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Improved Survival after Allogeneic Hematopoietic Stem Cell Transplantation in Recent Years. A Single-Center Study

Mats Remberger,[1,2] Malin Ackefors,[3] Sofia Berglund,[1,2] Ola Blennow,[3] Göran Dahllöf,[4] Aldona Dlugosz,[5] Karin Garming-Legert,[4] Jens Gertow,[1,2] Britt Gustafsson,[6] Moustapha Hassan,[7] Zuzana Hassan,[1,7] Dan Hauzenberger,[2] Hans Hägglund,[8] Helen Karlsson,[1,2] Lena Klingspor,[9] Gunilla Kumlien,[10] Katarina Le Blanc,[2,8] Per Ljungman,[8] Maciej Machaczka,[8] Karl-Johan Malmberg,[8] Hanns-Ulrich Marschall,[5] Jonas Mattsson,[1,2] Richard Olsson,[1,2] Brigitta Omazic,[1,2] Darius Sairafi,[1,2] Marie Schaffer,[2] Britt-Marie Svahn,[1] Petter Svenberg,[1,2] Lisa Swartling,[3] Attila Szakos,[11] Michael Uhlin,[1,2] Mehmet Uzunel,[1,2] Emma Watz,[10] Annika Wernerson,[11] Agneta Wikman,[10] Ann-Charlotte Wikström,[12] Jacek Winiarski,[6] Olle Ringdén[1,2]

We analyzed the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) over the past 2 decades. Between 1992 and 2009, 953 patients were treated with HSCT, mainly for a hematologic malignancy. They were divided according to 4 different time periods of treatment: 1992 to 1995, 1996 to 2000, 2001 to 2005, and 2006 to 2009. Over the years, many factors have changed considerably regarding patient age, diagnosis, disease stage, type of donor, stem cell source, genomic HLA typing, cell dose, type of conditioning, treatment of infections, use of granulocyte-colony stimulating factor (G-CSF), use of mesenchymal stem cells, use of cytotoxic T cells, and home care. When we compared the last period (2006-2009) with earlier periods, we found slower neutrophil engraftment, a higher incidence of acute graft-versus-host disease (aGVHD) of grades II-IV, and less chronic GVHD (cGHVD). The incidence of relapse was unchanged over the 4 periods (22%-25%). Overall survival (OS) and transplant-related mortality (TRM) improved significantly in the more recent periods, with the best results during the last period (2006-2009) and a 100-day TRM of 5.5%. This improvement was also apparent in a multivariate analysis. When correcting for differences between the 4 groups, the hazard ratio for mortality in the last period was 0.59 (95% confidence interval [CI]: 0.44-0.79; $P < .001$) and for TRM it was 0.63 (CI: 0.43-0.92; $P = .02$). This study shows that the combined efforts to improve outcome after HSCT have been very effective. Even though we now treat older patients with more advanced disease and use more alternative HLA nonidentical donors, OS and TRM have improved. The problem of relapse still has to be remedied. Thus, several different developments together have resulted in significantly lower TRM and improved survival after HSCT over the last few years.

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KEY WORDS: HSCT, GVHD, PBSC, Improved results

BACKGROUND

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for patients with hematologic malignancies, bone marrow failure syndromes, and some inherited disorders [1-3].

The main obstacles to success after HSCT are relapse of the underlying disease, graft-versus-host disease (GVHD), and infection [4-7]. To improve the results after HSCT, efforts have been made to solve these problems by earlier detection, reduction of incidents,

From the [1]Center for Allogeneic Stem Cell Transplantation; [2]Division of Clinical Immunology; [3]Department of Infectious Diseases; [4]Dental Medicine; [5]Gastroenterology; [6]Pediatrics; [7]Experimental Cancer Medicine; [8]Hematology; [9]Microbiology; [10]Transfusion Medicine; [11]Pathology; and [12]Biosciences and Nutrition, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden.

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Correspondence and reprint requests: Mats Remberger, PhD, Clinical Immunology, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden (e-mail: mats.remberger@ki.se).

