Bone Marrow Transplantation (2007) 40, 71–78

www.nature.com/bmt

ORIGINAL ARTICLE

Outcome of alveolar hemorrhage in hematopoietic stem cell transplant recipients

S Gupta[1], A Jain[2], CL Warneke[3], A Gupta[4], VR Shannon[5], RC Morice[5], A Onn[5], CA Jimenez[5], L Bashoura[5], SA Giralt[6], BF Dickey[5] and GA Eapen[5]

[1]*Department of Internal Medicine, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA;* [2]*Department of Internal Medicine, University of Iowa, Iowa, IA, USA;* [3]*Division of Quantitative Sciences, The University of Texas MD Anderson Cancer Center, Houston, TX, USA;* [4]*Department of Urology, The University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA;* [5]*Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and* [6]*Department of Blood and Marrow Transplantation, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Alveolar hemorrhage (AH) is a frequent, serious complication of hematopoietic stem cell transplantation (HSCT). To study the incidence of AH, its clinical course and outcomes in HSCT patients, a retrospective review of the records of all adult patients who underwent bronchoscopy between January 1, 2002 and December 31, 2004 was carried out and those who underwent bronchoscopy after HSCT identified. A total of 223 patients underwent bronchoscopy after HSCT for diffuse pulmonary infiltrates with respiratory compromise. Eighty-seven (39%) patients had AH. Of these, 53 had AH without any identified organism while 34 had an organism along with hemorrhage on bronchoalveolar lavage (BAL). Six-month survival rate of patients with AH was 38% (95% confidence interval: 27–48%). In 95 of the 223 patients, an organism was isolated from BAL. These patients had poor outcomes compared to patients in whom no organism was identified. Patients with both AH and an organism had the worst prognosis. Mortality of patients with AH is improving and long-term survival of patients with AH is feasible. Isolation of a microbial organism in BAL is a strong predictor of poor outcome.
Bone Marrow Transplantation (2007) **40,** 71–78; doi:10.1038/sj.bmt.1705695; published online 7 May 2007
Keywords: bronchoalveolar lavage; hemorrhage; infection; hematopoietic stem cell transplantation; factor VII; outcome assessment

Introduction

Pulmonary complications occur in up to 60% of all hematopoietic stem cell transplantation (HSCT) recipients, accounting for about 30% of all deaths. These include bacterial, fungal and viral pneumonias, noninfectious complications including bronchiolitis obliterans, lung injury due to drug toxicity or radiation and alveolar hemorrhage (AH).[1–10] The etiology of AH in transplant setting is poorly understood. Infections, cardiac causes including heart failure, toxicity from pretransplant thoracic radiation, chemotherapy, early marrow engraftment, GVHD and cytokines have been postulated to have a role in the pathogenesis of AH after HSCT.[11–18] The reported mortality associated with AH varies from 70 to 100%.[11–14]

Diffuse AH (DAH) was initially described in autologous HSCT recipients and since has been described with equal frequency in both allogeneic and autologous transplant recipients.[13,19–21] It is seen in 1–19% of HSCT recipients who undergo bronchoscopy.[3,4,12,22–24] DAH is characterized by the presence of multilobar pulmonary infiltrates and symptoms of pneumonia and hypoxia. In addition, a patient should have progressively bloodier return on bronchoalveolar lavage (BAL) from three separate subsegmental bronchi or the presence of $\geqslant$20% hemosiderin-laden macrophages in the absence of a clear cardiac or infective etiology for hemorrhage.[11–15,25] The BAL appearance and iron stain are complementary. Soon after the onset of hemorrhage, BAL may be bloody and the iron stain may be negative. After 48–72 h, the gross appearance of BAL may not remain bloody. At this time, presence of $\geqslant$20% hemosiderin-laden macrophages suggests AH. Smokers may have an elevated number of hemosiderin-laden macrophages and as such, using $\geqslant$20% hemosiderin-laden macrophages as the cutoff, decreases false-positive results.[11,26]

The bronchoscopic criteria used to diagnose DAH are nonspecific and may be seen in association with diffuse lung injury from a wide variety of causes including engraftment syndrome, idiopathic pneumonia syndrome, sepsis, etc. In the post-HSCT setting, DAH syndrome is a retrospective diagnosis. Prospectively in the clinical setting, patients usually present with clinical features of pneumonia and diffuse radiologic infiltrates leading to bronchoscopy. If the BAL is progressively bloody, a diagnosis of AH is made and later if no organism is isolated in BAL, it is labeled as DAH. At treatment initiation, one usually does not know

Correspondence: Dr GA Eapen, Department of Pulmonary Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA.
E-mail: geapen@mdanderson.org
Received 4 July 2006; revised and accepted 27 March 2007; published online 7 May 2007

whether an infectious agent is associated with the AH, as cultures are often delayed by several days. Therefore, definitive treatment decisions are based on finding AH on bronchoscopy in the appropriate clinical setting. We therefore sought to describe the incidence, clinical course and outcomes associated with the bronchoscopic diagnosis of AH in HSCT patients at our center.

Systemic high-dose steroids and platelet transfusions are typically used for the treatment of DAH.[19,27–29] Recent reports have suggested that the administration of recombinant factor VIIa to patients with DAH results in rapid clinical improvement and better survival.[30,31] We also studied the outcomes of patients who received factor VIIa for AH at our center.

