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ORIGINAL ARTICLE

Long-term results of a pilot study evaluating hyperbaric oxygen therapy to improve umbilical cord blood engraftment

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Abstract

Umbilical cord blood (UCB) transplantation is a promising option for hematopoietic stem cell transplantation in patients with hematologic malignancies who lack an HLA-matched sibling or well-matched unrelated donor; however, it has a higher incidence of delayed or failed engraftment because cell doses are low and bone marrow homing is inefficient. We have demonstrated that pre-treating irradiated immune-deficient mice with hyperbaric oxygen (HBO) prior to UCB CD34+ cell transplantation lowered host systemic erythropoietin (EPO) and improved UCB CD34+ cell homing and engraftment. These findings suggested that EPO-EPO-R signaling plays a role in UCB CD34+ homing and engraftment. In a pilot clinical trial, we showed that recipients of HBO therapy prior to UCB cell infusion had reduced systemic EPO, which was associated with improved kinetics of blood count recovery. Although early clinical outcomes at day 100 were encouraging, with improved overall survival, the long-term effects of HBO therapy on UCB-transplanted patients were not evaluated. In this study, we examined the long-term outcome of patients in our pilot study, compared with a historic control group, and correlated their clinical outcomes to serum EPO response to HBO. While 50% of HBO-treated patients received single UCB units, ~ 90% of the control patients received double UCB units. Although HBO patients had much better rates of survival at 6 months, their 1-year survival did not significantly differ from the control group. HBO-treated patients had on average lower relapse and non-relapse mortality rates, and less chronic graft versus host disease (GVHD), but had increased acute GVHD. However, these differences were not statistically significant, probably because of the small sample size. In the HBO-treated cohort, immune reconstitution analysis showed significant improvement in early B cell recovery, with a trend toward improvement in early NK cell recovery. When we evaluated the ratio of 8 h to baseline EPO levels, we found a non-significant trend toward lower EPO values in those who neither relapsed nor died by 1 year, compared to those who died or relapsed. This result suggests that EPO response to HBO may be associated with better outcomes. Disease progression-free survival was also improved in those who had more than 80% reduction in EPO levels in response to HBO. Our study highlights the long-term safety of HBO therapy when used prior to UCB transplantation. Future UCB transplant patients who receive HBO should have their serum EPO response measured, as it may be a marker of relapse/mortality.

Keywords Hyperbaric oxygen · Umbilical cord blood transplantation · Pilot study · Long-term results

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole curative option for many hematologic malignancies; it may represent one of modern medicine's most innovative achievements to date [1]. In consequence, patient access to HSCT has become an increasingly recognized need. However, only 30% of patients needing allogeneic HSCT have an adequately matched sibling [2]. Options for the remaining patients are limited to transplantation from well-matched unrelated donors [3], haploidentical donors [4], or

umbilical cord blood (UCB) from unrelated donors. Among these alternatives, UCB offers many advantages: it is easy to access, it entails no donor risks, and graft versus host disease (GVHD) is less common, despite HLA disparity [5, 6]. Nevertheless, transplantation with UCB has its own major drawbacks. The cell dose available for transplantation is limited [7] and bone marrow homing of UCB cells is impaired, so delayed or failed engraftment is more common in UCB transplantation than in other types of HSCT [8, 9].

Many approaches to improving homing and/or engraftment in UCB stem cell transplantation have been investigated. Most notable are bypassing the need for homing by direct administration of cord blood into the superior-posterior iliac crest under general anesthesia [10]; inhibiting CD26 peptidase activity, which is known to enhance CXCR4/SDF-1 mediated homing [11]; or inducing fucosylation [12]. Other strategies to overcome inadequate engraftment in UCB transplantation have been the use of double-cord blood transplants [13, 14], co-infusion of selected CD34+ cells from haploidentical donors [15], co-infusion of mesenchymal stem cells [16], and UCB stem and progenitor cell expansion [17].