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and improvement of treatments. This has resulted in considerable changes in the transplantation procedure, in treatment, and in patient selection. In particular, the introduction in the early 1980s of a combination of cyclosporine and methotrexate has significantly reduced severe aGVHD and improved TRM [8,9]. The number of patients eligible for HSCT has increased as a result of better treatments, and the introduction of less toxic reduced-intensity conditioning (RIC) regimens has made it possible to admit older patients and those with comorbidities [10-12]. During the late 1990s, the source of stem cells shifted from bone marrow to peripheral blood stem cells (PBSCs) [13-17]. Genomic tissue typing has been employed to better match unrelated donors [18,19]. The introduction of preemptive therapy for cytomegalovirus (CMV) infection has improved outcome and reduced the risk of fatal CMV disease [20,21]. In our unit, the use of liposomal amphotericin B after HSCT has significantly reduced the risk of invasive fungal infection from 11% to around 5% [22]. Today, there is a large number of efficient antifungals from which to choose. Other antiinfectious agents include new antibiotics and antiviral drugs such as acyclovir, ganciclovir, and foscarnet [23]. By the introduction of PCR methods to detect donor-recipient chimerism, there has been improved diagnosis of graft failure [24]. Minimal residual disease detection of threatening relapse has also been developed [25]. Donor lymphocyte infusions (DLIs) are used for treatment of graft failure and relapse [26,27]. Home care after HSCT has reduced aGVHD and improved survival; this is now routine practice in patients living within 2 hours' driving distance of our hospital [28]. In more recent years, mesenchymal stem cells have been introduced by us and have been shown to have an effect in some patients with severe aGVHD that is otherwise refractory to therapy [29]. A randomized study showed that the use of ursodiol not only reduced liver toxicity, it also improved survival [30]. Because of these improvements, patients with higher age, with more resistant underlying disease, and those with comorbidities have been accepted for HSCT. The range of diagnoses in patients admitted for HSCT has also changed over the years. Because the proportion of patients admitted with more advanced disease has increased with time, there was no apparent improvement in patient survival for a long time. It was therefore of interest to determine whether or not outcome after HSCT has improved over the last 2 decades.

PATIENTS AND METHODS

Patients

From January 1992 until December 2009, 1013 patients underwent HSCT at Karolinska University Hospital, Huddinge, Sweden. Patients transplanted for a solid tumor (n = 60) were excluded, as this is an experimental treatment with poor outcome [31]. In total, 953 patients were included in the study. Patient and donor characteristics are listed in Table 1.

There were 275 patients with acute myeloid leukemia (AML), 176 with acute lymphoid leukemia (ALL), 161 with chronic myeloid leukemia (CML), 24 with chronic lymphoid leukemia (CLL), 60 with lymphoma, 86 with myelodysplastic syndrome or myeloproliferative syndrome (MDS/MPS), 27 with multiple myeloma (MM), and 6 with myelofibrosis (MF). One hundred thirty-eight other patients had a nonmalignant disorder: severe aplastic anemia (n = 38), Fanconi anemia (n = 13), inborn error of metabolism (n = 80), or another nonmalignant disease (n = 7). The median age of all patients was 34 years (0-69), and there were 545 males and 408 females. There were 293 children under the age of 18 years (31%). Almost one-half of the patients (46%) had a malignancy beyond first complete remission or first chronic phase (CR1/CP1), and were considered to have high-risk disease.

The study was approved by the Ethics Committee at Huddinge University Hospital. All patients included in the study gave informed consent.

Donors

Donors were an HLA-identical sibling in 406 cases, at least an HLA-A, -B and -DR matched unrelated donor (MUD) in 419 cases, an allele-mismatched unrelated donor in 56 cases, or an antigen-mismatched related or unrelated donor in 13 and 54 cases, respectively. Five patients were transplanted from a syngeneic twin. There were 531 male and 410 female donors with a median age of 35 years (range: 0-71).

HLA Typing

All patients and donors were typed using PCR-SSP high-resolution typing for both HLA class I and II antigens (at the 4-digit level) [18,32-34].

Stem Cell Source

Bone marrow (BM) was given to 480 patients, and 429 patients received peripheral blood stem cells (PBSCs) from a granulocyte-colony stimulating factor (G-CSF) stimulated donor. In 44 cases, 1 (n = 33) or 2 (n = 11) cord blood units were used as grafts, mainly for patients lacking an acceptable related or unrelated donor. Median nucleated cell dose was 4.8×10^8/kg (range: 0.03-81.3). The $CD34^+$ cell dose was known in 703 transplants and was median 6.3×10^6/kg (0.03-68).

Conditioning

Conventional myeloablative conditioning was given to 704 patients and consisted of cyclophosphamide (Cy) at 60 mg/kg for 2 days in combination with 7.5-10 Gy single-fraction total body irradiation (sTBI)

Table 1. Characteristics of Patients Who Underwent Hematopoietic Stem Cell Transplantation from 1992 through 2009 at Karolinska University Hospital, Grouped According to Time Period