Patients and methods

The study was started in July 2005. Because most of the events occur within the first 6 months after HSCT, after obtaining approval from the institutional review board, we retrospectively reviewed records of all adult patients who underwent bronchoscopy between January 1, 2002 and December 31, 2004 at The University of Texas MD Anderson Cancer Center. From these records, we identified all patients who underwent bronchoscopy after HSCT. For these patients, the electronic clinical notes, bronchoscopy, radiology, pathology and microbiology reports were reviewed. Data were recorded for patients who underwent bronchoscopy for respiratory compromise with diffuse radiographic infiltrates. We reviewed patients undergoing bronchoscopy because evidence of AH on bronchoscopy is an essential feature for making the diagnosis of AH. The pediatric population was excluded because bronchoscopy is not routinely performed in children by our service.

The criteria for bronchoscopy included clinical suspicion of pneumonia with hypoxia and diffuse radiographic infiltrates. In patients with a platelet count of $<20\times10^9$/l or international normalized ratio (INR) >2, platelets or fresh-frozen plasma was transfused before BAL as per the institutional protocol. No patient underwent intubation for bronchoscopy. Bronchoscopy and BAL were performed in all patients with the above clinical findings who presented with hemoptysis. Bronchoscopy was carried out using the EVIS EXERA BF-P160 video bronchoscope (Olympus) under mild sedation using intravenous fentanyl and midazolam. Topical anesthesia was achieved with 2% methyl paraben free (MPF) lidocaine. With patients in a recumbent or semirecumbent position, the bronchoscope was wedged into an appropriate segment after airway inspection and BAL was carried out using 60–120 cc of 0.9% saline.

The patient characteristics including demography, underlying disease, pretransplant and pulmonary function tests (PFTs) were recorded. A history of tobacco or alcohol use during 1 year before presentation was considered positive. Accurate information about past tobacco or alcohol use or pack years of tobacco use was not available. We categorized and recorded disease status at HSCT based on the criteria published by Ho *et al*.[32] The preconditioning regimen, HLA matching, type of HSCT, neutrophil recovery, number of transplants received and history of GVHD were also extracted. The clinical presentation, interval time from HSCT to bronchoscopy, radiographic and BAL findings, thrombocytopenia ($<60\times10^9$/l), neutropenia (absolute neutrophil count $<0.5\times10^9$/l), or renal dysfunction (blood urea nitrogen >40 mg/dl or serum creatinine >1.5 mg/dl) at the time of bronchoscopy and the treatment given were recorded. High-dose steroid was defined as 250 mg/day or higher methylprednisolone or its equivalent.

For this report, a diagnosis of AH was made if the following criteria were fulfilled:

1. Symptoms (like dyspnea, cough, fever) and signs (like tachypnea, tachycardia) of pneumonia with respiratory compromise;
2. Evidence of widespread alveolar injury manifested by multilobar pulmonary infiltrates seen on chest radiographs;
3. No evidence of cardiogenic pulmonary edema based on clinical findings or cardiac echocardiography;
4. BAL showing progressively bloodier returns from three subsegmental bronchi or the presence of 20% or more hemosiderin-laden macrophages.

A diagnosis of DAH syndrome was made if the patients met the additional criterion of having no bacterial, viral or fungal pathogens detected in the BAL fluid.

All patients were followed for at least 6 months after bronchoscopy or until death. The last follow-up date was June 30, 2005. Data from index bronchoscopy for patients with AH and first bronchoscopy for respiratory compromise with diffuse infiltrates in non-AH patients were used for comparison. Data from the most recent HSCT, before the studied bronchoscopy, was used for patients undergoing more than one HSCT. Outcome measures analyzed included requirement for positive pressure ventilation over the 6 months following bronchoscopy, the length of index hospitalization and survival.

Microbiologic techniques

Gram stain and bacterial culture were performed on all BAL specimens. Fungal cultures, viral cultures and viral antigen studies for cytomegalovirus, respiratory syncytial virus and influenza A antigen were carried out on all samples. All samples were sent for cytologic studies using Papanicolaou, Giemsa and methenamine silver stains. Acid-fast stain was carried out on patients with a recent history of exposure to tuberculosis, gradual onset of symptoms, positive tuberculin test and otherwise when clinically indicated.

Statistical analysis

Pearson χ^2 tests, Fisher's exact tests and Wilcoxon rank sum tests were used in bivariate analyses to examine the association between AH diagnosis and demographic and clinical characteristics. We used logistic regression techniques to model the probability of AH (presence or absence) and computed odds ratios and the associated 95% confidence intervals (CI) for predictor variables. The variables analyzed included age, gender, race, prior

radiation therapy to lung, number of HSCTs, status of underlying disease at HSCT, FEV1/FVC <70 before HSCT, preconditioning regimen, type of HSCT, source of stem cells, renal dysfunction, thrombocytopenia, time to neutrophil recovery and presence of organism in BAL. Because the number of patients treated with a particular preconditioning regimens was small, the preconditioning regimens were analyzed in four groups: (1) busulfan + fludarabine; (2) melphalan + fludarabine; (3) carmustine (BCNU) + etoposide + cytarabine (Ara-C) + melphalan; and (4) others. The other regimens included busulfan + cyclophosphamide, cyclophosphamide + antithymocyte globulin, fractionated cyclophosphamide + vincristine + adriamycin + dexamethasone (hyper-CVAD), cyclophosphamide + fludarabine + alemtuzmab and melphalan. Analysis based on the underlying disease before HSCT could not be performed due to the small number of patients in each disease group.

