Bone Marrow Transplantation, (1999) 24, 81–87

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Follow-up of chimerism in children with hematological diseases after allogeneic hematopoietic progenitor cell transplants

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Summary:

Using *in situ* hybridization with an X and Y chromosome probe mixture, 106 bone marrow samples from 38 patients with malignant and non-malignant hematological diseases who received sex-mismatched allogeneic hematopoietic progenitor cell transplants (PCT) in a single institution within short-term intervals (1, 3, 6, 12, 24 and >24 months) have been sequentially studied. The patients received either HLA-identical ($n = 31$) or non-identical ($n = 7$) PCT. Twenty-six children showed donor chimerism, 10 children showed mixed chimerism (MC) and two children presented autologous reconstitution. Chimerism status with different parameters has been related (age, sex, donor, disease status before PCT, conditioning regimen, GVHD prophylaxis, relapse, GVHD and survival). Our results indicate that female patients ($P = 0.011$) and a less intensive conditioning regimen ($P = 0.039$) are significantly associated with the MC status. Mixed chimerism is not, *per se*, significantly associated with leukemia relapse but an increase of the MC is indicative of clinical relapse.

Keywords: FISH; mixed chimerism; minimal residual disease; conditioning regimen; relapse

Allogeneic hematopoietic progenitor cell transplants (PCT) are considered to be advantageous in particular malignant hematological diseases.[1–3] The success of this treatment modality is mainly affected by relapse or graft rejection. For patients receiving unmodified marrow transplants for leukemias in early stages, relapse rates range from <10% to 40%, depending on patient selection, treatment regimen, and the length of follow-up. Patients with more advanced states of leukemia have a higher relapse rate, approaching 50% to 70%, with T cell depletion (TCD) and absence of graft-versus-host disease (GVHD) being important prognostic factors for relapse.[4] Other factors responsible for relapse and/or graft rejection may be insufficient conditioning regimens or a deficient graft-versus-leukemia (GVL) effect, due to decreased numbers of donor effector cells or their functional ineffectiveness.[5]

The individual risk of relapse or graft rejection has been evaluated and correlated with the chimerism status after PCT.[6–8] Different methods have been applied for detecting chimerism: cytogenetics, restriction fragment length polymorphism (RFLP), fluorescence *in situ* hybridization (FISH) and PCR techniques.[9–19] Differing conclusions have emerged concerning the value of mixed chimerism (MC) for detecting impending relapse. While in severe aplastic anemia (SAA) or chronic myeloid leukemia (CML) the risk of relapse or graft failure were related to an increase of the frequency of host cells, the prognostic value for predicting relapse in patients with acute leukemias remains controversial.[5,6,20,21]

This paper describes a prospective study of chimerism in children with malignant and non-malignant hematological diseases who received a sex-mismatched PCT in a single institution. Sequential analysis of the individual chimerism status, by dual-color FISH technique on 106 bone marrow samples from 38 children, within short-term intervals was performed. In 10 of them, minimal residual disease (MRD) was studied. Chimerism has been correlated with different parameters (age, sex, HLA-donor, disease status before PCT, conditioning regimen, GVHD prophylaxis, relapse, GVHD and survival). The results indicate that female patients and a less intensive conditioning regimen are significantly associated with the MC status, and that only an increase of the host cells is indicative of clinical relapse.

Materials and methods

Patients

From September 1992 to April 1998, 38 children, 17 girls and 21 boys, from 1 to 14 years of age, who underwent a sex-mismatched PCT in the Unit of Bone Marrow Transplantation of the 'Hospital Materno-Infantil Vall d'Hebron' in Barcelona, were followed.