Year of HSCT	1992-1995	1996-2000	2001-2005	2006-2009
Number	188	269	279	217
Diagnosis:				
Acute leukemia	84 (45)	138 (51)	120 (43)	109 (50)
Chronic leukemia	53 (28)	69 (26)	43 (15)	19 (9)
MDS/MPS	10 (5)	14 (5)	30 (11)	32 (15)
Other hematol. malignancy	11 (6)	20 (7)	43 (15)	20 (9)
Nonmalignant disorders	30 (16)	28 (10)	43 (15)	37 (17)
High-risk disease	73 (39)	116 (43)	145 (52)	100 (46)
Age	28 (0.2-58)	32 (0-63)	35 (0-65)	39 (0.4-69)
Children (<18 years)	60 (32)	86 (32)	85 (30)	62 (29)
Sex (male/female)	118/70	145/124	161/118	121/96
Donor:				
HLA-identical, related	106 (56)	112 (42)	106 (38)	87 (40)
MUD	72 (38)	126 (47)	126 (45)	95 (44)
Mismatched	10 (5)	31 (12)	47 (17)	35 (16)
Female to male	58 (31)	46 (17)	55 (20)	38 (18)
NC dose ($\times 10^8$/kg)	2.3 (0.6-18.1)	4.1 (0.03-80)	8.6 (0.1-63.8)	9.8 (0.3-81)
Stem cell source:				
BM/PBSCs/CB	183/5/0	158/111/0	92/170/17	47/143/27
Conditioning:				
TBI-based MAC	143 (76)	175 (65)	54 (19)	43 (20)
Chemo-based MAC	42 (22)	77 (29)	100 (36)	70 (32)
RIC	3 (2)	17 (6)	125 (45)	104 (48)
ATG	87 (46)	179 (66)	211 (76)	157 (72)
GVHD prophylaxis:				
CsA or MTX	4	4	7	0
CsA/tacrolimus + MTX	183 (97)	234 (87)	220 (79)	151 (70)
CsA + MMF	0	6 (2)	22 (8)	0
TcD	0	13 (5)	1	0
Other*	1	12	29 (10%)	66 (30)
G-CSF	44 (23%)	237 (88%)	50 (18%)	39 (18%)

MDS/MPS indicates myelodysplastic syndrome/myeloproliferative syndrome; High-risk, beyond CR1/CP1; HLA, human leukocyte antigen; MUD, matched unrelated donor; NC dose, nucleated cell dose; BM, bone marrow; PBSCs, peripheral blood stem cells; CB, cord blood; TBI, total body irradiation; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; MMF, mycofenolate mofetile; TcD, T cell depletion; G-CSF, granulocyte-colony stimulating factor.
Absolute numbers or medians are presented. Figures in parentheses show percentage or range.
*CsA + Prednisolone, Tacrolimus + Sirolimus.

(n = 262), fractionated TBI (FTBI) at 3 Gy/day for 4 days (n = 157), and busulphan (Bu) at 4 mg/kg/day for 4 days (n = 270) [35]. Fifteen patients with severe aplastic anemia (SAA) and a sibling donor received Cy at 50 mg/m^2 for 4 days.

Reduced-intensity conditioning (RIC) was given to 249 patients and consisted of fludarabine (Flu) at 30 mg/m^2 for 3 to 6 days in combination with Bu at 4 mg/kg/day for 2 days (n = 125), FTBI at 3 Gy/day for 2 days and Cy at 60 mg/kg/day for 2 days (n = 49), Cy at 30 mg/kg/day for 2 days (n = 34), treosulphan at 12 to 14 g/m^2/day for 3 days (n = 25), or TBI (2 Gy) (n = 16) [12,36,37].

All patients with an unrelated donor or a nonmalignant disease received antithymocyte globuline (ATG, Thymoglobulin, Genzyme, Cambridge, MA [n = 490] or ATG-Fresenius, Fresenius, Gräfelfing, Germany [n = 39]), alemzumab (Genzyme) (n = 38), or Orthoclone OKT-3 (Ortho Biothech, NJ) (n = 66) for 2 to 5 days during conditioning.

GVHD Prophylaxis

Immunosuppressive treatment consisted of cyclosporine A (CsA) in combination with a short course of methotrexate (MTX) (n = 788), prednisolone (n = 53), or mycophenolate mofetil (MMF) (n = 28) [8,9]. Fifty-one patients received tacrolimus combined with sirolimus, and 10 received monotherapy with either CsA or MTX. The patients with a syngeneic twin donor received no immunosuppression, and 14 patients received a T cell-depleted graft [38].

During the first month, blood CsA levels were kept at 100 ng/mL in patients with malignancies when a sibling donor was used and at 200 to 300 ng/mL when an unrelated donor was used and also in patients with nonmalignant disorders regardless of donor [39]. In the absence of GVHD, CsA was discontinued after 3 to 6 months for patients with malignancies and after 12 to 24 months for patients with nonmalignant disorders.

Supportive Care

Supportive care has been described in detail previously [40-42].

Diagnosis and Treatment of GVHD

Both aGVHD and cGVHD were diagnosed on the basis of clinical symptoms and/or biopsies (skin, liver,

gastrointestinal tract, or oral mucosa) according to standard criteria [14]. The patients were treated for grade-I aGVHD with prednisolone, starting at 2 mg/kg/day, which was tapered after the initial response [41]. In more severe cases, ATG, methylprednisolone, MTX, psoralene and UV light (PUVA), or mesenchymal stem cells were used [8,29]. First-line therapy for cGVHD was CsA combined with corticosteroids [43]. In nonresponders, additional immune suppression was given.

Definitions

Neutrophil engraftment was defined as the number of days after HSCT until absolute neutrophil counts were $>0.5 \times 10^9$/L for 2 consecutive days. Platelet engraftment was defined as the first of 7 consecutive days with platelets $>30 \times 10^9$/L without transfusions.

Rejection was defined as >95% recipient chimerism in the CD33-positive cell population in peripheral blood and/or bone marrow 4 weeks after HSCT.

