Impact of detectable measurable residual disease on umbilical cord blood transplantation

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ABSTRACT

The impact of measurable residual disease (MRD) on cord blood transplantation (CBT) outcomes has remained debated. To address this issue, we assessed the impact of measurable MRD at CBT on outcomes in large cohort of patients with acute leukemia. Inclusion criteria included adult patients with acute myeloid (AML) or acute lymphoblastic leukemia (ALL), CBT as first allo-HCT in first or second complete remission (CR) at transplantation, and known MRD status at the time of CBT. Data from 506 patients were included in the analysis. Among them, 317 patients had AML and 189 had ALL. Positive MRD was reported in 169 (33%) patients while the remaining 337 patients were MRD negative at CBT. At 2 years, relapse incidence was 18% in patients with MRD negativity *versus* 33% in those with MRD positivity at transplantation (P<0.001). Two-year leukemia-free survival (LFS) and overall survival (OS) were 57% and 60%, respectively, in MRD negative patients, *versus* 38% (P<0.001) and 48% (P=0.004), respectively, in those with MRD positivity. There was no interaction between the impact of MRD on OS and LFS and diagnosis (i.e. ALL versus AML), single or double CBT, and reduced-intensity or myeloablative conditioning. On multivariate analysis, MRD positivity was associated with a higher risk of relapse (HR=1.8, P=0.003), comparable non-relapse mortality (P=0.44), worse LFS (HR=1.4, P=0.008) and a trend towards worse OS (HR=1.3, P=0.065). In conclusion, these data suggest that novel strategies that are aiming to achieve MRD negativity at CBT are needed for leukemic patients with positive MRD pre-CBT.

CONDENSED ABSTRACT

- Patients with detectable MRD pre-CBT have a higher risk of relapse than those without (HR=1.8, P=0.003).
- Patients with detectable MRD pre-CBT have a lower LFS than those without (HR=1.4, P=0.008).

BACKGROUND

Allogeneic hematopoietic cell transplantation (allo-HCT) is the treatment of choice for fit patients with high-risk acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL)[1-8]. Allo-HCT relies both on the conditioning regimen and on immune-mediated graft-versus-leukemia (GvL) effects for leukemia eradication[9-11]. Despite the rapid growth of T-cell replete HLA-haploidentical transplantation[12], umbilical cord blood transplantation (CBT) has remained an alternative option for patients with high-risk acute leukemia without an HLA-identical sibling donor[13-18].

During the last decade, several reports have highlighted the negative impact of detectable measurable residual disease (MRD) at transplantation on transplantation outcomes in patients transplanted from either HLA-matched related or unrelated donors, as well as in those given cells from HLA-haploidentical donors[19-25]. This remained true in patients in second complete remission (CR) at transplantation[26]. Interestingly, the negative impact of detectable MRD at transplantation holds true in large registry studies in which various techniques (depending on transplant center) were used for MRD detection[21,27,28]. However, a recent report has demonstrated a lower

incidence of relapse with double CBT than with HLA-matched transplantation among patients with detectable MRD at transplantation[15]. This is in line with emerging data suggesting higher GvL effects with CBT and one might question whether MRD has an impact on CBT outcome.

In this report, we assess the impact of detectable MRD at transplantation on CBT outcomes in a relatively large cohort of patients with acute leukemia in complete remission (CR) reported to the Acute Leukemia Working Party (ALWP) of the European society for Blood and Marrow Transplantation (EBMT) registry and to Eurocord.

METHODS

Patients and inclusion criteria

This is a retrospective study from the ALWP of the EBMT and from Eurocord. The EBMT registry is a voluntary working society of more than 500 transplant centers, participants of which are required once a year to report all consecutive hematopoietic stem cell transplantations and follow-up. Audits are routinely performed to check for data accuracy. Eurocord collects data on CBT carried out in more than 50 countries worldwide covering > 500 transplant centers (mainly EBMT).