Nineteen were treated for acute lymphocytic leukemia (ALL) (eight in first remission, seven in second remission and four in third remission), six for acute non-lymphocytic leukemia (ANLL) (five in first remission and one in second remission), one for chronic myeloid leukemia (CML) in chronic phase, three for myelodysplastic syndrome (MDS) associated with Fanconi's anemia (FA) (all of them in first

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Received 28 September 1998; accepted 9 February 1999

remission), one for Fanconi's anemia (FA), one for Diamond–Blackfan anemia (DBA), one for acquired aplastic anemia (AAA), two for severe combined immunodeficiency (SCID), one for leukocyte adhesion deficiency (LAD), one for hemophagocytic lymphohistiocytosis (HLH), and one for Hunter syndrome (HS). Thirty-one patients received PC from HLA-identical siblings and seven from HLA non-identical siblings. The clinical data and treatments are summarized in Table 1.

Cytogenetic and fluorescent in situ hybridization (FISH) studies

Bone marrow (BM) cells of the 38 patients were analyzed by cytogenetics at diagnosis, before PCT and at approximately 1, 3, 6,12, 24 and >24 months after PCT. The follow-up ranged from 1 to 33 months after transplantation. Whenever it was possible two short-term cultures (24 h) were performed, one of them with colcemid overnight. The chromosomes were G-banded with Wright stain. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature, 1991, 1995 (ISCN).[22,23] When available, 50 metaphases were studied.

Contemporaneously with cytogenetic analysis, each patient was examined using DNA probes for chromosomes X and Y, the hybridization targets for the chromosomes were satellite repeat clusters in the centromeric region and satellite II DNA at Yqh, respectively. The probes from VYSIS were labeled with red (CEP Spectrum orange) and green (CEP Spectrum green), and the hybridization was performed according to the protocol of VYSIS (Downers Grove, IL, USA). When available, 1000 cells per sample were analyzed. The X chromosome centromeres exhibited a red signal, the Yqh chromosome exhibited a green signal and the nuclei stained blue.

Control study

A parallel control study was performed by FISH on 16 000 interphase nuclei from bone marrow of 16 patients, eight females and eight males, who received an autologous PCT. In our study, the normal range was fixed up to $X + 3$ s.d., being $0.64 \pm 0.025\%$ for XX cells in males and $0.79 \pm 0.025\%$ for XY cells in females. A mixed chimerism status has been considered to exist when more than 1% of host cells was observed.

Classification of chimerism

A donor chimerism (DC) was considered when only donor cells were observed. When both host and donor cells were present, a mixed chimerism (MC) was considered. It could be a transient MC (tMC, decrease of host cells with or without their disappearance); a stable MC (sMC, similar number of recipient cells in the serial studies); or a recipient-growing MC (r-gMC, progressive increase of the number of recipient cells in the serial studies). When only host cells were detected the patient showed an autologous reconstitution (AR).

Statistical analysis

The relationship between clinical parameters and chimerism was determined using Fisher's exact test for qualitative variables, and the Mann–Whitney test for the quantitative variables. The leukemia-free survival (LFS) and the overall survival (OS) of the patients in each chimerism group were estimated by Kaplan–Meier analysis. Differences between survival curves were tested by the log-rank statistic. Results were considered significant when the P value was <0.05.

Results

Chimerism: FISH and cytogenetic analysis

FISH and cytogenetic analysis were performed on 106 samples from 38 patients with a mean follow-up of 16 months. Cytogenetic analysis was unsuccessful in 13 samples. Within the first 6 months after PCT cytogenetic analysis detected host cells in two patients both of whom showed graft failure and AR, with a range of 79.3% to 100% of host cells observed by FISH (Table 1). Excluding these patients, FISH detected within the first month after PCT a mean frequency of host cells of 6% in 14% (3/21) of patients studied and at 3 and 6 months after transplant a mean frequency of host cells of 3% in 25% (5/20) of patients examined (Table 2). One year post PCT the mean frequency of host cells detected was 14.5% and 40% (8/20) of patients showed mixed chimerism; in four of them FISH displayed more than 2.7% (range 2.7–22.2%) of host cells, cytogenetic analysis also detected mixed chimerism. Two years after PCT, FISH revealed a mean frequency of host cells of 3% in 29% (5/17) of patients studied; in one of them it was also detected by cytogenetics, FISH showed 5.4% of host cells. On the last examination, between 25 and 36 months post PCT, FISH analysis was performed in five patients, three of them (60%) showed an MC, with a mean frequency of 45.73% of host cells; cytogenetics detected host cells in two of them who showed hematological relapse; in these patients 37.8% and 97.8% of host cells were found by FISH.