Statistics

The analysis was performed on May 15, 2010. OS was calculated using the Kaplan-Meier method and compared with the log-rank test [44]. TRM, GVHD, graft failure, and relapse were estimated using a nonparametric estimator of cumulative incidence curves, taking competing events into consideration. Univariate and multivariate risk factor analyses for TRM and GVHD were performed using the proportional subdistribution hazard regression model developed by Fine and Gray. Multivariate modeling for OS and time-to-engraftment was performed using Cox regression models (to estimate hazard ratios [HRs]). Chronic GVHD was analyzed as a time-dependent covariate in the Cox regression model. In all multivariate analyses, HSCT during the years 2006 to 2009 were compared with HSCT during 1992 to 2005, and corrections were made for differences between the groups (age, donor, conditioning, stem-cell source, nucleated-cell dose, G-CSF, and disease stage). All *P* values were 2-tailed. Categoric parameters were compared using the chi-square test, and continuous variables were compared using the Mann-Whitney test. Analysis was performed using the cmprsk software package (developed by Gray, June 2001), Splus 6.2 software (Insightful, Seattle, WA) and Statistica software (StatSoft, Tulsa, OK).

RESULTS

The patients were divided into 4 groups depending on year of transplantation. Group 1 consisted of patients transplanted between 1992 and 1995 (n = 188), group 2 consisted of patients transplanted between 1996 and 2000 (n = 269), group 3 of patients transplanted 2001 to 2005 (n = 279), and group 4 of patients transplanted 2006 to 2009 (n = 217). There were considerable differences among these 4 groups concerning age, type of donor, nucleated cell dose, diagnosis, the use of G-CSF, stem cell source, and type of conditioning. This is illustrated in Table 1.

Engraftment

Graft failure occurred in 3.7%, 3.0%, 5.7%, and 6.0% of patients in the 4 groups ($P = .15$ globally comparing all 4 periods).

Time to neutrophil engraftment changed considerably over the years, as illustrated in Figure 1A. In the corrected multivariate analysis, HSCT during 2006 to 2009 (HR = 0.77, 95% CI: 0.66-0.91, $P < .001$) was found to be associated with slower neutrophil engraftment compared with HSCT during 1992 to 2005. Other factors associated with neutrophil engraftment were PBSCs (HR = 2.12, CI: 1.81-2.43, $P < .001$) G-CSF (HR = 1.26, CI: 1.10-1.45, $P = .001$), ATG (HR = 0.82, CI: 0.70-0.95, $P < .01$), and CB graft (HR = 0.42, CI: 0.28-0.62, $P < .001$).

Time to platelet engraftment ($>30 \times 10^9$/L) was shorter during the 2 last time periods compared with earlier periods ($P < .001$) (Figure 1B). However, in the corrected multivariate analysis, platelet engraftment in HSCT during 2006 to 2009 (HR = 0.92, CI: 0.78-1.09, $P = .35$) was not different from HSCT during 1992 to 2005. Factors associated to platelet engraftment were RIC (HR = 1.23, CI: 1.03-1.46, $P = 0.02$), PBSCs (HR = 2.07, CI: 1.76-2.43, $P < .001$), CB graft (HR = 0.52, CI: 0.37-0.74, $P < .001$), and G-CSF (HR = 0.62, CI: 0.54-0.72, $P < 0.001$).

GVHD

The incidence of aGVHD of grades II-IV increased from 13% (CI: 8%-18%) during 1992 to 1995 to 37% (30%-44%) during 2006 to 2009 (Figure 2A). In the corrected multivariate analysis, we found that HSCT during 2006 to 2009 (HR = 1.45, CI: 1.09-1.91, $P = .01$) was associated with a higher incidence of aGVHD II-IV compared with HSCT during 1992 to 2005. Other factors associated with aGVHD of grades II-IV in multivariate analyses were: MAC (HR = 1.81, CI: 1.35-2.43, $P < .001$), PBSCs (HR = 1.67, CI: 1.28-2.17, $P < .001$), mismatched donor (HR = 1.43, CI: 1.04-1.97, $P = .027$), and ABO blood group mismatch (HR = 1.20, CI: 1.05-1.38, $P = .01$).

The 1-year survival (95% CI) in patients with aGVHD of grades II-IV was 42% (22%-62%), 52% (42%-62%), 49% (39%-59%), and 73% (64%-82%) in the 4 respective groups ($P < .001$).

