Allogeneic and autologous bone marrow transplantation after consolidation therapy in high-risk acute myeloid leukemia in children. Towards a risk-oriented therapy

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Background and Objectives. Although chemotherapy in childhood acute myeloid leukemia (AML) has improved in the last decade, except for a group of better-risk patients (approximately one third), more than half the other patients relapse. The main objective of this study was to evaluate the results obtained with bone marrow transplants, either allogeneic (allo-BMT) or autologous (auto-BMT), following two intensive consolidation courses in a series of children with high-risk (HR) AML according to morphologic and early-response BFM criteria. A second objective was to compare the results of auto-BMT with those of allo-BMT.

Design and Methods. From April 1988 to May 2001, 79 children (< 15 years old) with *de novo* AML entered the prospective AML-88 trial in a single institution: 50 (63%) were qualified as having high-risk disease and are the subject of this study. After 1 or 2 induction courses, depending on early response, and two consolidations, patients with an HLA-identical sibling received an allo-BMT and all the others an auto-BMT. The conditioning regimen was cyclophosphamide and total body irradiation (TBI) in children over 3 years old and busulfan and etoposide in younger children. Bone marrow was purged with mafosfamide in auto-BMT and cyclosporine alone was given as graft-versus-host disease (GVHD) prophylaxis in allo-BMT.

Results. At the end of the chemotherapy phase (induction and consolidation), 46 of the 50 HR patients (92%) had attained complete remission (CR) after one (n=29), two (n=11) or three (n=6) courses; 2 more were in partial remission (PR) and 2 had died. The 48 patients in CR or PR received either an allo-BMT (17) or an auto-BMT (31). Hematologic reconstitution was significantly slower in auto-BMT recipients. Forty-one percent of patients who received allo-BMT suffered acute GVHD grades II-IV. Toxic deaths and relapse rates were 5.9% and 17.6%, respectively, in allo-BMT and 3.2% and 25.8%, respectively, in auto-BMT. Post-transplant 8-year event-free surviavl (EFS) was 74.5% (54-96) in allo-BMT and 74.2% (59-89) in auto-BMT. EFS and OS in all the series (50 patients) were 71% (59-83) and 73% (61-85), respectively, with a median follow-up of 7.2 years.

Interpretation and Conclusions. This study indicates that improved results in children with HR-AML can be obtained by either allo- or auto-BMT performed after two courses of intensive consolidation therapy provided good supportive therapy is given and transplant-related mortality (TRM) is minimized.

Key words: acute myeloid leukemia, children, high-risk, allogeneic bone marrow transplantation, autologous, bone marrow transplantation.

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Although chemotherapy in acute myeloid leukemia (AML) has improved, event-free survival (EFS) in the best series published in the last decade is under 50% when chemotherapy alone is given.[1,5] Complete remission can be obtained in 80%-85% of children, but the question of what post-remission treatment is best controversial. Consolidation with at least two courses of intensified chemotherapy consisting of cytarabine (Ara-C) combined with other drugs (daunorubicin, mitoxantrone, amsacrine or mitoxantrone) has proven to be very effective in a group of patients. Analysis of results of the AML-BFM 83 and 87 trials permitted identification of a low-risk (LR) group, comprising 32% of patients treated with chemotherapy alone, with a 5-year EFS of 68%. This group could be defined by morphologic and cytogenetic characteristics and by a good early response to induction treatment. For all the other patients (68% of the total), EFS was only 33%.[6] In the following study, AML BFM-93, the group of high-risk (HR) patients was treated separately, being given another course of intensification chemotherapy with high-dose Ara-C and mitoxantrone. With this second intensification cycle, in addition to a non-intensified consolidation course, EFS in this HR group increased to 44%.[7] Analysis of the results of the MRC-AML-10 trial in children identified three risk groups based on cytogenetics and response to a first course of induction therapy.[4] In this trial, including patients given chemotherapy alone and patients undergoing allogeneic or autologous bone marrow transplantation after 2 induction and 2 intensified consolidation courses, EFS of patients in HR, intermediate and LR groups was 32%, 54% and 59%, respectively, but in the published paper there was no mention of results obtained in the three risk groups with different post-remission treatments.

With the improvements recently reported in EFS and OS rates attained with chemotherapy alone in the lower-risk group of patients, present interest centers on knowing whether allogeneic bone marrow transplantation (allo-BMT) and autologous bone marrow transplantation (auto-BMT) performed after intensified consolidation treatment may really improve the survival rate in the high-risk patient group which, according to BFM criteria, constitutes two thirds of children with AML. No study has been published reporting the specific results obtained with transplants in this particular patient group.