Chimerism and post-PCT outcome

Two patients (cases 27 and 34) showed AR 3 months after PCT. FISH analysis revealed 100% and 99.9% host cells, respectively, and the patients died 3 and 6 months after transplant.

MC was found in 27.7% (10/36) of the cases (Table 1). In six patients (cases 1, 4, 15, 20, 26 and 28) an r-gMC was found. FISH analysis showed <3.9% host cells in cases 4, 26 and 28, all of whom are alive more than 2 years after PCT. The other three patients (cases 1, 15 and 20) relapsed between 9 and 33 months after transplant. Case 1, affected by FA with MDS showed 6% and 37% host cells, at 24 and 33 months after allo-PCT, respectively; case 15 affected by AML, showed DC (0.3%) and 97.8% host cells 3 and 9 months after transplant; case 20, affected by ALL, showed DC (0.5%) and 68.2% host cells at 1 and 9 months after PCT, dying 4 months after clinical relapse. Patients 1 and

Table 1 Clinical characteristics, FISH and cytogenetics results of childrens pre- and post PCT

No.	*Age*	*Disease*	*Status PCT*	*Conditioning regimen*	*Prophylaxis GVHD*	*GVHD A/C*	*Sex R/D*	*1 month*		*3 months*		*6 months*		*12 months*		*24 months*		*>24 months*		*Chimerism status*	*S mo/st*
								FISH Host cells (%)	*Kar*	*FISH Host cells (%)*	*Kar*	*FISH Host cells (%)*	*Kar*	*FISH Host cells (%)*	*Kar*	*Fish Host cells (%)*	*Kar*	*FISH Host cells (%)*	*Kar*		
1	6	FA/MDS	CR1	Cy+NI	CsA+ATG	I	F/M			3	XY			1.7	XY	6	XY	37.8	XX/XY	r-gMC	31/(R)A
2	8	ANLL	CR2	Cy+VP-16+ARAC	CsA	I	F/M			0	XY	0.4	XY	0.5	XY	0	XY	0	NM	DC	28/A
3	0.5	ANLL	CR1	Cy+VP-16+Bu	CsA+M	I	F/M			5.3	XY	4	XY	1	XY	1.5	XY	1.6	NM	MC(tMC)	54/A
4	8	ALL	CR1	Cy+VP-16+TBI	CsA+M	I	F/M			0.1	XY					1.1	XY			r-gMC	52/A
5	11	ALL	CR2	Cy+TBI	CsA	I	F/M			0.4	XY	0.1	XY	0.8	XY	0	XY			DC	55/A
6	4	CML	CP	Cy+TBI	CsA+M	I	F/M											0.2	XY	DC	124/A
7	11	FA/MDS	CR1	Cy+NI	CsA+MTX+ATG	II/L	F/M					2	XY	2	XY	2.5	NM			sMC	20/D
8	11	ALL	CR2	Cy+ARAC+TBI	CsA	II	M/F					4.2	NM	22.2	XX/XY	0	XX			DC(tMC)	54/A
9	13	ALL	CR2	Cy+VP-16+TBI	CsA	I/L	M/F	0	XX			0	XX	0.3	XX	0	XX			DC	40/A
