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Predictive Value of Respiratory Viral Detection in the Upper Respiratory Tract for Infection of the Lower Respiratory Tract With Hematopoietic Stem Cell Transplantation

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Background. Hematopoietic cell transplant (HCT) recipients are frequently infected with respiratory viruses (RVs) in the upper respiratory tract (URT), but the concordance between URT and lower respiratory tract (LRT) RV detection is not well characterized.

Methods. Hematopoietic cell transplant candidates and recipients with respiratory symptoms and LRT and URT RV testing via multiplex PCR from 2009 to 2016 were included. Logistic regression models were used to analyze risk factors for LRT RV detection.

Results. Two-hundred thirty-five HCT candidates or recipients had URT and LRT RV testing within 3 days. Among 115 subjects (49%) positive for a RV, 37% (42 of 115) had discordant sample pairs. Forty percent (17 of 42) of discordant pairs were positive in the LRT but negative in the URT. Discordance was common for adenovirus (100%), metapneumovirus (44%), rhinovirus (34%), and parainfluenza virus type 3 (28%); respiratory syncytial virus was highly concordant (92%). Likelihood of LRT detection was increased with URT detection (oods ratio [OR] = 73.7; 95% confidence interval [CI], 26.7–204) and in cytomegalovirus-positive recipients (OR = 3.70; 95% CI, 1.30–10.0).

Conclusions. High rates of discordance were observed for certain RVs. Bronchoalveolar lavage sampling may provide useful diagnostic information to guide management in symptomatic HCT candidates and recipients.

Keywords. diagnostics; hematopoietic stem cell transplantation; respiratory viruses.

Respiratory virus infections are a major cause of mortality and morbidity in hematopoietic cell transplant (HCT) recipients [1–3]. Although symptomatic patients are frequently tested for viruses in the upper respiratory tract (URT), lower respiratory tract (LRT) testing with bronchoscopy and/or bronchoalveolar lavage (BAL) is done less frequently, often only when prompted by clinical deterioration or for further evaluation of findings on radiologic imaging. Our study assessed the correlation between concurrent URT and LRT testing for respiratory viruses in HCT pretransplant candidates and posttransplant recipients.

Few studies have examined differences in respiratory viral detection by polymerase chain reaction (PCR) between upper and lower tract samples. In immunocompetent children with chronic respiratory symptoms, paired nasopharyngeal (NP) aspirate and BAL samples showed discordance in approximately one third of patients, with positive NP aspirate/negative BAL discordance being most common [4]. In a small study of adults, the majority of which were HCT recipients or had a hematologic malignancy, with matched NP and BAL specimens, PCR-based NP testing for respiratory viruses in patients with clinical evidence of LRT disease had a high negative predictive value (NPV) and a lower positive predictive value (PPV) [5]. A larger study that included mostly immunocompromised patients concluded that if a pathogen (a respiratory virus or 1 of 3 bacterial pathogens detected by a multiplex PCR panel) was already identified from an NP sample, BAL testing is unlikely to provide additional information; however, a significant number (20%) of subjects had a pathogen detected in the BAL without a positive NP sample [6]. Likewise, in a study of lung transplant recipients, viral detection exclusively in the LRT was reported, and thus the authors caution on the use of URT sampling alone to rule out LRT infection in this population [7].

Our study aimed (1) to describe discordance in HCT candidates and recipients and (2) to characterize specific viral, patient, and treatment risk factors that are associated with LRT detection. In addition, using quantitative PCR methodology,

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we aimed to define the role of viral load in respiratory virus LRT detection.

METHODS

Patients and Viral Testing

We retrospectively identified HCT pretransplant candidates (within 90 days of HCT) and posttransplant recipients who underwent a BAL ±1 and ±3 days from an NP aspirate with testing of respiratory specimens by multiplex PCR testing for 11 respiratory viruses (adenovirus A–F, human rhinovirus [HRV], influenza A and B, parainfluenza viruses [PIV] 1–4, human coronavirus, respiratory syncytial virus [RSV], and human metapneumovirus [HMPV]) between July 2009 and October 2016 [8]. In total, exactly 1000 HCT recipients underwent BAL respiratory virus testing during the study period. Clinically indicated bronchoscopy for LRT symptoms and/or radiographic abnormalities was determined by a pulmonologist. A BAL was generally performed to either rule-in respiratory viral involvement of the LRT and/or to rule out alternative causes for a LRT process. The BALs were collected per institutional standard practice using up to three 30-mL aliquots of normal saline. From this cohort, we then identified subjects who had URT viral PCR testing (from nasal wash or NP swab) within 3 days or within 1 day of the BAL. Only the first BAL per subject was included in the analysis.

