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Occurrence of graft-versus-host disease increases mortality after umbilical cord blood transplantation for acute myeloid leukaemia: a report from Eurocord and the ALWP of the EBMT

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Abstract. Baron F, Ruggeri A, Beohou E, Labopin M, Mohty M, Sanz J, Vigouroux S, Furst S, Bosi A, Chevallier P, Cornelissen JJ, Michallet M, Sierra J, Karakasis D, Savani BN, Gluckman E, Nagler A (University of Liege, Liege, Belgium; IUH University Paris VII; Hôpital Saint-Antoine; Hospital Saint Antoine, Paris, France; Hospital Universitario La Fe, Valencia, Spain; University Hospital of Bordeaux, Bordeaux; Institut Paoli Calmettes, Marseille, France; AOU Careggi, Florence, Italy; CHU Nantes, Nantes, France; Erasmus MC Cancer Institute, Department of Hematology, Rotterdam, The Netherlands; Centre Hospitalier Lyon-Sud, Lyon, France; Hospital Santa Creu i Sant Pau, Barcelona, Spain; Evangelismos Hospital, Athens, Greece; Vanderbilt University Medical Center, Nashville, TN, USA; IUH University Paris VII, Monaco city, Monaco; The Chaim Sheba Medical Center, Tel-Hashomer, Ramat-Gan, Israel). Occurrence of graft-versus-host disease increases mortality after umbilical cord blood transplantation for acute myeloid leukaemia: a report from Eurocord and the ALWP of the EBMT. *J Intern Med* 2017; https://doi.org/10.1111/joim.12696

Background. The efficacy of umbilical cord blood transplantation (UCBT) as treatment for acute myeloid leukaemia (AML) relies on immune-mediated graft-versus-leukaemia effects. Previous studies have suggested a strong association between graft-versus-host disease (GVHD) occurrence and graft-versus-leukaemia effects after allogeneic hematopoietic cell transplantation.

Methods. Here, we evaluated the kinetics of relapse rate in correlation with GVHD occurrence after UCBT. The kinetics of relapse rate over time in correlation to GVHD occurrence were assessed by calculating the relapse rate per patient-year within sequential 90-day intervals. The impact of GVHD on relapse and mortality was further studied in multivariate Cox models handling GVHD as a time-dependent covariate.

Results. The study included data from 1068 patients given single ($n = 567$) or double ($n = 501$) UCBT. The proportion of patients with grade II, III and IV acute GVHD was 20%, 7% and 4%, respectively. At 2 years, the cumulative incidence of chronic GVHD was 42%, the cumulative incidence of relapse was 32%, and overall survival was 32% as well. Relapse rates declined gradually over time during the first 30 months after transplantation. There was a possible suggestion that grade II–IV acute (HR = 0.8, $P = 0.1$) and chronic (HR = 0.65, $P = 0.1$) GVHD decreased relapse risk. However, grade II–IV acute GVHD significantly increased early (the first 18 months after UCBT) mortality (HR = 1.3, $P = 0.02$), whilst chronic GVHD increased each

early (HR = 2.7, $P < 0.001$) and late (HR = 4.9, $P < 0.001$) mortality after UCBT.

Conclusions. The occurrence of grade II–IV acute or chronic GVHD each increases overall mortality after UCBT for AML mitigating the possible graft-versus-leukemia effect of GVHD.

Keywords: acute myeloid leukaemia, graft-versus-host disease, graft-versus-leukaemia effects, umbilical cord blood transplantation.

Background

Allogeneic umbilical cord blood transplantation (UCBT) is a treatment option for many patients with acute myeloid leukaemia (AML) who lack an HLA-matched donor [1–4]. Its efficacy relies on immune-mediated graft-versus-leukaemia (GVL) effects [5–10]. Following allogeneic bone marrow (BM) or peripheral blood stem cell (PBSC) transplantation, there is a strong association between occurrence of graft-versus-host disease (GVHD) and a lower risk of AML relapse [11–16]. However, occurrence of GVHD has also been associated with higher risk of nonrelapse mortality, and thus only milder forms of GVHD (grade 1 acute and limited chronic) have been associated with improved overall survival [13].

Umbilical cord blood transplantation has been associated with a lower incidence of severe acute and/or chronic GVHD that would be expected based on the degree of donor-recipient human leukocyte antigen (HLA) mismatch [17]. Further, it has been suggested that acute GVHD following UCBT responds better to corticosteroids than acute GVHD following BM or PBSC transplantation [18, 19]. Three recent large studies assessed the impact of GVHD on outcomes after UCBT and yielded conflicting results [20–22]. Lazaryan *et al.* observed no association between acute (either grade II–IV or grade III–IV) or chronic (limited or extensive) GVHD and nonrelapse mortality after UCBT ($n = 711$) [20]. Acute GVHD did not impact the relapse incidence. In contrast, chronic GVHD was associated with a lower risk of relapse, but only in the subgroup of patients receiving double UCBT. More recently, Kanda *et al.* observed that acute and chronic GVHD each decreased the risk of relapse in a large cohort of UCBT recipients ($n = 2558$) [21]. However, only milder forms of GVHD (grade 1–2 acute and limited chronic GVHD) were associated with better survival due to a strong association between severe forms of GVHD and nonrelapse mortality. Finally, in the study by Chen *et al.*, grade III–IV acute GVHD was associated with worse survival, whilst occurrence of chronic GVHD did not have a significant impact on overall survival [22].