Allogeneic bone marrow transplantation performed from a matched sibling donor has proven to be more

effective than chemotherapy alone in several comparative studies in AML in children.[4,8-10] Results obtained with autologous bone marrow transplantation are more controversial: in three multicenter comparative studies results were discordant[4,9,11] but several institutional and multicenter non-comparative studies seem to indicate that results obtained with intensive consolidation chemotherapy may be improved with auto-BMT.[12-14] Nevertheless, no reports have been published on results of allo- and auto-BMT specifically in HR-AML in children.

Here we report our long-term results obtained in HR-AML patients with a regimen in which two intensified consolidation courses of treatment were followed by BMT, either allogeneic in patients with an HLA-identical sibling or autologous in the other patients.

Design and Methods

Patients

From April 1988 to May 2001, seventy-nine consecutive *de novo*, previously untreated AML patients under 15 years old were prospectively included in the AML-88 trial and treated in a single institution. Sixty-nine patients came from our region (Catalonia) and the other ten were referred from two hospitals of other regions; all the patients diagnosed as having AML in our institution as well as in the other centers were eligible for inclusion in this protocol except those with M3 subtype since 1993. No patient refused to participate in the trial. The diagnosis of AML was made according to morphologic FAB classification and immunologic and cytogenetic studies were performed. According to BFM criteria based on morphology and early response to induction treatment,[6] 50 patients (63%) were qualified as HR. The patients' main characteristics are shown in Table 1. The median age was 5 years with 32% of the patients under the age of 2. The median white cell count (WBC) was 14.6×10^9/L and 42% had more than 20×10^9/L. Extramedullary disease was present in 10% (2 in the central nervous system (CNS), 1 orbit sarcoma, 1 in skin and 1 in pericardium) and 43% had abnormalities in chromosomes 5, 7 or 11q23 or had complex karyotypes (18%).

Treatment protocol

This consisted of a chemotherapy phase and a transplant phase (Table 2).

Chemotherapy phase. As induction treatment, the DAE combination (daunorubicin, Ara-C and etoposide) was given on a 3-7-3-day basis (Table 2). No specific therapy for extramedullary disease was given. A bone marrow aspirate was performed on day 14. If the marrow contained more than 10% blasts, a second course of DAE (without intrathecal treatment) was immediately started.

Table 1. Patients' characteristics.

	High-risk group (n=50)	*Low-risk group (n=29)*
Age (years)		
Median (range)	5.14 (0.11-13.6)	6.98 (0.62-14,2)
Age <2y, n. (%)	16 (22)	3 (10.3)
Sex (male/female)	27/23	15/14
WBC ($\times10^9$/L)		
Median (range)	14.6 (1.5-540.0)	23.4 (3.6-254.0)
20-100, n. (%)	13 (26)	12 (41)
>100, n. (%)	8 (16)	3 (10)
FAB classification, n. (%)		
M1+M2	14 (28)	16 (55)
M3[1]		10 (35)
M4	10 (20)	3 (10)
M5	17 (34)	
M6	5 (10)	
M7	4 (18)	
Extramedullary disease, n. (%)	5 (10)	
Cytogenetics	(n=29)	(n=22)
normal karyotype	5	3
11q23 translocation	9	
t (8;21)		6
t (15;17)[1]		5
inv 16		3
abnormalities in 5 and 7	3	
trisomy 8	4	
trisomy 21	3	
complex karyotypes	3	
others	2	5
High-risk features[2]		
non-favorable cell morphology n. (%)	36 (70)	
early response not good	20 (40)	

[1]M3 (10 patients) were included before 1993; [2]according to the BFM classification.
Patients enrolled in the AML-88 trial: 79.
Patients qualified as high-risk according to morphology cytogenetics and early response to treatment (BFM criteria): 50 (63%).

Remission status was determined after recovery from myelosuppression following one or two induction courses. All patients in complete or partial remission at hematologic recovery, after 1 or 2 induction cycles, were given two intensified consolidation courses with moderately high doses of Ara-C together with mitoxantrone in the first intensification and amsacrine in the second.

All patients were given preventive CNS therapy with age-related doses of intrathecal methotrexate and Ara-C administered during induction and consolidation up to a total of six doses.

After recovery from the second intensification course, patients in CR or PR proceeded to the transplant phase. Histocompatibility typing of patients and family was performed after the first intensification therapy and, depending on the availability of an HLA-identical sibling, either an allo-BMT or auto-BMT was scheduled.

