Ann Hematol (1993) 67:111–114

Original article

Annals of
Hematology

Frozen vs. nonfrozen bone marrow for autologous transplantation in lymphomas: a report from the Spanish GEL/TAMO Cooperative Group*

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Received 24 March 1993 / Accepted 9 June 1993

Summary. To investigate the impact of frozen and nonfrozen bone marrow on engraftment kinetics and disease outcome, 94 patients with non-Hodgkin's lymphoma (NHL) autografted with frozen marrow (F group) were retrospectively compared with 38 who received marrow stored at 4° C or 10° C (NF group). The major end points of this study were time to hematopoietic recovery and early toxicity; disease response, disease-free survival (DFS), and relapse rate were also analyzed. Upon comparison of the NF and F groups, no significant differences were found in the period of time required to achieve a granulocyte count higher than 0.5×10^9/l (20 and 22 days, respectively, $p = 0.47$) or a platelet count higher than 20×10^9/l (28 and 27 days, respectively, $p = 0.54$). In addition, both groups behaved similarly in respect to toxic death (NF group 13%, F group 22%, $p = 0.36$), response rate (complete remission rate 78% in both groups), DFS (NF group 48%, F group 49%, $p = 0.66$), and relapse rate (NF group 30, F group 19%, $p = 0.37$). This study confirms that nonfrozen bone marrow is useful to support patients with NHL treated with myeloablative therapies.

Key words: Nonfrozen – Marrow – Autologous – Transplantation – Lymphomas

Introduction

In the past few years, high-dose chemoradiotherapy followed by autologous bone marrow transplantation (ABMT) has been increasingly employed as a rescue therapy for patients with non-Hodgkin's lymphoma (NHL) in relapse, still responding to chemotherapy [10]. Moreover, ABMT is being used as front-line therapy in patients with poor prognostic features [12, 16, 17]. As a consequence, the number of patients with lymphoma who are candidates for ABMT has dramatically increased.

Although the possibility of safely performing ABMT with nonfrozen marrow is well known [3–6, 15], frozen marrow is most frequently employed [1, 10]. To investigate the impact of nonfrozen marrow on engraftment kinetics and disease outcome, we compared 94 patients autografted with frozen marrow with 38 receiving marrow stored at 4° C or 10° C. All these patients were reported to the Registry of the Spanish GEL/TAMO Cooperative Group for Bone Marrow Transplantation in Lymphomas.

Patients and methods

Patients

From 1983 to 1992, 132 NHL patients received ABMT at 14 Spanish hospitals. Thirty-eight patients received marrow stored at 4° C or 10° C [nonfrozen (NG) group] and 94 frozen marrow [frozen (F) group]. Mean (±SD) age was 30 (±18) years in the NF group and 34 (±13) years in the F group. Sex distribution was 25 men (55%) and 13 women (34%) in the NF group and 63 men (67%) and 31 women (33%) in the F group. Histology in NF cases was low-grade (LG) lymphoma in four instances (11%), intermediate-grade (IG) in eight (22%), and high-grade (HG) in 26 (67%); in the F group the histological distribution was: eight cases of LG (8%), 35 of IG (37%), and 51 of HG (55%). Stage of the disease at diagnosis in the NF group was I or II in 12 patients (32%) and III or IV in 26 (68%); in the F group 23 patients had

* The activities of the Spanish Cooperative Group for Bone Marrow Transplants in Lymphomas (GEL/TAMO) are kindly supported by a grant from AMGEN, S.A., Spain
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stage I or II at diagnosis (24%), and 71 were in stage III or IV (76%). The phase of the disease at ABMT in the NF group was as follows: first complete remission (CR1) in 19 (50%), CR2 in four (10%), sensitive relapse (SR) in six (16%), and resistant relapse (RR) in nine (24%). In the F group, 37 patients were autografted in CR1 (40%), 13 in CR2 (14%), seven in other CR (7%), 18 in SR (19%), and 19 in RR (20%).

First-line treatment consisted of a single regimen in 60 (64%) and 28 (74%) patients of the NF and the F group, respectively. In the remaining cases more than one chemotherapy regimen was used as the initial therapy. Ninety percent of the patients in both groups had an ECOG performance status of 0–1 at ABMT.

