Clinical Significance of Human Coronavirus in Bronchoalveolar Lavage Samples from Hematopoietic Cell Transplantation Recipients and Patients with Hematologic Malignancies

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Summary: Coronavirus pneumonia is associated with high rates of oxygen use and mortality in hematopoietic cell transplant (HCT) recipients and patients with hematologic malignancies; mortality in HCT recipients is similar to that seen with RSV, influenza and parainfluenza virus.

Running title: Coronavirus in immunocompromised patient

ABSTRACT

Background: The possible role of human coronaviruses (HCoV) in lower respiratory tract disease (LRTD) in hematopoietic cell transplant (HCT) recipients and patients with hematologic malignancies (HM) has not been well studied**.**

Methods: We conducted a retrospective review of HCT/HM patients with HCoV detected in bronchoalveolar lavage (BAL). HCoV strains were identified in BAL samples using strain-specific PCR. Mortality rates were compared among HCT recipients with LRTD caused by HCoV, respiratory syncytial virus (RSV), influenza or parainfluenza virus (PIV) by multivariable Cox regression analysis.

Results: We identified 35 patients (37 episodes) with HCoV LRTD. Among 23 available BAL samples, 48% were strain OC43, 22% were NL63, 17% were 229E and 13% were HKU1. Overall, 21 patients (60%) required oxygen therapy at diagnosis and 19 (54%) died within 90 days of diagnosis. Respiratory co-pathogens were detected in 21 episodes (57%), including viruses (N = 12), fungi (N = 10), and bacteria (N = 8). Mortality rates were not different between patients with and without co-pathogens (p = 0.65). In multivariable models, mortality associated with HCoV LRTD was similar to that seen with RSV, influenza and PIV LRTD in HCT recipients (adjusted hazard ratio 1.34, 95% CI 0.66-2.71, p = 0.41 versus RSV, adjusted for cell source, cytopenia, co-pathogens, oxygen use and steroid use).

Conclusions: HCoV LRTD in patients with HCT or HM is associated with high rates of oxygen use and mortality. Mortality associated with HCoV LRTD in HCT recipients appears to be similar to that seen with RSV, influenza and PIV.

Key words: human coronavirus, bronchoalveolar lavage, lower respiratory tract disease hematopoietic cell transplant, hematologic malignancy

INTRODUCTION

Respiratory viruses can cause lower respiratory tract disease (LRTD) in immunocompromised hosts, which is associated with significant morbidity and mortality [1-4]. With the development and widespread use of new molecular diagnostic techniques, the clinical impact of previously under-diagnosed respiratory viruses in this population remains uncertain [5]. This is particularly true of human coronavirus (HCoV). In addition to Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses, four other strains of HCoV (229E, OC43, NL63 and HKU1) are now acknowledged to be human pathogens [6, 7]. Previous studies have demonstrated that HCoV is now the second most common virus identified from the upper respiratory tract in hematopoietic cell transplant (HCT) recipients [8]. Cases of fatal pneumonia related to HCoV without co-pathogens have also been reported mainly in HCT populations [9-12]. Two previous studies describe the possible role of HCoV in LRTD; however, these studies included only limited numbers of HCT recipients and patients with hematologic malignancies (HM) and outcome analyses could not be done [13, 14]. The purpose of this study was to describe the clinical characteristics and outcomes of HCT recipients and patients with HM with HCoV detected in the lower respiratory tract based on testing of bronchoalveolar lavage (BAL) fluid. Mortality rates were compared among HCT recipients with LRTD caused by HCoV, respiratory syncytial virus (RSV), influenza or parainfluenza virus (PIV).

METHODS

Study Design

We identified all HCT recipients and patients with HM with HCoV detected in clinical BAL samples from patients at the Fred Hutchinson Cancer Research Center, University of Washington or Seattle Children's Hospital from May 2006 through February 2016. We identified 3 additional HCT recipients with HCoV detected in BAL samples from a previously reported cohort [15]. Demographic and clinical data were collected from the above-mentioned institutions' databases and medical chart review. We also compared HCT recipients with HCoV LRTD to previously reported cohorts of HCT recipients with LRTD caused by RSV, influenza and PIV [16-18]. The study was approved by the Institutional Board Review at Fred Hutch.

Laboratory Testing and Definitions

Reverse transcription PCR (RT-PCR) was performed for HCoV on BAL samples, serum specimens, lung biopsy and autopsy samples according to a previously published protocol [19]. Viral load of HCoV was determined by quantitative RT-PCR using BAL samples. HCoV was identified from BAL specimens using the consensus HCoV assay, which is part of a multiplex PCR used to detect 12 respiratory viruses. Strain-specific PCR was performed using saved BAL samples as described previously [19]. We performed RT-PCR to detect HCoV RNA in frozen serum samples that were drawn between 23 days before and 23 days after the BAL. When adequate lung tissue was available, curls were cut from fresh frozen tissue or formalin fixed paraffin-embedded (FFPE) tissue blocks for RT-PCR. FFPE samples and frozen samples were extracted using RNAeasy FFPE kit and RNAeasy mini kit, respectively (Qiagen, Hilden,

Germany). All samples underwent fragment size analysis for quality with RT-PCR targeting amplicons from housekeeping genes with sizes ranging from 100 to 600 base pairs.