Cycle thresholds (Ct) were used as a proxy for viral load and were compared between upper and lower respiratory sample pairs that were positive for the same virus for a given subject. Patient charts were reviewed for steroid use, radiology results, presence of copathogens, and potential alternate diagnoses. Steroid dose was defined as the highest dose of steroid in milligrams/kilogram per day expressed in equivalent doses of prednisone in the 2 weeks preceding the BAL. Computed tomography of the chest obtained within 1 week of the BAL were reviewed. We chose to categorize imaging results for the presence versus absence of a solitary nodule because patients with a solitary nodule may carry an alternative diagnosis and be at lower risk for viral LRT involvement. Copathogens were defined as follows: (1) bacterial - >10 000 colony-forming units of a Gram-positive organism or any Gram-negative organism on BAL; (2) viral - any other respiratory virus detected in BAL by multiplex PCR or cytomegalovirus (CMV) shell vial positivity; and (3) fungal - positive serum or BAL galactomannan, BAL fungal PCR positivity, or BAL fungal culture positivity (excluding *Candida* species) [9, 10]. Alternate diagnoses included diffuse alveolar hemorrhage based on finding progressive bloody fluid return on BAL [11].

Statistical Analysis

Discordance was defined as either (1) a URT sample positive for a respiratory virus with the paired LRT sample negative for the same virus (termed positive/negative [P/N]) or (2) a URT sample negative for a respiratory virus with the paired LRT sample positive for the same virus (termed negative/positive [N/P]). Positive concordance was defined as both URT and LRT paired samples being positive for the same virus (P/P), and negative concordance was defined as both URT and LRT paired samples being negative for the same virus (N/N). The sensitivity and specificity for LRT detection by Ct value in the URT was plotted by generating a receiver operating characteristic (ROC) curve. The Ct value for subjects with negative testing in the URT was set at 40, above the upper limit of assay detection, to allow inclusion of these subjects in the ROC analysis. Positive and negative predictive values for LRT infection were plotted as a function of all possible Ct value cutpoints. Logistic regression models were used to evaluate odds ratios (OR) for the association between candidate risk factors and viral LRT detection. Patients with more than 1 virus detected in the URT (N = 12) were excluded from the logistic regression analysis. All patients in the cohort with LRT detection of adenovirus also had adenovirus testing of the plasma by PCR for viremia. Patients with disseminated adenovirus as evidenced by a positive plasma PCR at the time of LRT detection (N = 4) were excluded from the logistic regression and ROC analysis. Variables with $P \leq .2$ in univariable analysis were candidates for multivariable models and were retained in the models if they remained significant themselves or modified the effect of another factor (confounder). Covariates evaluated as candidate risk factors for inclusion in multivariable models are listed in Table 1. Statistical significance was defined as 2-sided $P < .05$. SAS version 9.4 TS1M3 (SAS Institute Inc., Cary, NC) was used for all statistical analyses.

RESULTS

Cohort Description

We identified 235 subjects with a BAL performed during the study period who had URT RV testing within 3 days of the BAL. Table 1 shows the demographic characteristics of the cohort. The majority of patients (63%) were between 21 and 60 years of age, and 60% were male. The majority of patients (84%) underwent allogeneic transplant. Only 14% had a BAL before HCT. The median number of days between BAL and nasal swab was 1 day. Table 1 also shows the demographics for a subset of 131 subjects in this cohort with URT and LRT testing within 1 day of each other; the subset is largely representative of the whole cohort.

Concordant Versus Discordant Results

Among the 235 sample pairs in the overall cohort, 49% (115 of 235) were positive for a respiratory virus in either the URT or LRT. Of these, 63% (73 of 115) were concordant positive for the same virus in both upper and lower tracts (P/P). Discordance was noted in 37% (42 of 115) of sample pairs. Among the discordant pairs, 60% (25 of 42) were positive in the URT but

Table 1. Demographics of Entire Cohort (N = 235) and of Subset With ±1 Day Between Upper and Lower Respiratory Tract Testing (N = 131)