Univariate logistic regression analyses were conducted for each predictor variable. Variables that were associated with AH occurrence at $P \leqslant 0.10$ and had less than 10% missing values were entered simultaneously for the full model. The full model was reduced using backward elimination to remove variables one at a time to arrive at a final reduced model. We constructed survival curves using the Kaplan–Meier method and compared the curves using log-rank test.

Univariate and multivariate Cox proportional hazards regression models were used to investigate whether any clinical factors were prognostic for survival. Time from bronchoscopy to last follow-up was used for overall survival time. The factors entered into the model were patient's age, gender, race, radiation therapy to lung before HSCT, pre-HSCT PFT, preconditioning regimens used for HSCT, use of total body irradiation during preconditioning, time to neutrophil recovery after HSCT, renal dysfunction, platelet count and absolute neutrophil count at the time of bronchoscopy, presence of AH and isolation of an organism in BAL. All reported *P*-values are two sided at a significance level of 5%. Analyses were performed using SAS for Windows (release 9.0; SAS Institute Inc., Cary, NC, USA).

Results

This study included 223 patients who underwent bronchoscopy between January 1, 2002 and December 31, 2004 for a clinical suspicion of AH. The follow-up period ranged from less than 1 to 42 months (median = 5 months). Thirty-four of the 223 patients were on mechanical ventilation before bronchoscopy. Of these, 21 were found to have AH. The neutrophil count recovered after a median of 12 days (range: 5–31) and platelet count recovered after a median of 14 days (range: 6–29).

Eighty-seven patients (39%) met the predefined diagnostic criteria for AH. The remaining 136 patients (61%) constituted the 'No AH' group. This group of patients had clinical and radiographic features suggestive of AH, but did not have bronchoscopic evidence of hemorrhage. Hemosiderin values were available for all 223 patients. The median levels were 20% (range: 0–95%) for patients with AH and 0% (range: 0–99%) for patients with no AH. Of the 87 patients with AH, 47 were diagnosed by progressively bloody BAL and 40 by hemosiderin-laden macrophages. The demographic and transplant characteristics of both groups are given in Table 1. All patients in the AH and 133 in the No AH group received high-dose steroids, which were discontinued in the No AH group after bronchoscopy. All patients received aggressive treatment with antimicrobials, blood product transfusion, intensive care and mechanical ventilation as necessary. Antimicrobial prophylaxis included a combination of oral trimethoprim–sulfamethoxazole and voriconazole. Intravenous vancomycin, meropenam and voriconazole were used for empiric antimicrobial coverage. Table 2 shows the underlying diseases for which HSCT was performed. Eighty-nine (40%) of the patients received more than one bronchoscopy and 15 patients (7%) underwent bronchoscopic biopsy along with BAL. No complications associated with bronchoscopic biopsy were noted. Patients with AH underwent bronchoscopy, a median of 3 days (range: 0–105), following HSCT while those with no AH underwent bronchoscopy, a median of 6 days (range: 0–187) after HSCT ($P = 0.008$). Hemoptysis was present in 35 (16%) of patients while 65 (29%) had pleural effusion on chest X-ray.

During the follow-up period, 132 (59%) patients died. The most frequent cause of death in both groups was respiratory failure (Table 3). Sixty-four percent of patients with AH required positive pressure ventilation within 6 months after bronchoscopy compared to 39% among patients without AH ($P = 0.0002$). Patients with AH (median = 3 months; 95% CI: 1, 5 months) had shorter overall survival than did patients without AH (median = 9 months, 95% CI: 5, 25 months; $P = 0.007$). We found no statistically significant differences in outcomes between patients with early (within 30 days of HSCT) and late onset of AH.

The outcomes were also studied based on the isolation of an organism in BAL. Twenty-five BALs in 18 patients (8%) underwent acid-fast staining. The stain was not positive in any of these patients. In 95 of the 223 patients (43%), an organism was isolated from BAL (Table 4). The organisms were isolated by culture (64%), cytology (21%), culture and cytology (11%), and positive viral antigen (4%). During follow-up, 65 (68%) of the 95 patients, found to have organisms on BAL died, and 67 (52%) deaths were observed among the remaining 128 patients. Patients in whom an organism was identified had shorter overall survival (median = 5 months, 95% CI: 2, 7 months) compared to that of patients in whom no organism was identified (median = 8 months, 95% CI: 5 months, data not attained; $P = 0.02$). The 6-month overall survival rate in patients with an isolated organism was 40% (95% CI: 30, 50) versus 56% (95% CI: 46, 64) in patients without an isolated organism.