Transplant phase. Conditioning for BMT (allo-BMT and auto-BMT) in patients over 3 years of age consisted of cyclophosphamide 120 mg/kg iv on 2 consecutive days followed by fractionated total body irradiation (TBI) 14 Gy in seven fractions over 3.5 days. A cobalt-60 source was used for irradiation and lung shielding was performed after 10 Gy. Younger children received busulfan, 16 mg/kg over 4 days, and etoposide iv 30 mg/kg in an 8-hour infusion instead of TBI. No pharmacokinetic adjustments for busulfan dosage were made.

Graft-versus-host disease prophylaxis in allo-BMT consisted of cyclosporine alone at a dose of 5 mg/kg iv in 24-hour infusion on day -1 and 3 mg/kg/day from day 0 to day +21, approximately, followed by oral administration over a 4-6-month period. Cyclosporine dose adjustments were made to maintain levels between 200 and 400 ng/mL during the first 3 months post-transplant. Marrow harvests for auto-BMT were obtained from iliac crests under general anesthesia after recovery from the second intensification course. A volume of 20 mL/kg was aspirated, filtered through 0.3 mm mesh, centrifuged at 850 g and the buffy coat harvested. When more than 1.5×10^8 mononucleated bone marrow cells were obtained, two thirds were incubated with mafosfamide at a constant dose of 50 μg $\times10^6$ cells for 30 minutes as described elsewhere[15] and one third was cryopreserved with DMSO as back-up marrow. Cryopreservation was performed by programmed slow cooling and the marrow cells were stored in liquid nitrogen at -196°C. On day 0 of auto-BMT, the marrow was thawed in a water-bath at 37°C and directly infused into the patient. The back-up marrow was infused if the level of 0.5×10^9/L neutrophils was not attained by day +45.

Low-dose heparin 1mg/kg/day in continuous i.v. infusion was used as hepatic veno-occlusive disease prophylaxis and all patients were nursed in laminar-air flow rooms with strict infection preventive measures including antifungal and antiviral prophylaxis with fluconazole and acyclovir, respectively. Intravenous non-specific immunoglobulin was administered weekly over three months to patients receiving an allo-BMT. G-CSF was not routinely used after chemotherapy or BMT. Cotrimoxazole was given throughout all the treatment and until one year after BMT.

Table 2. HIVH AML-88 Protocol.

Chemotherapy phase	
I. Induction: DAE combination (1 or 2 courses, see text)	
Daunorubicin	60 mg/m²/day i.v. push × 3 on days 1-3 (total 180 mg/m²)
Ara-C	100 mg/m²/day in continuous i.v. infusion on days 1-7 (total 700mg/m²)
Etoposide	100 mg/m²/day in 1 hr. i.v. infusion on days 1-3 (total 300 mg/m²)
	Intrathecal methotrexate + Ara-C in age-related doses* days 1 and 6
II. Consolidation & Intensification	
II.1. Ara-C	2.4 g/m²divided q12 hr i.v. over 2 hr × 4 days on days 1-4 (total 9.6 g/m²)
	Methotrexate 12 mg/m²/day i.v. push × 3 on days 1-3 (total 36 mg/m²)
	Intrathecal methotrexate + Ara-C in age-related doses* days 1 and 6
II.2.	Ara-C 2.4 g/m²divided q12 hr i.v. over 2 hr × 4 days on days 1-4 (total 9.6 g/m²)
	M-AMSA 100 mg/m²/day i.v. push × 3 on days 1-3 (total 300 mg/m²)
	Intrathecal methotrexate + Ara-C in age-related doses* days 1 and 6
	*< 12 months: methotrexate 6 mg, Ara-C 10 mg
	12-36 months: methotrexate 8 mg; Ara-C 15 mg
	> 36 months: methotrexate 10 mg, Ara-C 20 mg
Transplant phase	
Age under 3 years:	
	Busulfan 1 mg/kg/day oral divided q 6 hr x 4 days on days -8 to -5 (total 16 mg/kg)
	Etoposide 30 mg/kg in 6 hr i.v. infusion on day -4
	Cyclophosphamide 60mg/kg in 1 hr i.v. infusion ×2 on days -3 and -2 (total 120 mg/kg)
Age over 3 years:	
	Cyclophosphamide 60mg/kg in 1 hr i.v. infusion × 2 on days -6 and -5 (total 120 mg/kg) TBI 14 Gy in 7 fractions in 3.5 days on days -3 to 0