HCoV LRTD was defined as HCoV detection in a BAL sample from a patient with signs of LRTD (e.g. cough, dyspnea) or new pulmonary infiltrates. All BAL specimens underwent broad diagnostic tests including conventional cultures for bacteria, fungi, mycobacteria and viruses, shell vial culture for Cytomegalovirus, immunofluorescent-antibody staining for *Pneumocystis jirovecii* and Legionella, fungal PCR, Aspergillus galactomannan enzyme-linked immunosorbent assay and cytopathologic examination. HCoV was considered the sole respiratory pathogen if all above-mentioned microbiological test results on BAL specimens were negative. Pulmonary bacterial co-infection was defined as bacterial load of $>10^3$ colony-forming units per ml of BAL specimen with compatible radiological findings and clinical course. Any virus or fungus detected in BAL samples was considered a respiratory co-pathogen. Highest steroid doses in the 2 weeks prior to HCoV LRTD and cell counts most immediately prior to HCoV LRTD were recorded. Oxygen-free days and ventilator-free days are defined as days alive and free from oxygen support and mechanical ventilation, respectively [16]. Respiratory death was defined as any death occurring as a consequence of respiratory failure.

Morphologic re-review of available BAL samples, lung biopsies, and autopsy lung tissues was performed on hematoxylin and eosin stained sections by a board certified pathologist with expertise in transplant pathology.

Statistical Analysis

Patients' outcomes were compared using Chi-square or Fisher's exact test for categorical variables, and Wilcoxon rank-sum test for continuous variables, as appropriate. Summary of the various patient cohorts according to analysis type is shown in **Supplementary Figure 1**. Only the first episode of HCoV LRTD per subject was used for outcome analyses. We also excluded

two HCT recipients with a history of lung transplantation for outcome analyses except for evaluation of risk factors for mortality following HCoV LRTD. Univariable Cox proportional hazards models were used to evaluate risk factors for overall mortality by day 90 after the diagnosis of HCoV LRTD. Variables with p≤0.2 in the univariable models were candidates for multivariable models. Multivariable Cox regression model adjusted for respiratory viruses (HCoV, RSV, influenza and PIV), cell source, neutrophil counts, lymphocyte counts, monocyte counts, presence of co-pathogens, steroid dosage and oxygen use at diagnosis was performed. Patients with any respiratory viral co-pathogens were excluded for this analysis. The probability of overall mortality in HCT recipients by day 90 following the diagnosis of LRTD was estimated using the Kaplan-Meier method. The Log-rank test was used to compare mortality curves among subgroups. Two-sided *P* values <.05 were considered statistically significant. All statistical analyses were performed using SAS 9.4 for Windows (SAS Institute, Inc., Cary, NC).

RESULTS

Patient and Viral Characteristics

We identified a total of 35 patients (37 episodes) with HCoV detected by RT-PCR from BAL samples. **Table 1** shows characteristics of HCT recipients and patients with HM. Two patients developed HCoV LRTD twice. Two HCT recipients had a history of lung transplantation: one underwent lung transplantation for bronchiolitis obliterans related to previous HCT and the other received lung transplantation for cystic fibrosis before HCT. Only one pediatric patient (8 year old male) was identified in this cohort. The median time to HCoV LRTD after HCT in 28 recipients was 302 days (range, 8-7045): 20 (71%) and 12 (43%) patients developed HCoV LRTD > 100 days and > 365 days following transplant, respectively. All but one of the 20 patients with HCoV LRTD >100 days following transplant received either immunosuppressive

therapy or chemotherapy to control their underlying disorders (e.g., relapse of hematologic malignancy, graft versus host disease) prior to diagnosis of LRTD. Twenty-three recipients were transplanted after May 1, 2006 when respiratory viral PCR panel testing became routine. The median time to HCoV LRTD after HCT in these 23 patients was 340 days (range, 8-3618), which was similar to that of entire cohort. At the time of BAL, acute respiratory symptoms and new pulmonary infiltrates were present in the majority of episodes (**Table 2**). Among 23 available frozen BAL samples, 11 (48%) were identified as OC43, 5 (22%) as NL63, 4 (17%) as 229E and 3 (13%) as HKU1. The majority of episodes occurred in the winter and spring regardless of strain type (**Figure 1A**).

Other respiratory pathogens were detected in BAL samples in 21 episodes (57%), including viruses (12 episodes), fungi (10 episodes), and bacteria (8 episodes) (**Figure 1B**). Two or more other respiratory co-pathogens were detected in approximately half of these episodes (10/21). Two patients had respiratory co-pathogen as well as concomitant bacteremia/fungemia; one patient was found to have *staphylococcus aureus* in blood and BAL, and the other had both clostridium non-perfringens and *candida glabrata* in the blood only.