Patients were stratified further into four subgroups based on AH diagnosis and microbial organism status. Of the 87 patients with evidence of AH, 34 (39%) were found to have a microbial organism on BAL. Fifty-three (61%) patients had AH without any organism on BAL and thus met the

Table 1 Demographic and transplant characteristics of the patients undergoing bronchoscopy for respiratory compromise with diffuse infiltrates after stem cell transplant

	Alveolar hemorrhage (N = *87*)		*No alveolar hemorrhage* (N = *136*)		*Total patients* (N = *223*)		P-*value*
Age (years), median (minimum, maximum)	48	(22–76)	52	(18–77)	50	(18–77)	0.97[a]
Gender, *n* (%)							0.42[b]
Male	49	(56)	84	(62)	133	(60)	
Female	38	(44)	52	(38)	90	(40)	
Race, *n* (%)							0.36[c]
White	63	(72)	98	(72)	161	(72)	
Black	8	(9)	10	(7)	18	(8)	
Hispanic	12	(14)	26	(19)	38	(17)	
Others	4	(5)	2	(1)	6	(3)	
Current use of alcohol, *n* (%)	18	(21)	32	(24)	50	(22)	0.62[b]
Current smokers, *n* (%)	13	(15)	26	(19)	39	(17)	0.42[b]
Prior transplant[d], *n* (%)							0.73[b]
Allogeneic	9	(56)	13	(62)	22	(59)	
Autologous	7	(44)	8	(38)	15	(41)	
Type of HSCT, *n* (%)							0.02[b]
Allogeneic	74	(85)	98	(72)	172	(77)	
Autologous	13	(15)	38	(28)	51	(23)	
Preconditioning regimen, *n* (%)							0.0006[b]
BF	22	(25)	14	(10)	36	(16)	
MF	29	(33)	30	(22)	59	(26)	
BEAM	15	(17)	29	(21)	44	(20)	
Others	21	(24)	63	(46)	84	(38)	
TBI	3	(3)	20	(15)	23	(10)	0.006[b]
GVHD prophylaxis							
Tacrolimus	84	(97)	134	(99)	218	(98)	0.38[c]
Methotrexate	17	(20)	27	(20)	44	(20)	0.95[b]
Steroids	18	(21)	31	(23)	49	(22)	0.71[b]
ATG	9	(10)	15	(11)	24	(11)	0.87[b]
HLA match[e], *n* (%)							0.22[b]
Yes	59	(80)	85	(87)	144	(84)	
No	15	(20)	13	(13)	28	(16)	
Source of stem cells, *n* (%)							0.44[c]
Peripheral blood	54	(64)	94	(69)	148	(67)	
Bone marrow	29	(34)	36	(26)	65	(29)	
Cord blood	2	(2)	6	(4)	8	(4)	
Disease status at HSCT[f], *n* (%)							0.44[b]
Early	6	(7)	15	(12)	21	(10)	
Intermediate	32	(37)	50	(39)	82	(38)	
Advanced	48	(56)	63	(49)	111	(52)	
Early neutrophil recovery[g], *n* (%)	72	(84)	109	(82)	181	(83)	0.74[b]
Renal dysfunction[h], *n* (%)	35	(40)	22	(17)	57	(26)	0.0001[b]
Thrombocytopenia[i], *n* (%)	62	(71)	56	(43)	118	(54)	<0.0001[b]
Neutropenia[j], *n* (%)	35	(40)	28	(22)	63	(29)	0.003[b]
High dose steroids, *n* (%)	87	(100)	133	(98)	220	(99)	<0.0001[b]
Organism in BAL, *n* (%)	34	(39)	61	(45)	95	(43)	0.39[b]

Abbreviations: BAL = bronchoalveolar lavage; BEAM = carmustine (BCNU) + etoposide + cytarabine (Ara-C) + melphalan; BF = busulfan + fludarabine (other regimens included: busulfan + cyclophosphamide, cyclophosphamide + antithymocyte globulin, fractionated cyclophosphamide + vincristine + adriamycin + dexamethasone (hyper-CVAD), cyclophosphamide + fludarabine + alemtuzmab and melphalan); GVHD = graft versus host disease; HSCT = hematopoietic stem cell transplant; MF = melphalan + fludarabine; TBI = total body irradiation.

[a]Wilcoxon rank sum test.

[b]χ^2 test.

[c]Fisher's exact test.

[d]Thirty-seven patients had received prior stem cell transplant.

[e]The denominator for this group is the patient who received allogeneic transplant. The six HLA matched were A, B, C, DRB1, DRB3-5, DQB1.

[f]Disease status at HSCT was categorized into early, intermediate and advanced based on the criteria published by Ho *et al.*[32]

[g]<3 week after HSCT.

[h]Blood urea nitrogen >40 mg/dl or creatinine >1.5 mg/dl.

[i]Platelet count $<60 \times 10^9$/l.

[j]Absolute neutrophil count $<0.5 \times 10^9$/l.

Table 2 Indication for stem cell transplantation in the study population

	Alveolar hemorrhage (N = *87*)		*No alveolar hemorrhage* (N = *136*)		*Total patients* (N = *223*)	
	n	(%)	n	(%)	n	(%)
Acute lymphocytic leukemia	9	(10)	12	(9)	21	(9)
Acute myelogenous leukemia	27	(31)	19	(14)	46	(21)
Chronic myelogenous leukemia	8	(9)	16	(12)	24	(11)
Chronic leukocytic leukemia	6	(7)	10	(7)	16	(7)
Non Hodgkin's lymphoma	17	(20)	37	(27)	54	(24)
Hodgkin's disease	3	(3)	13	(10)	16	(7)
Multiple myeloma	9	(10)	14	(10)	23	(10)
Solid tumors[a]	1	(1)	6	(4)	7	(3)
Nonmalignant conditions[b]	7	(8)	9	(7)	16	(7)

Abbreviation: AH = alveolar hemorrhage.
[a]Solid tumors include renal cell, breast and ovarian carcinomas.
[b]Nonmalignant conditions include myelodysplastic syndrome and aplastic anemia.