Previous studies might have overestimated the beneficial impact of GVHD on relapse incidence given the tight association between GVHD and nonrelapse mortality (since relapse and nonrelapse mortality are competing events). In order to circumvent this potential limitation, Inamoto *et al.* proposed a new way of assessing the impact of covariate (including time-dependent covariate) on relapse incidence that is not affected by competing risk [23]. This method is based on the calculation of the rate of relapse per patient-year for each condition within sequential 90-day intervals after transplantation, and smoothed estimates of the event rates obtained by fitting a Poisson regression model to the observed numbers of events, using cubic spline terms for time. This method allows assessing the evolution of the hazard ratio for the risk of relapse over time and is not affected by competing risks. Here, we used this statistical approach to revisit the graft-versus-leukaemia effects after UCBT. Main observations were that although there was perhaps a suggestion (nonstatistically significant) for a lower incidence of relapse with the development of grade II–IV acute or chronic GVHD, grade II–IV acute GVHD increased the risk mortality the first 18 months after transplantation, whilst chronic GVHD increased the risk of mortality both before and particularly after 18 months following UCBT.

Methods

Data collection and definitions

This survey is a retrospective study performed by the Acute Leukemia Working Party (ALWP) of the European society for Blood and Marrow Transplantation (EBMT) and by Eurocord. EBMT registry is a voluntary working group of more than 500 transplant centres, participants of which are required once a year to report all consecutive stem cell transplantations and follow-up. Audits are routinely performed to determine the accuracy of the data. Eurocord collects data on UCBT performed in >50 countries worldwide and >500 transplant centres, mainly EBMT centres.

Inclusion criteria were adult (>18 years) patients, primary or secondary AML, and first single or double UCBT between 2004 and 2014.

RIC was defined as use of fludarabine (Flu) associated with <6 Gy TBI, or busulfan ≤8 mg kg^{-1}, melphalan ≤140 mg m^{-2} or other nonmyeloablative drugs, as previously reported [13, 24, 25]. Human leukocyte antigen (HLA) compatibility requirements followed the current practice of antigen level typing for HLA-A and HLA-B and allele level typing of HLA-DRB1. CB units were 4–6/6 HLA-A, HLA-B and HLA-DRB1 matched to the recipient and to the other unit in case of double UCBT in most patients. However, more recently, some centres are no longer matching the CB units between them with regard to HLA based on the study by Avery *et al.* [26]. HLA disparities between each unit and the recipient and between the two units were not necessarily at the same loci. Grading of acute and chronic GVHD was performed using established criteria [27].

For the purpose of this study, all necessary data were collected according to EBMT and Eurocord guidelines.

Ethics

The scientific boards of the ALWP of EBMT and of Eurocord approved this study.

Statistical analyses

Data from all patients meeting the inclusion/exclusion criteria were included in the analyses. Start time was date of transplant for all end-points. Neutrophil engraftment was defined as first of three consecutive days with a neutrophil count of at least 0.5×10^9 per L.

To evaluate the relapse incidence, patients dying either from direct toxicity of the procedure or from any other cause not related to leukaemia were censored. Nonrelapse mortality was defined as death without experiencing disease recurrence. Patients were censored at the time of relapse or of the last follow-up. Cumulative incidence functions were used for relapse incidence and nonrelapse mortality in a competing risk setting, since death and relapse were competing together.

For estimating the cumulative incidence of chronic GVHD, death was considered as a competing event.

Overall (OS) and leukaemia-free (LFS) survivals were estimated using the Kaplan–Meier estimates. Univariate analyses were done using Gray's test for cumulative incidence functions and log-rank test for OS and LFS.

Acute and chronic GVHD were assessed as time-dependent covariates. Patients at risk of relapse or mortality were classified in three mutually exclusive conditions based on their histories of acute GVHD or chronic GVHD as previously reported by Inamoto *et al.* [23]: grades 0–I acute GVHD without chronic GVHD (hereafter designated 'no GVHD'), grades II–IV acute GVHD without chronic GVHD (hereafter designated 'acute GVHD') or chronic GVHD with or without grade II–IV acute GVHD (hereafter designated 'chronic GVHD'). All patients were first classified in the 'no GVHD' group until the onset of acute GVHD or chronic GVHD. Patients with 'acute GVHD' were classified in that condition, regardless of whether acute GVHD had resolved, until the onset of chronic GVHD if it occurred. Patients with 'chronic GVHD' were classified in that condition thereafter. The impact of GVHD on relapse and mortality was illustrated by calculating the rate of relapse per patient-year for each condition within sequential 90-day intervals after transplantation, and smoothed estimates of the event rates obtained by fitting a Poisson regression model to the observed numbers of events, using cubic spline terms for time. In a second set of analyses, only patients who achieved stable engraftment were included in the analyses.

In addition to the analyses defined above, we also used time-dependent Cox models to assess the association between GVHD and risks of relapse, nonrelapse mortality and overall mortality.

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 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

Results

Outcomes after UCBT

The study included data from 1068 patients given single (n = 567) or double (n = 501) UCBT. Median patient age at transplantation was 45.5 (range, 18–73) years (Table 1).

Overall, grades II, III and IV acute GVHD were observed in 203 (20%), 77 (7%) and 42 (4%) patients, respectively. The 100-day cumulative incidence of grade II–IV acute GVHD was 31% (95% CI, 28–34%). Median time from transplantation to onset of grade II–IV acute GVHD was 29 (range, 4–229) days. In multivariate analyses, double UCBT was associated with a higher incidence of grade II–IV acute GVHD (HR = 1.5, 95% CI: 1.1–2.0, $P = 0.009$).