Respiratory co-pathogens were found in 82% (9/11) of episodes with OC43 and only 42% (5/12) of episodes with other strains ($p = 0.089$). The median viral loads of HCoV in BAL samples did not differ among strains (**Figure 2**)**.** No HCoV RNA was detected in serum samples prior to and following HCoV LRTD available from 21 episodes. Five lung biopsy samples and 4 lung autopsy samples were tested for RT-PCR among 6 patients (3 FFPE samples and 6 fresh frozen samples), all obtained within 67 days after LRTD diagnosis. Quality control fragment size analysis by RT-PCR of the RNA from these samples shows all FFPE specimens could be reliably amplified to 100 base pairs while all frozen specimens were reliably amplified to 600 base pairs. Only one sample (lung tissue) was positive for HCoV by RT-PCR, which had been obtained on the same day of BAL.

Outcomes

Patients' outcomes were summarized after excluding patients with second episodes of HCoV LRTD or a history of lung transplantation (**Tables 3 and 4**). Outcomes by day 28 and 90 after LRTD diagnosis were compared between HCT recipients and patients with HM. HCT recipients were more likely to have fewer oxygen and ventilator free days than patients with HM. Outcomes by day 28 and 90 after HCoV diagnosis were also compared between patients with and without respiratory co-pathogens with no statistical differences found.

Pathology Results

Twenty-eight patients had samples available for pathologic review, including 25 BAL samples, 5 lung biopsy samples, and 4 autopsy lung specimens; 6 of 7 patients without any other respiratory co-pathogens had either nonspecific findings of multinucleated giant cells or nuclear enlargement (**Supplementary Figures 2A and 2B**). Lung tissue from one patient was positive for HCoV by RT-PCR; the morphologic features noted in the lung biopsy were inflamed tissue with lymphocytes, neutrophils and cytologic atypia (**Supplementary Figures 3A and 3B**).

Comparison of Mortality with Other Respiratory Viruses and Risk Factors for Mortality

There were a total of 286 HCT recipients with a single respiratory virus identified in BAL samples for whom comparable clinical data were available (HCoV N = 18, RSV N = 113, influenza N = 36, and PIV N = 119); demographics of these are shown in **Supplementary Table 1**. Overall mortality rates by day 90 following viral LRTD caused by HCoV, RSV, influenza and PIV among HCT recipients without respiratory viral co-pathogens and without any co-pathogens were not different ($p = 0.78$ and $p = 0.47$, respectively) (**Figures 3A and 3B**). Furthermore, no difference was seen when the cohort was stratified by those with and without oxygen

requirement at the time of LRTD diagnosis (p=0. 78 and 0.78, respectively) (**Figures 3C and 3D**). Univariable Cox regression models were used to evaluate risk factors for overall mortality in HCT recipients with LRTD caused by HCoV, RSV, influenza or PIV without respiratory viral co-pathogens (**Table 5**). In multivariable models, cell source (bone marrow), respiratory bacterial or fungal co-pathogens, low neutrophil counts, low monocyte counts, steroid use, and oxygen requirement at diagnosis were associated with overall mortality (**Table 6**). Mortality due to HCoV LRTD was not significantly different from RSV LRTD [adjusted hazard ratio 1.34 (95% CI 0.66-2.71, p-value 0.41)]. Similarly, risk factors for overall mortality by day 90 in HCoV LRTD patients alone were evaluated using univariable and multivariable Cox regression models in HCT recipients: no risk factors significantly associated with mortality were found (**Supplementary Tables 2 and 3**).

DISCUSSION

In this study, we demonstrated that the presence of HCoV in BAL samples in immunocompromised hosts was significantly associated with high rates of respiratory support and mortality. HCT recipients appeared to be more affected than patients with HM. Although respiratory co-pathogens were frequently detected, the clinical outcomes of these patients were similar to those without co-pathogens. The mortality rate of HCT recipients by day 90 after developing HCoV LRTD was similar to rates seen with established respiratory pathogens including RSV, influenza and PIV (**Figure 3, Table 6**) [16-18]. All 4 HCoV strains were identified in BAL samples regardless of the presence of co-pathogens, and at least two HCoV strains were present nearly half of the year.