Table 3 Outcomes of patients with alveolar hemorrhage versus no alveolar hemorrhage

Outcome	*Alveolar hemorrhage* (N = *87*)		*No alveolar hemorrhage* (N = *136*)		*Total patients* (N = *223*)		P-*value*
PPV (*n*, %)	56	(64)	53	(39)	109	(49)	0.0002[a]
Cause of death[b]							
Respiratory failure	28	(50)	19	(26)	47	(37)	0.01[c]
Multiple organ failure	14	(25)	16	(22)	30	(23)	
Sepsis	0	(0)	8	(11)	8	(6)	
Disease progression	8	(14)	18	(25)	26	(20)	
GVHD	2	(4)	3	(4)	5	(4)	
Others	4	(7)	8	(11)	12	(9)	
Overall survival months (median, 95% CI)	3	(1, 5)	9	(5, 25)	6	(4, 10)	0.007[d]
1-Month survival (%, 95% CI)	55.2	(44.1, 64.9)	79.3	(71.5, 85.2)	69.9	(63.4, 75.4)	
6-Month survival (%, 95% CI)	37.8	(27.5, 48.1)	56.0	(47.2, 63.9)	48.9	(42.1, 55.4)	

Abbreviations: CI = confidence interval; GVHD = graft versus host disease; PPV = positive pressure ventilation within 6 months after bronchoscopy.
[a]χ^2 test.
[b]The cause of death was missing for four patients.
[c]Fisher's exact test.
[d]Log-rank test.

Table 4 Frequency of organisms isolated in bronchoalveolar lavage

MO in BAL	*AH + MO* (N = *34*)	*No AH + MO* (N = *61*)
Candida	11	21
Aspergillus sp.	7	13
Pseudomonas sp.	3	4
Staphylococcus sp.	3	9
Influenza A	4	5
RSV	1	6
CMV	2	3
Strongyloides	1	0
Toxoplasma	2	0

Abbreviations: AH = alveolar hemorrhage; BAL = bronchoalveolar lavage; CMV = cytomegalovirus; MO = microbial organism; RSV = respiratory syncytial virus.

predefined criteria for the diagnosis of DAH. Sixty-one of the 136 patients in the No AH group (45%) had a microbiologic organism isolated in BAL, while 75 patients (55%) did not have any organism on BAL. The prevalence of organisms in BAL did not differ significantly by AH diagnosis ($P = 0.4$). Patients with both AH and an organism had the worst outcomes (median survival = 1.5 months, 95% CI: 1, 4 months) (Figure 1 and Table 5). In pair-wise comparisons, the only statistically significant differences in overall survival were between patients who had AH and infection compared to each of the other three groups.

The hazard of death was evaluated using Cox proportional hazards analysis. In the multivariate reduced model, isolation of an organism in BAL (hazards ratio (HR) = 1.99, 95% CI: 1.38, 2.87), renal dysfunction (HR = 2.35, 95% CI: 1.60, 3.46) and neutropenia (HR = 2.77, 95% CI: 1.91, 4.01) were statistically significant predictors of decreased overall survival (Table 6). Of note, AH was not an independent predictor of survival time in the reduced multivariate model.

Recombinant factor VIIa was administered to 24 (28%) of the 87 patients with AH. Along with factor VIIa, 16 patients received platelet transfusions to keep platelet count above 50×10^9/l, four received desmopressin (1-deamino-8-D-arginine vasopressin,) and three received aminocaproic acid. Among patients who received factor VIIa, overall

survival, requirement for positive pressure ventilation, respiratory failure as the cause of death and duration of hospital stay after bronchoscopy were similar to those of other patients. No thrombotic complications were observed with the administration of factor VIIa in any of these patients.

Logistic regression was used to identify factors associated with diagnosis of AH among patients with suspected AH (Table 7). In the multivariate reduced model, renal dysfunction, thrombocytopenia and use of busulfan + fludarabine or melphalan + fludarabine for preconditioning were found to have significant associations with AH. Other demographic and clinical characteristics including age, gender, race, prior radiation therapy to lung, pre-HSCT PFT, number of HSCTs, status of underlying disease at HSCT, source of stem cells, other preconditioning regimens, time to neutrophil recovery and presence of organism in BAL were not significantly associated with AH.

Discussion

In this study, we found that AH developed in 39% of the patients for up to 15 weeks after HSCT. Patients with AH who had an organism isolated in the BAL had worse outcomes compared to patients whose BAL was sterile.

Our study has several strengths. First, it is among the largest series with a long follow-up of AH occurring after HSCT as compared to other studies.[4,14,20,22,32] Also, we have presented outcome data on patients with possible respiratory infection and have found that infection is an independent predictor of outcome. Other studies have excluded this group of patients.[14] In addition, we have used well defined criteria for the diagnosis of AH, whereas other similar studies have determined AH using less explicit criteria.[4,20,22] Our study is limited due to its retrospective nature and due to the possibility of referral bias. Furthermore, we cannot establish the actual incidence of AH after HSCT due to verification bias. Determining the incidence of AH would require performing bronchoscopy in all HSCT patients irrespective of their symptoms. Thus, the study can determine the incidence of AH only in patients undergoing bronchoscopy after HSCT. Also, we do not have data on patients who might have had a similar clinical presentation but did not undergo bronchoscopy. Thus, the incidence of AH is probably underestimated.