10	3	ALL	CR1	Cy+ARC+TBI	CsA	I	M/F	14.7						2.7	XY/XX	0.3	XX			DC(tMC)	55/A
11	6	NMD		Cy+Bu	CsA	I	F/M		NM					8	XX/XY	5.4	XX/XY			MC(tMC)	43/A
12	7	NMD		Cy+TBI	CsA+MTX	IV/E	F/M			0	NM	0.1	XY							DC	9/D
13	12	FA/MDS	CR1	Cy+NI	CsA+MTX	I	M/F	2.5						0.1	XX					DC	49/A
14	8	ALL	CR2	Cy+TBI	CsA	I	F/M		XY			0.1	XY	0.6	XY	0	XY			DC(tMC)	27/A
15	2	ANLL	CR1	Cy+Bu+VP-16	CsA	N	M/F	0.7				0.1	XX	0	XX	0.3	XX	97.8	XY	r-gMC	38/(R)A
16	5	NMD		Cy+Bu	CsA+MTX	I/L	F/M		XY	0.3	XY	0	XY							DC	13/D
17	5	ALL	CR2	Cy+TBI	CsA	N	M/F	0.2						0.1	XY	0.8	XY			DC	31/A
18	10	ALL	CR3	Cy+VP-16+TBI	CsA	II	M/F		XX	0.2	XX	0.3	XX							DC	9/GF
19	9	ANLL	CR1	Cy+TBI	CsA	N	M/F			0.6	XX	0	XX	0.8	XX	0.2	XX			DC	29/A
20	14	ALL	CR3	Cy+VP-16+TBI	CsA+MTX	II	F/M	0.5	NM	0.4	NM	0	NM	68.2	NM					r-gMC	13/(R)D
21	11	ALL	CR3	Cy+TBI	CsA	IV/E	M/F			0.6	XX	0.1	XX							DC	6/D
22	11	ALL	CR2	Cy+VP-16+TBI	CsA	I	F/M	0.1	NM	0	XY	0.1	XY			0.2	XY			DC	27/A
23	5	ANLL	CR1	Cy+VP-16+Bu	CsA+MTX	I	M/F	0	XX	0	XX	0	XX	0	XX					DC	15/A
24	3	ALL	CR2	Cy+VP-16+TBI	CsA	II	M/F	0.1	XX	0.8	XX	4.3	NM	10.4	XX/XY	0.4	XX			DC(tMC)	27/A
25	13	ALL	CR2	Cy+VP-16+TBI	CsA	III/L	M/F	0	NM	1.1	XX			0	XX	.2	XX			DC(tMC)	27/A
26	0.5	NMD		Cy+Bu	CsA+M	III	F/M	0.5	XY	3.9	XY									r-gMC	23/A
27	1	NMD		Cy+Bu	CsA+M	N	F/M	79.3	XX/XY	100	XX									AR(tMC)	6/D
28	4	ALL	CR1	Cy+VP-16+TBI	CsA	N	F/M	0	XY	0.2	XY	1.1	XY							r-gMC	18/A
29	9	ALL	CR3	Cy+VP-16+TBI	CsA+MTX	II	M/F	0	XX											DC	21/A
30	6	ALL	CR1	Cy+VP-16+TBI	CsA	N	M/F	0.5	XX	2.2	XX	0.1	XX	0.1	XX					CD(tMC)	19/A
31	14	NMD		Cy+Bu	CsA+MTX	N	F/M	0.4	XY	0.5	XY	0	XY	0.1	XY					DC	16/A
32	10	FA		Cy+TBI	CsA+MTX	N	M/F	0.	XX											DC	6/A
33	7	ANLL	CR1	Cy+Bu+VP-16+NI	CsA	III/E	F/M	0	XY											DC	6/D
34	0.5	NMD		Cy+Bu	CsA	N	M/F			99.9	XY	97.3	XY							AR(MC)	3/D
35	7	ALL	CR1	Cy+VP-16+TBI	CsA	III/E	M/F	0.4	XX											DC	7/D
36	2	ALL	CR1	Cy+Bu	CsA	I	M/F	1.6	XX											MC	4/A
37	5	ALL	CR2	Cy+VP-16+TBI	CsA+MTX	I	M/F	0	XX											DC	3/A
38	1	NMD		Cy+Bu	CsA	II	M/F	0	NM											DC	3/A