Inclusion criteria included adult patients (defined as ≥18 years of age at transplantation), with AML or ALL, single or double CBT as first allo-HCT from 2002-2017 in an EBMT-affiliated

center, in first or second CR at transplantation, and known MRD status at the time of transplantation.

Reduced intensity conditioning (RIC) was defined as regimens combining fludarabine with either < 6 Gy total body irradiation (TBI), ≤ 8 mg/kg busulfan, or ≤ 140 mg/m^2 melphalan or with other nonmyeloablative drugs as previously reported [29]. HLA-compatibility included antigen level typing for HLA-A and -B and allele level typing for HLA-DRB1. Cord blood units were generally 4–6/6 HLA-A, -B and -DRB1 matched to the recipient. Acute and chronic graft-versus-host disease (GVHD) were graded according to previously reported criteria [30].

MRD detection

Several techniques with various thresholds were used for MRD detection, depending of the centers. For AML patients, data on MRD methodology was available for 115 patients from 30 centers (supplemental table 1). Most centers (22/30) used multiparameter flow-cytometry (MFC) and PCR techniques with or without next generation sequencing (NGS). The most frequent threshold used was 10^{-5} for PCR techniques and 10^{-4} for MFC (supplemental table 1). For ALL patients, data on MRD methodology was available for 73 patients from 28 centers. Eighteen centers used MFC plus PCR techniques with or without NGS while 22 others used PCR techniques only. The most frequent threshold used was 10^{-5} for PCR techniques and 10^{-4} for MFC (supplemental table 1).

Statistical analyses

Analyses were carried out on data from all patients meeting the inclusion/exclusion criteria. Start time was the day of CBT for all endpoints. Patients were censored at the time of last follow-up. Neutrophil engraftment was defined as the first of 3 consecutive days with a neutrophil count of at least 0.5 x 10^9/L. Relapse was defined as the presence of 5% bone marrow blasts and/or reappearance of the underlying disease. Non-relapse mortality (NRM) was defined as death without evidence of relapse or progression. Overall survival (OS) was defined as the time from CBT to death, regardless of the cause. The primary endpoint of the study was leukemia-free survival (LFS) according to MRD status. Events in the composite endpoint LFS included relapse and death, whichever occurred first.

Cumulative incidence functions were used to estimate relapse incidence and NRM in a competing risk setting, because death and relapse compete with each other. To estimate the cumulative incidence of acute and chronic GVHD, we considered relapse and death to be competing events. Events in the composite endpoint GVHD-free and relapse free survival (GRFS) included grade III-IV acute GVHD, extensive chronic GVHD, relapse or death [31]. The Kaplan-Meier method was used to estimate OS, LFS and GRFS.

Univariate analyses were performed using Gray's test for cumulative incidence functions and the log-rank test for OS, LFS and GRFS. Multivariate Cox models were used to adjust the comparison of transplantation outcomes in patients with, *versus* without,

evidence of MRD at transplantation for possible imbalance between groups. Factors included in the Cox models comprised detectable MRD or not, diagnosis (AML *versus* ALL), age, year of transplantation, second *versus* first CR, myeloablative *versus* RIC regimen, double *versus* single CBT, *in vivo* T cell depletion or not, and center (frailty). All tests were two sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 19 (SPSS Inc, Chicago, IL), and R 3.4.3 (R Development Core Team, Vienna, Austria) software packages.

RESULTS

Patients

A total of 506 patients met the inclusion/exclusion criteria and were reported in the study (Table 1). Among them, 317 patients had AML and 189 had ALL (including 102 patients with Philadelphia chromosome positive (Phi-pos) ALL). Patients received either single (n=227) or double unit CBT (n=279). Disease status was first CR in 320 patients and second CR in the remaining 186 patients. MRD positivity was detected in 169 (33%) patients while the remaining 337 patients were MRD negative at CBT. MRD was more frequently detected in ALL than in AML patients (P=0.02), in patients given single CBT than in those receiving double CBT (P=0.02), and in those given *in vivo* T-cell depletion of the donor graft (P=0.006).