Variables	Categories	±3 Days (N = 235)	±1 Day (N = 131)
Gender	Female	93 (40%)	52 (40%)
	Male	142 (60%)	79 (60%)
Race	White	177 (75%)	98 (75%)
	Non-White	56 (24%)	33 (25%)
	Unknown	2 (1%)	
Recipient age at transplant	0–20	28 (12%)	18 (14%)
	>21 to <60	149 (63%)	79 (60%)
	≥60	58 (25%)	34 (26%)
Transplant number	1	186 (79%)	107 (79%)
	2	44 (19%)	26 (19%)
	3	5 (2%)	2 (1%)
Year of transplant	2009–2011	97 (41%)	103 (79%)
	2012–2013	70 (30%)	26 (20%)
	2014–2016	68 (29%)	2 (2%)
Donor type	Allo/Unrelated	197 (84%)	113 (86%)
	Auto	38 (16%)	18 (14%)
Conditioning regimen	Non-myeloablative	81 (34%)	48 (37%)
	Myeloablative without high-dose TBI (<1200)	107 (46%)	59 (45%)
	Myeloablative with high-dose TBI (≥1200)	47 (20%)	24 (18%)
Recipient CMV serostatus	-	78 (33%)	41 (31%)
	+	156 (66%)	90 (69%)
Donor CMV serostatus	-	150 (64%)	81 (62%)
	+	83 (35%)	50 (38%)
Day of BAL after transplant	Median (IQR)	49.0 (13.0–159.0)	57.0 (15.0–179.0)
Gap between BAL and nasal swab	Median (IQR)	1.0 (1.0–2.0)	1.0 (0.0–1.0)
	Mean (STD)	1.5 (0.9)	0.7 (0.4)
	Median (range)	1.0 (0.0–3.0)	1.0 (0.0–1.0)
Highest dose of steroids received in the 14 days before BAL[a]	0 to <1	181 (77%)	0.3 (0.0–1.0)
	1 to <2	33 (14%)	0.8 (1.8)
	≥2	19 (8%)	0.3 (0.0–13.5)
WBC on day of/closest day of BAL (cells/μL)	≤1000	73 (31%)	38 (29%)
	>1000	162 (69%)	93 (71%)
ANC on day of/closest day of BAL (cells/μL)	≤100	46 (20%)	24 (18%)
	100–500	27 (11%)	14 (11%)
	>500	162 (69%)	93 (71%)
Lymphocyte on day of/closest day of BAL (cells/μL)	≤100	64 (27%)	29 (22%)
	100–500	88 (37%)	55 (42%)
	>500	82 (35%)	47 (36%)
	Missing	1 (0%)	
Monocyte on day of/closest day of BAL (cells/μL)	≤100	88 (37%)	49 (37%)
	100–500	71 (30%)	42 (32%)
	>500	75 (32%)	40 (31%)
Imaging findings	All others	217 (92%)	124 (95%)
	Solitary nodule	8 (3%)	3 (2%)
	Missing	10 (4%)	4 (3%)
BAL before HCT	No	203 (86%)	119 (91%)
	Yes	32 (14%)	12 (9%)

Abbreviations: ALC, absolute lymphocyte count; allo, allogeneic stem cell transplant; AMC, absolute monocyte count; ANC, absolute neutrophil count; auto, autologous stem cell transplant; BAL, bronchoalveolar lavage; CMV, cytomegalovirus; HCT, hematopoietic cell transplant; IQR, interquartile range; STD, standard deviation; TBI, total body irradiation; WBC, white blood cell count.

[a]Equivalent dose of prednisone in mg/kg per day.

negative in the LRT (P/N), and 40% (17 of 42) were negative in the URT but positive in the LRT (N/P). Figure 1 shows the distribution of concordance/discordance for pairs with at least 1 test positive for individual viruses. In patients who underwent BAL within 3 days of an NP aspirate, discordance between URT and LRT results was observed at the highest rate for HMPV (9 positive pairs, 33% N/P and 11% P/N), HRV (44 positive pairs, 7% N/P and 27% P/N), PIV2 (2 positive pairs, 50% N/P), and