No. = number of patient; R/D = recipient/donor; F/M = female/male; PCT = progenitor cells transplant; GVHD = graft-versus-host disease; A/C = acute/chronic; L = limited; E = extensive; FA = Fanconi's anemia; MDS = myelodysplasic syndrome; ANLL = acute non-lymphoblastic leukemia; ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia; NMD = non-malignant disease; CR1 = first complete remission; CR2 = second complete remission; CR3 = third complete remission; CP = chronic phase; TBI = total body irradiation; Cy = cyclophosphamide; NI = nodal irradiation; Bu = busulfan; CsA = cyclosporine; MTX = methotrexate; M = another drug; FISH = fluorescence *in situ* hybridization; Kar = karyotype; MC = mixed chimerism; r-gMC = recipient-growing MC; TMC = transient MC; sMC = stable MC; DC = donor chimerism; S = survival; Mo = months; st = status post-PCT; A = alive; R = relapse; D = dead; GF = graft failure; AR = autologous reconstitution.

Table 2 Chimerism by FISH and cytogenetics analysis

Interval post transplant (months)	*FISH (% host cells) mean (range)*	*FISH (% Patients with MC)*	*Cytogenetics (% Patients with MC)*
1	6.26 (1.6–14.7)	14.3 (3/21)	0 (0/17)
3	3.1 (1.1–5.3)	25 (5/21)	0 (0/19)
6	3.1 (1.1–4.3)	25 (5/21)	0 (0/18)
12	14.52 (1–68.2)	40 (8/20)	21.1 (4/19)
24	3.30 (1.1–5.4)	29.4 (5/17)	6.2 (1/16)

MC = mixed chimerism.

15 received a second PCT and both remain in clinical remission at the time of writing. Two patients (cases 3 and 11) showed a tMC, with a decrease of host cells between 3 and >24 months post PCT; in case 3, FISH analysis detected, in several studies, 5%, 4%, 1%, 1.5% and 1.6% host cells, and in case 11, 14.7%, 8% and 5.4%. Both patients remain in clinical remission more than 40 months after transplant. In one patient (case 7) an sMC was found; FISH detected 2% host cells in three studies, between 3 and 24 months after transplant. The patient developed an acute and chronic GVHD and died 20 months after PCT. Case 36 was examined only once, 1 month after transplant, and showed an MC with 1.6% host cells. He is alive 4 months after PCT.

DC was found in 72.2% (26/36) of the cases (Table 1). Twenty patients (cases 2, 5, 6, 9, 12, 13, 16–19, 21–23, 29, 31–33, 35, 37 and 38) showed DC throughout the observation period with a mean follow-up of 23.3 months: 10 developed acute GVHD, one of whom (patient 18) died from a later graft failure 9 months after transplantation; six developed acute and chronic GVHD, five of whom died due to post-PCT complications and four cases did not develop GVHD. Six patients (cases 8, 10, 14, 24, 25 and 30) showed DC with a tMC; in these patients FISH detected the presence of an average number of 6.2% host cells (range 1.1–22%). Five of them developed acute GVHD, and one case (patient 25) developed a limited chronic GVHD; this patient showed 1.1% host cells 3 months after transplant and DC 6 months later. The six patients remain in complete continuous remission more than 24 months after allo-PCT.

Minimal residual disease

Ten patients (cases 1, 3, 6, 15, 19, 21, 29, 33, 35 and 36) with malignant diseases showed chromosome aberrations at diagnosis. All of them were studied for MRD by cytogenetic analysis. Only two patients relapsed (cases 1 and 15); patient 1 was diagnosed as having FA with MDS showing a 45,XX,–7 karyotype; 24 months post PCT only donor metaphases were present, while FISH showed 6% host cells. Five months later, the patient showed a 47,X,der(X),t(X;1)(q21;q23) del(X)(q22), der(1) t(1;7)(p32;q21),add(13)(q22),+mar karyotype in 14.5% of metaphases, and FISH detected 37.3% host cells. The patient relapsed clinically and is still alive 28 months after a second allo-PCT. Patient 15 was diagnosed with AML with a 46,XY,t(11;17)(q23;q21) karyotype and 6,12 and 24 months after transplant cytogenetic analysis detected only donor metaphases, while FISH showed 0.1%, 0% and 0.3% host cells, respectively. Nine months later FISH detected 97.8% host cells and all metaphases showed the karyotype observed at diagnosis in addition to other chromosome aberrations. He is alive 5 months after a second allo-PCT.