Engraftment and GVHD

At 60 days after CBT, neutrophil engraftment was achieved by 91% (95% CI 87%-94%) of MRD negative patients versus 85% (95% CI 79%-90%) of those with measurable MRD (P=0.05). The 100-day cumulative incidences of grade II-IV and of III-IV acute GVHD were 34% (95% CI 28%-39%) and 15% (95% CI 11%-19%) respectively, in MRD negative patients, *versus* 37% (95% CI 29%-44%, P=0.5) and 16% (95% CI 11%-22%, P=0.6). On multivariate analysis, the only factor associated with a lower incidence of grade II-IV acute GVHD was *in vivo* T cell depletion (HR=0.5, 95% CI 0.4-0.8, P=0.001) (Supplemental table 2).

The 2-year cumulative incidences of chronic and extensive chronic GVHD were 33% (95% CI 27%-38%) and 12% (95% CI 8%-16%) respectively in MRD negative patients, versus 25% (95% CI 18%-32%, P=0.2) and 13% (95% CI 8%-19%, P=0.4). On multivariate analysis, no factor was associated with chronic GVHD.

Relapse and NRM

At 2 years, relapse incidence was 18% (95% CI 14%-22%) in patients with MRD negativity *versus* 33% (95% CI 26%-41%, P<0.001) in those with MRD positivity at transplantation (Figure 1). This negative effect of MRD positivity on relapse was observed both in patients with AML and in those with ALL (independently of Phi

positivity), and was also observed both in patients in first CR and in those in second CR (Supplemental Tables 3-6). In the subgroup of patients with AML, poor risk cytogenetics (according to the MRC classification[32]) was associated with higher relapse incidence in comparison to good/intermediate risk cytogenetics (at 2-year 29% (95% CI, 16%-43%) vs 22% (95%CI, 15%-29%), P=0.02). Restricting the analyses in AML patients with good/intermediate risk cytogenetics (n=271), MRD positivity (n=82) remained significantly associated with a higher risk of relapse (28% (95% CI, 18%-38%) vs 16% (95% CI, 11%-22%), P=0.046; supplemental table 7). On multivariate analysis, MRD positivity was associated with an increased risk of relapse (HR=1.8, 95% CI 1.2-2.6, P=0.003) (Table 2). Other factors associated with risk of relapse included the use of RIC regimen (HR=1.8, 95% CI 1.2-2.9, P=0.01) and *in vivo* T cell depletion (HR=2.2, 95% CI 1.4-3.4, P<0.001).

LFS, GRFS

Two-year LFS was 57% (95%CI, 51%-63%) in MRD negative patients *versus* 38% (95%CI, 30%-45%) in those with MRD positivity at CBT (P<0.001) (Figure 1). There was no statistical interaction between any variable and the association of MRD status with LFS. Furthermore, as shown in Figure 2, detectable MRD at transplantation was associated with worse (or a trend towards worse) LFS in each assessed subgroup (single versus double CBT, AML versus ALL, Phi positive versus Phi negative ALL). Specifically,

among ALL patients, 2-year LFS was 55% (95%CI, 46%-65%) in MRD negative patients *versus* 33% (95%CI, 22%-44%) in MRD positive ones (P=0.002) while among AML patients the figures were 58% (95%CI, 51%-65%) *versus* 41% (95%CI, 31%-52%), respectively (P=0.02). Restricting the analyses to AML patients with good/intermediate risk cytogenetics[32], 2-year LFS was 59% (95% CI, 51%-66%) in MRD negative patients, versus 44% (95% CI, 32%-55%, P=0.019) in MRD positive ones. Further, interestingly, the negative impact of MRD positivity was at least as much marked in patients in second CR than in those in first CR (Figure 2). Importantly, MRD positivity remained associated with a worse LFS on multivariate analysis (HR=1.42, 95% CI1.1-1.84 P=0.008). Other factors associated with LFS included second *versus* first CR at transplantation (HR=1.33, 95%CI 1.01-1.76, p=0.04) and *in vivo* T cell depletion (HR=1.92, 95% CI 1.41-2.61, P=0.014) (Table 2).