The 2-year cumulative incidence of chronic GVHD was 42% (95% CI, 37–46%) with 50% limited and 50% extensive chronic GVHD. Median time from transplantation to onset of chronic GVHD was 147 (range, 61–1230) days. Amongst chronic GVHD patients, 101 (44%) had a prior history of grade II–IV acute GVHD. In multivariate analyses, patients with advanced disease had a lower incidence of chronic GVHD (HR = 0.5, 95% CI: 0.3–0.7, $P < 0.001$).

With a median follow-up of 35 months, 2-year incidences of relapse and nonrelapse mortality were 32% (95% CI, 29–35%) and 38% (95% CI, 35–41%), respectively. Two-year OS and LFS were 32% (95% CI, 30–36%) and 30% (95% CI, 27–33%), respectively.

Eight hundred and twenty-six patients (77%) achieved neutrophil engraftment. Restricting the analyses to the 826 patients with neutrophil engraftment, grades II, III and IV acute GVHD were observed in 176 (22%), 71 (9%) and 34 (4%) patients, respectively. The 100-day cumulative incidence of grade II–IV acute GVHD was 34% (95% CI, 31–38%). The 2-year cumulative incidence of chronic GVHD was 44% (95% CI, 39–49%) with 52% limited and 48% extensive chronic GVHD. Two-year incidences of relapse and nonrelapse mortality were 33% (95% CI, 30–36%) and 33% (95% CI, 29–36%), respectively. Two-year OS and LFS were 37% (95% CI, 34–41%) and 34% (95% CI, 31–38%), respectively.

Evolution of relapse rates after UCBT and impact of GVHD

As shown in Fig. 1, relapse rates per patient/year declined gradually over time during the first 30 months after transplantation; this was also the case when analyses were restricted to patients with neutrophil engraftment (Figure S1). In multivariate analyses, factors associated with higher relapse incidences included being transplanted in CR3+ (HR = 2.2, 95% CI: 1.3–3.7, $P = 0.003$) or in advanced disease (HR = 3.2, 95% CI: 2.4–4.3, $P < 0.001$) and poor risk cytogenetics (HR = 2.6, 95% CI: 1.3–5.5, $P = 0.01$), whilst there was a suggestion for a higher incidence of relapse in patients given grafts after RIC conditioning (HR = 1.3, 95% CI: 1.0–1.7, $P = 0.08$). Interestingly, the use of RIC conditioning was associated with a higher incidence of relapse the first 18 months after transplantation (HR = 1.4, 95% CI: 1.1–1.9, $P = 0.02$) but not thereafter (HR = 0.6, 95% CI: 0.3–1.3, $P = 0.2$).

Looking at the impact of GVHD on relapse, the relapse rate per patient/year from 3 to 6 months was lower in patients with acute GVHD than in patients without GVHD (Fig. 1). Thereafter, the relapse/rate per patient-year was comparable in acute GVHD and no GVHD patients. Regarding chronic GVHD, the relapse rate per patient/year tended to be lower than in the no GVHD and the acute GVHD group from 9 to 30 months after transplantation. In multivariate time-dependent Cox models, there was a suggestion for a lower incidence of relapse in patients with grade II–IV acute GVHD (HR = 0.78; 95% CI: 0.60–1.1, $P = 0.1$) and those with chronic GVHD (HR = 0.65; 95% CI: 0.37–1.12, $P = 0.1$). However, these associations did not reach statistical significance (Table 2) .

Separating grade II and grade III–IV acute as well as limited and extensive chronic GVHD, there was no statistically significant association between occurrence of GVHD (of any grade) and relapse risk (Table 3). Similar results were observed when the analyses were restricted to patients who achieved neutrophil engraftment (Table S1). However, there was a suggestion of an interaction between single or double UCBT and the impact of grade III–IV acute GVHD on the risk of relapse ($P = 0.07$), whilst no interactions were observed for grade II acute or chronic GVHD. Finally, the risk of relapse was similar in patients given 3/6 or 4/6 HLA-matched UCB versus those given 5/6 or 6/6 HLA-matched UCBT (HR = 1.2; 95% CI: 0.9–1.6, $P = 0.3$).

Evolution of nonrelapse mortality rates after UCBT and impact of GVHD

As shown in Fig. 2, nonrelapse mortality rates per patient/year declined gradually over time during the first 30 months after transplantation; this was also the case when analyses were restricted to patients with neutrophil engraftment (Figure S2).

