Diffuse alveolar hemorrhage is most often fatal and is affected by graft source, conditioning regimen toxicity, and engraftment kinetics

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ABSTRACT

Diffuse alveolar hemorrhage after hematopoietic stem cell transplantation is a frequently fatal complication with no standard therapy. Although significant changes in supportive and intensive care measures for patients undergoing hematopoietic stem cell transplantation have been made over the past decades, the impact of these changes on the incidence and outcome of patients with diffuse alveolar hemorrhage has not been examined. We analyzed 1228 patients who underwent allogeneic hematopoietic stem cell transplantation between 2008-2015 at the University of Minnesota to study the incidence, risk factors, and outcomes of diffuse alveolar hemorrhage. Diffuse alveolar hemorrhage developed in 5% of allogeneic hematopoietic stem cell transplant recipients, at a median of 30 days (range +3 to +168 days) after transplantation. The incidence of diffuse alveolar hemorrhage was significantly greater in recipients of umbilical cord blood than peripheral blood or bone marrow grafts (HR: 2.08, 95% CI: 1.16-3.74; P=0.01). In multivariate analysis, delayed neutrophil engraftment or primary graft failure was a risk factor for diffuse alveolar hemorrhage following peripheral blood or bone marrow hematopoietic stem cell transplants (HR: 5.51, 95% CI: 1.26-24; P=0.02) and delayed platelet engraftment was associated with significantly increased diffuse alveolar hemorrhage in umbilical cord blood transplant recipients (HR: 6.96, 95% CI: 2.39-20.29; P<0.05). Myeloablative regimens including total body irradiation were also risk factors for diffuse alveolar hemorrhage (HR: 1.8, 95% CI: 1.03-3.13, P=0.05) in both peripheral blood or bone marrow and umbilical cord blood hematopoietic stem cell transplants (HR: 1.87, 95% CI: 0.95-3.71). Patients with diffuse alveolar hemorrhage had an inferior 6-month treatment-related mortality (HR: 6.09, 95% CI: 4.33-8.56, P<0.01) and 2-year overall survival (HR: 4.16, 95% CI: 3.06-5.64; P<0.01) using either graft source. The etiology of diffuse alveolar hemorrhage is multifactorial, involving lung injury influenced by high-dose total body irradiation, graft source, and delayed engraftment or graft failure. The survival of patients with diffuse alveolar hemorrhage after hematopoietic stem cell transplantation remains poor. Clinical interventions or experimental studies (e.g., cell expansion for umbilical cord blood transplants or thrombopoietin use) that modulate these risk factors may limit the incidence and improve the outcomes of diffuse alveolar hemorrhage.

Introduction

Pulmonary complications occur frequently in patients after hematopoietic stem cell transplantation (HCT). Diffuse alveolar hemorrhage (DAH) is a serious pulmonary complication with a high mortality rate after HCT.[1,2] The incidence of DAH varies between 3% and 10% after allogeneic HCT.[3,4]

The clinical symptoms of DAH include cough, hypoxemia, fever, and rarely hemoptysis.[5] Chest radiography shows non-specific bilateral areas of ground-glass attenuation and patchy areas of consolidation.[6] Bronchoalveolar lavage findings are characteristic for the diagnosis of DAH with progressively bloodier lavage returns. The etiology of DAH remains unknown, but lung tissue injury, inflammation and cytokine release have all been implicated in the pathogenesis of DAH.[5] Some may require that no microorganism is isolated from the bronchoalveolar lavage fluid; however, DAH syndromes after allogeneic HCT have been shown to have similar clinical characteristics and presentation with or without an associated infection.[3,7]

The reported outcomes of patients with DAH after allogeneic HCT have been dismal. Generally, patients with DAH require intensive care unit support and often mechanical ventilation because of severe hypoxemia. The mortality rate can reach 70-100% in patients with DAH because of multiorgan failure and/or sepsis.[5,8-10]

Recent changes in allogeneic HCT and supportive care practices include: (i) more frequent use of alternative donor allografts [e.g., umbilical cord blood (UCB) and haploidentical donors]; (ii) use of reduced intensity conditioning for older patients or those with comorbidities; (iii) advances in the use of antibiotics targeting fungal and viral infection; and (iv) improvements in intensive care unit support. We therefore evaluated the impact of these changes on the contemporary incidence, risks, and outcomes of DAH after allogeneic HCT.