Two-year GRFS was 43% (95%CI, 37%-49%) in MRD negative patients *versus* 31% (95%CI, 24%-38%) in those with MRD positivity at CBT (P=0.005) (Figure 1). There was no statistical interaction between diagnostic group and the association of MRD status with GRFS. Specifically, among ALL patients, 2-year GRFS was 37% (95%CI, 28%-50%) in MRD negative patients *versus* 27% (95%CI, 16%-37%) in MRD positive ones (P=0.08) while among AML patients the figures were 46% (95%CI, 39%-53%) *versus* 35% (95%CI, 24%-45%), respectively (P=0.046). On multivariate analysis MRD positivity at transplantation was associated with worse GRFS (HR=1.3, 95%CI 1.03-1.65, P=0.029). No other factor was significantly associated with GRFS.

OS

Two-year OS was 60% (95%CI, 55%-66%) in MRD negative patients *versus* 48% (95%CI, 39%-56%) in those with MRD positivity at CBT (P=0.004) (Figure 1). There was no statistical interaction between diagnostic group and the association of MRD status with OS (Figure). Among ALL patients, the 2-year OS was 60% (95%CI, 50%-69%) in MRD negative patients *versus* 47% (95%CI, 35%-59%) in MRD positive ones (P=0.02) while among AML patients the figures were 60% (95%CI, 53%-67%) *versus* 48% (95%CI, 37%-59%), respectively (P=0.049). Restricting the analyses to AML patients with good/intermediate risk cytogenetics[32], 2-year OS was 62% (95% CI, 54%-69%) in MRD negative patients, versus 50% (95% CI, 37%-61%, P=0.034) in MRD positive ones. Further, interestingly, the negative impact of MRD positivity was as much marked in patients in second CR as it was in those in first CR (Figure 2). On multivariate analysis there was a trend for worse OS in patients with detectable MRD (HR=1.3, 95%CI 0.98-1.71, P=0.065). Other factors associated with OS included older age (per decade, HR=1.13, 95% CI 1.01-1.27, P=0.03), second *versus* first CR at transplantation (HR=1.45, 95%CI 1.08-1.94, p=0.01) and *in vivo* T cell depletion (HR=1.78, 95% CI 1.28-2.47, P<0.001) (Table 2).

At the time of analysis, death from the underlying disease of MRD negative and MRD positive patients was 14% and 18% respectively, while 5% and 11% respectively, died from GVHD.

DISCUSSION

Several reports have demonstrated the negative impact of detectable MRD at transplantation on transplantation outcomes in patients given grafts from HLA-matched or HLA-haploidentical donors[19,20,27,28]. However, the impact of detectable MRD at transplantation on CBT outcome remains debatable. Tucunduva *et al.* first reported that in the setting of Phi-positive ALL (n=98), detectable MRD (n=59) was associated with a higher risk of relapse following CBT [33]. In contrast, a study by Milano *et al.* of data from 137 patients with AML, ALL or myelodysplastic syndrome, reported that detectable MRD at transplantation (n=45) was not associated with a significantly higher risk of relapse following double CBT (HR=1.43, 95% CI : 0.58-3.57). The authors also reported a lower incidence of relapse with double CBT than with HLA-matched or HLA-mismatched transplantation, among MRD positive patients[15]. These data, which are in line with emerging data suggesting higher GvL effects in (double) CBT[34-36], prompted us to assess the impact of detectable MRD on CBT outcomes in a relatively large cohort of patients with AML or ALL in first or second CR at transplantation.