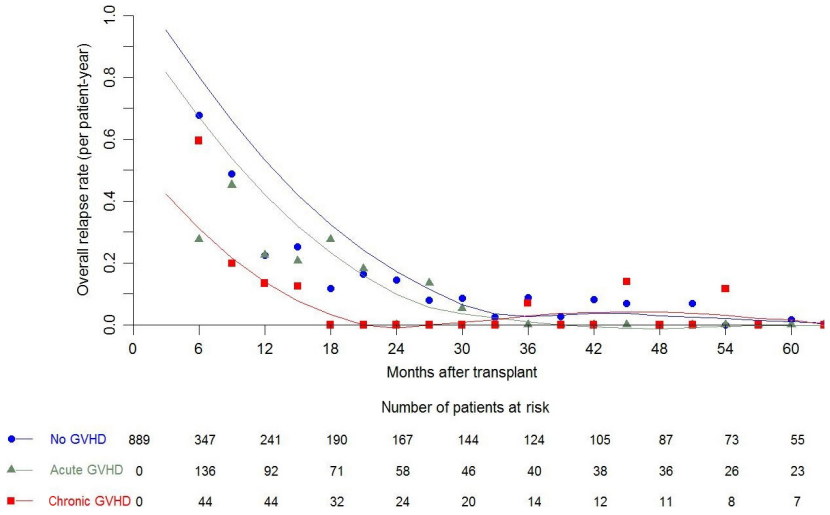

Fig. 1 *Evolution of relapse rates according to GVHD status. Rates were calculated within sequential 90-day intervals for patients without GVHD (shown in blue), for patients with grades II–IV acute GVHD (shown in green) or for patients with chronic GVHD (shown in red). Small symbols represent the actual relapse rates for each 90-day interval. The smoothed rates were plotted as curves for each condition.*

In multivariate analyses, factors associated with a higher incidence of nonrelapse mortality included higher patient age (HR = 1.02; 95% CI: 1.01–1.03, $P = 0.002$) and advanced disease (HR = 1.6; 95% CI: 1.2–2.2, $P < 0.001$), whilst the use of a RIC regimen was associated with a lower incidence of nonrelapse mortality (HR = 0.6, 95% CI: 0.4–0.7, $P < 0.001$). Each of these factors was significantly associated with early (≤18 months after UCBT) nonrelapse mortality, whilst none of them impacted late (>18 months after UCBT) nonrelapse mortality.

Looking at the impact of GVHD on nonrelapse mortality, occurrence of grade II–IV acute GVHD increased global (HR = 1.8, 95% CI: 1.4–2.3, $P < 0.001$) and early (HR = 1.8, 95% CI: 1.4–2.4, $P < 0.001$) nonrelapse mortality, whilst chronic GVHD was associated with higher global (HR = 2.7, 95% CI: 1.6–4.4, $P < 0.001$), early (HR = 2.3, 95% CI: 1.3–4.1, $P = 0.005$) and late (HR = 3.8, 95% CI: 1.2–11.4, $P = 0.02$) nonrelapse mortality.

Separating grade II and grade III–IV acute as well as limited and extensive chronic GVHD, there was a statistically significant association between occurrence of GVHD grade III–IV acute (HR = 4.1, 95% CI: 2.8–6.0, $P < 0.001$) and of extensive chronic (HR = 5.3, 95% CI: 3.6–7.9, $P < 0.001$) GVHD and the risk of nonrelapse mortality (Table 3). In contrast, grade II acute GVHD (HR = 0.8, 95% CI: 0.5–1.2, $P = 0.3$) as well as limited chronic (HR = 1.1, 95% CI: 0.6–2.1, $P = 0.7$) GVHD was not associated with nonrelapse mortality. Comparable results were observed when the analyses were restricted to patients who achieved neutrophil engraftment (Table S1). Finally, there was no interaction between single or double UCBT and the impact of GVHD on the risk of nonrelapse mortality.

Evolution of mortality after UCBT and impact of GVHD

As shown in Fig. 3, mortality rates per patient/year declined gradually over time during the first 30 months after transplantation; this was also the case when analyses were restricted to patients with neutrophil engraftment (Figure S3). In multivariate Cox analyses, higher patient age (HR = 1.01; 95% CI: 1.00–1.02, $P = 0.001$) and advanced disease (HR = 2.3; 95% CI: 1.8–2.8, $P < 0.001$) increased global (and early) mortality, whilst RIC regimen was associated with a lower risk of global (as well as early and late) mortality (HR = 0.8, 95% CI: 0.6–0.9, $P = 0.006$).

Looking at the impact of GVHD on mortality, grade II–IV acute GVHD increased the mortality rate per patient/year from 1 year to 21 months after transplantation, whilst chronic GVHD increased mortality throughout the study period. In multivariate time-dependent Cox models, grade II–IV acute GVHD significantly increased mortality (HR = 1.29; 95% CI: 1.05–1.57, $P = 0.01$). Separating early and late mortality, acute GVHD increased early but not late mortality. In contrast, chronic GVHD increased not only global (HR = 2.84; 95% CI: 2.2–3.7, $P < 0.001$) mortality but also early and

Table 1 *Patient and transplant characteristics*

	UCBT (n = 1068)
Median patient age, y (range)	45.5 (18–73)
Median follow-up, months (range)	35 (0–118)
Year of transplantation, median (range)	2011 (2004–2014)
Recipient gender M, # (%)	525 (49)
Karnofsky score, # (%)	
>80	572 (54)
≤80	301 (28)
Missing	195 (18)
Disease status, # (%)	
CR1	458 (43)
CR2	291 (27)
CR3+	42 (4)
>CR	209 (20)
Ukn	68 (6)
Cytogenetics, # (%)	
Good risk[a]	64 (6)
Intermediate risk[b]	527 (49)
High risk[c]	117 (11)
Not reported/failed	360 (34)
Graft type	
Single UCBT	567 (53)
Double UCBT	501 (47)
TNC, median (range)	4.2 (0.4–13.7)
Number of HLA disparities, # (%)	
0–1 mismatch	256 (24)
2–4 mismatches	594 (56)
Missing	218 (20)
Conditioning regimen, # (%)	
MAC	505 (47)
TCF	57 (5)
TBF	208 (19)
Other TBI-based	83 (8)
Other	157 (15)
RIC	546 (51)
TCF	319 (30)
TBF	38 (4)
Other TBI-based	55 (5)
Other	134 (12)
Missing	17 (1)
Recipient CMV seronegative, # (%)	267 (31.9)
ATG, # (%)	
Yes	476 (45)
No	394 (37)
Missing	198 (18)
Postgrafting immunosuppression, # (%)	
CSP alone	70 (7)
CSP + MMF	651 (61)
CSP + Predisolone	171 (16.0)
CSP + MTX	38 (4)
Other	138 (13)