Methods

All patients who received peripheral blood stem cells (PB), bone marrow (BM) or UCB HCT between 2008 and 2015 at the University of Minnesota were included. The data were prospectively collected in our institutional Blood and Marrow Transplant Database on patients who had provided written informed consent to Institutional Research Board-approved studies, supplemented by individual medical record review as needed.

Definitions

Patients received myeloablative or reduced intensity conditioning regimens (based on age ≥55 years or significant comorbidities, as previously described).[11] The dose of total body irradiation (TBI) differed, being 1320 cGy in fractionated doses for myeloablative conditioning and 200 cGy for reduced intensity conditioning regimens. UCB HCT grafts were matched at four to six loci of six HLA-A, -B (antigen level), and -DRB1 (allele level) to the recipient and in patients receiving two UCB units were generally similarly matched to each other.[12] HLA-matched was defined as 6/6 (or 4-5/6 + 5-6/6 for double UCB) and 8/8 for PB and BM graft sources with graft nucleated cell doses and CD34 content as previously described.[13,14] Standard risk was defined as patients with leukemia, lymphoma or other malignancy in first or second complete remission, chronic myelogenous leukemia in first chronic phase,

myelodysplastic syndrome without excess blasts, or nonmalignant diseases. All other patients were considered high risk.

DAH was diagnosed by strict clinical criteria and alveolar lavage in all patients during fiberoptic bronchoscopy. The criteria included acute onset of hypoxemia with presence of diffuse pulmonary infiltrates on a chest X-ray or computed tomography scan and the presence of progressively bloodier return with each subsequent bronchoalveolar lavage.[2,15] Aliquots of saline were successively instilled and aspirated and then visually examined to detect the presence of hemorrhage. The bronchoalveolar lavage fluid was submitted for cytological and microbiological examination. Cytological evaluation included specimen review using potassium hydroxide, Giemsa, and Papanicolaou stains. Appropriate microbiological and cytological examinations for bacteria, fungi, mycobacteria, *Pneumocystis jiroveci*, and viruses were also performed. For this study, we classified all patients as having DAH regardless of documented infection from bronchoalveolar lavage given our previous analysis demonstrating that infection-associated alveolar hemorrhage and DAH were related clinical syndromes with similar clinical presentation and risks.[3]

Supportive measures for the management of alveolar hemorrhage included correction of platelet and coagulation abnormalities, careful maintenance of fluid and electrolyte balance, and aggressive ventilator and oxygen support. All patients received prophylactic and empiric antimicrobial agents as clinically indicated. Corticosteroids, administered in most patients (>90%), consisted of a standard regimen of high-dose methylprednisolone, 500 mg twice a day for 3 days, followed by 250 mg twice a day for 3 days, 125 mg twice a day for 3 days, 60 mg twice a day for 3 days, and then 60 mg once a day tapered off over a 2-month period. Pediatric patients also received a similar dose and schedule of methylprednisolone with the dose of methylprednisolone adjusted per body surface area (i.e., starting dose 250 mg/m^2/dose intravenously, twice a day for 3 days).

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count ≥0.5×10^9/L. Event times for neutrophil and platelet recovery were measured from the date of transplantation and were censored for death or disease progression before day 21 without neutrophil recovery. Platelet engraftment was defined as the first day when the platelet count was >20×10^9/L and subsequently remained so without transfusions for 7 days. Neutrophil counts that never decreased below 0.5×10^9/L and platelet counts that never decreased below 20×10^9/L (without transfusion support) were defined as indicating engraftment at day +1 for either cell lineage. Primary graft failure was defined as a lack of neutrophil recovery at day 42 or less than 10% marrow reconstitution of donor origin, even with neutrophil recovery.

Statistical analysis

The baseline characteristics of the patients and their transplants and information on post-transplantation complications and outcomes were prospectively collected by the Biostatistical Support Group at the University of Minnesota using standardized collection procedures. Demographic and transplant characteristics were summarized by standard descriptive statistical methods. The statistical comparison of categorical variables was performed using a chi-square test, while the Kruskal-Wallis (Wilcoxon) rank-sum test was used for comparisons of continuous variables between patients with and without DAH.