Mean (±SD) number of nucleated cells infused was 3.2 (± 2)$\times 10^8$/kg in 34 cases of the NF group and 2.3 (± 2)$\times 10^8$/kg in 25 patients of the F group. In 61 cases of the F group, cellularity infused was given as number of mononucleated cells, with a mean±SD value of 1.3 (± 1.5)$\times 10^8$/kg. Data on cellularity infused were not available for four patients of the NF group (10%) and for eight of the F group (9%). Colony-stimulating factors (CSF) were given after ABMT to six patients of NF group (16%) and to 18 of the F group (20%).

Bone marrow harvesting and storage

Bone marrow was harvested from the posterior iliac crests with patients under a general anesthetic. In nonfrozen cases the aspirated marrow was heparinized and, after filtration, was placed in routine blood bank CPD bags in volumes of approximately 400 ml/bag. Culture medium was not added. The marrow was stored in a refrigerator at 4° C or 10° C for 24–56 h until reinfusion. In the frozen group, nucleated or mononucleated marrow cells were cryopreserved by means of controlled-rate freezing and stored in liquid nitrogen at −196° C. Bone marrow purging was performed in eight cases of the latter group.

Conditioning regimens

Conditioning therapy included chemotherapy (cyclophosphamide 120 mg/kg in two doses) and radiation [total body irradiation (TBI) in all but one instance] [3] in 30 (79%) cases from the nonfrozen group and in 47 (50%) from the frozen group.

In the nonfrozen marrow group, a single dose of irradiation (8–10 Gy) or the first dose of cyclophosphamide (CY) was administered 1–2 h after marrow aspiration. In patients receiving TBI first, CY was given 6 h later, with the interval between the first and second CY dose being 18–20 h. The marrow was reinfused 22 h after the last dose of CY (time between harvesting and marrow reinfusion 47–50 h). When CY was administered before TBI, the interval between the two doses of CY was 18–20 h, the patients were irradiated 24 h after the second CY dose, and marrow was reinfused 6 h later (time between harvesting and marrow reinfusion 49–52 h) [6].

Other preparative regimens in the NF group were CBV (CY 5 g/m^2, BCNU 600 mg/m^2, VP16 400 mg/m^2) in three patients [5], CBVbl (CY 3 g/m^2, BCNU 600 mg/sq.m., vinblastine 15 mg/m^2) in two cases, melphalan (140 mg/m^2) in another two [6], and high-dose BCNU (800 mg/m^2) in the remaining patient. In all the NF cases bone marrow was reinfused between 24 and 56 h after aspiration.

Patients in the F group conditioned only with chemotherapy received BEAM [11] in 24 cases, BEAC in 15 [11], CBV in six [18], busulphan and CY in one case [13], and CY plus high-dose cytarabine in one.

Statistical methods

Comparisons between groups were performed by the chi-square test with Yates correction, Fisher's exact test, and the Student's *t* test [2]. Actuarial curves of disease-free survival (DFS), probability of relapse (REL), probability of granulocyte recovery and platelet recovery were performed according to the Kaplan-Meier method [14]. Comparison between curves was performed using the generalized Mantel-Cox test. All *p*-values are two-sided.

To further evaluate the impact of bone marrow freezing on probability of granulocyte and platelet recovery, DFS, and relapse, multivariate analyses according the Cox model of multiple regression were performed [7]. Other variables also included in these analyses were: age, sex, histology, stage, and serum LDH at diagnosis, number of front-line regimens, status of the disease and performance status at ABMT, and conditioning regimen. For statistical calculations the BMDP package was used [8].

Results

The two groups did not differ significantly in terms of age, sex, histology, stage at diagnosis, serum LDH at diagnosis, first-line chemotherapy, performance status at ABMT, and status of the disease at ABMT. Patients of the NF group were more frequently conditioned with chemotherapy plus TBI than patients of the F group ($p=0.004$).

Hematological recovery

Data on hematological recovery are summarized in Table 1. In the whole series, a granulocyte count higher than 0.5×10^9/l was attained in a median time of 21 days (range, 10–103 days). The lower 25th percentile of granulocyte recovery had its upper limit at 16 days; patients who lived longer than 16 days and did not reach a granulocyte count higher than 0.5×10^9/l were considered engraftment failures. Median time to granulocyte recovery was 20 days in the NF group (range: 12–103) and was 22 days in the F group (range: 10–48; $p=0.47$) (Fig. 1). None of the 37 patients in the NF group surviving more than 16 days failed to achieve a granulocyte count higher than 0.5×10^9/l; in the F group, four patients of the 89 (4.5%) surviving longer than 16 days failed to attain a granulocyte count higher than 0.5×10^9/l ($p=0.45$). Delayed engraftment, defined as granulocyte recovery beyond the median for the whole series (21 days), was observed in 13 of 37 patients (35%) of the NF group and in 41 of 85 of the F group (48%) ($p=0.25$).