MAC, myeloablative conditioning; RIC, reduced-intensity conditioning (as defined previously[25]); Y, year; M, male; CR, complete remission; #, number of patients; TCF, total body irradiation (TBI), cyclophosphamide and fludarabine; TBF, thiotepa, busulfan and fludarabine; ATG, antithymocyte globulins; TNC, total nucleated cells; CSP, cyclosporine A; MMF, mycophenolate mofetil.
[a]defined as t(8;21), t(15;17), inv or del (16), or acute promyelocytic leukaemia, these abnormalities only or combined with others; [b]defined as all cytogenetics not belonging to the good or high risk (including trisomias); [c]defined as 11q23 abnormalities, complex karyotype, abnormalities of chromosomes 5 and 7.

late mortality. Separating grade II and grade III–IV acute as well as limited and extensive chronic GVHD, there was a statistically significant association between occurrence of grade III–IV acute (HR = 2.8, 95% CI: 2.0–3.9, $P < 0.001$) and of extensive chronic (HR = 2.3, 95% CI: 1.7–3.0, $P < 0.001$) GVHD and the risk of mortality (Table 3). In contrast, grade II acute (HR = 0.8, 95% CI: 0.6–1.1, $P = 0.2$) as well as limited chronic (HR = 0.9, 95% CI: 0.7–1.3, $P = 0.6$) GVHD was not associated with overall mortality. Comparable findings were observed when the analyses were restricted to patients who achieved neutrophil engraftment (Table S1).

Impact of ATG

We restricted the analyses assessing the impact of ATG on UCBT outcomes in the subgroup of patients with sustained engraftment in order to avoid the possible confounding impact of ATG on neutrophil engraftment. In multivariate analyses, the use of ATG was associated with a lower incidence of grade II (HR = 0.4, 95% CI: 0.3–0.6,

Table 2 *Multivariate Cox models I (3 groups of GVHD)*

	Relapse			Nonrelapse mortality			Mortality		
Variable	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*
Overall									
GVHD									
aGVHD only grade 0–I (ref)	–	–	–	–	–	–	–	–	–
aGVHD only grade II-IV	0.78	0.58, 1.06	0.11	1.8	1.40, 2.31	<0.001	1.29	1.05, 1.57	0.01
cGVHD with or without aGVHD	0.65	0.37, 1.12	0.12	2.69	1.61, 4.48	<0.001	2.84	2.19, 3.67	<0.001
Patient age at transplant	1	1.00, 1.01	0.34	1.02	1.01, 1.03	0.002	1.01	1.00, 1.02	0.001
Disease status at transplant									
CR1 (ref)	–	–	–	–	–	–	–	–	–
CR2	1.1	0.81, 1.51	0.53	1.03	0.78, 1.36	0.85	1.09	0.88, 1.35	0.41
CR3+	2.17	1.29, 3.65	0.003	0.83	0.43, 1.58	0.57	1.27	0.85, 1.90	0.25
Advanced disease	3.21	2.40, 4.28	<0.001	1.64	1.22, 2.20	<0.001	2.25	1.83, 2.77	<0.001
Cytogenetic									
Good (ref)	–	–	–	–	–	–	–	–	–
Interm	1.92	0.97, 3.80	0.06	0.58	0.38, 0.89	0.01	1	0.69, 1.45	0.99
Poor	2.64	1.26, 5.53	0.01	0.59	0.34, 1.03	0.06	1.05	0.68, 1.63	0.82
NA/failed	1.48	0.71, 3.06	0.29	0.88	0.55, 1.38	0.57	1.15	0.77, 1.71	0.49
Graft typeDCBT versus SCBT	1	0.78, 1.28	0.98	0.8	0.63, 1.01	0.07	0.85	0.72, 1.02	0.07
Conditioning typeRIC versus MAC	1.28	0.97, 1.68	0.08	0.57	0.43, 0.74	<0.001	0.77	0.63, 0.93	0.006
Before 18 months									
GVHD									
aGVHD only grade 0–I (ref)	–	–	–	–	–	–	–	–	–
aGVHD only grade II-IV	0.81	0.59, 1.11	0.2	1.83	1.41, 2.38	<0.001	1.29	1.05, 1.59	0.02
cGVHD with or without aGVHD	0.63	0.34, 1.17	0.14	2.32	1.28, 4.20	0.005	2.69	2.03, 3.57	<0.001
Patient age at transplant	1	0.99, 1.01	0.63	1.02	1.00, 1.03	0.004	1.01	1.00, 1.02	0.009
Disease status at transplant									
CR1 (ref)	–	–	–	–	–	–	–	–	–
CR2	1.01	0.72, 1.42	0.96	0.99	0.73, 1.32	0.92	1.03	0.82, 1.29	0.8
CR3+	1.76	0.96, 3.23	0.07	0.92	0.48, 1.76	0.79	1.21	0.78, 1.88	0.4
Advanced disease	3.19	2.36, 4.30	<0.001	1.64	1.21, 2.22	0.001	2.26	1.83, 2.79	<0.001
Cytogenetic									
Good (ref)	–	–	–	–	–	–	–	–	–
Interm	1.72	0.83, 3.57	0.15	0.55	0.35, 0.86	0.008	0.94	0.64, 1.38	0.74
Poor	2.43	1.11, 5.31	0.03	0.52	0.29, 0.94	0.03	0.97	0.61, 1.53	0.89
NA/failed	1.26	0.58, 2.74	0.56	0.78	0.49, 1.25	0.3	1.03	0.68, 1.55	0.89
G raft typeDCBT versus SCBT	1.03	0.79, 1.33	0.85	0.79	0.62, 1.01	0.06	0.85	0.71, 1.02	0.08
Conditioning typeRIC versus MAC	1.41	1.05, 1.88	0.02	0.55	0.42, 0.73	<0.001	0.8	0.65, 0.97	0.03
After 18 months									
GVHD									
aGVHD only grade 0–I (ref)	–	–	–	–	–	–	–	–	–

