MAJOR ARTICLE

Respiratory Syncytial Virus in Hematopoietic Cell Transplant Recipients: Factors Determining Progression to Lower Respiratory Tract Disease

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(See the editorial commentary by Ljungman on pages 1151–2)

Background. Respiratory syncytial virus (RSV) lower respiratory tract disease (LRD) is a life-threatening complication in hematopoietic cell transplant (HCT) recipients. Lymphopenia has been associated with an increased risk of progression from upper respiratory tract infection (URI) to LRD.

Methods. This study retrospectively analyzed the significance of lymphocyte engraftment dynamics, lung function, smoking history, corticosteroids, antiviral treatment, viral subtypes, and RSV-specific neutralizing antibodies for the progression to LRD in 181 HCT recipients with RSV URI.

Results. In multivariable models, smoking history, conditioning with high-dose total body irradiation, and an absolute lymphocyte count (ALC) $\leq 100/mm^3$ at the time of URI onset were significantly associated with disease progression. No progression occurred in patients with ALCs of $>1000/mm^3$ at URI onset. Lymphocyte engraftment dynamics were similar in progressors and nonprogressors. Pre- and posttransplant donor and posttransplant recipient RSV subtype-specific neutralizing antibody levels, RSV viral subtypes, and corticosteroids also were not significantly associated with LRD progression.

Conclusions. Host and transplant related factors appear to determine the risk of progression to LRD more than viral factors. Dysfunctional cell-mediated immunity appears to be important in the pathogenesis of progressive RSV disease after HCT. A characterization of RSV-specific T-cell immunity is warranted.

Keywords. Respiratory syncytial virus; Hematopoietic cell transplantation; Respiratory tract disease; Respiratory virus.

Infection caused by respiratory viruses is a threat for hematopoietic cell transplant (HCT) recipients. Respiratory syncytial virus (RSV) can cause serious progressive disease in both allogeneic and autologous transplant recipients [1–3] with mortality as high as 20%–40% with current treatment interventions [4]. Although treatment options such as antiviral agents, steroids, and immunoglobulin are frequently used, there is no definitive treatment for RSV disease in the transplant population [4–10]. RSV infection in HCT recipients can present as a self-limited upper respiratory tract infection (URI) or can progress to more severe lower respiratory tract disease (LRD), a condition associated with a high mortality or late airflow obstruction among survivors [11]. Because RSV LRD is a potentially life-threatening disease, identification of patients who are more likely to progress to severe LRD is important. Lymphopenia has been identified as important risk factor for progression in several studies [1, 12, 13], but the significance of other factors that could plausibly be associated with disease progression, including lymphocyte engraftment dynamics, viral subtypes, neutralizing antibody titers, lung function, and smoking history has not been determined.

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The purpose of this study was to comprehensively determine the significance of host, viral, and treatment factors for the progression to RSV LRD in patients who presented with RSV URI.

MATERIALS AND METHODS

Patients

Patients who underwent allogeneic, syngeneic, or autologous HCT at Fred Hutchinson Cancer Research Center and the Seattle Cancer Center Alliance in Seattle, Washington, from November 1988 to June 2011 were analyzed. Patients with respiratory symptoms had respiratory secretions analyzed for respiratory viruses according to institutional protocols and received standardized care for RSV disease [14]. Patients with RSV URI received high dose intermittently (6 g/day, 60 mg/mL for 2 hours every 8 hours) or low dose continuously (6 g/day, 20 mg/mL for 18 hours/day) ribavirin at the discretion of the attending physician, with or without immunoglobulin or a monoclonal antibody directed against the fusion protein of RSV, palivizumab. Use of steroids, intravenous immunoglobulin (IVIG), and palivizumab at the time of RSV infection was also analyzed. Steroids were given to patients with acute graft vs host disease (GVHD) and IVIG was given mainly for GVHD prophylaxis and low immunoglobulin levels (maximum 500 mg/kg/week). After January 1993, routine IVIG administration was discontinued. Only patients who signed a consent allowing retrospective research (>95%) were included in the study, and the study was approved by Fred Hutchinson Cancer Research Center Institutional Review Board.

Definition of RSV Respiratory Tract Infection

Patients who developed respiratory tract illness due to confirmed RSV infection were considered to have RSV infection. Only patients who presented with RSV URI within 100 days post HCT were included for the analysis. RSV URI was defined as respiratory infection confined to the nose, throat, and sinuses as confirmed by detection of RSV without any evidence of lower respiratory tract involvement. RSV LRD was defined as detection of RSV in the lower respiratory tract by bronchoalveolar lavage (BAL) or biopsy in patients with lower respiratory tract symptoms (cough, oxygen requirement, wheezing) or radiographic new infiltrates. Patients who had URI only were defined as nonprogressors and patients who had URI initially that subsequently progressed to LRD were defined as progressors. Patients who presented with RSV LRD concurrently with URI (defined as development of LRD within 2 days of RSV URI) were excluded.