SARS and MERS are recognized as highly human-pathogenic coronavirus, causing acute, severe, frequently fatal LRTD [20-22]. Although four other strains of HCoV (229E, OC43,

NL63 and HKU1) are also human pathogens, the clinical impact of HCoV LRTD remains unclear especially in immunocompromised patients [4, 14, 23]. A previous prospective study with weekly nasal surveillance sampling during the first 100 days after HCT demonstrated prolonged HCoV shedding in the upper respiratory tract (> 3 weeks) in half of subjects including asymptomatic patients [8]. In addition, respiratory co-pathogens were identified in more than half the episodes in this study (57%). Given the prolonged asymptomatic shedding and frequent detection of respiratory co-pathogens, attributing poor clinical outcomes to HCoV in lower respiratory tract may be difficult. In the current study, follow-up BAL procedures were not performed to assess prolonged shedding in the lower respiratory tract; however, a prior study that included only 4 patients with cancer demonstrated only one out of 10 cases had HCoV detected in follow-up BAL specimens, arguing against asymptomatic prolonged shedding in the lower respiratory tract [14]. More data are needed to define shedding duration in the immunocompromised population. In our study, clinical outcomes including intensity of respiratory support, days alive without hospitalization, and mortality were not significantly different between patients with and without other co-pathogens, suggesting HCoV in lower respiratory tract can contribute to severity of LRTD regardless of co-pathogens. Lung tissue from one patient was positive for HCoV by RT-PCR. This patient subsequently developed prolonged oxygen requirement, which also supports the potential pathogenicity of HCoV.

To further demonstrate the clinical significance of of HCoV LRTD in HCT recipients, we compared mortality rates in HCT recipients with HCoV LRTD to other respiratory viruses using multivariable Cox regression analysis and found mortality rates in HCoV LRTD were comparable to those seen with RSV, influenza and PIV. Given the common perception of HCoV as a relatively benign pathogen based on limited data [5, 8], our data are somewhat surprising. The adverse impact of oxygen requirement at the time of diagnosis on subsequent clinical outcome has been suggested [16, 24]. Once substantial acute lung injury occurs, clinical

outcome can potentially be affected by inflammation rather than virus itself. Therefore, mortality rates by day 90 following LRTD caused by HCoV, RSV, influenza and PIV were also compared according to oxygen requirement at the time of LRTD diagnosis; no statistically significant difference was found. These data combined suggest that HCoV LRTD is significantly associated with poor clinical outcome in this immunocompromised population.

Previous studies mainly in immunocompetent hosts have not demonstrated a distinct association between particular HCoV strains in upper respiratory tract samples and disease severity in LRTD [25-29]. Although there are case reports with each strain identified in lower respiratory tract as a sole pathogen in HCT recipients, systematic data are limited [9-12]. This is the first study to describe that all four HCoV strains can be detected in BAL samples with and without any other respiratory co-pathogens in a large immunocompromised population. However, the few instances of each strain limited our ability to examine if specific HCoV strains are associated with increased disease severity in LRTD. Further studies with larger sample sizes will help to characterize the role of particular HCoV strains.

Our study showed relatively late presentation of HCoV LRTD following transplant. The median time to HCoV LRTD following HCT was 302 days, which was longer than median days to LRTD caused by RSV (52.5 days), PIV (78 days) and influenza (95 days) [16, 17, 30]. Since this may in part be due to the fact our cohort included some patients who were transplanted prior to the introduction of routine HCoV PCR testing, we separately analyzed patients who underwent transplantation after introduction of a respiratory viral PCR panel in 2006 and determined the median time of diagnosis remained similar (340 days). The majority of patients who developed HCoV LRTD > 100 days following transplant had received either chemotherapy or immunosuppressive therapy as predisposing factors. This does not explain why there is a relatively lower incidence of HCoV LRTD early after transplant. Differences in infection control practices early after transplant and factors that affect progression to LRTD early versus late

after transplant may play a role. Further studies are needed to determine why HCoV often causes LRTD late after transplant.

This study evaluated the largest cohort of HCoV LRTD confirmed with BAL in patients with HCT and HM by HCoV strain-specific and quantitative PCR in BAL samples. In addition, RT-PCR was performed on serum specimens, lung biopsy and autopsy samples. The main limitation of this study was the relatively small sample size, which prevented us from detecting small differences and performing multivariable analyses to evaluate risk factors for mortality in patients with HCoV LRTD. Another limitation is the fact that BAL samples were available in only two-thirds of the patients for strain identification, which limited our ability to compare clinical and virological differences among each HCoV strain. Among a total of 9 lung biopsy and autopsy samples, only one lung biopsy sample, which was taken on the same day as the BAL, was positive for HCoV by RT-PCR. The lower rate of detection may be due to the fact that the negative samples were obtained from 19 days to 67 days after the diagnosis of LRTD, suggesting that the timing of collecting samples may have been too late to identify HCoV in lung specimens. Furthermore, not all samples were optimally preserved for RT-PCR, and thus the sensitivity for HCoV may have been suboptimal.

We demonstrated high rates of respiratory support including oxygen use and mechanical ventilation requirement as well as a high mortality in immunocompromised patients with HCoV identified in lower respiratory tract. Mortality rates associated with HCoV LRTD in transplant recipients were similar to those seen with other respiratory viral pathogens including RSV, influenza and PIV. Thus, we conclude that HCoV appears to be a significant respiratory pathogen in the populations studied. This is an important observation because HCoVs are highly prevalent in immunocompromised hosts. The appreciation of HCoV as an important lower respiratory tract pathogen could impact clinical management including risk stratification in future studies and provide a rationale to develop antiviral therapies. Further studies are needed to

clarify if particular HCoV strains and viral load are correlated with clinical outcome and to identify risk factors for progression to LRTD.