A cumulative incidence estimator was used to calculate the probabilities of neutrophil engraftment, DAH and infection, reflecting non-event deaths as a competing risk. The cumulative incidence of treatment-related mortality was also calculated, reflecting relapse as a competing risk.[16] Fine and Gray regression analysis was used to compare the differences between cumulative

incidence curves for the endpoints of neutrophil engraftment, treatment-related mortality, DAH and infection.[17] The Kaplan-Meier method was used to estimate the probabilities of disease-free survival and overall survival through 2 years after allogeneic HCT and the log-rank test was used for univariate comparisons.[18] A Cox proportional hazard regression model was used to estimate differences between the adjusted survival curves,[19] with DAH being treated as a time-dependent variable.

Factors that were considered in regression models included gender (male *versus* female), age at transplant (0-35 years *versus* ≥35 years), recipient's cytomegalovirus serostatus (positive *versus* negative), intensity of the transplant conditioning regimens (myeloablative *versus* reduced intensity), graft source (PB/BM *versus* UCB), TBI use (yes *versus* no), composite factor of TBI and conditioning intensity, diagnosis (malignant *versus* nonmalignant), neutrophil engraftment (treated as a time-dependent variable), platelet engraftment (treated as a time-dependent variable), disease risk (standard risk *versus* high risk) and greatest mismatch for HLA disparity considering the worst matched of the two UCB units (matched *versus* mismatched). Factors with a univariate *P*-value <0.15 or those with established potential clinical importance were included in the multivariate analysis. Prognostic factor models for all clinical outcomes were built using a backward selection method (*P*<0.05 was considered significant for remaining in the model). All statistical analyses were implemented using Statistical Analysis System statistical software version 9.3 (SAS Institute Inc., Cary, NC, USA). The cut-off significance level for all *P* values was 0.05.

Results

A total of 1228 patients undergoing allogenic HCT were included in the study: 658 received PB/BM grafts and 570 received UCB grafts. There were significant differences between PB/BM recipients and UCB graft recipients regarding patient- and disease-characteristics (*Online Supplementary Table S1*). The median total nucleated cell count was higher and the median time-to-engraftment was shorter in PB/BM graft recipients than in UCB graft recipients.

DAH was diagnosed in 59 patients (5%) (Table 1) at a median of 30 days (26 days for BM/PB and 33 days for UCB grafts) after HCT. The median time from HCT to neutrophil engraftment was 19 days (14 days for BM/PB and 22 days for UCB) while that for platelet engraftment was 63 days (20 days for BM/PB and 84 days for UCB grafts). Fifty-one percent of patients had DAH within the 2 weeks preceding or after neutrophil engraftment. Seventy-nine percent of patients with DAH had no platelet engraftment at the time of DAH.

The patients with DAH had a median age of 32 years, 66% were male, 85% had a malignant disease, 42% had a history of smoking, 5.1% had a history of pre-HCT lung disease, 54% were seropositive for cytomegalovirus, 59% received myeloablative conditioning, 86% received TBI, 64% were HLA-matched and 20% had a sibling donor. At the time of DAH, the median platelet count was 24×10^9/L (range, 1.0-114), 34% had renal dysfunction (creatinine >1.3 mg/dL), 34% had abnormal liver function (alanine transaminase >35 U/L), 8% had an elevated international normalized ratio >1.5, 25% had a prolonged partial thromboplastin time (≥37 s, although in most cases it was <40 s), and no tested patient (n=37) had a low fibrinogen level <180 mg/dL. Forty-six percent of patients had fever,

27% had a documented systemic infection, and in 19% of cases an infectious organism was isolated from the bronchoalveolar lavage fluid along with the findings of DAH. In patients with DAH, the incidence of grade II-IV acute graft-*versus*-host disease (GvHD) by day +100 was 36%, with most cases of grade II-IV GvHD (69.5%) occurring

Table 1. Patient and transplant characteristics.