Virology and Microbiology Procedures

Respiratory specimens (nasopharyngeal wash/swab and BAL samples) were studied for viral antigen detection by direct fluorescent antibody (DFA) staining for RSV, influenza A, B, parainfluenza 1, 2, 3, and adenovirus, human metapnuemovirus (since April 2007); conventional and shell vial centrifugation viral cultures were performed as a standard practice for all patients throughout the study period [6, 11]. After 2006, upper respiratory tract samples were tested by polymerase chain reaction (PCR) instead of conventional methods, whereas BAL samples were tested both by conventional methods and PCR [15].

RSV Subtyping by Molecular Methods

Cultured RSV isolates were stored at −70°C. Total nucleic acids were extracted from the samples and tested for RSV RNA by a real-time reverse transcription polymerase chain reaction (RT-PCR) assay as described elsewhere [16]. The RSV type specific assay contained one primer set to amplify a 94 bp region of the RSV polymerase gene from both types A and B, a 5′ 6FAM-labeled probe specific for RSV type A, and a 5′ VIC-labeled probe specific for RSV type B. Both probes were labeled on the 3′ end with MGBNFQ.

RSV Neutralization Assays

We tested available sera from a prospectively collected serum repository (mostly collected from 1990s). Pretransplant sera from both donors and HCT recipients and weekly posttransplant sera close to URI event (within 2 weeks before and 1 week after) were analyzed. The sampled sera were stored at or below −20°C. Measurement of serum neutralizing-antibody titers to RSV was performed using an established microneutralization method for RSV A (MNA) and RSV B (MNB) strains in the laboratory of Dr Walsh at the University of Rochester [17, 18]. In brief, serum dilutions were incubated with 75 plaque-forming units (PFU) of RSV A2 strain (group A virus) or RSV B1 strain (group B virus) for 30 minutes at room temperature, followed by the addition of 1.5×10^4 HEp-2 cells in 96-well culture plates. After 3 days, the quantity of RSV antigen was determined by EIA using monoclonal antibody (mAb) to the RSV F protein. The neutralization titer was defined as the serum dilution that results in a 50% reduction in color development.

Statistical Analysis

The probability of progression from upper to lower RSV infection was estimated using cumulative incidence. Death was treated as a competing risk for progression. Differences in the proportions of patients who progressed to LRD were evaluated for statistical significance with logistic regression models. The following variables were considered potential predictors of progression: patient age, sex, smoking status, donor type, cell source, donor and recipient CMV serostatus, year transplant performed, total body irradiation (TBI) conditioning, presence of acute GVHD, lymphocyte count at the time of RSV URI onset, pretransplant lung function, RSV subtype, time from transplant to RSV infection, ribavirin treatment, steroid

treatment at varying doses, RSV antibody levels and weekly IVIG infusion. Grades 2–4 acute GVHD was considered present if it was diagnosed before or up to 2 days after the diagnosis of upper RSV infection. Patients who received an autologous or syngeneic transplant were categorized separately for analysis of acute GVHD as a risk factor for progression. The antibody levels were classified according to RSV subtype. All covariates with at least 90% complete data were candidates for the primary analysis via multiple logistic regression models. A variable was retained in the multivariable model if its univariate association with progression resulted in a P value $\leq .1$. Ribavirin treatment was retained in all models because it was a predictor of interest. Covariates missing for more than 10% of the data were not included in the first round of multivariable modeling, but were subsequently examined in subsets of patients with available data.

The statistical significance of differences in absolute lymphocyte counts (ALCs) between the times of URI onset and LRD onset for progressors, and the times of URI onset and 7 days later for nonprogressors, was evaluated with the Wilcoxon signed rank test. These differences in ALC values and the slopes (change in ALC/days between URI and LRD or 7) were then compared by progression status via t-test. Reported P values are 2-sided and based on the Wald statistic. No adjustments were made for multiple comparisons. Statistical analyses were performed using SAS Version 9 (SAS Institute, Inc, Cary, NC).