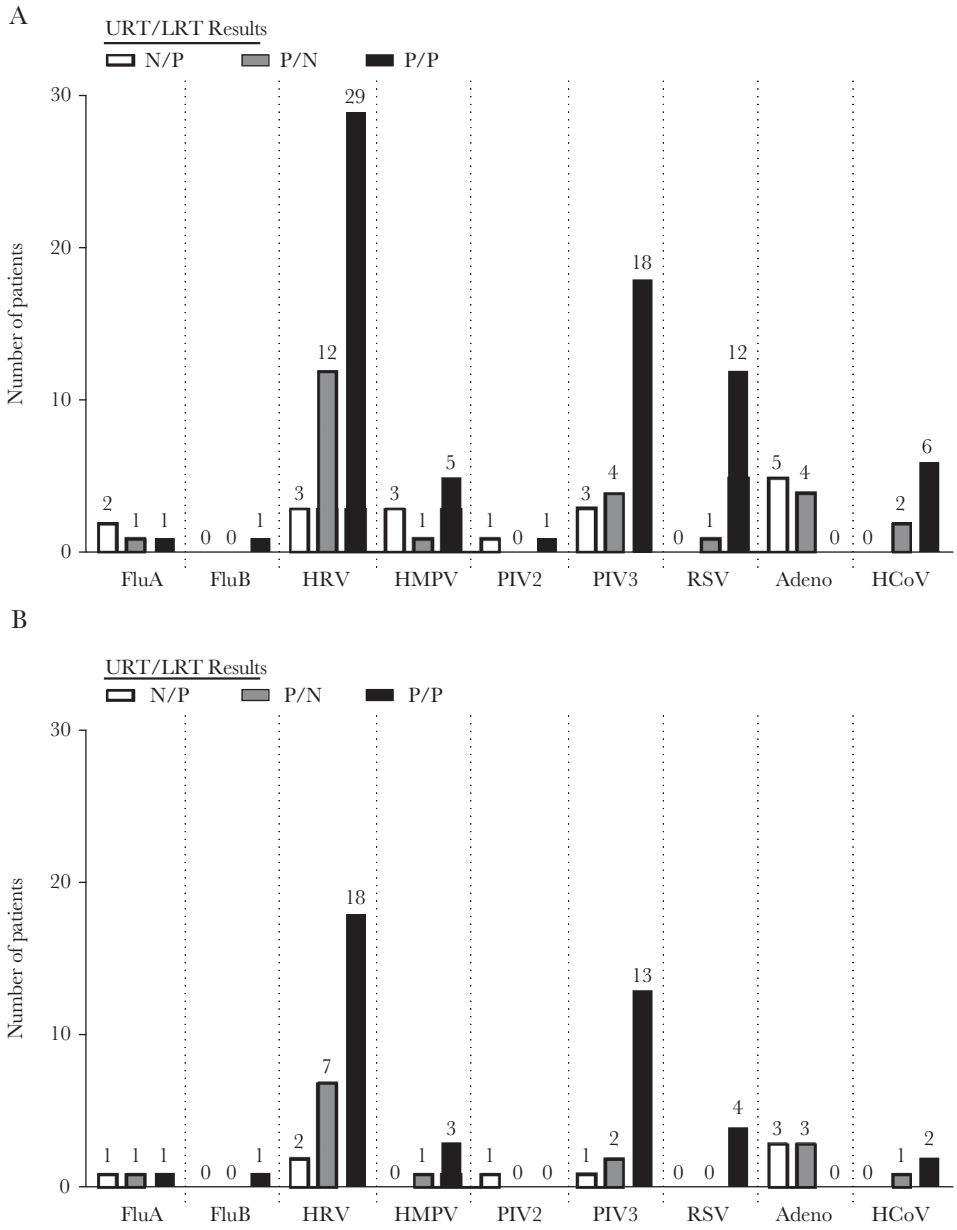

Figure 1. Results of upper respiratory tract (URT) and lower respiratory tract (LRT) sample testing with concordance or discordance by specific virus (represented as result from URT/LRT with N = negative and P = positive). Data are shown for subjects with a bronchoalveolar lavage ±3 days (A) or ±1 day (B) from the URT test. Sample pairs negative in both URT and LRT (N/N) are not represented here. Adeno, adenovirus; FluA, influenza A; FluB, influenza B; HCoV, human coronavirus; HMPV, human metapneumovirus; PIV, parainfluenza viruses 1–4 ; HRV, human rhinovirus; RSV, respiratory syncytial virus.

PIV3 (25 positive pairs, 12% N/P and 16% P/N) (Figure 1A). All 9 pairs with a positive adenovirus result were discordant (56% N/P and 44% P/N). Respiratory syncytial virus had the highest frequency and percentage of concordant results (13 positive pairs, 92% concordant with 8% P/N and no N/P pairs). Similar patterns of discordance were observed when analyzing patients who underwent BAL within 1 day of an NP aspirate (Figure 1B) and when analyzing the subset of patients who underwent BAL after a positive NP aspirate (Supplemental Figure 1). The Ct values of all viruses grouped together between the URT and LRT in concordant pairs were not significantly different when the BAL was performed either ±3 days ($\Delta Ct_{URT\text{-}LRT} = -0.1$, $P = .90$) or ±1 day ($\Delta Ct_{URT\text{-}LRT} = -0.57$, $P = .56$) from collection of the URT specimen.

The distribution of copathogens and alternate diagnoses was examined for concordant positive pairs and discordant pairs (Figure 2). *Aspergillus fumigatus*, another respiratory virus, or bacteria were the most commonly identified copathogens. No significant differences were observed in the proportion of copathogens or alternate diagnoses in subjects with discordant testing versus concordant positive results by Fisher's exact test.

Risk for Lower Respiratory Tract Detection

In a univariable analysis of risk factors for LRT detection, detection of virus in the URT (OR = 54.9; 95% confidence interval [CI], 22.4–135) and recipient CMV seropositivity (OR = 2.44; 95% CI, 1.20–4.76) were associated with an increased risk for LRT detection (Table 2). Of note, factors not found to be associated with LRT detection included conditioning regimen, transplant type (allogeneic versus autologous), steroid use, presence of a solitary nodule on imaging, or lymphocyte, neutrophil, monocyte, or overall white blood cell counts. In a multivariable analysis, detection of virus in the URT (OR = 73.7; 95% CI, 26.7–204) and recipient CMV seropositivity (OR = 3.70; 95% CI, 1.30–10.0) remained strongly associated with an increased risk for LRT detection (Table 2). An analysis of the subset of patients who underwent BAL within 1 day of the NP aspirate and a separate analysis of the subset of only HCT recipients who underwent BAL after transplantation yielded similar results (Supplementary Tables 1 and 2). The proportion of patients with a positive LRT sample was similar between patients with URT testing before LRT testing (28% or 57 of 201) versus after LRT testing (28% or 5 of 18). Thirty-day mortality in patients with positive respiratory viral testing in the LRT was 23.0% (14 of 61) compared with 15.2% (24 of 158) in those with negative testing, although this difference was not statistically significant ($P = .23$).