Table 1. Hematological recovery

	NF (Nonfrozen) group	F (frozen) group
Whole series (132 patients)		
Engraftment failure[a]	0 of 37 (0%)	4 of 89 (4.5%)
Delayed engraftment[b]	13 of 37 (35%)	41 of 85 (48%)
Median time to granulocytes $>0.5\times 10^9$/l (range)	20 days (13–103)	22 days (10–48)
Median time to platelets $>20^9$/l (range)	28 days (12–300)	27 days (10–157)

SD, Standard deviation; CR, complete remission

[a] Absence of granulocyte recovery in patients who lived longer than 16 days (25th percentile time for granulocyte recovery in the whole series)

[b] Granulocyte recovery after 21 days (median time for granulocyte recovery in the whole series)

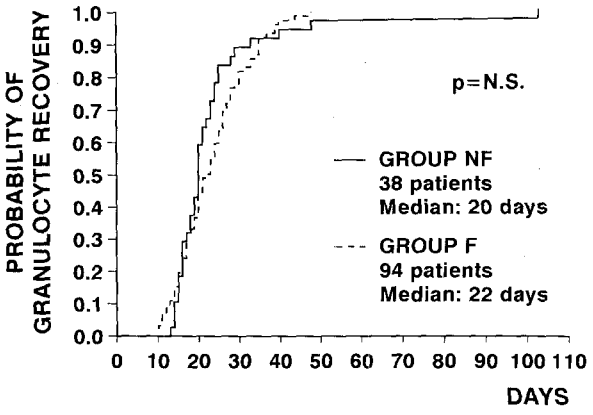

Fig. 1. Actuarial probability of recovering a granulocyte count higher than 0.5×10^9/l

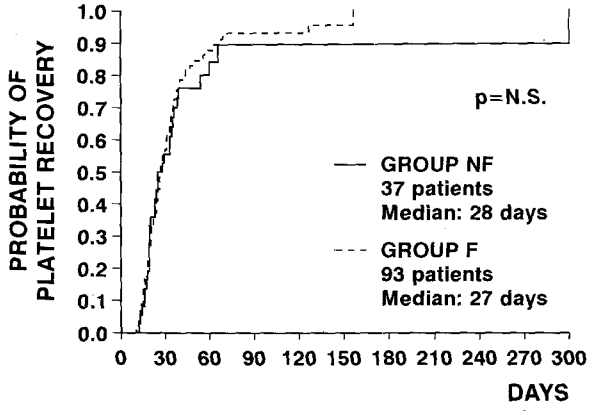

Fig. 2. Actuarial probability of recovering a self-sustained platelet count higher than 20×10^9/l

Data on platelet recovery were available in 130 patients. A platelet count higher than 20×10^9/l was achieved in a median time of 27 days (range 10–300). There were no significant differences in platelet recovery between the NF group (median 28 days, range 12–300) and the F group (median 27 days, range 10–157) (Fig. 2).

Early toxicity

Severe infections (sepsis or pneumonia) developed in nine of 32 (28%) evaluable patients of the NF group and in 33 of 86 (38%) of the F group. Cardiac toxicity was documented in one case (3%) of the NF group and in four (5%) of the F group. Liver veno-occlusive disease was reported in two of 31 patients (6%) of the NF group and in two of 88 (2%) of the F group. Interstitial pneumonitis was diagnosed in one of 31 cases (3%) of the NF group and in one of 88 (1%) of the F group. None of these differences was statistically significant.

Toxic deaths

Five of 38 patients of the NF group (13%) and 20/91 (22%) of the F group died within 6 months after ABMT due to procedure-related complications ($p = 0.36$). In three cases of the F group it was not specified whether death was due to ABMT-related complications. Causes of toxic death are shown in Table 2. There was one death due to infection in the NF group (2.6%) vs ten of 90 evaluable patients (11%) in the F group ($p = 0.22$).