Table 2 (*Continued*)

	Relapse			Nonrelapse mortality			Mortality		
Variable	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*
aGVHD only grade II-IV	0.58	0.22, 1.55	0.28	1.58	0.62, 4.02	0.33	1.26	0.62, 2.56	0.53
cGVHD with or without aGVHD	0.84	0.22, 3.13	0.79	3.76	1.24, 11.44	0.02	4.87	2.41, 9.85	<0.001
Patient age at transplant	1.02	0.99, 1.05	0.21	1.02	0.99, 1.05	0.24	1.03	1.00, 1.05	0.04
Disease status at transplant									
CR1 (ref)	–	–	–	–	–	–	–	–	–
CR2	1.94	0.81, 4.62	0.14	1.63	0.67, 3.97	0.28	1.92	0.99, 3.72	0.05
CR3+	4.32	1.41, 13.21	0.01	0	0.00, Inf	1	1.24	0.45, 3.44	0.68
Advanced disease	2.52	0.79, 8.04	0.12	1.68	0.47, 6.02	0.42	2.33	0.97, 5.56	0.06
Cytogenetic									
Good (ref)	–	–	–	–	–	–	–	–	–
Interm	3.06	0.40, 23.21	0.28	1.42	0.18, 11.46	0.74	1.57	0.36, 6.81	0.55
Poor	2.93	0.28, 30.67	0.37	2.9	0.29, 28.98	0.37	1.94	0.37, 10.25	0.43
NA/failed	3.83	0.45, 32.41	0.22	4.83	0.55, 42.11	0.15	3.55	0.76, 16.51	0.11
Graft typeDCBT versus SCBT	0.83	0.40, 1.72	0.62	1.01	0.43, 2.37	0.99	0.9	0.50, 1.61	0.72
Conditioning typeRIC versus MAC	0.59	0.26, 1.34	0.2	0.63	0.23, 1.73	0.37	0.5	0.26, 0.97	0.04

GVHD, graft-versus-host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; CR, complete remission; DCBT, double umbilical cord blood transplantation; SCBT single umbilical cord blood transplantation; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning.

$P < 0.001$) and grade III–IV (HR = 0.5, 95% CI: 0.3–0.8, $P = 0.01$) acute GVHD. In contrast, there was no impact of ATG on chronic GVHD (HR = 1.5, 95% CI: 0.9–2.4, $P = 0.1$). Importantly, there was no association between ATG and the risk of relapse (HR = 0.9, 95% CI: 0.6–1.3, $P = 0.5$) (Table S1). However, ATG was associated with a significantly higher incidence of nonrelapse mortality (HR = 2.2, 95% CI: 1.5–3.4, $P < 0.001$) translating to worse OS (HR = 1.4, 95% CI: 1.1–1.9, $P = 0.02$). Specifically, 49 out of 315 patients (16%) not given ATG died within the first 100 days after transplantation because of GVHD [$n = 15$ (5%)], AML [$n = 13$ (4%)], infection [$n = 11$ (3%)], second malignancy [$n = 1$ (0.5%)] or other [$n = 9$ (3%)]. During the same time period, 74 out of 379 ATG patients (20%) died because of GVHD [$n = 12$ (3%)], AML [$n = 22$ (6%)], infection [$n = 20$ (5%)], second malignancy [$n = 3$ (1%)], haemorrhage [$n = 3$ (1%)] or other [$n = 14$ (4%)].

Discussion

A few recent studies have assessed the impact of GVHD on outcomes after UCBT. These studies assessed the impact of GVHD on transplantation outcomes throughout the study period and reported conflicting results [20–22]. Here we assessed the impact of GVHD on early (the first 18 months after transplantation) and late (thereafter) transplantation outcomes in a large cohort of AML patients given single or double UCBT by calculating the rate of relapse per patient-year for each condition within sequential 90-day intervals after allo-SCT (this method allows assessment of the evolution of the HR for the risk of relapse over time and is not affected by competing risks [23]) and by performing conventional Cox models where acute and chronic GVHD were handled as time-dependent covariates. Several observations were made.