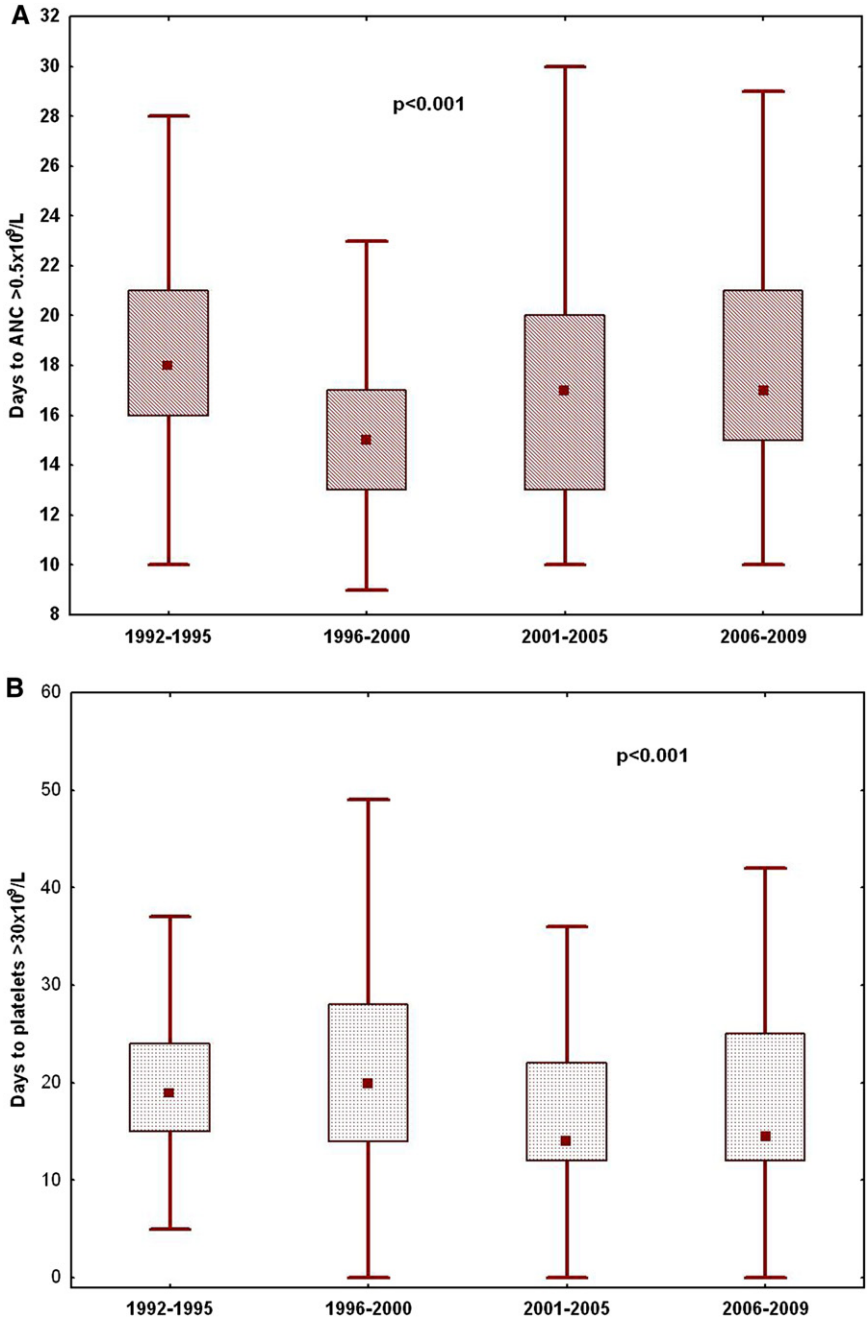

Figure 1. Box plot of time to neutrophil (A) and platelet (B) engraftment after allogeneic HSCT over 4 different time periods. Median, 25% to 75% interquartile range and nonoutlier range. The *P* value refers to a global comparison of all 4 groups. ANC indicates absolute neutrophil count.

The incidence of cGVHD decreased from 47% (95% CI: 40%-54%) to 30% (95% CI: 23%-37%) over the entire study period ($P < .001$ comparing all 4 periods) (Figure 2B). In the corrected multivariate analysis, HSCT during 2006 to 2009 (HR = 0.67, CI: 0.49-0.92, $P = .013$) was associated with less cGVHD compared with HSCT during 1992 to 2005.

Other factors associated with cGVHD were patient age (by decades) (HR = 1.18, CI: 1.11-1.26, $P < .001$), HLA-identical related donor (HR = 1.53, CI: 1.21-1.93, $P < .001$), TBI-based conditioning (HR = 1.73, CI: 1.36-2.21, $P < .001$), and G-CSF (HR = 1.39, CI: 1.09-1.76, $P < 0.01$).

TRM

TRM improved significantly over the entire study period ($P < .001$ comparing all 4 periods) (Figure 3A). The 1-year TRM (95% CI) was 22% (16%-28%), 22% (17%-27%), 19% (14%-24%), and 13% (9%-17%) in the 4 groups, respectively. In the corrected multivariate analysis, HSCT during 2006