In the remainder of the patients cytogenetic analysis detected only donor cells, five of whom (patients 3, 6, 19, 29 and 36) are alive, while three (patients 21, 33 and 35) developed a chronic GVHD and died between 6 and 7 months after PCT.

Correlation between chimerism and clinical–biological parameters

The patients who showed autologous reconstitution were excluded from the statistical analysis. MC status has been considered to exist when MC was observed in all the samples analyzed or when MC was found in the last study. Table 3 shows the correlation between the chimerism status and some clinical–biological parameters. MC patients had a median of age lower than DC patients (5 years *vs* 8.5 years) but these differences were not significant. Recipient sex differences between MC and DC patients were significant: 80% (8/10) of patients with MC were female, while 69% (18/26) of the patients with DC were male ($P = 0.011$). Differences between malignant and non-malignant diseases were comparable in both chimeric groups. However, patients with high risk malignant diseases (in second or in third remission) generally showed DC, but the differences were not significant. HLA differences between recipient and donor were not significant in both chimeric groups. DC was predominantly detected in patients conditioned with TBI plus chemotherapy, 84% (21/25), and MC occurred in patients receiving exclusively chemotherapy, 54% (6/11), these differences being significant ($P = 0.039$). However, DC occurred more often in patients who received only CsA as GVHD prophylaxis, 76% (16/21), than in patients who received CsA with another drug, 66% (10/15).

Seventy-two percent of the patients that have not relapsed are alive after a mean follow-up of more than 2 years. Of these, 77% (20/26) showed DC and 23% (6/26) MC. Three patients (8%) relapsed, and all developed an r-gMC. One patient (2.6%) showed DC and died of multiorgan failure after graft failure. Clinical acute GVHD grade 0–I was diagnosed in 58% (21/36) of the patients, seven with MC and 14 with DC. Clinical acute GVHD grade II–IV developed in 42% (15/36) of the patients, three of whom showed MC; clinical chronic GVHD was diagnosed in 22% (8/36) of the patients. In only one was a MC detected. These differences were not significant. The median survival was higher in the MC group (45 months) than the DC group (30 months).

The prognostic influence of MC on overall survival (OS) and leukemia-free survival (LFS) in malignant disease patients was estimated by Kaplan–Meier curves. No significant differences were found between children with DC and those with MC with respect to OS ($P = 0.314$) or LFS ($P = 0.670$). The 2-year probabilities of OS for patients with DC was 65.9%, and for patients with MC it was 65.6%. The 2-year probabilities of LFS for patients with DC was 75%, and for patients with MC it was 51%.