A ROC curve was generated to summarize sensitivity and specificity of varying Ct cutpoints in the URT for the presence of LRT infection. A Ct cut point of 27.5 in the URT had a sensitivity of 70% and a specificity of 98%, whereas a cutpoint of 32.0 had a sensitivity of 80% and a specificity of 96% (Figure 3A). Using a Ct cutoff of 27.5 in the URT with a prevalence of LRT infection of 28% (61 of 219) in the cohort, the PPV was 94% and the NPV was 90% for LRT infection (Figure 3B). With higher Ct values in the URT, the PPV declined whereas the NPV for LRT infection increased. For comparison, any positive test in the URT had a PPV of 76% and a NPV of 95%.

DISCUSSION

In the present study, we characterized the rates of discordance in respiratory viral detection between matched URT and LRT samples in a large cohort of HCT candidates/recipients who underwent BAL for suspected LRTI and had concomitant URT testing. We demonstrate high discordance rates for HRV, HMPV, PIV3, and adenovirus between the URT and LRT. Furthermore, we identified risk factors for detection of respiratory viruses in the lungs of HCT candidates and recipients, including viral detection in the NP and recipient CMV seropositivity.

Before the advent of molecular testing, a high rate of discordance was observed with rapid antigen detection assays which had a sensitivity of 15% in URT specimens and 89% in LRT specimens from immunocompromised adults [12]. Discordance between molecular diagnostic testing of URT and LRT specimens has also been reported in other studies, particularly in immunocompromised populations, where 79%–86% of patients with a positive LRT specimen had a concordant URT specimen [6, 13, 14]. The positive and NPVs of URT testing were 86%–88% and 89%–94%, respectively. In a different study of HCT recipients with HMPV or RSV detected in the LRT, 33% had a discordant negative test for HMPV, whereas no patients had a discordant negative test for RSV [2]. In the present study, we found high levels of discordance

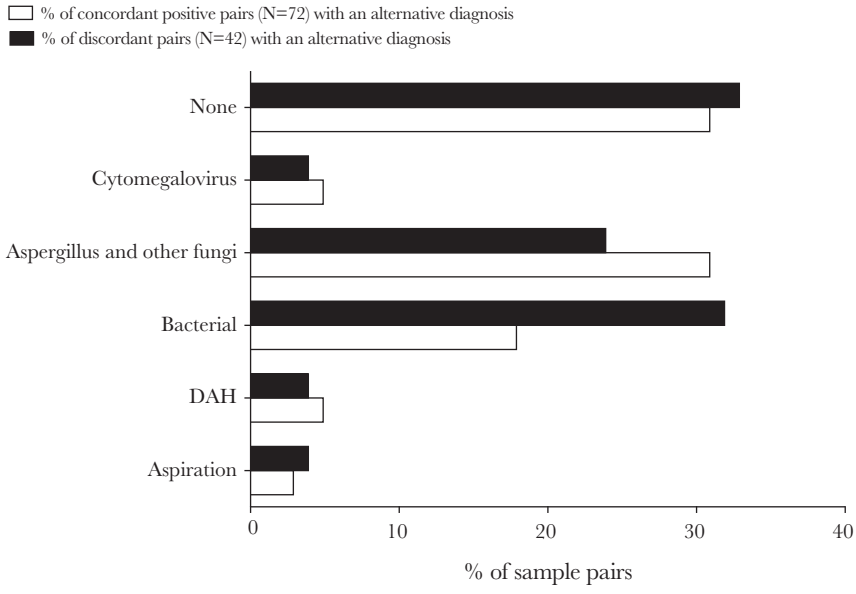

Figure 2. Distribution of copathogens and alternate diagnoses in subjects with concordant positive pairs (N = 73) and discordant pairs (N = 42). No significant differences between copathogens and alternate diagnoses in subjects with concordant P/P versus discordant results was observed by Fisher's exact test. DAH, diffuse alveolar hemorrhage.