Recently, we have demonstrated that UCB $CD34^+$ cell exposure to erythropoietin (EPO) impairs its in vitro transmigration and promotes erythroid differentiation. Furthermore, lowering EPO in the host under hyperbaric conditions at the time of UCB infusion improves the early bone marrow homing of UCB cells [18]. In a previous preclinical study using a murine model, we demonstrated that hyperbaric oxygen (HBO) therapy prior to UCB cell infusion significantly improved engraftment of UCB $CD34^+$ human cells in the bone marrow of sub-lethally irradiated immune-deficient mice [18]. These encouraging in vivo experiments laid the foundation for our first clinical trial in humans, where we tested HBO therapy as a means to optimize engraftment in human subjects undergoing UCB transplantation. Fifteen patients with hematologic malignancies were enrolled and treated with HBO, and their day 100 outcomes were recorded. We demonstrated that HBO exposure in the clinical UCB transplantation setting was very well tolerated and was associated with significant reduction in serum EPO levels. Furthermore, patients who underwent HBO treatment showed an earlier time to neutrophil recovery, higher rates of platelet engraftment, and improved 100-day survival, compared to historic controls [19]. However, the long-term effects of HBO therapy on UCB-transplanted patients were not evaluated. In the current study, we retrospectively evaluated the same patients enrolled in our pilot clinical trial to assess their clinical outcome at 6-month and 1-year post-transplant. Our clinical follow-up study confirms the long-term safety of HBO therapy when used in UCB transplantation. We provide preliminary evidence that HBO improves early B cell recovery, and that reducing EPO levels by HBO may be associated with positive clinical outcomes.

Methods

We retrospectively evaluated patients enrolled in our pilot clinical trial conducted at the University of Kansas Cancer Center between April 2013 and March 2015. In that study, eligible patients were 17–70 years of age with a hematological malignancy for which allogeneic UCB transplantation was indicated. A total of 18 patients were screened and 15 were enrolled and treated [19]. We evaluated the 6-month and 1-year outcomes of patients who had undergone HBO, and compared the results to those of historic patients who had received standard UCB transplantation for hematologic malignancies between 8/12/2008 and 3/3/2015. We excluded patients with lymphoma, chronic lymphocytic leukemia, chronic myeloid leukemia, and myelodysplastic syndrome (MDS), because these diagnoses were rare in the HBO and control cohorts.

As previously described [19], patients enrolled in the pilot clinical trial received HBO therapy 6 h prior to UCB infusion on day 0 of their transplant. HBO therapy involved breathing 100% oxygen for 90 min in a mono-place hyperbaric chamber following compression to 2.5 ATA (atmospheric absolute). Subjects had received either myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) and were infused with either a single or double UCB unit, based on the clinical context. Clinical outcomes evaluated included incidence and severity of GVHD, steroid dependence, mortality, and overall survival. In addition, charts of HBO and control patients were reviewed for neutrophil and platelet count recovery. To evaluate immune reconstitution in the HBO and control cohorts, we collected the absolute numbers of CD3, CD4, and CD8 for T cell recovery, CD19 for B cell recovery, and CD16/65 for NK cell recovery.

Blood samples were drawn from patients undergoing HBO therapy at baseline (prior to HBO therapy) and at 6-h, 8-h, 24-h, and 48-h post-treatment. EPO levels in serum samples were measured by enzyme-linked immunosorbent assay (ELISA). Since maximum EPO reduction was detected at 8 h from the start of HBO therapy, we calculated the ratio of 8 h to baseline EPO levels for each patient, and correlated it with progression-free survival over the first year after transplant.

Statistical methods

The study population was described by summary statistics, including counts, proportions, medians, and ranges. Categorical variables were compared using Fisher's exact test, and continuous variables were compared using the non-parametric Wilcoxon rank-sum test. The Kaplan-Meier graphs summarized overall survival (OS) and progression-free survival (PFS), and the log-rank test compared survival between treatment groups. Since competing risks were present, cumulative incidence functions were used to summarize secondary outcomes in graphs of time to non-relapse mortality (NRM) and acute

GVHD, with inference based on Gray's test. Gray's test was also used to compare the cumulative incidence of infection-related mortality between the two groups. Confidence intervals presented are two-sided, and tests were performed at the two-sided 0.05 level. SAS version 9.4 was used for all analyses unless otherwise indicated. For the immune reconstitution analysis, the non-parametric Kruskal-Wallis test was used to compare the ranks of different cell subsets between both groups on days 100, 180, and 365 post-transplant, using GraphPad Prism.