RESULTS

One hundred eighty-one patients including 147 allogeneic and 34 autologous HCT recipients were evaluated (Table 1). Two patients received syngeneic transplants, and 7 patients were treated with tandem transplants (autologous HCT prior to allogeneic HCT, classified as allogeneic for analysis). The median age was 40.0 years (range, 0.3–72.8 years). The age distribution is as follows: 6 patients were younger than 2 years of age, 20 were 2–14 years, 15 were 15–24 years, 89 were 25–49 years, and 51 were 50 year old or older. One hundred fifty-three patients were white (85%). Eighty one recipients (45%) were CMV negative, and 82 donors (45%) were CMV negative. Forty patients (22%) received HCT during 1988–1993, 42 (23%) during 1994–1996, 50 (28%) during 1997–2001, and 49 (27%) during 2002–2011. The distribution of transplant year was similar between autologous and allogeneic transplants ($P = .19$).

Among the 181 patients studied, 138 patients (76%) had URI only (nonprogressors) and 43 patients (24%) had URI initially and then progressed to LRD (progressors). RSV URI occurred at median post-transplant day 49 (range 1–100). The cumulative incidence of progression at day 40 post-URI diagnosis was 24% (95% confidence interval [CI], 18%–30%, Figure 1*A*). Progression to LRD occurred in 36 allogeneic HCT recipients (24%) and in 7 autologous recipients (21%). Progression to LRD occurred at a median of 7 days after RSV URI onset (range, 2–38 days). There were no progression in the 6 patients who were <2 years old.

RSV isolates from 76 patients were available for additional virus culture and subtyping by RT-PCR (49 with stable URI and 27 with progression to LRD; Table 1). For RSV subtype specific antibodies measurement, sera from 36 donors and 40 recipients pretransplant were available. Sera taken near the time of onset of RSV URI from 38 recipients posttransplant were also tested (25 with stable URI and 13 with progression to LRD).

Risk Factors for Progression from Upper to Lower Respiratory Tract Infection

In a univariate model (Table 1), smoking status, transplant year, high TBI dose, conditioning regimen, lymphocyte count at URI onset, and time of URI onset were sufficiently predictive of progression to LRD to qualify for the multivariable model.

In a multivariable model (Table 2, left side), including 175 patients with 41 progressors, positive smoking status, TBI of 1200 cGy or more, and a lymphocyte count of 100/mm^3 or less at the time of RSV URI onset were significant risk factors for progression to LRD. Of note, treatment with high-dose ribavirin did not reach statistical significance when included in the model. To determine if risk factors were different when considering only LRD events confirmed by radiographic infiltrates, an additional model was fit (Table 2, right side). In that subgroup of 31 patients who progressed to radiographically proven LRD, only a lymphocyte count of 100/mm^3 or less at the time of RSV URI onset remained a significant risk factor for progression to LRD. The probability of progression to LRD from RSV URI by lymphocyte counts at URI onset is shown in Figure 1*B*.

To determine whether the high-dose ribavirin treatment effect varied based on baseline lymphocyte count we performed a stratified analysis (Supplementary Figure 1*A* and 1*B*). There appeared to be stronger trend towards a treatment effect in the low lymphocyte strata (total lymphocyte count <300/mm^3), but neither result reached statistical significance.

We also fit multivariable models to examine the effect of corticosteroid dose at the time of URI diagnosis. When we adjusted for lymphocyte count, smoking status, high-dose ribavirin and TBI, steroid dose was not associated with progression to LRD (data not shown).

Because the stored RSV culture isolates were further subtyped by quantitative real-time RT-PCR in 76 patients, we could identify each RSV infection episode by RSV subtypes (36 patients with subtype A and 40 patients with subtype B; Table 1). RSV subtype was not significantly associated with progression in univariate analysis (Table 1). RSV subtype-specific neutralizing antibody (RSV A vs RSV B) levels were examined in donors and in recipients, pretransplant, and

Table 1. Patient Demographic and Clinical Characteristics, With Univariate Odds Ratios (95% Confidence Intervals) for Progression From URI to LRD