First, there was a suggestion that both grade II–IV acute and chronic GVHD perhaps slightly decreased the incidence of relapse although it did not reach statistical significance (perhaps due to the relatively low number of patients in the two GVHD groups). Interestingly, our data suggest that the protective effects of acute and chronic GVHD on the risk of relapse do not occur at the same timing. Specifically, acute GVHD seemed to protect against GVHD only the first 6 months after

Table 3 *Multivariate Cox models II (five groups of GVHD)*

	Relapse			Nonrelapse Mortality			Mortality		
Variable	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*	HR	95% CI for HR	*P*
GVHD									
aGVHD only grade 0–I (ref)	–	–	–	–	–	–	–	–	–
aGVHD only grade II	0.85	0.58, 1.23	0.39	0.77	0.47, 1.23	0.27	0.81	0.60, 1.10	0.19
aGVHD only grade III-IV	0.71	0.34, 1.45	0.34	4.06	2.75, 6.00	<0.001	2.80	2.02, 3.87	<0.001
cGVHD limited with or without aGVHD	0.97	0.64, 1.48	0.89	1.12	0.62, 2.05	0.70	0.91	0.65, 1.27	0.58
cGVHD extensive with or without aGVHD	0.70	0.40, 1.21	0.21	5.30	3.55, 7.91	<0.001	2.25	1.69, 3.00	<0.001
Patient age at transplant	1.00	0.99, 1.01	0.71	1.02	1.01, 1.03	0.002	1.01	1.01, 1.02	<0.001
Disease status at transplant									
CR1 (ref)	–	–	–	–	–	–	–	–	–
CR2	1.10	0.79, 1.53	0.56	1.03	0.75, 1.40	0.87	1.07	0.85, 1.35	0.57
CR3+	1.92	1.10, 3.33	0.02	1.03	0.54, 1.98	0.93	1.43	0.94, 2.18	0.09
Advanced disease	3.73	2.74, 5.09	<0.001	1.43	1.03, 1.98	0.03	2.21	1.77, 2.77	<0.001
Cytogenetic									
Good (ref)	–	–	–	–	–	–	–	–	–
Interm	1.95	0.94, 4.03	0.07	0.85	0.53, 1.36	0.49	1.18	0.78, 1.78	0.43
Poor	2.88	1.33, 6.28	0.008	0.67	0.36, 1.25	0.21	1.09	0.68, 1.77	0.71
NA/failed	1.28	0.59, 2.80	0.53	1.02	0.61, 1.70	0.94	1.11	0.72, 1.72	0.64
Graft type : DCBT versus SCBT	0.98	0.75, 1.27	0.85	0.90	0.68, 1.18	0.44	0.93	0.77, 1.13	0.48
Conditioning type : RIC versus MAC	1.44	1.07, 1.95	0.02	0.61	0.45, 0.83	0.002	0.81	0.65, 1.01	0.06
ATG used : yes versus no	1.04	0.77, 1.39	0.81	1.65	1.22, 2.25	0.001	1.37	1.11, 1.70	0.004

GVHD, graft-versus-host disease; aGVHD, acute GVHD; cGVHD, chronic GVHD; CR, complete remission; DCBT, double umbilical cord blood transplantation; SCBT single umbilical cord blood transplantation; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning; ATG, anti-thymocyte globuline.

transplantation, whilst perhaps a more durable protection occurred with chronic GVHD.

Secondly and more importantly, occurrence of both acute and chronic GVHD increased overall mortality. Interestingly, separating early (≤18 months after UCBT) and late mortality, acute GVHD increased early but not late mortality, whilst chronic GVHD increased both. Interestingly, the negative impact of acute GVHD was restricted to grade III–IV acute GVHD (there was no impact of grade II acute GVHD), whilst the negative impact of chronic GVHD was limited to extensive chronic GVHD. These results differ from those recently reported by Kanda *et al.* who observed better overall survival associated with the development of grade I–II acute GVHD and/or limited chronic GVHD [21] and those reported by Chen *et al.* who observed no impact of chronic GVHD on overall survival [22]. These differences could by partly due to the fact that both adults and children were included in the study by Chen *et al.* and that the analysis of the impact of chronic GVHD on survival in the Kanda *et al.* study was stratified for prior history of acute GVHD. Importantly, our observations suggest that strategies aimed at better preventing GVHD are also requested in the UCBT setting. In our study, the use of ATG was associated with a lower incidence of grade II–IV and grade III–IV acute GVHD but not with chronic GVHD (in contrast to what has been observed when peripheral blood stem cells are used as stem

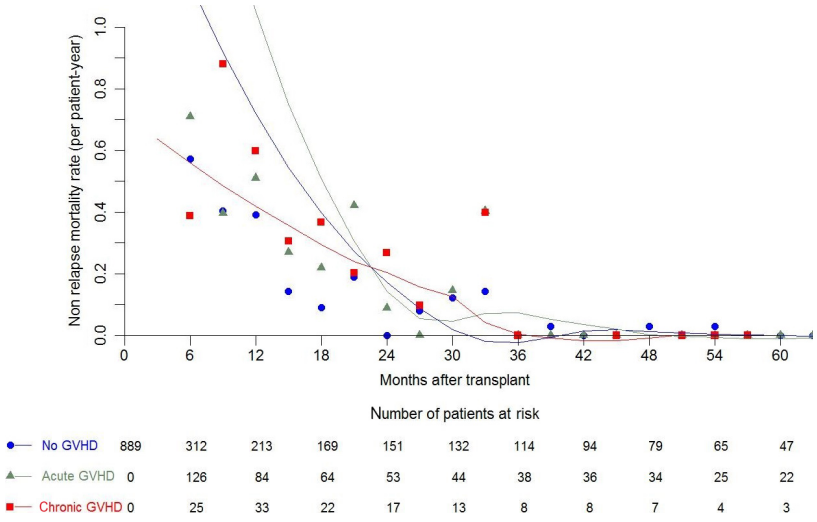

Fig. 2 *Evolution of nonrelapse mortality rates according to GVHD status. Rates were calculated within sequential 90-day intervals for patients without GVHD (shown in blue), for patients with grades II–IV acute GVHD (shown in green) or for patients with chronic GVHD (shown in red). Small symbols represent the actual relapse rates for each 90-day interval. The smoothed rates were plotted as curves for each condition.*