Results

Study population

Fourteen patients with a median age of 45 (range 17–70, 64.3% M) underwent HBO therapy. Their outcome was compared to that of 41 control patients with a median age of 47 (range 18–71, 53.7% M). The median follow-up interval was 759 days in the HBO group and 421 days in the control group. Most patients had AML (85.7% in the HBO group vs 78.1% in the control group). Distribution of preparative regimens (myeloablative vs non-myeloablative) was comparable between the groups: 57.1% of the HBO group vs ~66% of the control group underwent non-myeloablative regimens. Even though the two groups were balanced with respect to age, gender, underlying disease, and preparative regimen, a greater number of patients in the HBO cohort had single-unit UCB transplantation (7/14) compared to the controls (4/41) ($p = 0.003$, Table 1). Our choice of single vs double units for UBC transplantation was individualized to each subject, based on published guidelines [20].

Blood count recovery

In the HBO group, 14/14 subjects experienced neutrophil recovery. The median time to recovery was 15 days. In the historic controls, 36/41 subjects recovered neutrophils with a median time to neutrophil recovery of 19.5 days. No significant difference in rates or timing of neutrophil recovery was detected ($p = 0.31$ and $p = 0.43$, respectively).

In the HBO group, 14/14 subjects experienced platelet recovery. The median time to platelet recovery was 36.5 days. In the historic controls, 29/41 subjects recovered platelets with a median time to platelet recovery of 38 days. No significant difference in timing of platelet recovery was detected ($p = 0.96$), but rates of platelet recovery were significantly different (100% in HBO vs 70.7% in historical controls, $p = 0.02$).

Survival and mortality

Survival was compared at two clinically meaningful time points: 6 months and 1 year. Six-month survival in the HBO group was 100%, compared to 67.0% in the control group (95% CI 50.1–79.4%, $p < 0.0001$, Fig. 1). Mortality in the control group was attributable to relapse (53.8%), infection (30.8%), severe refractory GVHD (7.7%), and cardiac failure related to preparative regimen (7.7%) (Table 2).

Despite these significant differences between groups in overall survival rates at 6 months, survival through 1 year was not significantly different ($p = 0.43$, Fig. 1). One-year overall survival in the HBO group was 62.3% (95% CI 31.7–82.4%), compared to 56.5% (95% CI 39.6–70.3%) in the control group. In the HBO group, five deaths were observed over 1 year of follow-up, compared to 17 deaths in the control group. We found no significant difference between groups in the cumulative incidence of relapse ($p = 0.48$) or non-relapse mortality (NRM) ($p = 0.20$) through 1-year post-

Table 1 Patients' characteristics

	HBO cohort ($N = 14$)	Control cohort ($N = 41$)	p value
Age (median (range))	45 (17–70)	47 (18–71)	0.39
Gender (N (%))			0.55
Male	9 (64.3%)	22 (53.7%)	
Female	5 (35.7%)	19 (46.3%)	
Diagnoses (N (%))			0.71
AML	12 (85.7%)	32 (78.1%)	
ALL	2 (14.3%)	9 (22.0%)	
Single vs double cords (N (%))			0.003
Single	7 (50.0%)	4 (9.8%)	
Double	7 (50.0%)	37 (90.2%)	
Preparative regimen (N (%))			0.75
Myeloablative	6 (42.9%)	14 (34.2%)	
Non-myeloablative	8 (57.1%)	27 (65.9%)	