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Conflict of Interest statement. Michael Boeckh received research support and served as a consultant for Gilead Sciences, Merck, Ansun Bioscience, and Aviragen Therapeutics; and as consultant for Humabs Biomed. Janet A. Englund received research support from GlaxoSmithKline, Gilead, Pfizer, and Chimerix, and as a consultant for Pfizer and GlaxoSmith Kline (Data Safety Monitoring Board). Cecilia Yeung received a research grant from Gilead Sciences for unrelated research. All other authors declare no relevant conflict of interest.

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Figure 1A. Human coronavirus strain.

Seasonal distribution of human coronavirus lower respiratory tract disease.

Figure 1B. Respiratory co-pathogens in human coronavirus lower respiratory tract disease

Abbreviations**:** NT, nontypeable strain due to unavailable sample; RhV, rhinovirus; RSV, respiratory syncytial virus; HMPV, human metapnuemovirus; CMV, cytomegalovirus; PIV, parainfluenza virus; ADV, adenovirus; *A. fumigutus*, *A*spergillus *fumigatus*; *C. neoformans*, *Cryptococcal neoformans*; PJP, *P*neumocystis jiro*veci*; *P. aeruginosa*, *P*seu*domonas aeruginosa*; *S. aureus*, *S*taphy*lococcus aureus*; *B. cepacia*, *B*urkh*olderia cepacia*; *H. influenza*, *Haemophilus influenza*; VGS, Viridans group streptococcus; *E. faecium*, *E*nterococcus *faecium*

Each color indicates category of co-pathogen as follows: white (viruses), grey (fungi) and dark grey (bacteria).

Respiratory viral co-pathogens were detected in 12 patients, fungal co-pathogens were detected in 10 patients and bacterial co-pathogens were detected in 8 patients. The number of patients (N=30) with other respiratory co-pathogens does not equal the sum of detections for each respiratory co-pathogens (N=36) owing to codetections of multiple co-pathogens in some subjects.

Figure 2. Viral load of human coronavirus in bronchoalveolar lavage samples

The bars indicate median values and first and third quartiles.

Figure 3. Kaplan-Meier oveall survival curve by day 90 after diagnosis of lower respiratory tract disease without respiratory viral co-pathogens according to respirtory virus classification in hematopoietic cell transplanat recipients. A, Kaplan-Meier oveall survival curve in overall cohort (n = 286) (Log-rank test, p=0.78). **B, Kaplan-Meier oveall survival curve in patients without other co-pathogens (n = 173)** (Log-rank test, p=0.47)**. C, Kaplan-Meier oveall survival curve in patients with oxygen requirement at diagnosis (n = 178)** (Log-rank test, p=0.78). **D, Kaplan-Meier oveall survival curve in patients without oxygen requirement at diagnosis (n = 108)** (Log-rank test, p=0.78). Abbreviations: RSV, respiratory syncytial virus; PIV, parainfluenza virus; Flu, influenza virus; HCoV, human coronavirus.

Table 1. Characteristics of All Patients With Human Coronavirus Lower Respiratory Tract Disease

Characteristic		Total No. (%) (n = 35)	Hematopoietic cell transplant recipients No. (%) (n = 28)	Hematologic malignancy patients No. (%) (n = 7)
Female		10 (29)	10 (36)	0
Age, median (range)		53 (8-68)	53.5 (24-67)	52 (8-68)
Underlying pulmonary disorders [a]		10 (29)	10 (36)	0
Immunosuppressive therapy or chemotherapy		34 (97)	27 (96)	7 (100)
Transplant year	1996-2006		6 (21)	
	2007-2015		22 (79)	
Transplant Number ≥ 2			10 (36)	
Cell source	Cord		1 (3)	
	Bone marrow		6 (21)	
	PBSC		21 (75)	
Donor type	Autologous		4 (14)	
	Related		10 (36)	
	Unrelated		14 (50)	
Days between transplant and HCoV LRTD, median (range)			302 (8-7045)	

All values are indicated as No. (%) unless they are indicated specifically in table.

Abbreviations: HCoV, human coronavirus; PBSC, Peripheral blood stem cell; LRTD, lower respiratory tract disease.