Table 3 Correlation between chimerism status and clinial–biological parameters

	MC	*DC*	*P*	*OR*
Age of patient				
median (range)	5.0 (0.5–14.0)	8.5 (1.0–14.0)	NS	
Sex of patient				
Female	8 (80.0%)	8 (30.8%)	0.011	9.00 (2.06–39.40)
Male	2 (20.0%)	18 (69.2%)		
Disease				
MD	7 (70.0%)	21 (80.8%)	NS	0.56 (0.14–2.25)
NMD	3 (30.0%)	5 (19.2%)		
Status before PCT				
CR1	5 (83.3%)	7 (35.0%)	NS	9.29 (1.31–65.94)
CR2 or CR3	1 (16.7%)	13 (65.0%)		
Donor				
HLA-identical	8 (80.0%)	23 (88.5%)	NS	1.17 (0.16–8.73)
HLA non-identical	2 (20.0%)	3 (11.5%)		
Conditioning				
TBI + chemotherapy	4 (40.0%)	21 (80.0%)	0.039	0.16 (0.04–0.61)
Chemotherapy	6 (60.0%)	5 (19.2%)		
GVHD prophylaxis				
CsA	5 (50.0%)	16 (61.5%)	NS	0.63 (0.18–2.15)
CsA + M	5 (50.0%)	10 (38.5%)		
Status after PCT	6 (60.0%)			
Alive	3 (30.0%)	20 (77.0%)		
Relapse				
Graft failure	1 (10.0%)	1 (3.8%)	NS	1.90 (0.04–13.14)
Death by other complications		5 (19.0%)		
aGVHD				
0–I	7 (70%)	14 (53.8%)	NS	2.00 (0.54–7.39)
II–IV	3 (30%)	12 (46.2%)		
cGVHD				
Yes	1 (12.5%)	7 (33.3%)	NS	0.29 (0.04–1.94)
No	7 (87.5%)	14 (66.7%)		
Survival				
median (range)	44.6 (5.4–83.8)	30.1 (22.7–37.5)	NS	

P = value; NS = not significant; OR = overall risk; MC = mixed chimerism; DC = donor chimerism; MD = malignant disease; NMD = non-malignant disease; CR1 = first complete remission; CR2 = second complete remission; CR3 = third complete remission; TBI = total body irradiation; CsA = cyclosporine; CsA + M = cyclosporine plus other drug; PCT = progenitor cells transplant; GVHD = graft-versus-host disease, aGVHD = acute GVHD; cGVHD = chronic GVHD.

Discussion

FISH with X and Y chromosome probes and cytogenetic analysis have been used to study 38 patients who received allo-PCT for different hematological diseases. The presence of host cells was observed by both techniques during the follow-up. A high percentage of host cells was found at 1, 3 and 6 months post PCT, between 3% and 6%, according to previous observations that showed a frequency of recipient cells between 1% and 7% in the early post-transplant period.[9,10] In our series this frequency decreases during the first, and second year post transplant, showing an average of 14% and 3.3% host cells, respectively. These results corroborate the observations of Bernasconi *et al*,[9] Palka *et al*[10] and Wessman *et al*,[17] who found >1% of host cells 2 years after transplantation. The high frequency of host cells (46%) at >24 months after PCT is due to two of five patients studied in this period showing clinical relapse.

Host cells were observed in 11% of the cases by cytogenetics, while by FISH they were detected in 28%. The sensitivity of cytogenetics increases with the number of metaphases analyzed. Palka *et al*,[10] analyzing between 15 and 20 metaphases, detected MC only when >10% of host cells were observed by FISH. In our study analyzing 50 metaphases, MC was detected, while by FISH only 2.7% of host cells were found.

On the other hand, MRD was evaluated by cytogenetics in 10 patients with malignant diseases and chromosome aberrations at diagnosis. Eight patients in clinical remission showed a normal karyotype. MRD was observed in two patients: one patient (case 1), with a 45,XX,–7 karyotype at diagnosis of AF with SMD, showed a complex karyotype indicative of a clonal evolution of the disease, associated with AML; the other patient (case 15,) affected by AML M5, at relapse showed the same chromosome aberration, t(11;17)(q23;q21), present at diagnosis in addition to other aberrations. Therefore, our results suggest that the disappearance of the original abnormal clone is associated with the induction of clinical remission and that the reappearance of an abnormal karyotype is prognostically unfavorable.[12,24,25]

Regarding the prognostic value of the presence of host cells, it was found, as in previous studies,[9,10] that the progressive increase of host cells is related to clinical and hematological relapse, whereas patients who developed a tMC remain in clinical remission. Our results are in contrast to those of Bourhis *et al*,[26] who showed that patients with ⩾5% host cells had a higher probability of relapse. We

detected more than 5% (5.3–22%) host cells in four patients and no patient relapsed. On the other hand, two patients relapsed directly from a DC status (cases 15 and 20). In each of these cases the interval between the detection of DC and the relapse was more than 6 months. This agrees with the results obtained by quantitative PCR analysis where some patients relapsed without a prior MC status.[5] The authors postulate that these patients may have developed a stage of tMC, but the critical time-point of molecular confirmation by PCR was missed because the time intervals in the individual follow-up were too long (>6 months).