Criteria for response

Early death was defined as a death occurring before response to therapy could be established. Patients were considered to be in complete remission (CR) when all extramedullary disease had resolved, the neutrophil count was higher than 1.5×10^9/L, platelet count greater than 100×10^9/L and there was normal bone marrow cellularity with trilineage hematopoiesis and less than 5% blast cells. Partial response (PR) was considered as a decrease in blast cells in bone marrow (to 5%-25%) and no blast cells in peripheral blood. Early bone marrow response was classed as good if the bone marrow aspirate at day 14 contained less than 10% blast cells. Relapse was established by the reappearance of more than 5% blast cells in the bone marrow aspirate. Event-free survival (EFS) was defined as the time between diagnosis and relapse, death from any cause or the patient being alive in first CR at last follow-up.

Implant failure was considered if the level of 0.5 $\times10^9$/L neutrophils was not attained by day +45 post-infusion.

Post-transplant EFS was the EFS time since the transplant was performed. Overall survival (OS) was measured from the time of entry into the protocol to the time of death or last follow-up. Analysis of

EFS and OS probabilities according to the type of transplant (allo- or auto-BMT) was made by intention to treat since the HLA typing results were known. All relapse and survival data were updated on January 31, 2002 and all follow-up data were censored at this point.

Assessment of late toxicity and quality of life

Post-transplant long-term side effects were assessed by evaluation of growth, pubertal development, hormonal studies, echocardiography (every 6 months in the first two years and then anually thereafter), respiratory function tests and ophthalmologic examination. The quality of life was evaluated with the Spanish version of the PEDQOL (Pediatric questionaire of quality of life) described by Calaminus *et al.*[16]

Statistical analysis

All patients were assessed for initial response to treatment (CR or PR). The χ^2 test or Fisher´s exact test was applied to assess the relationship between the type of transplant and clinical characteristics of the patients. The relationship between numeric variables and transplant type was compared by the non-parametric Mann-Whitney U test. EFS and OS curves were estimated according to the Kaplan-Meier method[17] and compared by the log-rank test.[18] Cox regression analysis was used for calculation of estimates of relative risk for OS and EFS. Confidence intervals were calculated at the 95% confidence level. All allografted patients were considered assessable for acute GVHD and the occurrence of chronic GVHD was evaluated in patients who survived at least 100 days after allo-BMT. Results are expressed as probability and 95% confidence limits. The statistical studies were performed using the SPSS (*Statistical Package for Social Sciences*).[19]

Ethical considerations

This protocol was approved by the local Ethics and Research Committee and informed consent for all the procedures was obtained from parents or legal guardians of the children.

Results

Chemotherapy phase (Table 3)

One early death due to intracranial hemorrhage occurred on day 12; 80% of the patients attained CR after one (29 patients) or two (11 patients) courses of induction treatment. The other 9 patients (18%) had a partial response (PR), or failure (more than 25% blast cells) after two courses. All living patients received intensification chemotherapy. At the end of this phase, six more patients attained CR, 40 maintained CR status, 2 were in PR with 5% and 10% blast cells in bone marrow, and one died after disease progression. The median time between the intensification courses was 22 days (19-31).

Table 3. Results in chemotherapy phase.

Induction	
Patients initiating treatment	50
Early death	1 (2%)
Patients in CR	40 (80%)
*after one course	29
*after two courses	11
Failures or PR	9 (18%)
Consolidation and intensification	
Patients receiving treatment (40 in CR and 9 in PR or F)	49
Progression and death	1
Patients maintaining CR	40
Patients attaining CR in this phase	6
Patients in PR (5-10% blast cells in BM)	2

Transplant phase

In the 46 patients (92%) who attained CR after 1 to 3 courses of chemotherapy and the 2 patients in PR, HLA family typing showed that 17 had an HLA-identical sibling donor and 31 had no donor. In no case did infection or toxicity preclude the BMT; consequently, there was 100% compliance with intention to transplant. Clinical characteristics (Table 4) of the 17 patients who received an allo-BMT and the 31 patients who underwent an auto-BMT were similar except that the 2 patients with PR received an allo-BMT and the 3 patients with abnormalities in chromosomes 5 and 7 received an auto-BMT.

The number of courses (for induction and consolidation) was the same in patients receiving either of the two transplant modalities. The median interval between diagnosis and transplant was 4.5 months and between remission and transplant 3 months; in no instance was this interval longer than 6 months.