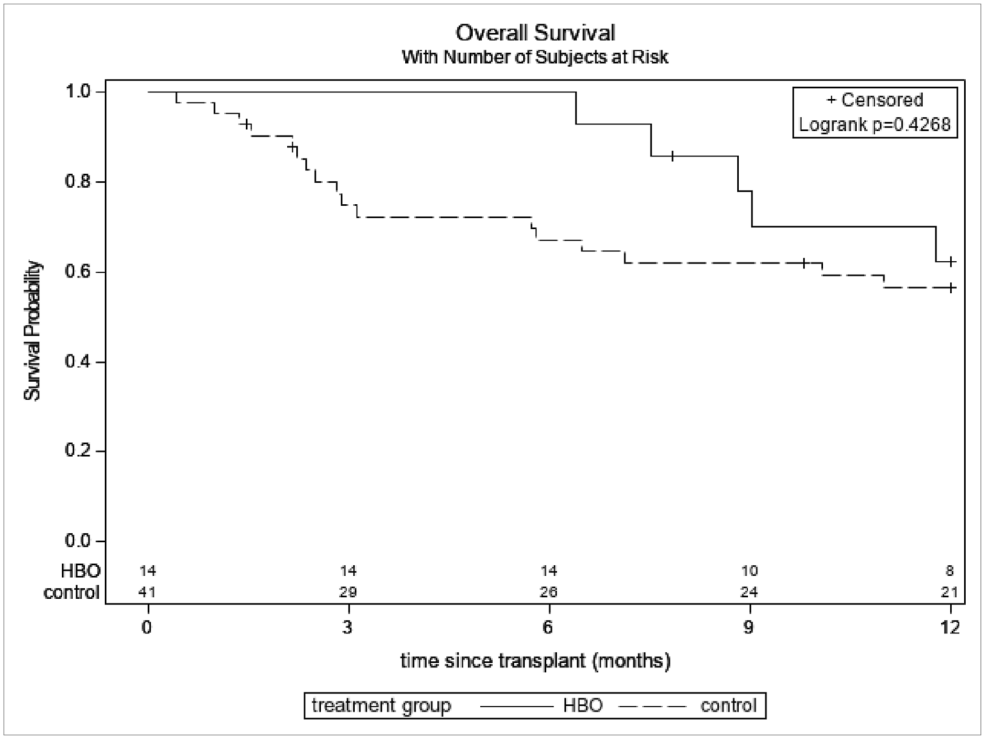

Fig. 1 Overall survival in HBO group, shown in solid line, compared to our control group, shown in dotted line

transplant. The 1-year cumulative incidence of relapse was 28.6% (95% CI 8.2–53.4%) in the HBO group compared to 39.8% (95% CI 24.5–54.7%) in the control group. The 1-year cumulative incidence of NRM was 7.9% (95% CI 0.4–31.5%) in the HBO group compared to 22.4% (95% CI 10.9–36.4%) in the control group (Table 3). The sole non-relapse-related death in the HBO group occurred on day 269 post-transplant and was attributable to pneumonia, septic shock,

Table 2 Clinical outcomes at 6 months

	HBO cohort ($N = 14$)	Control cohort ($N = 41$)	p value
Overall survival	100%	67.0% (50.1%, 79.4%)	< 0.0001
Cause of death			
Relapse	N/A	7 (53.8%)	
Infection	N/A	4 (30.8%)	
GVHD	N/A	1 (7.7%)	
Preparative regimen-related	N/A	1 (7.7%)	
Acute GVHD	85.7% (46.6%, 96.9%)	77.1% (57.6%, 88.5%)	0.45
Grade III/IV acute GVHD	21.4% (4.5%, 46.4%)	34.3% (19.0%, 50.1%)	0.33
Grade of acute GVHD			0.33
I	4/12 (33.3%)	4/26 (15.4%)	
II	5/12 (41.7%)	10/26 (38.5%)	
III	3/12 (25.0%)	7/26 (26.9%)	
IV	0	5/26 (19.2%)	
Steroid usage (conditional on 6-month survival)			1.00
Steroid-free	11/14 (78.6%)	19/26 (73.1%)	
On steroids	2/14 (14.3%)	5/26 (19.2%)	
Steroid data missing	1/14 (7.1%)	2/26 (7.7%)	

Data shown are estimated survival probabilities, or cumulative incidences, with 95% confidence intervals. Counts are shown with associated within cohort proportions

Table 3 Clinical outcomes at 1-year

A-Relapse and GVHD at 1-year:			
Outcome	HBO Cohort (N=14)	Control Cohort (N=41)	*p*-value
Overall Survival	62.3% (31.7%, 82.4%)	56.5% (39.6%, 70.3%)	0.43
Relapse	28.6% (8.2%, 53.4%)	39.8% (24.5%, 54.7%)	0.48
NRM	7.9% (0.4%, 31.5%)	22.4% (10.9%, 36.4%)	0.20
Acute GVHD	85.7% (46.6%, 96.9%)	77.1% (57.6%, 88.5%)	0.45
Chronic GVHD (conditional on 12 month survival)	4/8 (50.0%)	12/21 (57.1%)	1.00
B- Cause of Death at 1-year:			
Cause of Death	HBO Cohort (N=14)	Control cohort (N=41)	*p*-value
Relapse	3 (60.0%)	8 (47.1%)	1.00
Graft Failure	1 (20.0%)	2 (11.8%)	
Infection	1 (20.0%)	5 (29.4%)	
GVHD	0	1 (5.9%)	
Drug Toxicity	0	1 (5.9%)	

Data shown are estimated survival probabilities, or cumulative incidences, with 95% confidence intervals. Counts are shown with associated within cohort proportions

and ensuing acute respiratory failure. The cumulative incidence of mortality from infections between the two groups was not significantly different ($p = 0.56$, Fig. 2).