[a] Bronchiolitis obliterans (n=4), lung transplantation for bronchiolitis obliterans (n=1), lung transplantation for cystic fibrosis (n=1), radiation pneumonia (n=2), Asthma (n=1), prolonged acute respiratory distress syndrome (n=1), diffuse alveolar hemorrhage (n=1), cystic fibrosis (n=1)

Table 2. Presentation of Human Coronavirus Lower Respiratory Tract Disease Episodes

	Total [a] No. (%) (n = 37)	Hematopoietic cell transplant recipients No. (%) (n = 30)	Hematologic malignancy patients No. (%) (n = 7)
Respiratory symptoms[b]	34 (92)	27 (90)	7 (100)
Abnormal lung examination[c]	25 (68)	21 (70)	4 (57)
Abnormal findings on chest imaging[d]	34 (92)	27 (90)	7 (100)
HCoV strains			
OC43	11 (30)	10 (33)	1 (14)
NL63	5 (14)	4 (13)	1 (14)
229E	4 (11)	4 (13)	0
HKU1	3 (8)	3 (10)	0
Unknown	14 (38)	9 (30)	5 (71)
Respiratory co-pathogen	21 (57)	18 (60)	3 (43)
None	16 (43)	12 (40)	4 (57)
Viruses	5 (13)	3 (10)	2 (29)
Bacteria	4 (11)	4 (13)	
Fungi	4 (11)	4 (13)	
Multiple	8 (22)	7 (23)	1 (14)
Quantitative viral load, median (range) Log10 copies/ml	5.4 (2.4-9.0)	5.3 (2.4-7.8)	6.1 (3.4-7.4)
WBC count ≤ 1,000 x10^6 cells/L	11 (30)	7 (23)	4 (57)
Lymphocyte count ≤ 300 x10^6 cells/L	19 (51)	15 (50)	4 (57)

Neutrophil count ≤ 500 x10^6 cells/L	14 (38)	9 (30)	5 (71)
Monocyte count ≤ 300 x10^6 cells/L	24 (65)	19 (63)	5 (71)
Steroid dose [e]			
No	14 (38)	7 (23)	7 (100)
≤ 1mg/kg	13 (35)	13 (43)	0
> 1mg/kg	10 (27)	10 (33)	0
Oxygen requirement at diagnosis	23 (62)	20 (67)	3 (43)

All values are indicated as No. (%) unless they are indicated specifically in table.

Abbreviations: HCoV, human coronavirus; WBC, White blood cell.

[a] Two patients had separated HCoV LRTD episodes. The first patient developed LRTD 361 days and 415 days following HCT, respectively. The second patient developed LRTD 425 days before and 11 days after HCT, respectively.

[b] Cough or dyspnea

[c] Crackles, wheeze, rhonchi, or decreased breath sound

[d] Any new abnormal lung findings except for single nodule

[e] Max daily dose within 2 weeks prior to diagnosis

Table 3. Outcome of Patients With Human Coronavirus Lower Respiratory Tract Disease

	Total (n = 33)	Hematopoietic cell transplant recipients (n = 26)	Hematologic malignancy patients (n = 7)	P value
Outcome by day 28 after diagnosis				
Mechanical ventilation requirement (%)	7 (21)	7 (27)	0	0.30
Oxygen-free days	17.0 (11.8)	15.0 (12.2)	24.7 (5.6)	0.04
Ventilator-free days	22.1 (9.7)	20.5 (10.4)	28.0 (0.0)	0.03
Days alive without hospitalization	11.7 (10.9)	10.5 (10.7)	16.1 (11.5)	0.22
Outcome by day 90 after diagnosis				
Number of any death (%)	18 (55)	16 (62)	2 (29)	0.20
Number of respiratory death (%)	10 (30)	9 (35)	1 (14)	0.40

All values are presented as mean (standard deviation) unless they are indicated specifically in table.

Table 4. Outcome of Human Coronavirus Lower Respiratory Tract Disease With and Without Respiratory Co-pathogens

	Total (n = 33)	HCoV as sole respiratory pathogen (n = 14)	HCoV coinfected with other respiratory pathogens (n = 19)	P value
Outcome by day 28 after diagnosis				
Mechanical ventilation requirement (%)	7 (21)	2 (14)	5 (26)	0.67
Oxygen-free days	17.0 (11.8)	19.0 (11.3)	15.6 (12.2)	0.36
Ventilator-free days	22.1 (9.7)	24.4 (8.1)	20.4 (10.6)	0.16
Days alive without hospitalization	11.7 (10.9)	13.4 (11.0)	10.4 (11.0)	0.43
Outcome by day 90 after diagnosis				
Number of any death (%)	18 (55)	7 (50)	11 (58)	0.65
Number of respiratory death (%)	10 (30)	2 (14)	8 (42)	0.13

All values are presented as mean (standard deviation) unless they are indicated specifically in table.