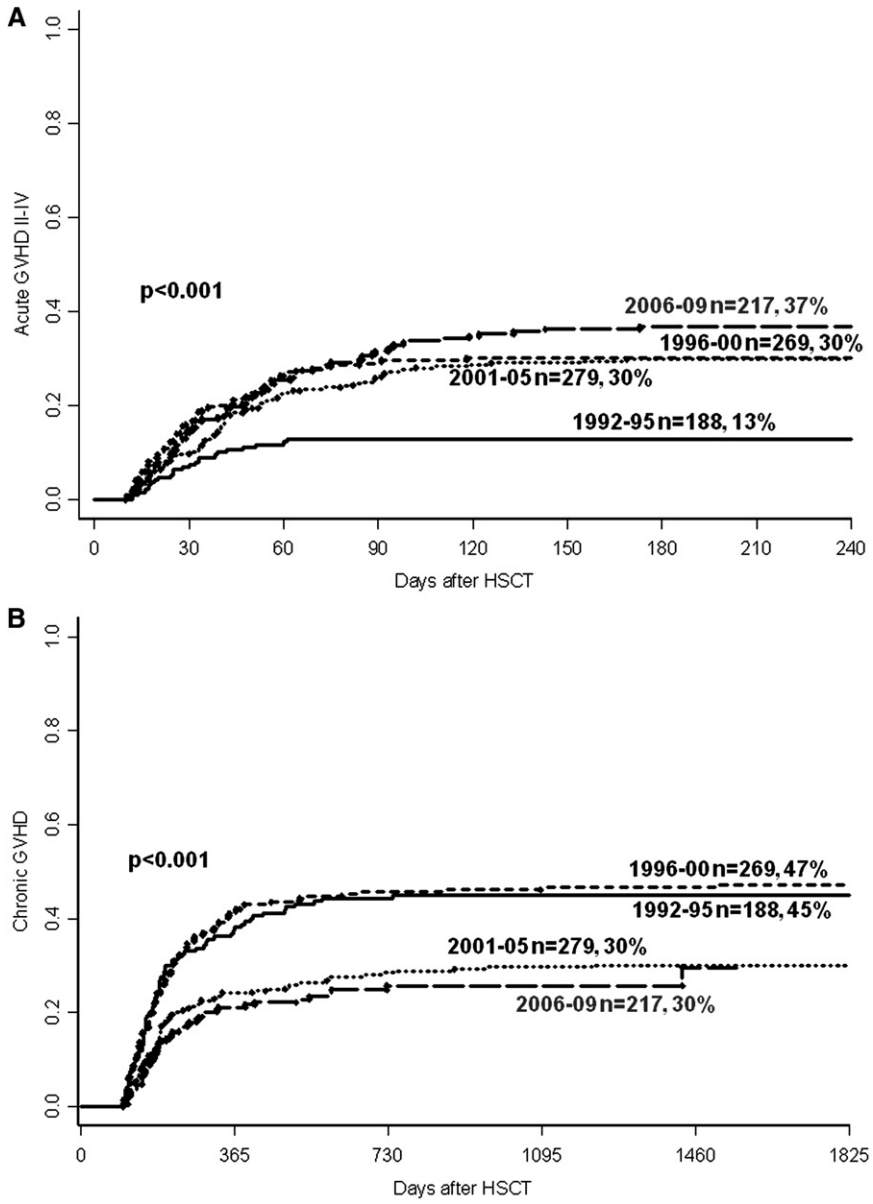

Figure 2. Cumulative incidence of aGVHD of grades II-IV (A) and cGVHD (B) after allogeneic HSCT over 4 different time periods. The *P* value refers to a global comparison of all 4 groups.

to 2009 (HR = 0.63, CI: 0.43-0.92, $P = .02$) was found to be associated with lower TRM compared with HSCT during 1992 to 2005.

Other factors associated with TRM was patient age (by decades) (HR = 1.18, CI: 1.10-1.28, $P < .001$), acute leukemia (HR = 0.65, CI: 0.50-0.86, $P = .002$), HLA-identical related donor (HR = 0.60, CI: 0.46-0.78, $P < .001$), RIC (HR = 0.64, CI: 0.46-0.88, $P = .007$), and home care (HR = 0.55, 0.34-0.91, $P = .02$).

OS

The 3-year OS (95% CI) improved over the years: 53% (46%-60%) during 1992 to 1995, 57% (51%-63%) during 1996 to 2000, 60% (54%-66%) during 2001 to 2005, and 71% (65%-77%) during 2006 to 2009 ($P = .0015$ comparing all 4 periods) (Figure 3B). In the corrected multivariate analysis, HSCT 2006 to 2009 was associated with less mortality (HR = 0.59, CI: 0.44-0.79, $P < .001$) compared with HSCT during 1992 to 2005.

Other factors associated with mortality were patient age (by decades) (HR = 1.13, CI: 1.06-1.21, $P = .003$), malignant disease (HR = 2.14, CI: 1.40-3.29, $P < .001$), unrelated or mismatched donor (HR = 1.43, CI:1.17-1.73, $P < .001$), high-risk disease (HR =1.54, CI: 1.26-1.88, $P < .001$), and low NC dose (HR = 1.02, CI: 1.00-1.03, $P = .03$).

A comparison of the various outcome variables during the different time periods are shown in Table 2.