Characteristics	N (% of Total)	Limited URI N (% of Subset)	Progression to LRD N (% of Subset)	OR (95% CI)	*P* Value[a]
Age, years[b]					.18
<25	41 (23)	35 (85)	6 (15)	1.0	
25–49	89 (50)	63 (71)	26 (29)	2.4 (.9–6.4)	
≥50	51 (28)	40 (78)	11 (22)	1.6 (.5–4.8)	
Sex					.19
Female	73 (40)	52 (71)	21 (29)	1.0	
Male	108 (60)	86 (80)	22 (20)	0.6 (.3–1.3)	
Smoker					.04
No	105 (58)	86 (82)	19 (18)	1.0	
Yes	63 (35)	43 (68)	20 (32)	2.1 (1.0–4.4)	
Missing	13 (7)	9 (69)	4 (31)	–[c]	
Donor					.45
Matched-related	62 (34)	51 (82)	11 (18)	1.0	
Mismatched-related	15 (8)	10 (67)	5 (33)	2.3 (.7–8.1)	
Unrelated	72 (40)	52 (72)	20 (28)	1.8 (.8–4.1)	
Autologous	32 (18)	25 (78)	7 (22)	1.3 (.4–3.8)	
Underlying diagnosis[d]					.70
Allogeneic					
Acute leukemia	75 (41)	59 (79)	16 (21)	1.0	
Chronic leukemia	45 (25)	31 (69)	14 (31)	1.7 (.7–3.9)	
Lymphoma	11 (6)	8 (73)	3 (27)	1.4 (.3–5.8)	
Other	18 (10)	15 (83)	3 (17)	0.7 (.2–2.9)	
Autologous				1.0 (.4–2.8)	
Acute leukemia	3 (2)	3 (100)	0	–[e]	
Chronic leukemia	1 (1)	1 (100)	0	–[e]	
Lymphoma	12 (7)	9 (75)	3 (25)	–[e]	
Other	16 (9)	12 (75)	4 (25)	–[e]	
Cell source					.13
PBSC	64 (35)	53 (83)	11 (17)	1.0	
BM or cord blood	117 (65)	85 (73)	32 (27)	1.8 (.8–3.9)	
Recipient CMV serostatus					.66
Negative	81 (45)	63 (78)	18 (22)	1.0	
Positive	100 (55)	75 (75)	25 (25)	1.2 (.6–2.3)	
Donor CMV serostatus					.41
Negative	83 (46)	61 (73)	22 (27)	1.0	
Positive	63 (35)	50 (79)	13 (21)	0.7 (.3–1.6)	
Autologous	32 (18)	25 (78)	7 (22)	–[c]	
Missing	3 (2)	2 (67)	1 (33)	–[c]	
Transplant year					.08
1988–1993	40 (22)	29 (72)	11 (28)	1.0	
1994–1996	42 (23)	31 (74)	11 (26)	0.9 (.4–2.5)	
1997–2001	50 (28)	34 (68)	16 (32)	1.2 (.5–3.1)	
2002–2011	49 (27)	44 (90)	5 (10)	0.3 (.1–1.0)	
TBI conditioning					.02
No	72 (40)	60 (83)	12 (17)	1.0	
Low (200 cGy)	17 (9)	16 (94)	1 (6)	0.3 (.1–2.6)	
High (1200–1575 cGy)	92 (51)	62 (79)	30 (33)	2.4 (1.1–5.2)	

Table 1 continued.

Characteristics	N (% of Total)	Limited URI N (% of Subset)	Progression to LRD N (% of Subset)	OR (95% CI)	*P* Value[a]
Conditioning regimen[d]					.02
Myeloablative allogeneic					
Chemo and high dose TBI	86 (48)	57 (66)	29 (34)	1.0	
Chemo alone or low dose TBI	47 (26)	41 (87)	6 (13)	0.3 (.1–0.8)	
Nonmyeloablative allogeneic	16 (9)	15 (94)	1 (6)	0.1 (.1–1.0)	
Autologous	32 (18)	25 (78)	7 (22)	0.6 (.2–1.4)	
GVHD prophylactic regimen					.53
Allogeneic					
Calcineurin inhibitor + MTX	101 (56)	76 (75)	25 (25)	1.0	
Calcineurin inhibitor + MMF	18 (10)	16 (89)	2 (11)	0.4 (.1–1.8)	
Other	30 (17)	21 (70)	9 (30)	1.3 (.5–3.2)	
Autologous	32 (18)	25 (78)	7 (22)	0.9 (.3–2.2)	
Acute GVHD					.28
Grade 0 or 1	52 (29)	42 (81)	10 (19)	1.0	
Grade 2, 3 or 4	95 (52)	69 (73)	26 (27)	1.6 (.7–3.6)	
Lymphocytes/mm^3 at URI					.001
>500	56 (31)	50 (89)	6 (11)	1.0	
101–500	84 (46)	65 (77)	19 (23)	2.4 (.9–6.5)	
≤100	35 (19)	19 (54)	16 (46)	7.0 (2.4–20.6)	
Missing	6 (3)	4 (67)	2 (33)	–[c]	
Lung function: FEV1/FVC					.60
N	153				
Median (range)	0.78 (0.52–1.11)	–	–	0.9 (.6–1.4)	
RSV posttransplant					.10
>30 d	124 (69)	99 (80)	25 (20)	1.0	
≤30 d	57 (31)	39 (68)	18 (32)	1.8 (.9–3.7)	
RSV subtype					.70
A	36 (20)	24 (67)	12 (33)	1.0	
B	40 (22)	25 (62)	15 (38)	1.2 (.5–3.1)	
Ribavirin					.26
No	112 (62)	86 (77)	26 (23)	1.0	
Low dose	37 (20)	25 (68)	12 (32)	1.6 (.7–3.6)	
High dose	32 (18)	27 (84)	5 (16)	0.6 (.2–1.8)	
Steroids mg/kg/d					.64
None	69 (38)	54 (78)	15 (22)	1.0	
≤1	57 (31)	45 (79)	12 (21)	1.0 (.4–2.3)	
1–2	35 (19)	24 (69)	11 (31)	1.7 (.7–4.1)	
>2	14 (8)	10 (71)	4 (29)	1.4 (.4–5.2)	
Weekly IVIG transfusion					.19
No	147 (81)	115 (78)	32 (22)	1.0	
Yes	34 (19)	23 (68)	11 (32)	1.7 (.8–3.9)	