The contribution of respiratory infections in the causation of AH and clinical significance of isolating an organism in BAL has not been well characterized. In our study, a substantial proportion of the AH cases had evidence of an organism in their BAL. Despite aggressive antimicrobial prophylaxis, the mortality of patients with an organism isolated in BAL was significantly higher. In general, patients who developed infections had poorer survival and patients who had both AH and an organism in BAL fared the worst. Thus, patients who develop infections have poorer survival. But, many of the isolated organisms are colonizers of the upper respiratory tract in immunocompromized patients and their isolation does not necessarily mean infection.

Most reports have described poor survival (usually less than 20–25% 6-month survival) for patients with DAH.[11,13,14] Contrary to this, in our patient population with AH (including infections), the estimated survival using the Kaplan–Meier method at 30 days was 55% and at 6-months it was 38%. These outcomes are better than previous published reports. The exact reasons for the improved survival are unclear. However, the better out-

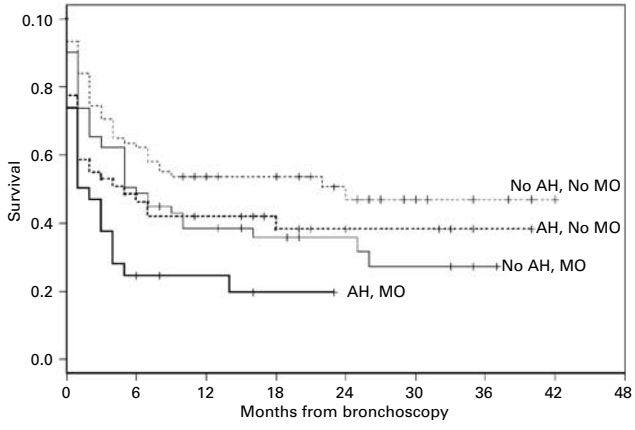

Figure 1 Kaplan–Meier survival curves for the four subgroups: (1) patients without alveolar hemorrhage (AH) and any microbial organism (MO) in BAL, (2) patients with AH but no microbial organism in BAL, (3) patients without AH but having microbial organism in BAL, (4) patients with both AH and MO in BAL.

Table 5 Outcomes of alveolar hemorrhage diagnosis and the presence of a MO in BAL

Outcome	*Alveolar hemorrhage*				*No alveolar hemorrhage*				P-*value*
	MO (n = *34*)		*No MO* (n = *53*)		*MO* (n = *61*)		*No MO* (n = *53*)		
PPV (*n*, %)	26	(76)	30	(57)	27	(44)	26	(35)	0.004[a]
Respiratory failure cause of death[b] (*n*, %)	12	(48)	16	(52)	11	(30)	8	(23)	0.046[a]
Overall survival months (median, 95% CI)	1.5	(1, 4)	5	(1, NA)	6	(3, 16)	24	(7, NA)	0.002[c]
1-Month survival (%, 95% CI)	50	(32.4, 65.3)	58.5	(44.1, 70.4)	73.8	(60.8, 83.0)	83.9	(73.3, 90.5)	
6-Month survival (%, 95% CI)	24.6	(11.5, 40.3)	46.3	(32.3, 59.2)	48.6	(35.6, 60.5)	62.1	(50.0, 72.0)	

Abbreviations: BAL = bronchoalveolar lavage; CI = confidence interval; MO = microbial organism; NA = not attained; PPV = positive pressure ventilation within 6 months after bronchoscopy.
[a]χ^2 test.
[b]The cause of death was missing for four of the 132 deaths.
[c]Log-rank test.

Table 6 Cox proportional hazards regression analysis for overall survival among patients undergoing bronchoscopy for respiratory compromise with diffuse infiltrates after hematopoietic stem cell transplant

	Univariate HR (95% CI)	P-*value*	*Multivariate HR (95% CI)*	P-*value*
Organism on BAL	1.48 (1.05, 2.08)	0.026	1.99 (1.38, 2.87)	0.0002
Renal dysfunction[a]	2.15 (1.49, 3.11)	<0.0001	2.35 (1.60, 3.46)	<0.0001
Neutropenia[b]	2.62 (1.82, 3.77)	<0.0001	2.77 (1.91, 4.01)	<0.0001
Thrombocytopenia[c]	2.19 (1.51, 3.18)	<0.0001		
Alveolar hemorrhage	1.56 (1.10, 2.20)	0.012		
Neutrophil recovery >3 weeks after HSCT	1.56 (1.02, 2.37)	0.039		

Abbreviations: BAL = broncho alveolar lavage; CI = confidence intervals; HR = hazards ratio; HSTC = hematopoietic stem cell transplantation.
[a]Blood urea nitrogen >40 mg/dl or creatinine >1.5 mg/dl.
[b]Absolute neutrophil count $<0.5 \times 10^9$/l.
[c]Platelet count $<60 \times 10^9$/l.