The conditioning regimen consisted of total body irradiation and cyclophosphamide in 52% of patients (Table 5) and busulfan and cyclophosphamide in 48% (patients under 3 years of age). In 2 patients (4%) who had echocardiographic abnormalities, the conditioning regimen consisted of etoposide and melphalan. Twenty-four patients received purged bone marrow and the other seven received either both purged and unpurged because of implant failure or unpurged alone because of the low number of cells collected.

The number of mononuclear cells infused was significantly higher in allo-BMT than in auto-BMT.

Table 4. Characteristics of patients in the two transplant groups.

	Allo-BMT (n=17)	*Auto-BMT (n=31)*
Characteristics at diagnosis		
Age in years, median (range)	4.6 (0.4-135)	5.6 (0.11-13.6)
Sex (male/female)	10/7	17/14
WBC ($\times10^9$/L), median (range)	19.0 (1.5-220)	13.5 (1.5-540.0)
FAB subtypes		
M1+M2	4	10
M4+M5	10	16
M6+M7	3	5
Cytogenetics		
Unfavorable	2	4
N. of chemotherapy courses until CR	1.47 (0.72)	1.42 (0.67)
Patients in CR at transplant	15	31
Patients in PR at transplant	2	
Interval between diagnosis and transplant in months, median (range)	4.4 (2.6-7)	4.8 (3.7-6.5)
Interval between CR and transplant in months, median (range)	3.0 (1-6)	3.2 (2.5-5)

Table 5. Characteristics of transplants and outcome in the two transplant groups.

	Allo-BMT n=17	*Auto-BMT n=31*	*Total n=48*
Conditioning regimen			
• with TBI	11	14	25 (52%)
• without TBI	6	17	23 (48%)
Mononuclear cells infused ($\times10^8$/kg) median (range)	2.5 (0.8-8.7)	1.1 (0.3-7.4)	
Hematologic reconstitution			
• days for neutrophils >0.5×10^9/L	17 (12-43)	40 (7-120)	
• days for platelets >20×10^9/L	20 (10-99)	56 (21-130)	
Acute GVHD grades II-IV	7 (41%)	-	
Toxic deaths	1 (5.9%)	1 (3.2%)	2 (4%)
Relapses	3 (!7.6%)	8 (25.8%)	11 (23%)
Deaths (total)	3 (17.6%)	8 (25.8%)	11 (23%)
Alive in first CR	13	22	35
Alive in second CR	1	1	2
Disease-free survival at 10 years (Kaplan Meier estimate)	0.745 (C.I. 0.54-0.96)	0.743 (C.I. 0.59-0.89)	0.744 (C.I. 0.62-0.86)

Post-transplant outcome (Table 5)

Hematologic reconstitution was significantly slower in patients receiving an auto-BMT. The median time to attain 0.5×10^9/L neutrophils and 20×10^9/L platelets was 17 and 20 days respectively, in allo-BMT versus 40 and 56 days in auto-BMT. In auto-BMT, patients who received more than 1×10^8 MNC/kg attained 0.5×10^9 neutrophils at a median time of 22 days compared with 46 days in patients who received less than 1×10^8/kg (p=0.02). The average number of RBC and platelet transfusions was significantly higher in auto-BMT (p=0.01). In 4 patients, the marrow was not treated with mafosfamide because of the low number of cells harvested and in another 3 patients the unpurged back-up bone marrow was infused.

In allo-BMT, acute GVHD grades II-IV appeared in 41% of patients and 3 patients of 16 at risk (17%) developed chronic GVHD (three limited and one extensive). One patient died of bronchiolitis obliterans four months after transplant and 3 relapsed after 11, 18 and 32 months. Two of these patients died after disease progression but one attained a second remission and received an allo-BMT from the same donor and remained in second CR 30 months later. In total, at the time of data analysis 14 patients were alive, 13 in first CR and 1 in second CR. Post-transplant 8-year EFS was 74.5% (CI:.54-96) (Figure 1). The two patients transplanted in PR were alive in first CR.

In auto-BMT, only one (3%) of the 31 patients died, without relapsing, of septic shock. Eight patients (26%) had relapses (7 in bone marrow and one extramedullary) 4 to 18 months after transplant (median: 6 months); seven of these died shortly after relapse, but one, after achieving a second remission, received an allo-BMT from an unrelated matched donor and remained in second CR 36 months later. The EFS was higher in the twenty-four patients who received purged bone marrow than in the seven who received unpurged bone marrow (p= 0.04) (Table 6). In total, at the time of data analysis, 23 patients were alive and in CR (22 in first CR and 1 in second CR) and post-transplant EFS was 74.3% (CI: 59-89) (Figure 1).

