients who were selected, because the patients were thought to benefit from one conditioning rather than the other. This selection introduces an obvious bias, with younger and fitter patients selected for MAC. This may be the reason that the MAC group had faster engraftment of neutrophils. This contrasts with a previous retrospective analysis in AML patients receiving grafts from unrelated donors, where the RIC patients

Figure 4: a. Probability of survival in patients selected for treatment with myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) (p = 0.08). Five-year survival was 67% in the MAC group and 48% in the RIC group. Multivariate analysis (RH 1.39, p = 0.36). b. Probability of leukemia-free survival (LFS) in patients with acute myeloid leukemia (AML) selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) (p = 0.017). Six-year LFS was 60% in the MAC group and 37% in the RIC group. Multivariate analysis (RH 1.61, p = 0.14). c. Probability of leukemia-free survival in patients with acute myeloid leukemia (AML) selected for myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) with intermediate-risk cytogenetics (p = 0.07). Five-year survival was 61% in the MAC group and 44% in the RIC group.

had faster neutrophil engraftment [6]. Despite the faster neutrophil engraftment in the MAC group, time to discharge was the same in the two study groups (20 days). The reason that the MAC patients were not discharged earlier despite faster neutrophil engraftment is probably due to the more toxic regimen giving rise to more mucositis, as was found in the two randomized studies [7,8]. Because of this, the MAC patients needed more total parenteral nutrition and it took some days before oral intake was sufficient for discharge [8]. More transfusions of erythrocytes and platelets were needed in the MAC patients, which is in keeping with the results of the randomized toxicity study.

In the present study, where less fit patients were selected for RIC, the incidence of CMV reactivation was the same as in the MAC group. This has also been seen in another study comparing HSCT patients selected for RIC or MAC [36]. It contrasts with the results of the randomized study with similar, fit patients where CMV infection was significantly less common in RIC patients than in MAC patients [8]. This finding highlights the fact that by selecting for MAC or RIC, these are different patient populations which of course influence outcome in various ways. With less fit patients in the RIC group, the risk of CMV infection is the same as with more fit patients treated with MAC.

Acute GVHD was more common in the MAC group than in the RIC group, both in univariate analysis and multivariate analysis, taking into consideration differences between the two groups such as differences in age, unrelated donor transplants, female donor to male recipient, and use of ATG (Table 2). RIC has previously been reported to be associated with less acute GVHD than MAC [4,9-11]. One reason for the increased risk of GVHD using MAC may be that tissue toxicity induced by chemoradiotherapy causes release of cytokines that are of importance for the development of GVHD [37,38]. Another reason for the reduced risk of acute GVHD using RIC rather than MAC may be that mixed donor T-cell chimerism is more common with RIC [8,15] and that donor T-cell chimerism is more associated with acute GVHD than mixed chimerism [14]. Animal studies have also shown that mixed chimerism is associated with a reduced risk of GVHD [39,40]. In the present analysis, mixed recipient-donor chimerism had no effect on GVHD in uni- or multivariate analysis. This highlights the fact that RIC is associated with a reduced risk of acute GVHD despite the fact that RIC patients are older than the MAC patients and that acute GVHD is otherwise more severe in older patients. Thus, toxicity induced by conditioning was more important for the development of acute GVHD than achieving full donor T-cell chimerism.

The incidence of chronic GVHD was not significantly different in the RIC and MAC groups (Figure 3). This is in keeping with the results of most of the other studies [3-8,11,41]. However, a few studies have shown that RIC patients had less chronic GVHD than MAC patients [4,9]. There is a correlation between acute and chronic GVHD, which would speak in favor of a reduced risk of chronic GVHD in patients treated with RIC rather than MAC [42-44]. However, the incidence of chronic GVHD also increases with increasing age. Thus, it is probable that these two factors counterbalance each other-with no difference in incidence of chronic GVHD between patients treated with RIC and MAC, as seen in this study.

TRM was similar in the two groups (Figure 2a). This study demonstrates that selection of RIC for patients with high age or comorbidity gives low TRM: 13% at three years, which was similar to that for MAC (Figure 2c). This is supported by the results of the two randomized studies [7,8]. However, one study in HLA-identical siblings found a lower probability of TRM in the RIC patients than in the MAC patients [6]. A study from the Center for International Blood and Marrow Transplant Research (CIBMTR) found that there was no significant difference in TRM using MAC, RIC, or non-myeloablative conditioning [5]. A study from the European Group for Blood and Marrow Transplantation (EBMT) using unrelated donors found that in patients over 50 years of age, TRM was significantly higher in the MAC group than in the RIC group, whereas in patients less than 50 years of age there was no significant difference in TRM using RIC or MAC [6].

Relapse was significantly more common in the RIC group in univariate analysis, but not in multivariate analysis when adjusting for differences in patient characteristics between the groups. Several large retrospective studies have shown that relapse is probably higher with RIC than with MAC [4-6]. This might be expected, because with more chemoradiotherapy, leukemia cells are killed more efficiently, which is supported by prospective randomized studies [45]. However, a randomized study showed a similar incidence of relapse in patients who were randomized to RIC or MAC [7]. However, the low probability of relapse in the MAC group, 24% at five years, suggests that MAC may be selected in young patients with an expected high risk of relapse. There are data that show that the GVL effect may be more effective using RIC than using MAC [46]. A large study from the CIBMTR recently suggested that in patients with AML or myelodysplastic syndrome, the GVL effect using RIC results in improved survival and LFS, both short-term and long-term, compared to MAC. These data suggested that RIC should be selected more often as conditioning, even for good-risk patients with AML and MDS. The randomized toxicity study also showed that RIC should be selected more often, because this results in significantly less toxicity and suffering than with MAC [8].

Overall survival and LFS were encouraging in the MAC patients. The LFS at six years was 60% (Figure 4b). The corresponding LFS in the RIC patients was only 37%, but this was a selected group with co-morbidities and high age and it was therefore acceptable. ATG decreased survival, and especially LFS, due to an increased risk of relapse. Using unrelated donors, ATG is beneficial because it reduces acute GVHD and also TRM [47]. Especially when using RIC and HLA-identical sibling transplants, ATG should not be given and a higher PBSC cell dose should be aimed for to reduce the risk of graft failure and relapse, and perhaps improve LFS [25]. When using an unrelated donor, the ATG dose may be lower in RIC patients than in MAC patients to maintain a strong GVL effect.

This is a registry retrospective analysis and therefore, the data has to be interpreted with caution. Even if all known risk-factors were included in the multivariate analysis, there may be unknown factors of importance which were not included.

To conclude, when we selected AML patients of high age, and with comorbidity, for RIC, TRM was low and similar to that in more fit patients treated with MAC. One reason for this may be that the risk of acute GVHD was reduced with RIC. We also found that conditioning, i.e. RIC, was more important than mixed chimerism for reduction of acute GVHD. Although there was higher relapse with RIC in univariate analysis, there was no significant difference in the multivariate analysis. Survival and LFS with RIC, although inferior to that with MAC, was acceptable in this high-risk category. Thus, selection for RIC or MAC based on age and comorbidity appears valid according to the results of the present study.

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