Acute GVHD

All acute GVHD episodes occurred during the first 6 months of transplant, so the cumulative incidences of acute GVHD at 6 months and 1 year were equivalent. The 1-year cumulative incidence of acute GVHD was 85.7% (95% CI 46.6–96.9%) in the HBO group compared to 77.1% (95% CI 57.6–88.5%) in the control group ($p = 0.45$, Fig. 3). In the HBO group, the proportions of subjects (total $n = 12$) who experienced acute GVHD grades I, II, III, and IV were 33.3%, 41.7%, 25.0%, and 0%, respectively. In comparison, in the control group, the proportions of patients (total $n = 26$) who experienced acute GVHD grades I, II, III, and IV were 15.4%, 38.5%, 26.9%, and 19.2%, respectively. Statistically, the severity of GVHD was not significantly different between the groups ($p = 0.33$). Of the 14 surviving HBO patients at 6-month post-transplant, 11 (78.6%) remained steroid-free, compared to 19 of the 26 surviving control subjects (73.1%) ($p = 1.00$, Table 2).

Chronic GVHD

Four of the eight HBO subjects alive at 1-year post-transplant had chronic GVHD (50.0%) compared to 12 of the 21 surviving control subjects (57.1%) ($p = 1.00$, Table 3). Extensive chronic GVHD was seen in 75.0% (3/4) of HBO group patients, compared to 38.4% (5/13) in the control group ($p = 0.22$).

Secondary graft failure

No patients in either group experienced secondary graft failure.

Immune reconstitution

We found a trend toward delayed CD3 and CD8 cell recovery in the HBO group ($p = 0.07$ and 0.07, respectively) up to day 180 (Fig. 4). By day 365 post-transplant, no significant differences in CD3 and CD8 recovery were observed between both groups. CD4 cell recovery was significantly less in the HBO group after day 100 ($p = 0.048$). On the other hand, we found a significant delay in reconstitution of CD19 cells in the control group compared to the HBO group ($p < 0.001$) on day +100 post-transplant. Also, a trend toward early reconstitution of NK cells (CD16/65) was seen in the HBO group, but it did not reach statistical significance ($p = 0.091$) (Fig. 4).

Correlation between EPO reduction in response to HBO therapy and clinical outcomes in the HBO cohort

Exploratory analyses were performed to investigate the association between EPO reduction in response to HBO therapy and clinical outcomes in the HBO group. For the whole HBO cohort, median EPO level was 33.38 mU/mL at baseline (prior to HBO) and 9.98 mU/mL at 8 h, with a median 8-h/baseline EPO ratio of 0.57. We used ratios of 8-h/baseline EPO to split the HBO cohort into a high-reduction group (> 80%, $n = 4$) and a low-reduction group (< 80%, $n = 10$). The progression-free survival (PFS) was