Table 7 Results from logistic regression model for factors associated with alveolar hemorrhage

Independent variable	*Univariate OR (95% CI)*	P-*value*	*Multivariate reduced OR (95% CI)*	P-*value*
Renal dysfunction[a]	3.37 (1.80, 6.30)	0.0001	3.69 (1.84, 7.39)	0.0002
Thrombocytopenia[b]	3.28 (1.84, 5.85)	<0.0001	2.75 (1.48, 5.11)	0.0014
Preconditioning				
BF	4.71 (2.05, 10.84)	0.0003	5.00 (2.04, 12.25)	0.0004
MF	2.90 (1.43, 5.90)	0.0033	3.13 (1.43, 6.84)	0.004
BEAM	1.55 (0.70, 3.44)	0.279	1.32 (0.55, 3.14)	0.531
Others	1.00		1.00	
Allogeneic HSCT	2.21 (1.10, 4.44)	0.026		
No TBI	4.95 (1.43, 17.21)	0.012		
FEV1/FVC ⩾70	2.98 (0.82, 10.77)	0.097		
DLCO <80	1.65 (0.95, 2.86)	0.076		
Neutropenia[c]	2.45 (1.35, 4.46)	0.003		

Abbreviations: BEAM = carmustine (BCNU) + etoposide + cytarabine (Ara-C) + melphalan; BF = busulfan + fludarabine (other regimens included: busulfan + cyclophosphamide, cyclophosphamide + antithymocyte globulin, fractionated cyclophosphamide + vincristine + adriamycin + dexamethasone (hyper-CVAD), cyclophosphamide + fludarabine + alemtuzmab and melphalan); CI = confidence intervals; DLCO = diffusion capacity for carbon monoxide; FEV1 = forced expiratory volume in 1 s; FVC = forced vital capacity; HSCT = hematopoietic stem cell transplant; MF = melphalan + fludarabine; TBI = total body irradiation; OR = odds ratio.
[a]Blood urea nitrogen >40 mg/dl or creatinine >1.5 mg/dl.
[b]Platelet count $<60 \times 10^9$/l.
[c]Absolute neutrophil count $<0.5 \times 10^9$/l.

comes in our patients may be related to the use of improved diagnostic and supportive medical technologies coupled with a proactive management strategy practiced at our institution.

Though most (87%) of AH cases occurred within 3 weeks after transplant, patients developed AH up to 15 weeks after HSCT. Similar to some previous studies, these results suggest that AH is a common cause of pulmonary infiltrates after HSCT and its occurrence is not limited to certain periods after the transplant.[9] Thus, AH should be included in the differential diagnosis of late-onset pulmonary complications.

Factor VIIa use was not associated with improved outcomes in these patients. This must be interpreted with caution due to the possibility of selection bias whereby sicker patients might have been more likely to receive factor VIIa. But it is important to note that out of 24 patients who received factor VIIa, none developed any thrombotic complications.

Conclusions

We present a large series on stem cell transplant patients undergoing bronchoscopy for respiratory compromise with diffuse radiographic infiltrates. AH is a common cause of pulmonary infiltrates. The occurrence of AH is not limited to certain periods after HSCT and should be included in the differential diagnosis of late-onset pulmonary complications. The mortality of these patients is improving and long-term survival of patients with DAH is feasible. Isolation of a microbial organism in BAL is a strong adverse prognostic marker.

References

1 Breuer R, Lossos IS, Berkman N, Or R. Pulmonary complications of bone marrow transplantation. *Respir Med* 1993; **87**: 571–579.