Table 2. Univariate and Multivariate Analyses of Risk Factors for Respiratory Viral Detection in the LRT Among HCT Candidates or Recipients[a] (N = 219)

		Univariate Analysis		Multivariate Analysis	
Covariates	Categories	OR (95% CI)	*P* Values	OR (95% CI)	*P* Values
Gender	Female	0.87 (.47–1.59)	.642		
	Male	1			
Race	White	1			
	Non-White	1.50 (.76–2.96)	.246		
	Unknown	N/A	.989		
Recipient age at transplant	0–20	1			
	>21 to <60	0.70 (.29–1.71)	.438		
	≥60	1.00 (.38–2.66)	1		
Year of transplant	2009–2011	1		1	
	2012–2013	0.92 (.46–1.83)	.811	1.46 (0.47–4.50)	.139
	2014–2016	0.47 (.22–1.01)	.053	0.42 (0.14–1.31)	.057
Transplant number	1	1			
	2	1.14 (.54–2.42)	.729		
	3	4.14 (.67–25.6)	.126		
Donor type	Allo/Unrelated	0.73 (.34–1.57)	.424		
	Auto	1			
Conditioning regimen	Non-myeloablative	1			
	Myeloablative without high-dose TBI (<1200)	0.79 (.41–1.53)	.491		
	Myeloablative with high-dose TBI (≥1200)	0.74 (.31–1.74)	.487		
Donor CMV serostatus	-	1			
	+	1.33 (.72–2.44)	.355		
Recipient CMV serostatus	-	1		1	
	+	2.44 (1.20–4.76)	.013	3.70 (1.30–10.0)	.015
Gap between BAL and nasal swab (in days)	as continuous	1.11 (.81–1.52)	.519		
Highest dose of steroids received in the 14 days before BAL[b]	0 to <1	1			
	1 to <2	1.16 (.51–2.63)	.718		
	≥2	1.11 (.37–3.34)	.847		
Highest dose of steroids received in the 14 days before BAL[b]	as continuous	1.30 (.96–1.77)	.094	1.02 (0.62–1.67)	.94
WBC on day of/closest day of BAL (cells/μL)	≤1000	1			
	>1000	1.02 (.54–1.94)	.943		
ANC on day of/closest day of BAL (cells/μL)	≤100	1			
	>100	1.20 (.56–2.56)	.637		
Lymphocyte on day of/closest day of BAL (cells/μL)	≤100	1			
	>100	1.23 (.63–2.43)	.546		
Monocyte on day of/closest day of BAL (cells/μL)	≤100	1			
	>100	0.90 (.49–1.66)	.743		
Imaging findings	All others	1			
	Solitary nodule	1.99 (.43–9.17)	.376		
Respiratory viral detection in the URT	Negative	1		1	
	Positive	54.9 (22.4–135)	<.001	73.7 (26.7–204)	<.001

Abbreviations: ALC, absolute lymphocyte count; allo, allogeneic stem cell transplant; AMC, absolute monocyte count; ANC, absolute neutrophil count; auto, autologous stem cell transplant; BAL, bronchoalveolar lavage; CI, confidence interval; CMV, cytomegalovirus; HCT, hematopoietic cell transplant; LRT, lower respiratory tract; N/A, not applicable; OR, odds ratio; TBI, total body irradiation; URT, upper respiratory tract; WBC, white blood cell count.

[a]Twelve patients with more than 1 respiratory virus in the URT were excluded from the analysis. Four patients with adenovirus detected in the plasma at the time of diagnosis of LRT involvement by BAL were also excluded from the analysis.

[b]Equivalent dose of prednisone in mg/kg per day.

with 37% of sample pairs among subjects with positive testing showing discordance between URT and LRT testing for any virus. Although the sample sizes were too small for each virus to perform statistical testing, discordance rates were highest for HMPV, HRV, adenovirus, and PIV3. Of note, this discrepancy was present also among patients with URT specimens obtained within 1 day of the BAL. Human metapneumovirus LRT disease is associated with high mortality in HCT recipients, and our results suggest that a negative URT specimen may not be sufficient to rule out LRT infection [2]. We noted that adenovirus testing showed discordance in every subject with the virus. Reactivation in other tissues followed by viremia and dissemination to the lungs may