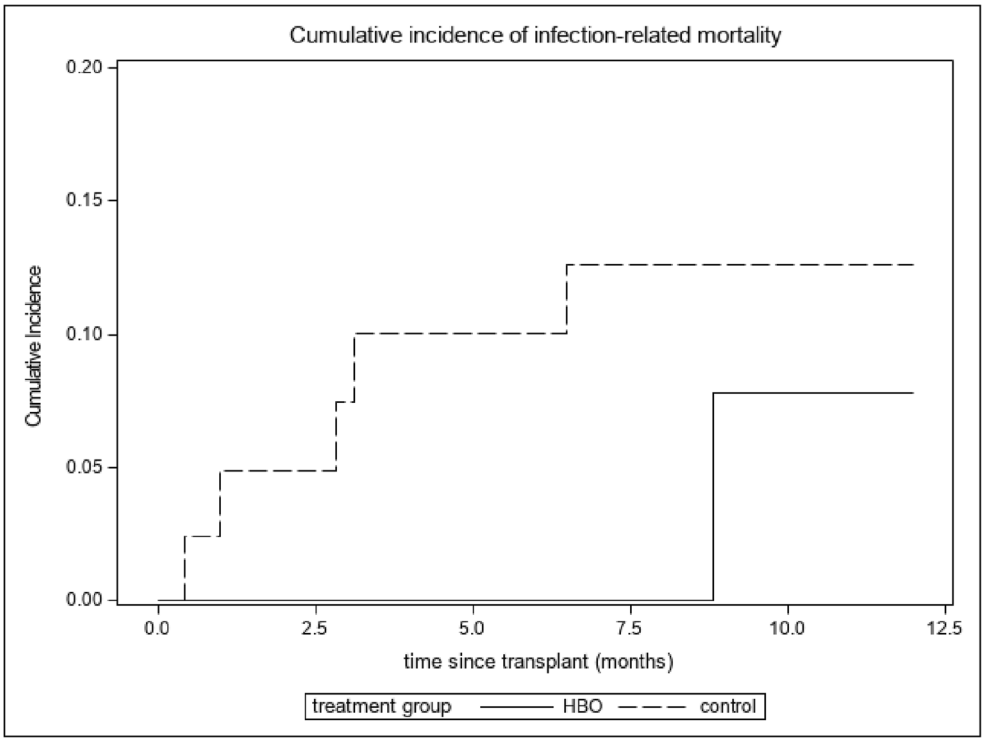

Fig. 2 Cumulative incidence of infection-related mortality in HBO group (solid line) compared to the control group (dotted line). ($p = 0.58$)

better, though not significantly at the 0.05 level, in patients who had high EPO reduction ($p = 0.09$, Fig. 5). Furthermore, excluding the data from one subject who was lost to follow-up prior to 1 year, we compared the median ratio of 8-h/baseline EPO between subjects who died or relapsed prior to 1 year and subjects who were alive and relapse-free at 1 year. The median ratio was 0.45 (range 0.27–0.81) in survivors who neither relapsed nor died at 1 year ($N = 8$), compared to 0.91 (range 0.38–0.95) in those who died or relapsed ($N = 5$, $p = 0.09$). While this finding indicates a trend toward poorer outcomes for subjects with higher ratios, and therefore lower reductions in

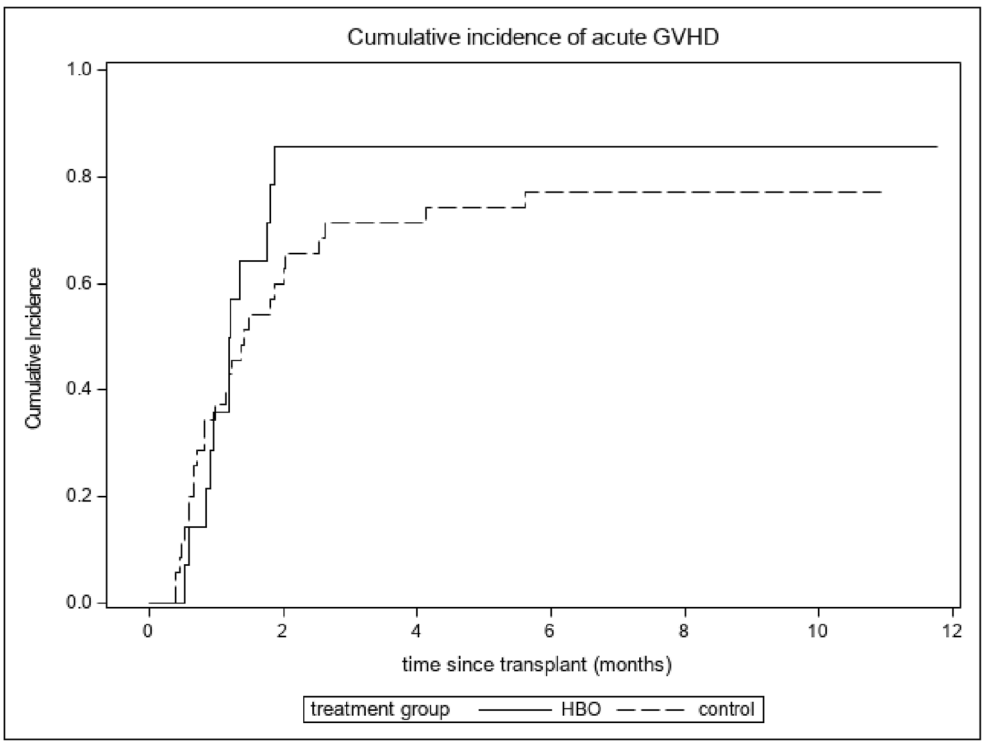

Fig. 3 Cumulative incidence of acute graft versus host disease (GVHD) in HBO group (solid line) compared to the control group (dotted line)