		No DAH n (%)	DAH n (%)	*P* value
Number		1169 (95%)	59 (5%)	
Sex	Male	708 (61)	39 (66)	0.40
	Female	461 (39)	20 (34)	
Age at HCT (years)	0-35	591 (51)	31 (52)	0.77
	≥35	578 (49)	28 (47)	
Malignant disease	Yes	830 (71)	50 (85)	**0.02**
	No	339 (29)	9 (15)	
Disease risk	Standard	800 (68)	36 (61)	0.23
	High	369 (32)	23 (39)	
Recipient CMV serology	Positive	651 (56)	32 (55)	0.87
	Negative	506 (44)	26 (45)	
Conditioning intensity	MAC	534 (46)	35 (59)	**0.04**
	RIC	635 (54)	24 (41)	
TBI	Yes	865 (74)	51 (86)	**0.03**
	No	304 (26)	8 (14)	
Conditioning intensity & TBI	MAC TBI	339 (29)	28 (47)	**0.01**
	MAC no TBI	195 (17)	7 (12)	
	RIC	635 (54)	24 (40)	
Graft source	BM/PB	639 (55)	19 (32)	**<0.01**
	UCB	530 (45)	40 (68)	
Graft source	PB	342 (53)	10 (53)	0.97
	BM	297 (47)	9 (47)	
Graft source & conditioning intensity	BM/PB-MAC	261 (49)	10 (29)	**0.02**
	UCB-MAC	273 (51)	25 (71)	
Graft cell dose infused				
TNC x10⁸/kg, median (range)	BM/PB	5.22 (0.01-35.50)	3.10 (0.02-12.57)	**0.01**
	UCB	0.46 (0.03-99.01)	0.39 (0.21-2.04)	**0.01**
CD34 dose, median (range)		2.67 (0.01-64.46)	0.95 (0.02-10.14)	**<0.01**
HLA matching	M BM/PB	543 (46%)	13 (22%)	**<0.01**
	MM BM/PB	96 (8%)	6 (10%)	
	UCB	530 (45%)	40 (68%)	
Post-transplant events				
Neutrophil engraftment (n)		1099	45	
Median days (range)		14 (0-42)	19 (0-41)	**<0.01**
Platelet engraftment (n)		984	22	
Median days (range)		30 (0-175)	63 (0-168)	**<0.01**
Engraftment day +1				
Neutrophils, n (%)	Yes	96 (8.2%)	1 (1.6%)	**0.04**
	No	1073 (91.8%)	58 (98.4%)	
Platelets, n (%)	Yes	18 (1.5%)	1 (1.6%)	0.61
	No	1151 (98.5%)	58 (98.4%)	
Graft failure	Yes	51 (4%)	11 (19%)	**<0.01**
	No	1118 (96%)	48 (81%)	
Acute GvHD grade II-IV (n)		383	23	
Median days (range)		35 (11-174)	34 (14-148)	0.40
<day 100 (n=360)		360 (31)	21 (36)	0.13

BM: bone marrow; CMV: cytomegalovirus; DAH: diffuse alveolar hemorrhage; GvHD: graft-*versus*-host disease; HCT: hematopoietic stem cell transplantation; M: matched; MAC: myeloablative conditioning; MM: mismatched; TNC: total nucleated cell count; PB: peripheral blood; RIC: reduced-intensity conditioning; TBI: total body irradiation; TRM: treatment-related mortality; UCB: umbilical cord blood. Significant differences are shown in bold.

prior to DAH. Most patients (92%) received high-dose steroids for the treatment of DAH and seven patients (12%) additionally received an anti-tumor necrotizing factor: etanercept in six cases and infliximab in one. In three patients, anti-tumor necrotizing factor was mainly used for the treatment of concurrent severe gut GvHD. Seventy-five percent of the patients required mechanical ventilator support.

Risk factors

DAH occurred more often in patients who received UCB grafts, TBI at a myeloablative dose, and HLA-mismatched donor grafts: it was also more common among those who had a malignant disease (Table 1). The median time to platelet engraftment (63 days *versus* 30 days) and to neutrophil engraftment (19 days *versus* 14 days) was significantly delayed in the group with DAH compared with the group that did not develop DAH ($P<0.01$ for each). Primary graft failure was significantly more frequent in the DAH group (Table 1). Only 1.6% of patients who never experienced severe thrombocytopenia had DAH compared with 8.2% of patients with severe thrombocytopenia ($P=0.04$). The median graft cell dose was significantly lower in the group that had DAH (3.1×10^8/kg) than in the group that did not develop DAH (5.22×10^8/kg) ($P<0.01$) among PB/BM HCT recipients, whereas among UCB HCT recipients it was lower in those with DAH than in those without (0.39×10^8/kg *versus* 0.46×10^8/kg, respectively; $P<0.01$) (Table 1). Sex, age, disease risk, recipient cytomegalovirus serology, and the incidence of acute GvHD were all similar between the groups with and without DAH.