Post-transplant EFS for the 48 patients receiving either an allo-BMT or an auto-BMT was 74.4 %, with no differences between the two transplant modalities. The median post-transplant follow-up for surviving patients was 82 months (3 - 158).

Overall results and risk factor analysis

Overall survival probability of the whole HR-AML series at 10 years was 73% (C.I.61-85) (Figure 2) and 10-year EFS 71% (C.I.59-83). The median follow-up for all living patients was 87 months (6-162).

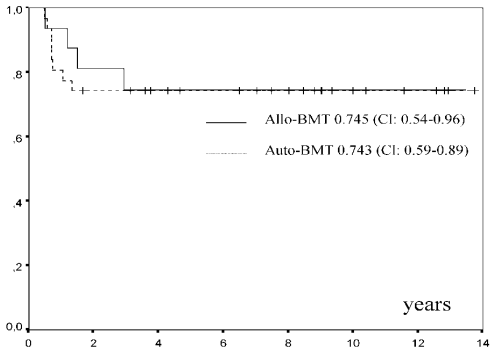

Figure 1. Actuarial curves of post-transplant event-free survival for high-risk AML children receiving either auto-BMT or allo-BMT.

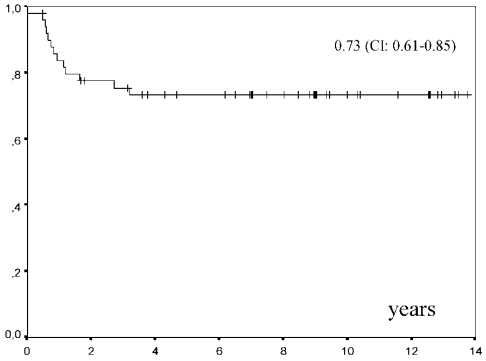

Figure 2. Actuarial curve of overall survival for all high-risk patients entered in the AML-88 trial (n=50).

Table 6. Analysis of risk factors for OS and EFS.

	OS		EFS	
Risk factor	*RR*	*p*	*RR*	*p*
Age < 2 yr.	0.9 (0.3-3.1)	0.95	1.2 (0.4-.5)	0.76
WBC > 100×10^9/L	4.1 (1.3-12.6)	0.01	4.1 (1.4-13.4)	0.01
M5 (*vs* others)	1.9 (0.6-5.8)	0.23	2.3 (2.8-6.4)	0.12
Unfavorable cytogenetics (*vs* others)	1.2 (0.4-3.6)	0.75	1.3 (0.4-4.0)	0.76
>1 chemotherapy cycle to attain CR	0.9 (0.3-2.8)	0.78	0.8 (0.2-2.5)	0.65
Chemotherapy (*vs* TBI)	2.6 (0.8-8.8)	0.10	2.9 (0.9-9.1)	0.07
No purging in auto-BMT	3.5 (0.9-13.1)	0.06	3.9 (1.0-14.7)	0.04

Univariable analysis of risk factors for OS and EFS showed that the parameters with statistical significance were a WBC higher than 100×10^9/L at diagnosis for both OS and EFS and no-purging in auto-BMT for EFS. All the other possible risk factors studied, i.e. age, FAB subtype, cytogenetics, conditioning regimen, number of chemotherapy courses to attain CR and number of MNC infused showed no differences or did not reach statistical significance (Table 6). In particular, the outcome of patients with unfavorable cytogenetics (abnormalities in chromosomes 5 or 7 or complex karyotypes) was similar to that of the other patients.

Toxicity and quality of life

During the chemotherapy phase, complications included febrile neutropenia in 60% and bacteriemia in 30% of the courses, but no mortality due to infections occurred. The median cumulative dose of anthracyclines received was equivalent to 360 mg/m^2 of daunorubicin (amsacrine doses not included). Two patients had severe toxic myocardiopathy before transplantation and received conditioning with melphalan and etoposide; both tolerated the procedure and remained alive. Overall transplant-related mortality was 4% (5.9% in the allo-BMT group and 3.2% in the auto-BMT one). No case of veno-occlusive disease was seen and only three patients developed pneumonia. Mucositis was frequent but not severe. One patient died from bronchiolitis obliterans in the context of chronic GVHD and another had chronic restrictive lung disease. With regards to long-term side effects, impaired growth velocity was observed in 11, two of whom received treatment with recombinant human growth hormone. Subclinical hypothyroidism was detected in 7 patients. Hypogonadism with abnormal hormone values was found in 9 of 21 girls and 4 of 16 boys. All the patients with hypogonadism received hormone replacement therapy.