Abbreviations: BM, bone marrow; CMV, cytomegalovirus; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GVHD, graft vs host disease; IVIG, intravenous immunoglobulin; LRD, lower respiratory disease; MMF, mycophenolate mofetil; MTX, methotrexate; N, number; PBSC, peripheral blood stem cell; RSV, respiratory syncytial virus; TBI, total body irradiation; URI, upper respiratory infection;

[a] *P* value represents a global test for heterogeneity in risk of progression.

[b] Median age of allogeneic and autologous recipients were 36 years and 40 years ($P = .31$).

[c] Subgroup excluded from analysis.

[d] Underlying diagnosis and risk-based conditioning regimens were categorized as previously reported [1, 3].

[e] Autologous disease groups combined for analysis due to small numbers.

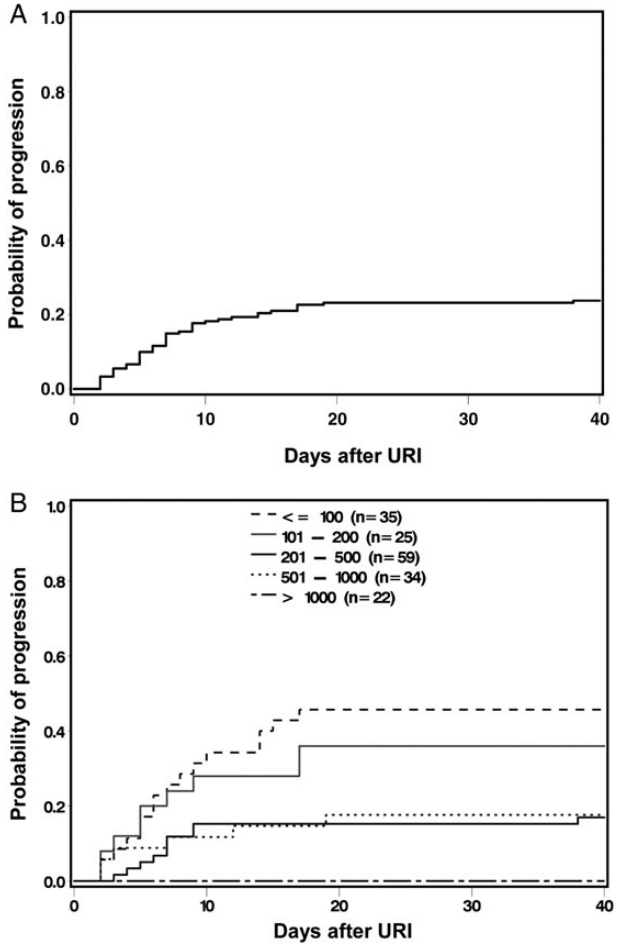

Figure 1. *A*, Probability of progression to LRD from time of upper RSV infection. *B*, Probability of progression to LRD from upper RSV infection by lymphocyte counts at URI. Abbreviations: LRD, lower respiratory disease; RSV, respiratory syncytial virus; URI, upper respiratory tract infection.

posttransplant close to the time of RSV URI, and none of these showed a statistically significant association with progression (Table 3). Similarly, weekly IVIG infusion did not impact risk of progression (Table 1).