Table 5. Univariable Cox Regression Analysis for Overall Mortality by Day 90 after Diagnosis of Lower Respiratory Tract Disease (n = 286)

Covariates	Categories	Hazard ratio (95% CI)	P-value
Cell source	Peripheral blood stem cell	1	
	Bone marrow	1.69 (1.22-2.36)	<.01
	Cord	0.51 (0.16-1.62)	0.25
Transplant year	1993-2006	1	
	2007-2015	0.87 (0.60-1.25)	0.45
Respiratory co-pathogen	None	1	
	Non-respiratory virus [a]± Bacteria/Fungi	1.61 (0.93-2.80)	0.09
	Bacteria/Fungi	1.54 (1.09-2.19)	0.02
Days between transplant and diagnosis	<=30	1	
	31-365	0.94 (0.65-1.34)	0.71
	>365	0.55 (0.32-0.94)	0.03
White blood cell counts, 10^6 cells/L	<=1.0	1.69 (1.21-2.37)	<.01
	>1.0	1	
Neutrophil counts, 10^6 cells/L	<0.5	1.76 (1.26-2.47)	<.01
	>=0.5	1	
Lymphocytes counts, 10^6 cells/L	<0.3	1.63 (1.17-2.29)	<.01
	>=0.3	1	
Monocyte counts, 10^6 cells/L	<0.3	2.38 (1.53-3.70)	<.01

	>=0.3	1	
Steroid use within 2 weeks before diagnosis	No	1	
	<1mg/kg	0.96 (0.62-1.47)	0.85
	1-2mg/kg	1.48 (1.00-2.20)	0.05
	>2mg/kg	2.23 (1.16-4.27)	0.02
Oxygen use at Diagnosis	No	1	
	Any	2.51 (1.72-3.66)	<.01
Respiratory virus	Respiratory syncytial virus	1	
	Parainfluenza	1.16 (0.81-1.68)	0.42
	Influenza	1.08 (0.63-1.84)	0.78
	Human coronavirus	1.32 (0.69-2.53)	0.40

[a] Cytomegalovirus, Herpes simplex virus, Human herpesvirus 6 and Epstein-Barr virus

Table 6. Multivariable Cox Regression Analysis for Overall Mortality by Day 90 after Diagnosis of Lower Respiratory Tract Disease (n = 286)

Covariates	Categories	Adjusted hazard ratio (95% CI)	P-value
Cell source	Peripheral blood stem cell	1	
	Bone marrow	1.64 (1.13-2.40)	0.01
	Cord	0.74 (0.23-2.41)	0.62
Respiratory co-pathogen	None	1	
	Non-respiratory virus [a]± Bacteria/Fungi	1.80 (1.00-3.26)	0.05
	Bacteria/Fungi	1.66 (1.12-2.45)	0.01
Neutrophil counts, 10^6 cells/L	<0.5	1.61 (1.00-2.58)	0.05
	>=0.5	1	
Lymphocytes counts, 10^6 cells/L	<0.3	0.95 (0.62-1.45)	0.81
	>=0.3	1	
Monocyte counts, 10^6 cells/L	<0.3	1.87 (1.12-3.13)	0.02
	>=0.3	1	
Steroid use within 2 weeks before diagnosis	No	1	
	<1mg/kg	1.27 (0.77-2.08)	0.35
	1-2mg/kg	1.38 (0.87-2.20)	0.18

	>2mg/kg	2.40 (1.15-5.03)	0.02
Oxygen use at diagnosis	No	1	
	Any	3.00 (1.98-4.53)	<.01
Respiratory virus	Respiratory syncytial virus	1	
	Parainfluenza	1.13 (0.77-1.67)	0.52
	Influenza	0.88 (0.47-1.66)	0.70
	Human coronavirus	1.34 (0.66-2.71)	0.41

[a] Cytomegalovirus, Herpes simplex virus, Human herpesvirus 6 and Epstein-Barr virus

Figure 1

Figure 1A.

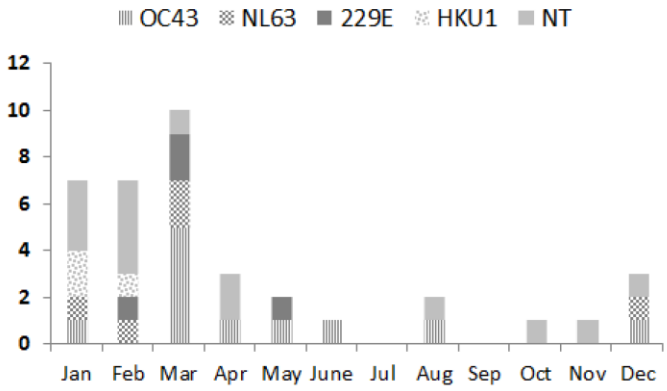

Figure 1B.