Quality-of-life evaluation showed physical, emotional, cognitive and social functioning in transplanted patients comparable to that in the control group (study ongoing for the whole series; results will be published elsewhere).

Discussion

Since unfavorable cytogenetic findings are rare in children (abnormalities in chromosome 5 or 7 are observed in less than 4% of children with AML[20,21] and chromosome studies had not been completed in

all our patients,we adopted the BFM criteria to classify our patients into risk groups and analyze their outcome. The frequency of HR patients in our series (63%) was similar to that found in the BFM studies.[6,7]

The scheme of induction and consolidation therapy was similar to, but not the same as, that used in the MRC-AML-10 trial and BFM-93 trial (HR arm). In our group of HR patients we obtained 80% CR after induction treatment and 92% CR with the subsequent intensified consolidation course, a figure similar to that obtained in all children in the MRC-AML-10 trial.[4]

Our experience with the AML-88 trial was the object of a preliminary report[13] and, according to a recent update, the overall survival of the series of 79 consecutively-diagnosed patients was 76% at 10 years (C.I. 64%-88%) after a median follow-up of 72 months. In the present study, the estimated 10-year OS for the 50 HR patients entered in the study was 73% (C.I. 61%-85%) and 10-year EFS was 71% (C.I.59%-83%). There was a low number of toxic deaths (6%) and the relapse rate was under 20%. Post-transplant EFS was similar for patients receiving either an allo-BMT or an auto-BMT: 74.5% vs 74.4%. The relapse rates were 17.6% vs 25.8%; no relapses were observed after 32 months in patients receiving an allo-BMT and after 18 months in the recipients of an auto-BMT.

Results in patients receiving an allo-BMT were similar to those reported by Michel *et al.*[8] and better than other series including all AML patients. One factor favoring these better results was the low number of toxic deaths: 5.9% in our series, 3% in the French series but 14% to 28% in other pediatric reports.[9,22-27]

Auto-BMT does, in fact, constitute a supra-intensive post-remission treatment and, though published results are discordant, several multicenter and institutional studies in adults and children have shown it to be more effective than chemotherapy alone for preventing relapses,[4,12-14,24-26] and with good supportive therapy the risk of toxic death is less than 5% in children.[4,13,14] Results obtained with auto-BMT in our HR group of patients compare favorably with those reported in other series which also included good-risk patients.[4,9,11,12] The only reference in the literature to results obtained in HR-AML children with auto-BMT as post-consolidation treatment is a report by Bonetti *et al.*, of a multicenter Italian study.[14] In this report, 53 children in first CR received an auto-BMT after a conditioning regimen consisting of TBI and melphalan. Most had been given first-line chemotherapy according to protocol AIEOP-LAM 92 P/M and, in 45, *in vitro* bone marrow purging was performed with standard-dose mafosfamide before infusion. Five-year disease-free survival (DFS) after transplant was 68% with a median follow-up of 40 months and, according to the risk groups following BFM criteria, the rate of DFS was 63% in the HR group and 77% in the standard group. In the MRC-AML-10 trial, overall 7-year DFS in the 50 children (including all risk groups) allocated to receive an auto-BMT was 68%. In the Italian and British studies, relapse rates were, respectively, 26% and 31% and the proportion of toxic deaths, respectively, 2% and 4% in all risk groups.[4,14] In the POG and the CCG studies,[9,11] no differences in terms of DFS were observed between patients who received an auto-BMT and those who received additional intensive chemotherapy but no distinction was made between low risk and high risk groups.

In our opinion, factors which may influence outcome in children with AML in first CR receiving an auto-BMT are previous induction and consolidation chemotherapy, conditioning regimen, quality of supportive therapy and, possibly, whether the infused marrow is purged or not.