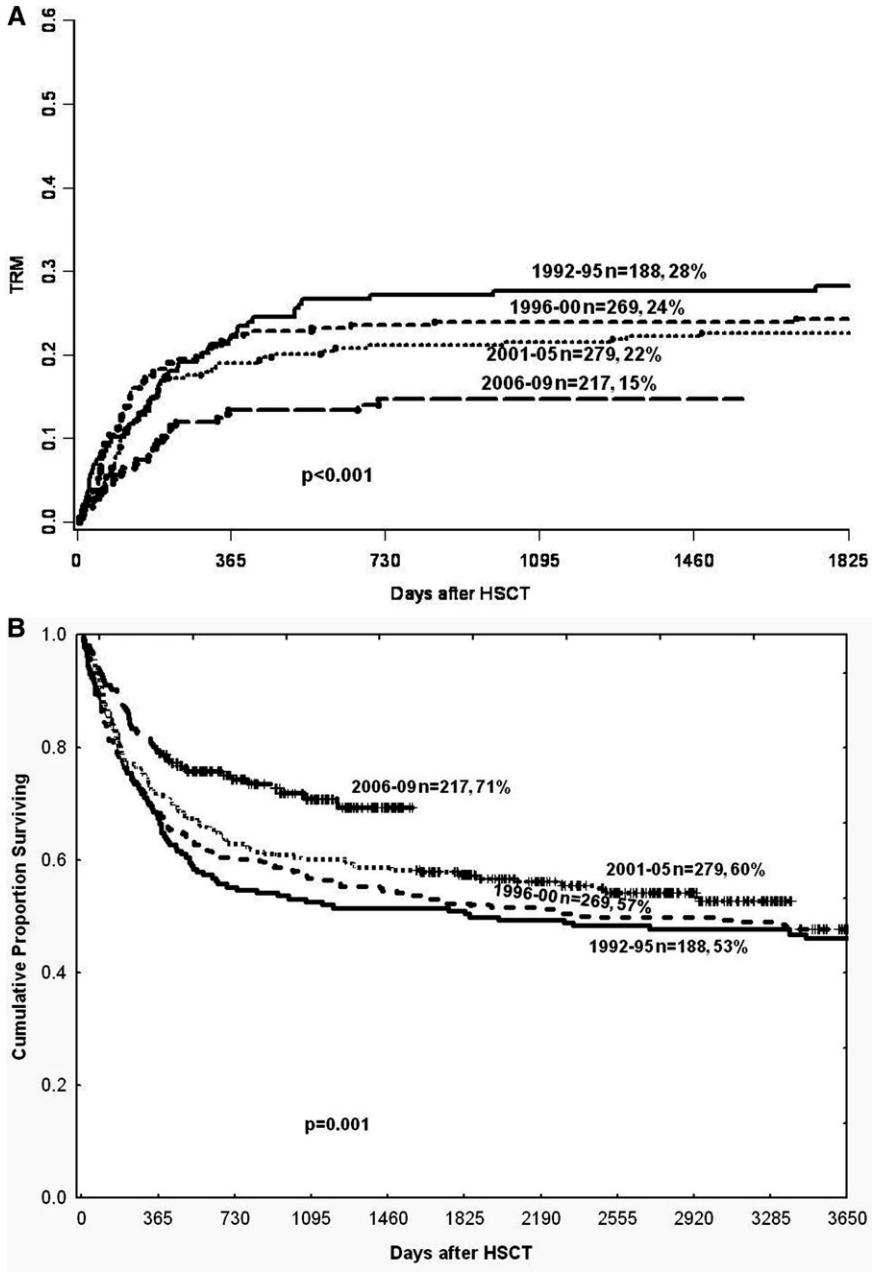

Figure 3. Cumulative incidence of TRM (A) and probability of survival (B) 3 years after allogeneic HSCT over 4 different time periods. The *P* value refers to a global comparison of all 4 groups.

Relapse

In patients with hematologic malignancies, the incidence of relapse has not changed over the years: It was approximately 25% in all 4 groups. When corrected for differences among the 4 groups, no difference in relapse incidence among the 4 groups was seen.

DISCUSSION

During the past, decades HSCT has changed considerably with respect to stem cell source, conditioning, choice of donor, stem cell dose, prophylaxis, and treatment of infections. These changes have been made to improve morbidity and survival after HSCT. At the same time, the indications for HSCT have changed and as a consequence of using milder conditioning (RIC), older patients and patients with more comorbidities have been accepted for HSCT [10-12]. This may have affected the results in this cohort of patients. Considering all these circumstances, has the OS really improved? It was for this reason that we decided to study the results from the last 2 decades and to determine whether there have been significant improvements in outcome.