Table 2. Causes of toxic death

	NF (nonfrozen) group	F (frozen) group
Evaluable	38	91
Infection	1 (2.6%)[a]	10 (11%)
Liver VOD	1 (2.6%)	3 (3.3%)
Other liver toxicity	—	1 (1.1%)
Heart toxicity	1 (2.6%)	4 (4.4%)
Neurologic toxicity	—	1 (1.1%)
Interst. pneumonitis	1 (2.6%)	—
Unspecified	1	1
Total	5 (13%)	20 (22%)

[a] All percentages are referred to number of evaluable patients

Disease response and treatment outcome

Response of NHL to ABMT was evaluated 3 months after the procedure. Twenty patients (five in the NF and 15 in the F group) were not evaluable for disease response due to early death. No significant differences in terms of disease response were observed between the 33 evaluable patients of the NF group and the 79 evaluable cases of the F group. Seventy-two patients transplanted in CR remained in this status after BMT (21 in the NF group, 51 in the F group). Sixteen patients autografted with active disease reached a CR after transplant: five (15%) in the NF group and 11 (14%) in the F group. One patient of the NF group (3%) and four of the F group (5%) autografted with active disease reached a partial remission. Thirteen of 79 (15%) patients in the NF group and in six of 33 (18%) in the F group failed to respond.

There were no differences in the DFS of the two groups ($p = 0.66$; Fig. 3). At 3 years, the actuarial probability of DFS for the NF group was 48% (95% CI: 31–65%; median, 26 months) and 49% (95% CI: 38–60%; median 20 months) for the F group.

At 3 years the probability of relapse was 30% in the NF group (95% CI: 12–48%) and 19% in the F group (95% CI: 7–30%; $p = 0.37$).

Multivariate analyses

Bone marrow freezing was not a significant predictive variable in any of the multivariate analyses performed. Thus, marrow freezing did not influence granulocyte or platelet recovery, probability of DFS, or relapse.

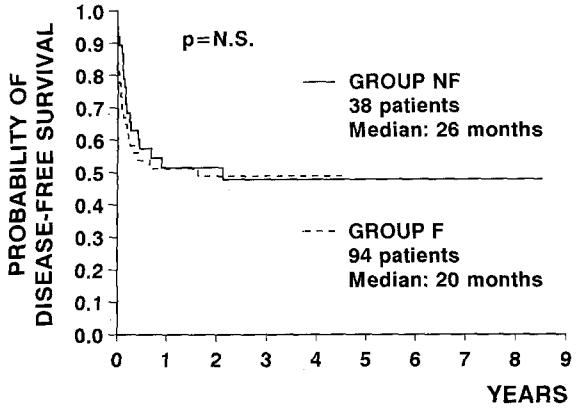

Fig. 3. Actuarial probability of disease-free survival according to whether the bone marrow is frozen

Discussion

In the past 10 years, autologous bone marrow has been used increasingly to overcome the bone marrow toxicity associated with high-dose chemoradiotherapy in the treatment of various neoplasms. Although the role of ABMT in lymphoma treatment is not yet completely defined, an increasing number of patients with lymphoma are being treated with such a procedure [10]. The usual sources of hematopoietic cells used are bone marrow or peripheral blood. Although the possibility of safely performing ABMT with nonfrozen marrow is well known [3–6, 15], frozen marrow is more frequently used [1, 10]. This allows the administration of a wider variety of conditioning regimens, facilitates the manipulation of bone marrow before reinfusion, and allows a more convenient and flexible transplant schedule. In this regard, it should be noted that if for any reason the transplant has to be delayed, nonfrozen marrow is lost.

For all these reasons, it may be questioned whether nonfrozen bone marrow still has a role in supporting myeloablative therapies. A randomized trial comparing autologous transplants with frozen marrow, nonfrozen marrow and peripheral blood would be of interest but has never been undertaken. Therefore, although retrospective, our analysis is of interest. In this study the usefulness of nonfrozen marrow for ABMT in NHL is confirmed. Indeed, no differences were found between patients who received frozen and those who received nonfrozen marrow in regard to the main endpoint analyzed, hematopoietic recovery. Also, disease response to treatment and disease outcome were similar in the two groups. The relatively high toxic death rate (19%) observed in this study may be explained by the high proportion (45%) of patients with advanced disease who were autografted.

The analysis of this series on the basis of conditioning regimens (chemotherapy + radiotherapy vs. chemotherapy), the single difference between the F and the NF group, did not show any difference. Furthermore, multivariate analyses including conditioning regimen and other variables confirmed that bone marrow freezing had no significant predictive value.

Finally, our results may be of interest considering the burden, both physical and financial, that the increasing number of autologous bone marrow transplants poses. The lack of facilities for the freezing of bone marrow should not be considered a limiting factor for ABMT in NHL. Additionally, since autologous transplants from nonfrozen bone marrow are less costly than those performed from frozen marrow, restrictions to ABMT programs in some settings may thereby be overcome.

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