DAH was observed in 7% (40/570) of the UCB graft recipients (Table 2). These UCB graft recipients received myeloablative TBI more often than UCB graft recipients without DAH did (90% *versus* 76%; $P=0.05$), more often had double UCB grafts (90% *versus* 62%; $P<0.01$) and received fewer cells/kg recipient weight [0.39×10^8/kg (range, 0.21-2.04×10^8/kg) *versus* 0.46×10^8/kg (range, 0.03-99.01×10^7/kg; $P<0.01$]. Neutrophil and platelet engraftment failure (by the time of death or DAH) was strongly associated with DAH (30% *versus* 9%, and 70% *versus* 21%, respectively, in the groups with and without DAH) (Table 2).

Multivariate analysis showed that UCB HCT recipients had a 2-fold higher incidence of DAH than PB/BM HCT recipients (HR: 2.08, 95% CI: 1.16-3.74; $P=0.01$). Delayed neutrophil engraftment or graft failure was a risk factor for DAH in PB/BM HCT recipients (HR: 5.51, 95% CI: 1.26-24; $P=0.02$) whereas delayed platelet engraftment was associated with significantly increased DAH in UCB HCT recipients (HR: 6.96, 95% CI: 2.39-20.29; $P<0.05$) (Table 3A,B). Two different engraftment models were tested because neutrophil engraftment was strongly correlated with platelet engraftment. TBI at a myeloablative dose was also a risk factor for DAH (HR: 1.8, 95% CI: 1.03-3.13; $P=0.05$), an effect that was more pronounced in UCB HCT recipients (HR: 1.87, 95% CI: 0.95-3.71; $P=0.08$) than in BM/PB HCT recipients (HR: 1.63, 95% CI: 0.62-4.31; $P=0.60$) (Table 3A,B).

Treatment-related mortality and survival

DAH was associated with higher treatment-related mortality at 6 months (HR: 6.09, 95% CI: 4.33-8.56; $P<0.01$) and a lower overall survival at 2 years (HR: 4.16,

95% CI: 3.06-5.64; $P<0.01$) following HCT with either graft source (PB/BM or UCB). UCB was also a risk factor for poorer treatment-related mortality at 6 months (HR: 1.43, 95% CI: 1.08-1.9; $P=0.01$) and overall survival (HR: 1.22, 95% CI: 1.02-1.48; $P=0.03$).

A total of 44 patients with DAH (74.5%) required intubation. Of the DAH patients requiring intubation, 54% and 66% died 30 and 60 days after intubation while only 13% and 26% of DAH patients not requiring intubation died by 30 and 60 days after the diagnosis of DAH ($P=0.01$). Among the intubated patients, UCB HCT recipients had a higher mortality at 6 months compared with the BM/PB graft recipients (84% *versus* 56%; $P=0.05$) (*Online Supplementary Figure S1*). Of seven patients who received an anti-tumor necrotizing factor drug, only one (14%) survived.

Table 2. Characteristics of patients receiving umbilical cord blood hematopoietic stem cell transplantation.