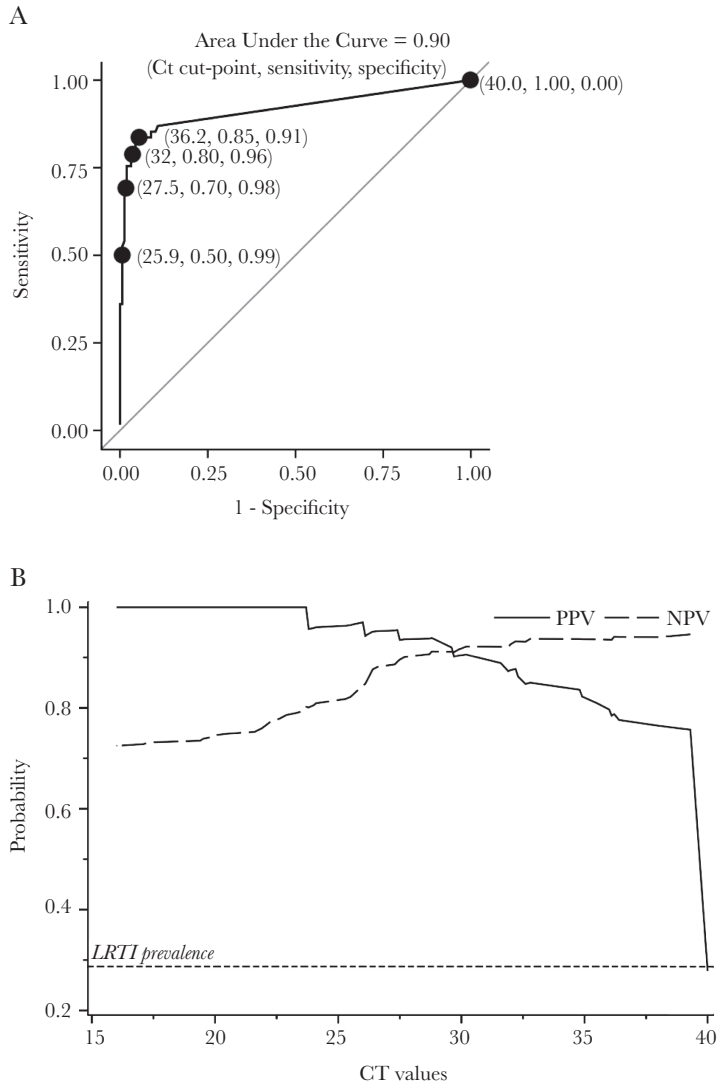

Figure 3. Sensitivity, specificity, and predictive values for lower respiratory tract (LRT) infection based on cycle threshold (Ct) values in the upper respiratory tract (URT). (A) Receiver operating characteristic (ROC) curve of Ct values in the URT. The Ct values for patients with negative testing in the URT was set to 40, above the upper limit of assay detection. (B) Positive and negative predictive values (PPV and NPV, respectively) for LRT infection based on Ct values in the URT. Patients with adenovirus detected in the plasma at the time of diagnosis of LRT involvement by bronchoalveolar lavage (N = 4) were excluded from the analysis.

have contributed to this finding. In contrast, RSV testing showed an approximately 100% concordance, suggesting that patients with RSV detected in the URT and clinical/radiographic evidence of LRT involvement may be presumed to have RSV in the LRT. These patients could be treated accordingly for RSV pneumonia and also be enrolled for clinical trials. We have previously categorized LRT infection with respiratory viruses into groups depending on viral detection in the LRT, where proven LRTI is defined as a positive LRT sample (BAL, lung biopsy, or autopsy specimen) with radiographic abnormality, probable LRTI is defined as a positive LRT sample without radiographic abnormality, and possible LRTI is defined as a positive URT sample with radiographic abnormality but no LRT sampling. We have shown that subjects with possible LRTI have outcomes more similar to URT infection for both PIV and RSV, and that subjects with proven/probable LRTI have worse outcomes including need for oxygen, oxygen-free days, and mortality [15, 16]. These results suggest that LRT testing to stratify patients into possible versus proven/probable LRTI can provide useful prognostic information in HCT recipients. This may become increasingly important because new antivirals are in development, many of which are being evaluated depending on the site of infection (upper versus lower tract). Our study included 17 patients positive for a respiratory virus in the LRT but negative in the URT. Five viruses, namely, adenovirus influenza A, PIV2, PIV3, HMPV, and HRV, had N/P discordance. Thus, proximal URT testing did not identify the LRT pathogen in these cases, including viruses that may warrant treatment with current antivirals (influenza A and adenovirus) as well as viruses for which specific antiviral therapy is being developed (PIV and HMPV).

The probability of detecting virus in the LRT was increased in patients with virus detected in the URT. Other studies have found an association between higher respiratory viral loads and more severe disease manifestations [17–19]. We found that lower Ct values in the URT were associated with higher PPVs for LRT infection. However, the NPV appeared to plateau such that the NPV at a Ct value of 27.5 was similar to the NPV of a negative test in the URT (90% vs 95%, respectively). It is important to note that because even a negative PCR in the URT is not fully predictive, Ct values for viruses detected in the URT cannot be used on their own to rule out LRT involvement.

We also found that CMV-seropositive HCT recipients had an increased risk for LRT respiratory virus detection. We have previously reported recipient CMV seropositivity as a risk factor for respiratory virus acquisition and progression to LRT infection after HCT [20, 21]. Another study reported an association between CMV reactivation and RSV infection after HCT with the development of severe pneumonia [22]. Cytomegalovirus seropositivity and reactivation have been associated with increased morbidity, mortality, and graft-versus-host disease after HCT [23–25]. The pathogenesis of CMV infection and disease is complex with several immunomodulating interactions between CMV and the immune system, including effects on human leukocyte antigen expression and cytokine production (reviewed in reference [26]). Our findings here suggest that increased risk of LRT infection may be another indirect effect of CMV.