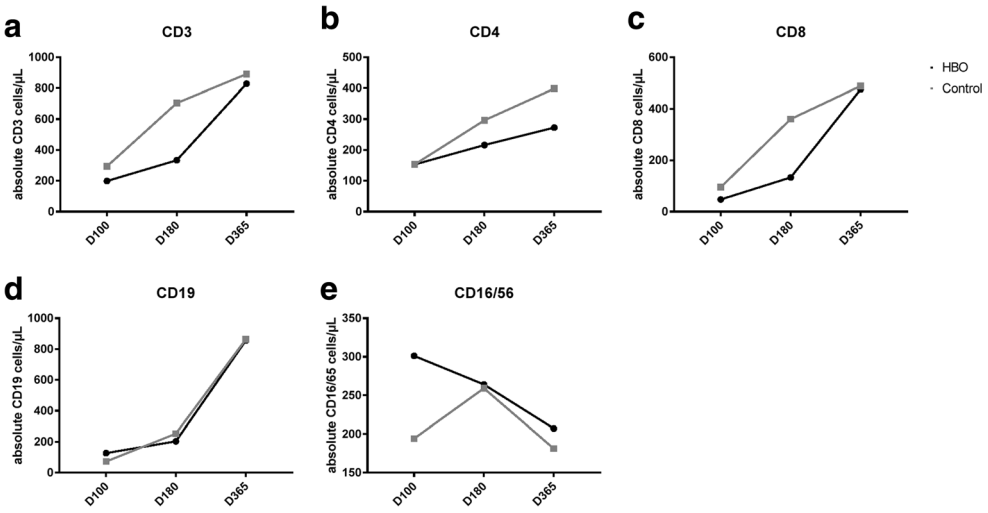

Fig. 4 Immune reconstitution in the HBO cohort compared to the control cohort. Median absolute CD3 (**a**), CD4 (**b**), CD8 (**c**), CD19 (**d**), and CD16/56 (**e**) cells on days 100, 180, and 365 post-transplant. The sample sizes for post-transplant days 100, 180, and 365 were 11 vs 25, 10 vs 21, and 6 vs 14 for the HBO (black line) and control (gray line) cohorts, respectively

EPO, the small sample size limits the power of these exploratory analyses).

Discussion

In the present study, we extend our follow-up of a cohort of subjects who underwent HBO treatment and UCB transplantation, examining their 6-month and 1-year outcomes. First, the results show the long-term safety of this intervention. Most important, we saw no deaths in the HBO cohort at 6 months. This milestone is important as most UCB transplant-related deaths occur in the first 6 months, usually secondary to infections [21]. We found that HBO treatment was associated with significantly better survival at 6 months, but not at 1 year. In exploratory analyses, we also found preliminary evidence of a positive correlation between EPO reduction following HBO therapy and clinical outcomes.

However, this study is limited because it is retrospective in nature and the historical controls were derived from a larger timespan (5 years vs 2 years in the case of the HBO cohort) during which patients were treated. This difference could potentially explain some of the observed differences.

Although our HBO-treated patients demonstrated a higher rate of GVHD than the control group, they had a trend toward earlier presentation and less severity with no HBO patients