Lymphocyte Engraftment Dynamics

The effect of immune reconstitution on progression was examined in 43 progressors and 138 nonprogressors by comparing changes in ALC over time. In progressors, ALC at URI onset and LRD onset were compared. Because the median time for progression from URI to LRD was 7 days, the ALC at the time of URI onset and 7 days later were compared in nonprogressors. In the progressors, the median ALC at URI and LRD onset were 140/mm^3 and 198/mm^3, respectively (Supplementary Figure 2*A*). In nonprogressors, the median ALC at URI onset and URI 7 days were 400/mm^3 and 453/mm^3 (Supplementary Figure 2*B*).

Table 2. Multivariable Odds Ratios for Progression From URI to LRD (N = 175)

	All LRD		Radiograph-positive LRD	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Smoker				
No	1.0		1.0	
Yes	2.5 (1.1–5.6)	.03	2.0 (.8–4.8)	.14
Missing	1.7 (.4–7.2)	.50	2.3 (.5–10.4)	.28
TBI conditioning				
None or low (200 cGy)	1.0		1.0	
High (1200–1575 cGy)	2.5 (1.1–5.6)	.03	2.1 (.8–5.2)	.12
Lymphocytes/mm^3 at URI				
>500	1.0		1.0	
101–500	2.1 (.7–5.8)	.17	3.0 (.8–11.4)	.11
≤100	6.0 (1.9–18.9)	.002	10.3 (2.5–41.8)	.001
Ribavirin				
None or low dose	1.0		1.0	
High dose	0.5 (.2–1.5)	.21	0.5 (.2–1.7)	.29

Abbreviations: CI, confidence interval; LRD, lower respiratory tract disease; OR, odds ratio; URI, upper respiratory infection.

The median (range) change in ALCs in progressors vs nonprogressors were −6/mm^3 (−410 to 780/mm^3) and 29/mm^3 (−3854 to 2070/mm^3), respectively (Figure 2*A*). ALC changes as a rate per day were also examined by calculating the slopes of ALC changes as (ALC at LRD onset–ALC at URI onset) divided by days between URI and LRD in progressors, and (ALC at URI d7–ALC at URI onset) divided by 7 in nonprogressors. The median (range) slopes in progressors vs nonprogressors were −2/mm^3 day^{-1} (−48 to 220/mm^{3}day^{-1}) vs 4/mm^{3}day^{-1} (−551 to 296/mm^3 day^{-1}) (Figure 2*B*). There were no significant differences between the groups in change in ALC (P = .52) or slope of ALC (P = .96).

DISCUSSION

RSV infection in patients undergoing HCT can result in respiratory failure and death if LRD develops [19]. RSV disease progression is not universal, with observed rates of progression ranging from 0% up to 60% (mean 38%) [10, 20, 21]. The identification of risk factors associated with progression and assessment of potential treatment modalities are important when caring for these patients. This study was designed to determine the significance of viral subtype, type-specific neutralizing antibodies and other previously untested factors that could be associated with progression to lower respiratory tract disease. We found that smoking, conditioning with high-dose TBI, and

Table 3. Virus Subtype and Neutralizing Antibody Titers Pretransplant Donors and Pre-and Posttransplant Recipient Sera Close to the Time of URI Onset, With Univariate Odds Ratios (95% Confidence Intervals) for Progression From URI to LRD

	N	Median (range)	OR (95% CI)	*P* Value
Pre-transplant donor Ab log_2	36	10.8 (7.8–13.4)	0.9 (.5–1.6)	.66
Pre-transplant recipient Ab log_2	40	10.0 (7.4–12.9)	1.0 (.6–1.7)	.95
Post-transplant recipient Ab log_2	38	10.0 (7.4–12.5)	0.8 (.4–1.3)	.35

Abbreviations: Ab, antibody; CI, confidence interval; LRD, lower respiratory tract disease; N, number; URI, upper respiratory infection.

Data are shown from only available viruses and sera samples for further virus subtyping or subtype specific antibody titer measurement.

absolute lymphocyte counts (ALCs) $\leq 100/mm^3$ at the time of URI onset were significantly associated with progression (Table 2), whereas the viral subtype, RSV-specific neutralizing antibodies, lung function, and lymphocyte engraftment dynamics appeared similar between progressors and nonprogressors (Table 1 and 3 and Figure 2*A* and 2*B*).