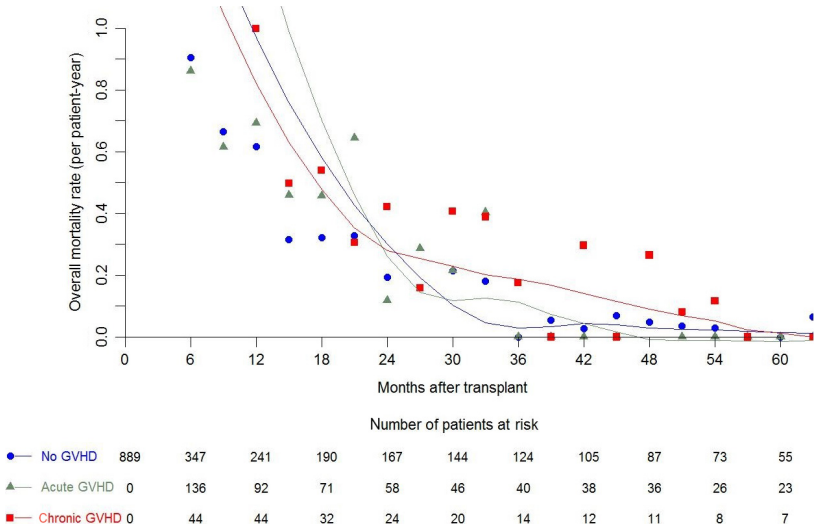

Fig. 3 *Evolution of mortality rates according to GVHD status. Rates were calculated within sequential 90-day intervals for patients without GVHD (shown in blue), for patients with grades II–IV acute GVHD (shown in green) or for patients with chronic GVHD (shown in red). Small symbols represent the actual relapse rates for each 90-day interval. The smoothed rates were plotted as curves for each condition.*

cell source [28]). Notably, despite preventing acute GVHD the use of ATG was associated with higher nonrelapse mortality leading to worse OS. This is in concordance with the results of previous studies reported by the French group of bone marrow transplantation [29, 30]. These results are also in accordance with those reported by Admiraal *et al.* who demonstrated that reducing the exposure of ATG after UCBT (allowing early CD4+ T cell recovery as observed in the PBSCT setting [31]) improved outcomes in paediatric UCBT [32]. Importantly, a recent study has observed that administration MMF at the dose of 3 g day^{-1} (instead of 2 g day^{-1}) decreased the incidence of grade II–IV acute GVHD without affecting infection-related mortality of other transplantation outcomes in the setting of double UCBT following RIC conditioning [33].

Our study also confirms observations from our group that the incidence of grade II–IV acute GVHD is higher in double than in single-UCBT recipients [34]. However, as also observed both in patients transplanted myeloablative or RIC conditioning, other transplantation outcomes were not significantly improved in patients given two cord blood units [34, 35].

Besides advanced disease status and poor risk cytogenetics, this study also observed a higher risk of disease relapse in patients transplanted following RIC versus myeloablative conditioning, as

previously reported [25]. However, interestingly, the conditioning intensity impacted the risk of relapse only the first 18 months after UCBT but had no impact thereafter. This suggests that graft-versus-leukaemia effects are the main mechanisms protecting for late relapses after UCBT. Other approaches to decrease relapse incidence after CBT might include posttransplant administration of disease-targeted medications [36–39] or of chimeric antigen receptor T cells [40].

Being a registry based study, there are some limitations in our study including heterogeneity in term of conditioning regimen and GVHD prophylaxis (we tried to assess this limitation by performing multivariate analyses), and missing data on comorbidity other than Karnofsky score, minimal residual disease and response of GVHD to therapy.

Conclusions

In summary, our study showed that despite potentially increasing graft-versus-leukaemia effects, occurrence of each grade II–IV acute or chronic GVHD increases overall mortality after UCBT for AML. This demonstrates that strategies aimed at better preventing GVHD are also requested in the UCBT setting.

Author's contribution

FBa wrote the manuscript, designed the study and interpreted the data; EB and ML designed the study, analysed and interpreted the data, and edited the manuscript; EG and AN designed the study, interpreted the data and edited the manuscript; MM and BS helped in the study design and edited the manuscript; MM, JS, SV, SF, AB, PC, JJC, MM, JS and DK reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

Conflict of interest statement

The authors declare that they have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Multivariate Cox models assessing the impact of GVHD (5 GVHD groups) in patients with neutrophil engraftment (n = 826).

Figure S1. Evolution of relapse rates according to GVHD status in patients with neutrophil engraftment.

Figure S2. Evolution of nonrelapse mortality rates according to GVHD status in patients with neutrophil engraftment.

Figure S3. Evolution of mortality rates according to GVHD status in patients with neutrophil engraftment.