Table 2. Comparisons of Various Outcome Variables after HSCT during Four Different Time Periods

SCT years	Mortality	TRM	aGVHD II-IV	cGVHD	Neutrophil Engraftment	Platelet Engraftment
1992-1995	1.0	1.0	1.0	1.0	1.0	1.0
1996-2000	0.82 (0.57-1.16)	0.79 (0.48-1.28)	1.86 (1.03-3.35)	0.87 (0.4-1.39)	1.57 (1.25-1.96)	0.69 (0.55-0.87)
2001-2005	0.77 (0.57-1.06)	0.76 (0.46-1.23)	3.60 (2.04-6.35)	0.41 (0.24-0.69)	0.78 (0.60-1.03)	0.83 (0.63-1.08)
2006-2009	0.46 (0.31-0.70)	0.38 (0.22-0.66)	4.35 (2.37-7.99)	0.34 (0.16-0.70)	0.52 (0.38-0.71)	0.62 (0.46-0.83)

TRM indicates transplant-related mortality; aGVHD, acute graft-versus-host disease; cGVHD, chronic GVHD; SCT, stem cell transplantation; HSCT, hematopoietic stem cell transplantation.
The earliest period (1992-1995) was used as a reference group and the analyses were corrected for differences between the groups. Hazard ratio (HR) and 95% confidence interval (CI) are given for each comparison.

During recent years, we have transplanted older patients, treated more patients beyond CR1, used a wider range of donors, and given higher cell doses, more PBSC and CB grafts, more RIC, and less TBI-based conditioning. The use of G-CSF has also changed over the years. Neutrophil engraftment was slower during the last period (2006-2009) when corrected for all differences in treatment during the years. Initially, G-CSF was used to speed up the engraftment. However, when we found that it increased the risk of aGVHD, it was discontinued [45,46].

The incidence of aGVHD of grades II-IV has increased during recent years in multivariate analysis corrected for differences between the groups. One possibility is that we have been more aggressive in making the diagnosis of GVHD, for example, by endoscopy. Other changes in practice that were not corrected for in the multivariate analysis may have affected the incidence of GVHD. In most studies, aGVHD II-IV has been associated with an inferior survival [47]. However, survival in patients with moderate-to-severe GVHD has improved significantly in recent years. The use of mesenchymal stem cells may improve short-term survival in patients with GVHD of grades III-IV [29]. Prophylaxis using new antifungals in patients with moderate and severe aGVHD may have improved their survival in the last few years.

After correcting for differences between the groups, the incidence of cGVHD over the years has apparently decreased. The fact that the incidence of cGVHD has decreased in recent years is surprising, because our aim has been to induce cGVHD in patients with hematologic malignancies to better induce a graft-versus-leukemia effect [39]. One factor in more recent years that might have counterbalanced this effect is that we use more CB, which reduces the risk of cGVHD.

TRM decreased significantly during the last time period. Several factors may have contributed to the improved TRM, although not detected in the multivariate analysis. One such factor is the introduction of targeted Bu levels, which reduces toxicity. Although Epstein-Barr virus-associated lymphoma is a relatively rare cause of death after HSCT, it can now be treated with B cell antibodies and EBV-specific cytotoxic T cells; and with such treatment, most patients survive [48,49]. Hemorrhagic cystitis (HC) is another rare complication that has been successfully treated in a few patients using mesenchymal stem cells [50]. Although only relatively few patients will be helped by each of these new interventions, taken together they may improve TRM, as seen in the last period. In keeping with this, after correcting for all differences between groups, we found improved TRM in the last period.

Detection and treatment of relapse has changed over the years. The introduction of chimerism analysis in leukemia-specific cell lineages and monitoring of CML and Ph1+ ALL patients with BCR/ABL PCR have made it possible to detect and treat relapse at an earlier stage. Early treatment of relapse with DLI may improve response and survival [25]. Despite the wider use of DLI for correction of chimerism, the probability of relapse has not decreased in recent years. The reason for this may be that cGVHD has decreased and it has a major influence on relapse in patients with leukemia [51-53]. Other possible reasons are fewer patients transplanted for CML in recent years, because this disease is very sensitive to DLI, and more patients transplanted for high-risk AML and MDS.

Most important, OS survival has improved significantly during recent years, even though we now transplant older patients, treat more high-risk disease, and use more alternative donors—all factors that were associated with inferior survival in the multivariate analysis. This may have been balanced by a higher nucleated cell dose during the last time period. However, survival improved during the last period, even when corrected for all differences between the groups. This indicates that other factors were associated with the improved survival. Such factors may be better diagnosis, prophylaxis, and treatment of infections; improved care of the patients; and a better understanding of the importance of nutritional status [54]. Furthermore, all improvements that have collectively reduced TRM have also improved survival because the risk of relapse was unchanged. In addition to this, accumulated experience locally and an

increased level of clinical knowledge and competence among our staff have also contributed. Moreover, integration of our own research in the field as well as input from other units in the Centre for International Blood and Marrow Transplant Research and the European Group for Blood and Marrow Transplantation have helped us to treat HSCT in the best way possible. Thus, several factors together have most likely contributed to the improved overall survival. Our results are consistent with 2 very recently published papers addressing basically the same issue [55,56].

We conclude that TRM and overall survival have improved in recent years. The reasons for this might be improved diagnosis, prophylaxis, and treatment of infections; improved treatment of GVHD; improved care of the patients; and a general increase in the knowledge and skill of the staff.

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