First, we observed that detectable MRD at CBT was associated with higher risk of relapse leading to worse LFS, worse GRFS and a suggestion for worse OS. This impact of detectable MRD was observed in all subgroups but perhaps appeared to be less marked among patients with Phi-positive ALL, possibly because many of these patients might have received a tyrosine kinase inhibitor after CBT (or possibly due to a lack of

statistical power in subgroup analyses). Interestingly, the impact of detectable MRD at CBT was at least as strong in patients in CR2 at transplantation as in those in first CR. Nevertheless, despite detectable MRD at transplantation being associated with poorer CBT outcomes it should be stressed that CBT outcomes among MRD positive patients remained encouraging with approximately half of the patients still alive 2 years after CBT.

This study also confirms several observations made previously regarding CBT for acute leukemia such as similar LFS with both RIC and MAC regimens [29,37], similar LFS with double or single unit CBT [10,38], and a detrimental impact of *in vivo* T-cell depletion on CBT outcome [18,39,40].

There are some limitations to our study including the fact that MRD assessment was not standardized and that the method of MRD detection varied among centres, or the fact that we lack data on the use of post-transplant anti-leukemia agents (such as tyrosine kinase inhibitor in case of Phi-positive ALL or FLT3-mutated AML[5,41,42], or hypomethylating agents[43,44]).

CONCLUSIONS

In summary, our results indicate that among acute leukemia patients undergoing CBT, achieving MRD negativity at time of transplantation is associated with a lower risk of relapse translating into better LFS. Novel strategies are needed for those leukemic patients with MRD positivity pre-CBT aiming at either achieving MRD negativity at CBT

or preventing relapse following CBT by receiving post-transplant anti-leukemic drugs and/or immunotherapy and thus further improving CBT results.

DECLARATIONS

Ethics approval and consent to participate

The scientific board of the ALWP of the EBMT approved this study.

Consent for publication

NA

Availability of data and materials

ML, AN and MM had full access to all the data in the study (available upon data specific request).

Competing interests

Frédéric Baron has received travel grants from Celgene, Abbvie, Novartis and Sanofi as well as honoraria from Merck and Abbvie. The remaining authors declare that they have no relevant conflict of interest.

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Authors' contributions

FBa wrote the manuscript, designed the study, and interpreted the data; ML designed the study, analyzed and interpreted the data, and edited the manuscript; AR, AN and MM designed the study, interpreted the data and edited the manuscript; EG helped in the study design and edited the manuscript; JS, SR, HLW, MP, JMR, ED, AR, PSR and TR reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

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FIGURE LEGEND

Figure 1. Impact of MRD on transplantation outcomes. A) relapse. B) non relapse mortality. C) leukemia-free survival. D) GVHD-free and relapse-free survival. E) overall survival.

Figure 2. Forest-plot showing the impact of detectable MRD (versus not) on leukemia-free survival (A, LFS) and overall survival (B, OS) in various subgroups.

Table 1. Patient and transplant characteristics

	MRD neg (n=337)	MRD pos (n=169)	Pvalue [1]
Median patient age, y (range)	43 (18-70)	41 (18-66)	0.46
Patient sex, # (%)			0.73
Male	168 (50)	87 (51)	
Female	169 (50)	82 (49)	
KPS, # (%)			0.51
< 80	11 (4)	4 (2)	
>= 80	291 (96)	156 (98)	
Missing	35	9	
Diagnosis, # (%)			0.02[3]
AML	223 (66)	94 (56)	
Good risk[2]	23	20	
Intermediate risk[2]	87	43	
Poor risk[2]	34	12	
NA / failed	79	19	
ALL	114 (34)	75 (44)	
Phi neg B ALL	16	8	
Phi pos B ALL	53	49	
T ALL	19	9	
ALL missing	26	9	
Status at transplantation, # (%)			0.42
CR1	209 (62)	111 (66)	
CR2	128 (38)	58 (34)	
Follow-up, mo (IQR)	40 (19-74)	53 (19-70)	
Conditioning regimen, # (%)			0.10
Myeloablative	202 (60)	114 (67)	
TBI based	105 (31)	56 (33)	
TBF	70 (21)	43 (25)	
BuCy	12 (4)	4 (2)	
Other / missing	15 (4)	11 (7)	
Reduced-intensity	135 (40)	55 (33)	
TBI based	123 (36)	46 (27)	
Other / missing	12 (4)	9 (5)	
In vivo TCD, # (%)			0.006
Yes	203 (64)	82 (51)	
No	116 (36)	80 (49)	
Missing	18	7	
Units of CBT			0.02
Single	139 (41)	88 (52)	
Double	198 (59)	81 (48)	
Postgrafting immunosuppression, # (%)			0.9
CSP alone	69 (22)	41 (25)	
CSP + MMF +/- MTX	211 (66)	102 (63)	
Other	40 (12)	19 (11)	
Missing	17	7	