To date, lymphopenia is the only established risk factor for progression of RSV URI to LRD in HCT recipients. We validated this association in statistical models that adjusted for other important risk factors and defined, for the first time to our knowledge, a protective level of the ALC. In our study, we determined that lymphocyte counts $>1000/mm^3$ at the time of URI onset were associated with complete protection from disease progression. Although absolute levels of lymphocytes appeared to be extremely predictive for subsequent LRD, lymphocyte engraftment dynamics had no apparent effect. A potential role of lymphocytes in immune reconstitution has been suspected as risk factor for RSV LRD development because severe RSV disease is known to occur more frequently if infection occurs before engraftment [22]. Immune reconstitution inflammatory syndrome (IRIS) is a well-known phenomenon observed as clinical deterioration in the setting of infection in the face of immune restoration by antiretroviral therapy in certain individuals infected with human immunodeficiency virus (HIV) [23–25]. IRIS has also been reported in non-HIV settings such as HCT, solid organ transplant recipients, autoimmune diseases, or cancer therapy with multiple pathogens [26, 27].

The existence of the immune reconstitution phenomenon in the context of RSV disease progression in HCT recipients has not been systematically analyzed. Because lymphocyte counts were found to be the critical risk factor for progression, we examined the effect of lymphocyte count changes on progression from URI to LRD to test whether progression to LRD was associated with increasing lymphocyte counts and thus a form of IRIS. The possibility of IRIS due to respiratory virus infection

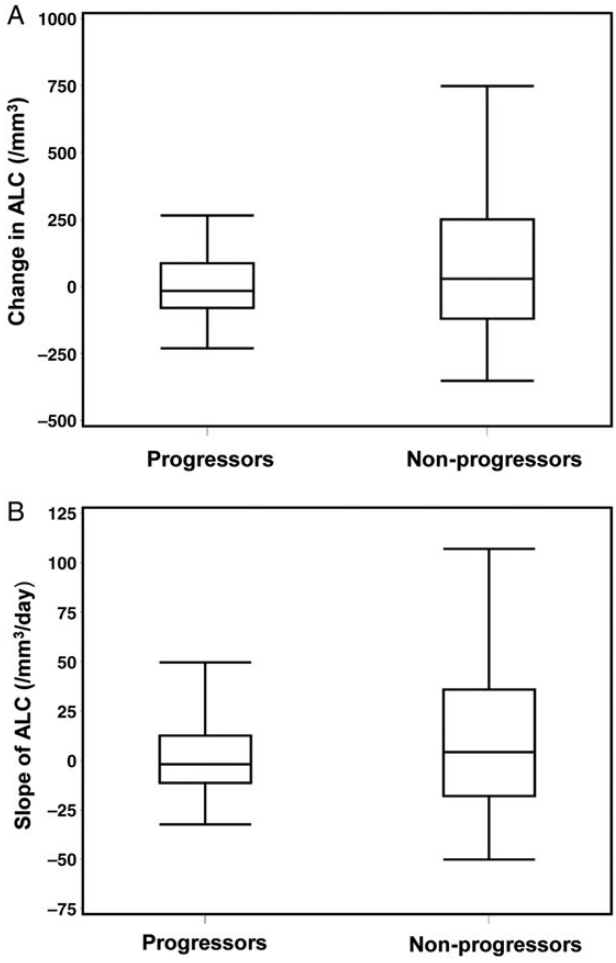

Figure 2. *A*, Box plots for the changes in lymphocyte counts in progressors and nonprogressors. Changes were calculated as (ALC at LRD onset − ALC at URI onset) in progressors and (ALC at URI d7 − ALC at URI onset) in nonprogressors. Median values for the changes in ALCs were $-16/mm^3$ (range -410 to $780/mm^3$) for the progressors and $29/mm^3$ (range -3854 to $2070/mm^3$) for the nonprogressors ($P = .52$). *B*, Box plots for the slope of lymphocyte counts in progressors and nonprogressors. Slopes were calculated as (ALC at LRD onset − ALC at URI onset) ÷ days between URI and LRD in progressors and (ALC at URI d7 − ALC at URI onset) ÷ 7 in nonprogressors. Median values for the changes in ALCs were $-2/mm^3$ (range -48 to $220/mm^3$) for the progressors and $4/mm^3$ (range -551 to $296/mm^3$) for the nonprogressors ($P = .96$). Abbreviations: ALC, absolute lymphocyte count; LRD, lower respiratory tract disease; URI, upper respiratory infection.

(5 patients with parainfluenza and 1 patient with RSV) has been reported in severe combined immunodeficiency disease (SCID) patients who presented with post-engraftment pneumonitis after HCT [26, 28]. However, we did not observe differences in lymphocyte engraftment dynamics between progressors and nonprogressors (Figure 2*A* and 2*B*), suggesting that profound lymphopenia and the associated immune defect rather than an IRIS phenomenon is responsible for progression to LRD.