Several studies in patients suffering aplastic anemia or chronic leukemia found evidence that the risk of relapse or graft failure was increased when MC was detected. This evidence is not clear for patients with acute leukemia, probably because relapses in these cases generally take place within a shorter time-period in comparison to cases with chronic leukemias.[20,21] According to this, the relapse in a patient with MDS associated with FA who showed a clear r-gMC with a leukemia transformation could be predicted; however, relapse in two patients with acute leukemia could not be predicted, probably because the time intervals of examination were too long (>6 months).

A significant association between chimerism status and the female sex was found. The incidence of MC was not significant between the group of patients with an HLA-identical donor and the group of patients with an HLA non-identical donor. These results do not agree with those of Leeuwen *et al*,[6] perhaps due to sample size. A significant relationship between MC and the type of conditioning regimen was found. Recently, a relationship between the TBI dose and the incidence of MC has been suggested.[20,27] Leeuwen *et al*[6] observed a significant relationship between the low TBI doses and MC in children conditioned with equal doses of Cy plus varying doses of TBI. Generally, in infants less than 2 years old, Bu is used instead of TBI. In our series Bu is used in very young children (<2 years), and the majority showed MC. Recently, it was shown that the systemic exposure to equal doses of Bu is less in children as compared with adults because of differences in pharmacokinetics. This finding may explain the occurrence of MC in three infants less than 2 years of age after BuCy4 treatment as opposed to DC observed in a youngster 16 years of age.[28] Ugozzoli *et al*[19] observed DC in 16 patients (ages 1.5 to 14 years) after BuCy4 treatment.

Significant differences in the frequency of MC between the group which received only CsA for GVHD prophylaxis and the group which received CsA with another drug have not been found. The use of CsA in GVHD prophylaxis was associated with a significantly increased incidence of MC, but the influence of the other variables cannot be rejected, because the majority of these patients received less than 8 Gy total dose of TBI.[6] On the other hand, in our series as in others,[5,6,9,20] the incidence and severity of acute and chronic GVHD was low in patients with MC. Moreover, it has been reported that patients with MC did not develop severe acute or chronic GVHD which was considered to be associated with the GVL effect, and this suggests that deficient immunological effector mechanisms may be responsible for the recurrence of pathological cells.[5,11]

In our series, both OS and LFS were not significantly different between the MC and DC groups. The overall survival was similar in the DC group and in the MC group (65.9% *vs* 65.6%). In patients with CML, OS was significantly superior in MC than in DC and it has been suggested that this survival advantage of MC is probably related to a lower incidence of GVHD.[21] On the other hand, LFS was higher in the DC group than in the MC groups, 75% *vs* 51%, but MC was not significantly associated with graft failure or leukemia relapse.[6,20] As has been postulated, the only predictive factor for leukemia relapse was a progressive increase of host cells.[9,10]

In summary, the present study confirms the usefulness of sequential FISH studies in the assessment of bone marrow engraftment stability and for early detection of an increase in host cells. In addition, our results indicate that there is a presence of host cells 1 and 2 years after allo-PCT, more frequently in patients who do not receive TBI as the conditioning regimen, but only the serial increase of host cells is related to the clinical relapse. On the other hand, in acute leukemia the relapse is developed in a short time-period, suggesting the performance of a more exhaustive follow-up.

Acknowledgements

We would like to thank Albert and Dr M Martín (Unidad de Estadística, Facultad de Medicina, UAB) for their valuable help with the statistical management of the data. This study was supported by the 'Fondo de Investigaciones sanitarias', project number 95/0072–01.

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