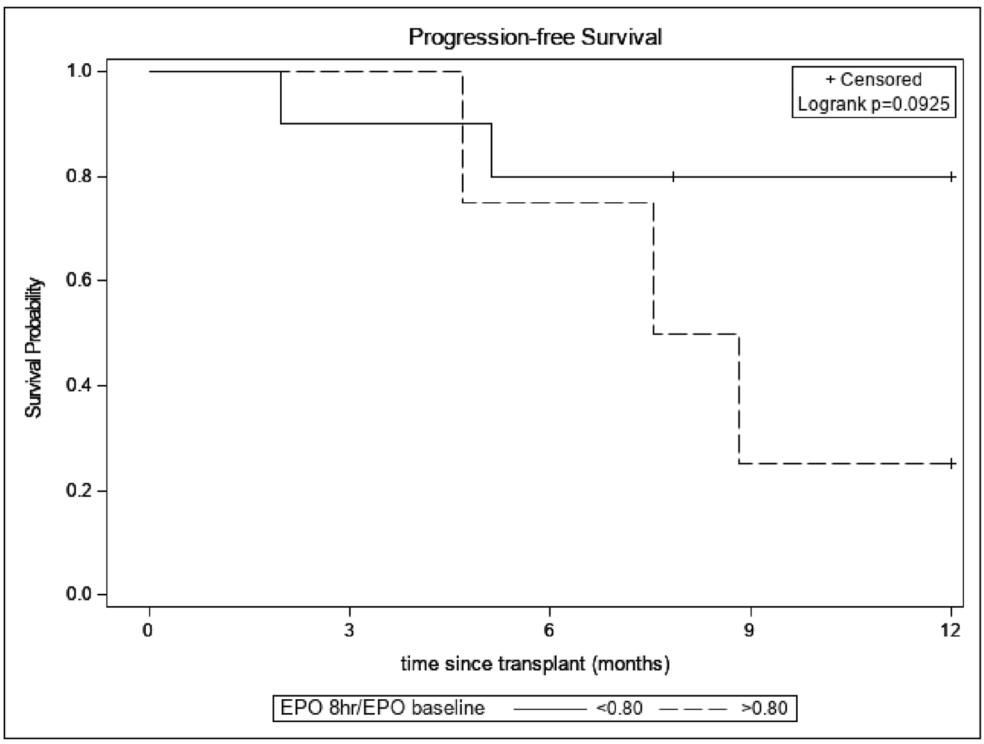

Fig. 5 EPO reduction in response to HBO and progression-free survival. Progression-free survival based on the ratio of 8-h/baseline EPO, which was used to divide the HBO cohort into a high-reduction group (> 0.80 reduction, $n = 4$, dotted line) and a low-reduction group (< 0.80 reduction, $n = 12$, full line)

with grade IV GVHD. The earlier onset of acute GVHD in patients receiving HBO therapy might potentially be attributed to earlier onset of immune reconstitution driven by the graft. However, our immune reconstitution analysis did not capture early recovery of immune function in the first 60 days, when all our HBO patients developed acute GVHD.

Earlier immune reconstitution might explain the absence of infection-related mortality in the HBO cohort at 6 months. Interestingly, our immune reconstitution analysis showed improved B cell and NK cell recovery, but delayed T cell recovery, in the HBO cohort. Higher NK cell number early post-transplant was reported to be associated with improved transplant-related mortality [22]. One explanation for delayed T cell recovery in our HBO cohort is because our HBO cohort had significantly more single-cord blood transplants, while our control cohort had more double-cord blood transplants. Other studies have suggested that improved T cell recovery, especially CD4+ T cells, occurs in double- vs single-cord blood transplants [23]. Also, more patients in our HBO cohort had acute GVHD and required steroids for treatment, which might explain their delayed T cell recovery. Nonetheless, a higher proportion of our HBO group was steroid-free at 6 months, thus avoiding the numerous side effects and complications of steroids, including infections.

In our study, we saw apparent differences in disease relapse, but they were not statistically significant in this small sample. This observation might be related to the improved NK cell recovery seen in our HBO cohort, given that lower rates of relapse have been reported in association with higher early NK cell recovery [22]. In addition, HBO effects on EPO might play a role in the reduced relapse rate in our HBO cohort, as explained below.

Our finding of a correlation between EPO reduction following HBO therapy and positive clinical outcomes underscores the benefits of targeting EPO with HBO, as patients who neither relapsed nor died at 1 year had a 55% median drop in EPO from baseline (prior to HBO treatment), while patients who either died or relapsed had only a 9% median drop in EPO from baseline. This finding suggests that recipients of HBO and UCB transplantation should have their serum EPO response analyzed as a potential predictor of relapse/mortality. Takeshita et al. not only demonstrated the expression of EPO-R in almost 60% of acute leukemia cells, but also reported that in vitro treatment of these cells with EPO leads to their proliferation [24]. Furthermore, they showed that patients expressing EPO-R and exhibiting an in vitro response to the addition of EPO had a significantly higher propensity toward earlier relapse [25]. These findings are consistent with our finding that higher EPO levels may predict relapse.

Despite the lack of statistical significance for most of the examined clinical endpoints, we would like to point out that most of our HBO-treated patients received single UCB units for their transplantation, while most of control group patients received double UCB units, a difference that was statistically significant. The use of fewer UCB units in our HBO-treated, UBC-transplanted patients, if this does not compromise clinical outcomes, is likely to reduce treatment costs, which is an important consideration given the high cost of UCB transplantation [26].

In summary, the long-term follow-up of our pilot study investigating HBO as a modality to improve engraftment following UCB transplantation confirms the long-term safety of this intervention. Our assessment of long-term outcomes and the effects of EPO response to HBO therapy on these outcomes have produced complex results that will we will explore further in a prospective randomized phase II study of this adjunctive treatment for patients who will receive UCB transplantation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments, or comparable ethical standards.

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