RSV-specific antibodies play an important role in the prevention of RSV disease in young children [29, 30]. Palivizumab prophylaxis has been documented to prevent serious RSV disease in young children with underlying cardiac and pulmonary disease [31] but has shown only very limited success in treatment situations [6, 19, 32–35]. In elderly immunocompetent persons, having RSV antibody level below 10 $\log_2$ was associated with more hospitalization and ICU care [36]. A recent retrospective pooled review on management of RSV infections in adult recipients of HCT showed that treatment interventions (such as aerosolized ribavirin alone or ribavirin combined with immunoglobulin, RSV specific immunoglobulin, or palivizumab) reduced the progression by almost one-third compared to no treatment [20]. Another small retrospective analysis of palivizumab given for RSV URI did not show a beneficial effect [8, 10]. Overall, the importance of serum RSV-Ab level in protecting the HCT patient from RSV disease progression is not known, and there is presently no good evidence supporting its use in patients with RSV URI [37].

Based on the above data, we hypothesized that differences in RSV antibody level would influence the progression outcome during RSV infection. The effect of preexisting RSV antibody level in donors and recipients before HCT and posttransplant period near the time when RSV infection occurred was examined. Our analyses did not show any role of preexisting antibody levels in the progression of RSV disease from upper to lower tract infection (Table 3). Although we did not observe differences in antibody levels between progressors and nonprogressors, this study is the first to our knowledge to measure the RSV A and B subtype specific antibody level in HCT recipients and their donors. Optimal RSV-specific antibody levels in HCT recipients or donors for the prevention of progression in HCT recipients are not known, but these data will provide reference for future studies.

Differences in RSV disease severity based on subtypes A and B have been suggested in children with primary infection in the literature [38]. RSV A and B typically cocirculate in the community, and both types are also detected in immunocompromised populations [16, 39, 40]. We correlated RSV subtypes with disease progression in our subjects, demonstrating that RSV subtype was not significantly associated with progressive disease (Table 1). Although we were only able to perform this analysis in a relatively small subset of patients, we were unable to detect any trends. Therefore, if an effect of subtype on disease progression is present, it is likely to be small.

Active smoking before HCT was a risk factor for progression in this study. This is a novel observation for the immunocompromised patient population and a potentially modifiable factor. Household smoke has long been identified as a risk for RSV bronchiolitis in the pediatric population [41, 42] but has not been studied systematically in immunocompromised adult populations.

We also examined the role of steroid treatment at varying doses, presence of varying degrees of GVHD, time from transplantation to RSV infection, and other transplant and host factors but found no statistically significant associations. The lack of association with steroid dose is important in clinical practice. Physicians often wonder whether small doses of steroids can be given to ease obstructive patterns or if a rapid taper should be initiated if patients are already receiving steroids (mostly for GVHD). Although this was not a randomized trial, our data would suggest that moderate doses of systemic corticosteroids may not be associated with progression to LRD.

This study did not find a statistically significant effect of high-dose ribavirin when given for RSV URI. However, the number of patients treated was relatively small, and the trend was consistent with other reports in the literature [43–45]. Preemptive aerosolized ribavirin has not been evaluated in adequately powered randomized controlled trials in immunocompromised patients, although recent analyses suggested a beneficial effect [2, 20]. In addition, there are a few case series studies reporting the effect of oral or intravenous ribavirin treatment, which needs further evaluation [46, 47]

Our study has several strengths and limitations. Although we included clinical data from a large number of patients who initially presented with RSV URI, sera for RSV specific antibody test were available only in a subset of recipients and donors (less than half), and RSV virus subtype was also only available in 76 patients. Therefore, smaller effect sizes associated with RSV subtypes or RSV-specific antibody levels may have been missed. Despite these limitations, this is the first study to our knowledge to examine RSV subtype- specific antibody levels in HCT recipients with concurrent virus subtyping. In addition, this study examined the effect of immune reconstitution, although only by analyzing overall lymphocyte engraftment dynamics. We were unable to evaluate the effects of specific T-cell subsets because these data were not available, and there were no cells available for study.

In conclusion, among 181 HCT recipients who presented with RSV URI, we identified smoking, conditioning with high-dose TBI (1200–1575 cGy), and ALC of $\leq 100/mm^3$ at the time of URI onset as significant risk factors associated with progression. We also observed that ALC of $>1000/mm^3$ was completely protective against RSV disease progression. The importance of lymphocyte counts and the lack C of importance of antibody levels suggest that a characterization of RSV-specific cell-mediated immunity is warranted.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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