Furthermore, we observed a trend towards decreased risk of detecting virus in the LRT in patients transplanted between 2014 and 2016 and a trend towards decreased risk in patients transplanted between 2012 and 2013 compared to 2009 and 2011. It is possible this could have been secondary to a decrease in virus detected in the URT in the later years (31% between 2009 and 2011, 24% between 2012 and 2013, and 17% between 2014 and 2016) because a negative result in the URT was strongly associated with a lower risk for LRT detection. This reduction in URT respiratory viral detection may also have been secondary to improvements in infection control practices. Alternatively, the trend towards reduced risk of LRT detection in later years may be a reflection of practice changes either with (1) delaying transplants in patients with positive testing for respiratory viruses in the URT or (2) fewer bronchoscopies being performed now compared to in the past [27]. This could have led to a sampling bias in more recent years in which a bronchoscopy was more often performed when lung disease due to an alternative, nonrespiratory viral, process was suspected.

We also sought to understand the differences in viral load observed in concordant upper and lower samples. There was no significant difference between viral loads in the upper versus lower tract when analyzing all viruses together in patients with a BAL within 3 days or 1 day of URT testing. One argument against early bronchoscopy to test for viral LRT involvement includes the notion that viruses in the URT may be "pushed" into the LRT during the procedure itself. Lower respiratory tract positivity could also simply reflect upper tract secretions that are aspirated during the procedure, similar to the finding of oral flora in bacterial cultures of a BAL. The finding of several cases of positive testing in the nose yet negative testing in the lungs argues against the "pushing down" of viruses from the URT to the LRT during bronchoscopy and against the detection of viruses in the LRT as an artifact of contamination from the URT during bronchoscopy. The sample size was too small to support analysis of Ct values for individual viruses.

Our study has several limitations. First, even though our sample size was relatively larger than other studies of immunocompromised patients with paired URT and LRT sampling, we could not evaluate risk factors for discordance for individual viruses. Second, data were collected retrospectively, and, therefore, need for sampling of the URT and LRT was determined by the clinician. The patient population was limited to those who underwent BAL within 1 or 3 days of an NP aspirate because viral detection in the LRT was an outcome measure, and therefore risk factors may differ with patients who undergo only URT testing. Third, our primary analysis focused on HCT recipients who had undergone BAL within 3 days from an NP aspirate. This could be considered too long a time period. More important, however, the results from this analysis were similar to that for patients who had undergone BAL within 1 day of an NP aspirate. Fourth, differences in sampling from the LRT by BAL and URT by swabbing may contribute to differences in Ct values. The higher collection volume for BALs compared with NP aspirates (approximately 30 vs 5 mL) would generally lead to higher Ct values in LRT specimens due to greater dilution. Fifth, antiviral therapy in patients with influenza or RSV detected in NP aspirates may have affected the detection of these viruses in the BAL. However, we found that almost every case of RSV infection was concordant and only 1 case of influenza was discordant P/N. Sixth, because copathogens and alternative diagnoses were identified in many patients, we cannot definitively conclude whether LRT symptoms, signs, or radiographic abnormalities were caused specifically by the respiratory virus, the copathogen, and/or a concomitant noninfectious process. Finally, Ct values may not be as generalizable between different assays compared with a true viral load measured in copies/milliliter. Even though most currently available commercial PCR assays do not include Ct values, our study shows the predictive value of these results for LRT infection.

CONCLUSIONS

In summary, our data demonstrate discordance between URT and LRT respiratory virus detection for several common respiratory viruses. We suggest that early LRT viral testing could provide useful diagnostic information that may affect management of respiratory viral infections in certain HCT candidates and recipients.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Supplemental Figure 1. Results of upper respiratory tract (URT) and lower respiratory tract (LRT) sample testing among patients with positive URT testing before undergoing bronchoalveolar lavage (BAL). Concordance or discordance is shown by specific virus (represented as result from URT/LRT with N = negative and P = positive). Data are shown for subjects with a BAL ±3 days from the URT test. Adeno, adenovirus; FluA, influenza A; FluB, influenza B; HCoV, human coronavirus; HMPV, human metapneumovirus; PIV, parainfluenza viruses 1–4; HRV, human rhinovirus; RSV, respiratory syncytial virus.

Notes

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Author contributions. J. B. and M. V. designed and performed the research, collected data, analyzed data, and wrote the manuscript; H. X. and W. L. performed statistical analyses, generated tables and figures, and critically reviewed the manuscript; S. A. P. and G.-S. C. contributed to the analysis plan and critically reviewed the manuscript; M. M., J. A. H., K. R. J., and A. P. L. critically reviewed the manuscript; M. J. B. and A. W. designed and performed the research, analyzed data, provided resources, and wrote the manuscript.

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