The effectiveness of intensified induction and consolidation chemotherapies has been demonstrated in AML patients of all ages in several studies.[2,3,4,7,27-29] In particular, intensification of induction therapy in poor early responders and administration of at least two courses of consolidation including Ara-C at intermediate or high doses (1 to 3 g/m^2 per dose) associated with other active drugs (mitoxantrone, amsacrine, etoposide) appears to be necessary to obtain lower levels of minimal residual disease (MRD). All our patients received at least 3 cycles of chemotherapy (1 induction and 2 consolidation), and indeed the 20% of children who had not attained CR after 2 induction cycles received 4 cycles; of note, six patients achieved CR only after the first intensified consolidation course. The attempt to eradicate residual leukemia from the body is made through the administration of conditioning regimens in both transplant modalities and through the graft-versus-leukemia effect in allo-BMT. There do not appear to be differences in effectiveness between several regimens (TBI + cyclophosphamide, TBI + melphalan, TBI + etoposide, busulfan + cyclophosphamide + etoposide)[4,8,9,13,14,21,22,26] although, according to a recent study of the EBMT Acute Leukemia WP, the BAVC combination produced results clearly inferior to all the others.[30] Even melphalan alone has been used in auto-BMT with good results.[12,31]

The combination used by us in young children (busulfan, etoposide and cyclophosphamide) was equally effective as TBI- cyclophosphamide in terms of relapse rate and DFS (*data not shown*).

Using genetically-marked grafts, it has been shown that leukemia cells in the graft may contribute to relapse.[32] However, the relative importance of re-infused leukemic cells in terms of relapse rate remains unknown and there is no con-

clusive evidence that purging is effective. Nevertheless, comparative data from EBMT and IBMT registries appear to indicate that purging of bone marrow with either mafosfamide or 4-hydroxyperoxycyclophosphamide (4-HPC) has an influence on relapse rate, leukemia-free survival and overall survival.[22,25,29,33-36] In a recent report of the American Autologous Bone Marrow Registry (ABMTR), 294 patients were studied: 211 received an autograft that had been purged by 4-HPC and 83 received an unpurged autograft. Patients receiving purged autografts had a lower risk of treatment failure: 3-year probabilities of DFS were 56% and 31% after purged and non-purged auto-BMT, respectively, in patients in first remission.[34] No comparative studies in children have been published, but data from the EBMT-Acute leukemia WP are similar to those of the ABMT registry.[30,34,35] In our study, most patients received purged marrow alone, but because of the low number of patients receiving unpurged marrow, differences observed in relapse rates were not statistically significant.

The kinetics of engraftment after auto-BMT are particularly slow in AML.[25] Purging with cyclophosphamide derivatives may further delay engraftment.[34,36] In the report of the ABMTR, the median duration of post-transplant neutropenia (less than 0.5×10^9/L) was 40 days after purged transplants versus 29 days after unpurged auto-BMT, and the median time to achieve a platelet count over 20×10^9/L was 51 days and 41 days, respectively.[34] In our series, the median duration of severe neutropenia and thrombocytopenia was 40 and 56 days, respectively. The dose of cells infused influenced the time of hematologic recovery. In this respect, the use of peripheral blood stem cells for autologous transplantation (auto-PSCT) may permit faster engraftment.[37,38] A multicenter trial comparing purged auto-BMT with purged auto-PSCT would be highly appropriate.

We conclude that intensified induction and consolidation chemotherapy followed by an allo- or auto-BMT, as performed in our trial, is both tolerable and efficacious treatment for children with HR-AML. These results obtained in our institution should be confirmed in a multicenter trial. Late effects and quality of life evaluation show that most patients enjoy a normal life. Consequently, we postulate giving treatment to children with AML according to specific subtypes and risk groups. Patients with M3 subtype are at present treated with excellent results with therapies which basically combine anthracyclines and all-trans-retinoic acid. CR are obtained in 90% of patients and 70% to 80% remain disease-free at 5 years.[39,40] The M7 subtype in patients with Down´s syndrome is another example of a subtype in which transplantation is not required.[41] Low-risk patients, as defined by BFM or MRC criteria, have a good response after 2 or 3 post-remission consolidation courses.[4,6] For the HR group (2/3 of patients in the BFM classification), the strategy described in the present study appears to be a good option.

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Pre-publication Report & Outcomes of Peer Review

Contributions

JJO was responsible for the design of the protocol and wrote the paper. PB performed the studies at diagnosis and took care of the patients during the chemotherapy phase. TO and CDH performed the transplants and followed the patients clinically. CDH and LLA were responsible for the data handling and statistical analysis. ALL evaluated the late toxic effects. MT and LLM were responsible for marrow purging and other marrow manipulations.

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In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Aggressive chemotherapy is effective in only one third of children with acute meyloid leukemia.

What this study adds

This study confirms that both autologous and allogeneic bone marrow transplantation performed after two courses of chemotherapy improve outcome of children with acute meyloid leukemia.