[1], calculated with χ^2 statistics for categorical variables and Mann-Whitney test for continuous variables; [2], cytogenetic risk was categorized according to the MRC classification[32]; [3], refers to the comparison of AML versus ALL; Y, year; mo, month; KPS, Karnofsky score, AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; Phi, Philadelphia chromosome; TBI, total body irradiation; TBF, thiotepa, busulfan and fludarabine; BuCy, busulfan and cyclophosphamide; IQR, interquartile range; CR, complete remission; TCD, T-cell depletion; CBT, cord blood transplantation; #, number of patients; tacro, tacrolimus; CSP, cyclosporine A; MMF, mycophenolate mofetil; MTX, methotrexate.

Table 2: Multivariate analyses for CBT outcomes.

	RELAPSE		NRM		LFS		OS		GRFS	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
MRD detectable vs not	1.78 (1.22-2.58)	0.003	1.15 (0.80-1.66)	0.44	1.42 (1.1-1.84)	0.008	1.3 (0.98-1.71)	0.065	1.3 (1.03-1.65)	0.029
ALL vs AML	1.33 (0.88-2.03)	0.181	1.14 (0.77-1.69)	0.521	1.22 (0.92-1.63)	0.168	1.09 (0.8-1.48)	0.592	1.21 (0.94-1.57)	0.135
Age (per 10 years)	0.99 (0.85-1.16)	0.91	1.16 (1-1.35)	0.047	1.08 (0.97-1.2)	0.169	1.13 (1.01-1.27)	0.031	1.04 (0.95-1.15)	0.403
Year of CBT	0.99 (0.93-1.06)	0.82	0.96 (0.91-1.02)	0.207	0.98 (0.94-1.02)	0.273	0.97 (0.925-1.01)	0.17	0.99 (0.95-1.03)	0.594
CR2 vs CR1	1.31 (0.87-1.99)	0.193	1.35 (0.93-1.98)	0.119	1.33 (1.01-1.76)	0.043	1.45 (1.08-1.94)	0.013	1.26 (0.99-1.62)	0.064
RIC vs MAC	1.84 (1.16-2.92)	0.01	0.829 (0.52-1.32)	0.43	1.23 (0.89-1.7)	0.221	1.06 (0.75-1.5)	0.741	0.93 (0.69-1.24)	0.616
DCBT vs SCBT	1.0 (0.65-1.52)	0.992	0.71 (0.48-1.05)	0.089	0.83 (0.63-1.11)	0.216	0.75 (0.55-1.02)	0.067	0.91 (0.70-1.18)	0.476
in vivo TCD	2.18 (1.39-3.41)	0.001	1.69 (1.11-2.58)	0.014	1.92 (1.41-2.61)	<0.001	1.78 (1.28-2.47)	0.001	1.17 (0.89-1.55)	0.261
Centre (frailty)		0.924		0.357		0.905		0.701		0.898

NRM, non relapse mortality; LFS, leukemia-free survival; OS, overall survival; GRFS, GVHD-free and relapse free survival; MRD, minimal residual disease; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CBT, cord blood transplantation; CR, complete remission; RIC, reduced-intensity conditioning regimen; MAC, myeloablative conditioning regimen; dCBT, double unit CBT; sCBT, single unit CBT; TCD, T-cell depletion; HR, hazard ratio; CI, confidence interval.