2 Cordonnier C, Bernaudin JF, Bierling P, Huet Y, Vernant JP. Pulmonary complications occurring after allogeneic bone marrow transplantation. A study of 130 consecutive transplanted patients. *Cancer* 1986; **58**: 1047–1054.
3 Dunagan DP, Baker AM, Hurd DD, Haponik EF. Bronchoscopic evaluation of pulmonary infiltrates following bone marrow transplantation. *Chest* 1997; **111**: 135–141.
4 Feinstein MB, Mokhtari M, Ferreiro R, Stover DE, Jakubowski A. Fiberoptic bronchoscopy in allogeneic bone marrow transplantation: findings in the era of serum cytomegalovirus antigen surveillance. *Chest* 2001; **120**: 1094–1100.
5 Kotloff RM, Ahya VN, Crawford SW. Pulmonary complications of solid organ and hematopoietic stem cell transplantation. *Am J Respir CritCare Med* 2004; **170**: 22–48.
6 Krowka MJ, Rosenow III EC, Hoagland HC. Pulmonary complications of bone marrow transplantation. *Chest* 1985; **87**: 237–246.
7 Nusair S, Breuer R, Shapira MY, Berkman N, Or R. Low incidence of pulmonary complications following nonmyeloablative stem cell transplantation. *Eur Respir J* 2004; **23**: 440–445.
8 Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG *et al*. Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. *Br J Haematol* 1998; **100**: 680–687.
9 Roychowdhury M, Pambuccian SE, Aslan DL, Jessurun J, Rose AG, Manivel JC *et al*. Pulmonary complications after bone marrow transplantation: an autopsy study from a large transplantation center. *Arch Pathol Lab Med* 2005; **129**: 366–371.
10 Soubani AO, Miller KB, Hassoun PM. Pulmonary complications of bone marrow transplantation. *Chest* 1996; **109**: 1066–1077.
11 Afessa B, Tefferi A, Litzow MR, Krowka MJ, Wylam ME, Peters SG. Diffuse alveolar hemorrhage in hematopoietic stem cell transplant recipients. *Am J Respir Crit Care Med* 2002; **166**: 641–645.
12 Huaringa AJ, Leyva FJ, Giralt SA, Blanco J, Signes-Costa J, Velarde H *et al*. Outcome of bone marrow transplantation patients requiring mechanical ventilation. *Crit Care Med* 2000; **28**: 1014–1017.
13 Jules-Elysee K, Stover DE, Yahalom J, White DA, Gulati SC. Pulmonary complications in lymphoma patients treated with high-dose therapy autologous bone marrow transplantation. *Am Rev Respir Dis* 1992; **146**: 485–491.
14 Lewis ID, DeFor T, Weisdorf DJ. Increasing incidence of diffuse alveolar hemorrhage following allogeneic bone marrow transplantation: cryptic etiology and uncertain therapy. *Bone Marrow Transplant* 2000; **26**: 539–543.
15 Robbins RA, Linder J, Stahl MG, Thompson AB, Haire W, Kessinger A *et al*. Diffuse alveolar hemorrhage in autologous bone marrow transplant recipients. *Am J Med* 1989; **87**: 511–518.
16 Cooke KR, Kobzik L, Martin TR, Brewer J, Delmonte J, Crawford JM *et al*. An experimental model of idiopathic pneumonia syndrome after bone marrow transplantation: I. The roles of minor H antigens and endotoxin. *Blood* 1996; **88**: 3230–3239.
17 Weisdorf DJ. Diffuse alveolar hemorrhage: an evolving problem? *Leukemia* 2003; **17**: 1049–1050.
18 Wojno KJ, Vogelsang GB, Beschorner WE, Santos GW. Pulmonary hemorrhage as a cause of death in allogeneic bone marrow recipients with severe acute graft-versus-host disease. *Transplantation* 1994; **57**: 88–92.
19 Metcalf JP, Rennard SI, Reed EC, Haire WD, Sisson JH, Walter T *et al*. Corticosteroids as adjunctive therapy for diffuse alveolar hemorrhage associated with bone marrow transplantation. University of Nebraska Medical Center Bone Marrow Transplant Group. *Am J Med* 1994; **96**: 327–334.
20 Nevo S, Swan V, Enger C, Wojno KJ, Bitton R, Shabooti M *et al*. Acute bleeding after bone marrow transplantation (BMT)- incidence and effect on survival. A quantitative analysis in 1402 patients. *Blood* 1998; **91**: 1469–1477.
21 Sisson JH, Thompson AB, Anderson JR, Robbins RA, Spurzem JR, Spence PR *et al*. Airway inflammation predicts diffuse alveolar hemorrhage during bone marrow transplantation in patients with Hodgkin disease. *Am Rev Respir Dis* 1992; **146**: 439–443.
22 Capizzi SA, Kumar S, Huneke NE, Gertz MA, Inwards DJ, Litzow MR *et al*. Peri-engraftment respiratory distress syndrome during autologous hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001; **27**: 1299–1303.
23 Glazer M, Breuer R, Berkman N, Lossos IS, Kapelushnik J, Nagler A *et al*. Use of fiberoptic bronchoscopy in bone marrow transplant recipients. *Acta Haematologica* 1998; **99**: 22–26.
24 Quabeck K. The lung as a critical organ in marrow transplantation. *Bone Marrow Transplant* 1994; **14** (Suppl 4): S19–S28.
25 Corso S, Vukelja SJ, Wiener D, Baker WJ. Diffuse alveolar hemorrhage following autologous bone marrow infusion. *Bone Marrow Transplant* 1993; **12**: 301–303.
26 De Lassence A, Fleury-Feith J, Escudier E, Beaune J, Bernaudin JF, Cordonnier C. Alveolar hemorrhage. Diagnostic criteria and results in 194 immunocompromised hosts. *Am J Respir Crit Care Med* 1995; **151**: 157–163.
27 Chao NJ, Duncan SR, Long GD, Horning SJ, Blume KG. Corticosteroid therapy for diffuse alveolar hemorrhage in autologous bone marrow transplant recipients. *Ann Inter Med* 1991; **114**: 145–146.
28 Haselton DJ, Klekamp JG, Christman BW, Barr FE. Use of high-dose corticosteroids and high-frequency oscillatory ventilation for treatment of a child with diffuse alveolar hemorrhage after bone marrow transplantation: case report and review of the literature. *Criti Care Med* 2000; **28**: 245–248.
29 Raptis A, Mavroudis D, Suffredini A, Molldrem J, Rhee FV, Childs R *et al*. High-dose corticosteroid therapy for diffuse alveolar hemorrhage in allogeneic bone marrow stem cell transplant recipients. *Bone Marrow Transplant* 1999; **24**: 879–883.
30 Hicks K, Peng D, Gajewski JL. Treatment of diffuse alveolar hemorrhage after allogeneic bone marrow transplant with recombinant factor VIIa. *Bone Marrow Transplant* 2002; **30**: 975–978.
31 Pastores SM, Papadopoulos E, Voigt L, Halpern NA. Diffuse alveolar hemorrhage after allogeneic hematopoietic stem-cell transplantation: treatment with recombinant factor VIIa. *Chest* 2003; **124**: 2400–2403.
32 Ho VT, Weller E, Lee SJ, Alyea EP, Antin JH, Soiffer RJ. Prognostic factors for early severe pulmonary complications after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 223–229.