	No DAH	DAH	*P* value
Number	N=530	N=40	
Sex			**0.74**
Male	317 (60%)	25 (63%)	
Female	213 (40%)	15 (38%)	
Age at HCT (years)			**0.17**
Median (range)	28 (0-73)	32 (2-72)	
Malignant disease			**<0.01**
Yes	401 (76%)	37 (92%)	
No	129 (24%)	3 (8%)	
Disease risk			0.21
Standard	381 (72%)	25 (63%)	
High	149 (28%)	15 (38%)	
Conditioning intensity			0.18
MAC	273 (52%)	25 (63%)	
RIC	257 (48%)	15 (38%)	
TBI			**0.05**
Yes	405 (76%)	36 (90%)	
No	125 (24%)	4 (10%)	
Conditioning intensity & TBI			**0.04**
MAC TBI	177 (33%)	21 (53%)	
MAC no TBI	86 (18%)	4 (10%)	
RIC	272 (48%)	15 (37%)	
UCB units			**<0.01**
Single	199 (38%)	4 (10%)	
Double	331 (62%)	36 (90%)	
TNC x 10⁸/kg			
N	530	40	**0.01**
Median (Range)	0.46 (0.03-99.01)	0.39 (0.21-2.04)	
TNC x 10⁸/kg by unit number			
Single, median (range)	0.59 (0.03-14.28)	0.35 (0.21-0.37)	**0.03**
Double, median (range)	0.43 (0.19-99.01)	0.41 (0.23-2.04)	0.22
ANC engraftment			
Median days (range)	18 (0-42)	22 (0-41)	0.24
Platelet engraftment			
Median days (range)	44 (0-175)	84 (48-168)	**<0.01**
Graft failure			**<0.01**
Yes	36 (7%)	9 (23%)	
No	494 (93%)	31 (78%)	

ANC: absolute neutrophil count; DAM: diffuse alveolar hemorrhage; HCT: hematopoietic stem cell transplant; MAC: myeloablative conditioning; RIC: reduced intensity conditioning; TBI: total body irradiation; TNC: total nucleated cell count; UCB: umbilical cord blood; Significant differences are shown in bold.

Discussion

We observed that even in this recent era, the overall incidence of DAH was 5% for all patients, similar to the incidence recorded in earlier studies.[5,20,21] We found that myeloablative TBI, UCB HCT, and delayed engraftment or graft failure were significant risk factors for DAH.

Thrombocytopenia is important in hemorrhagic complications after allogeneic HCT;[22,23] however, its importance in DAH is controversial.[15,24,25] The relation of platelet recovery with DAH is not direct. In our study, both the severity and duration of thrombocytopenia were significantly associated with DAH. However, the risks of DAH cannot be fully explained solely by low platelet counts given that the median platelet count was >20x10⁹/L in our patients with DAH. Robbins *et al.* also showed that platelet transfusions did not prevent the development and/or progression of DAH.[15] Patients with DAH more often had malignant disease and myeloablative conditioning, but reduced intensity conditioning was not associated with DAH.

We also found that myeloablative conditioning containing TBI was a risk factor for DAH. These findings strongly suggest that direct lung injury at the time of conditioning by myeloablative dose TBI predisposes to DAH after HCT. Others have suggested that irradiation induces cellular damage and plays an important direct role in lung injury[26] and the association with high-dose TBI during conditioning contributes to the risk of DAH.[15,26-28] Moreover, TBI-induced lung injury may prolong or deepen thrombocytopenia given that in human and some animal studies the lung can contribute to platelet production.[29-31] TBI may also damage the vascular and rheological microenvironments of the pulmonary capillaries which may be more hemostatic than the microenvironments of pulmonary epithelial tissues.[32]

A longer duration of thrombocytopenia was associated with increased DAH in our study. It is well-known that UCB HCT is associated with delayed engraftment/graft failure compared with other related or unrelated grafts.[12,15,33-35] Moreover, UCB HCT recipients also received more myeloablative conditioning with TBI, another risk factor for DAH. Overall, we found that UCB grafts were associated with more DAH than PB/BM grafts. In multivariate analysis, UCB HCT was confirmed as an independent risk factor for DAH.

Delayed neutrophil engraftment was another risk factor for DAH, particularly for patients receiving PB/BM grafts. It is known that a higher number of stem cells/total nucleated cells expedites engraftment.[36] Patients with DAH received fewer total nucleated cells and more often had delayed engraftment than patients without DAH in both the UCB and PB/BM HCT recipients. Among the patients undergoing single unit UCB HCT, the association

Table 3A. Multivariate analysis of risks for diffuse alveolar hemorrhage: neutrophil engraftment.

Variables	All HCT		UCB HCT		PB/BM HCT	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	RR (99% CI)	*P* value
Conditioning		0.05		0.08		0.60
RIC	1.00		1.00		1.00	
MAC no TBI	0.83 (0.36-1.93)		0.69 (0.23-2.04)		1.08 (0.28-4.16)	
MAC TBI	1.80 (1.03-3.13)		1.87 (0.95-3.71)		1.63 (0.62-4.31)	
Graft source		0.01	--	--	--	--
BM/PB	1.00		--		--	
UCB	2.08 (1.16-3.74)		--		--	
ANC engraftment*		0.07		0.41		0.02
Yes	1.00		1.00		1.00	
No	2.16 (0.93-5.01)		1.57 (0.53-4.62)		5.51 (1.26-24.00)	

*As a time-dependent variable.

Table 3B. Multivariate analysis for diffuse alveolar hemorrhage: platelet engraftment.

Variables	All HCT		UCB HCT		PB/BM HCT	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	RR (99% CI)	*P* value
Conditioning		0.05		0.08		0.60
RIC	1.00		1.00		1.00	
MAC No TBI	0.78 (0.34-1.79)		0.65 (0.22-1.91)		1.10 (0.32-3.80)	
MAC TBI	1.76 (1.02-3.04)		1.78 (0.91-3.48)		1.64 (0.62-4.32)	
Graft source		0.07	--	--	--	--
BM/PB	1.00		--		--	
UCB	1.65 (0.96-2.83)		--		--	
Platelet engraftment*		<0.01		<0.01		0.15
Yes	1.00		1.00		1.00	
No	4.44 (2.10-9.42)		6.96 (2.39-20.29)		2.26 (0.74-6.90)	

*As a time-dependent variable. ANC: absolute neutrophil count; BM: bone marrow; HCT: hematopoietic stem cell transplantation; HR: hazard ratio; MAC: myeloablative conditioning; PB: peripheral blood; RIC: reduced intensity conditioning; TBI: total body irradiation; UCB: umbilical cord blood.

between lower infused cell dose and DAH was even more evident.

Most cases of DAH occurred within 2 weeks of neutrophil engraftment. This suggests that a sudden neutrophil influx may contribute to lung injury.[1,7,15,37-39] This phenomenon can even occur in a periengraftment period when patients still have neutropenia.[15,38,40]

Older age (>40 years),[3,5,15] severe acute GvHD,[3,7,41] and compromised renal function[15,41] have each been reported as risk factors for DAH. In our study, neither age nor acute GvHD was recognized as a risk factor. Kidney dysfunction was observed in one-third of the patients with DAH. Clinically apparent coagulopathy, as determined by standard tests, was uncommon in our DAH patients, as in earlier studies.[42] Pretransplant respiratory infections have also been described as risk factors for DAH after HCT.[10] Although there is no study specifically evaluating the association between smoking and pulmonary complications among HCT recipients, tobacco use and prior lung disease have not been reported as risk factors for DAH.[43] Likewise, no correlation was found between smoking status, bronchiolitis index determined by bronchoscopy, or inflammatory cell fractions, and the likelihood of developing DAH in autologous HCT recipients.[44] We observed that almost half of the patients with DAH had a history of smoking but were rarely diagnosed with a specific lung disease prior to their allogeneic HCT.

The therapy of DAH remains empirical and thus inadequate, due in part to the unknown pathogenesis of the condition. Because the immune response and inflammation are suggested to contribute to the pathogenesis of DAH, steroid treatment and mechanical ventilator support for acute respiratory failure are used commonly, although often unsuccessfully, for therapy.[21,25,38,45] Etanercept or other anti-cytokine agents,[46] drugs targeting coagulopathy such as aminocaproic acid and recombinant factor VIIa,[47-49] and prophylactic use of an interleukin-1 receptor antagonist to prevent GvHD[50] have also been used. All led to poor outcomes in patients with DAH and the mortality rates for this syndrome remain very high[3,9,37,38,51-53] due to respiratory failure, sepsis and multi-organ failure.[38,54]

Our study confirms that the pathogenesis of DAH is complex, affected by conditioning regimen, graft source, and engraftment kinetics and that the outcome remains poor, particularly for patients requiring intubation/mechanical ventilation. Improved management of DAH awaits better understanding of the complex relationship of these multiple risk factors and the definition of the best strategy to expedite engraftment and limit lung injury. Formal testing in comparative trials of therapeutic strategies is needed to validate approaches and limit the high mortality of this devastating syndrome.

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