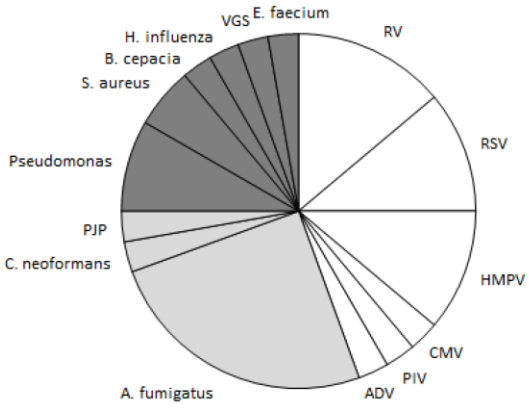

Figure 2

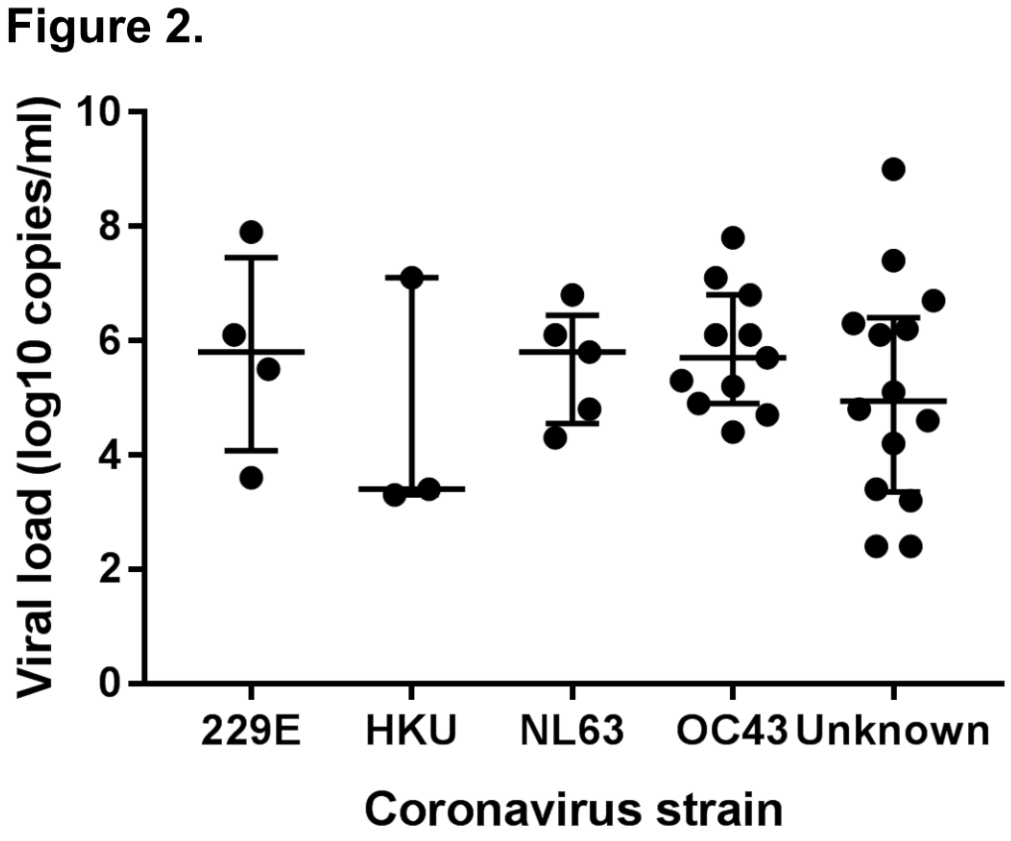
Figure 2.
Viral load (log10 copies/ml)
10
8
6
4
2
0
229E
HKU
NL63
OC43
Unknown
Coronavirus strain

Figure 3

Figure 3A.

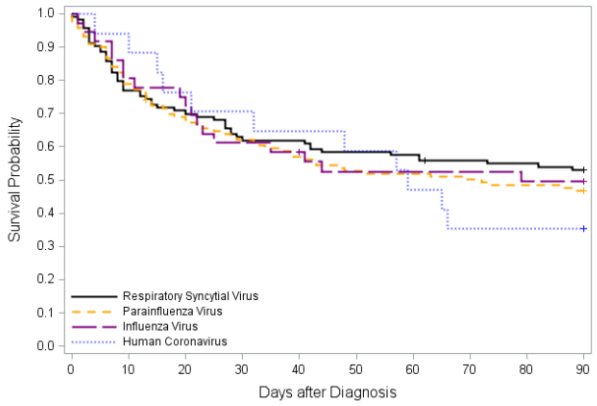

Figure 3B.

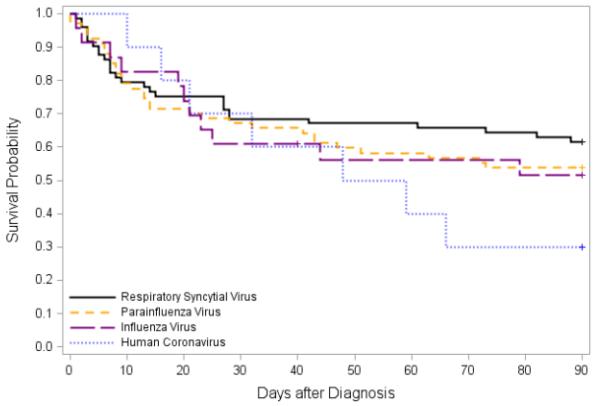

Figure 3C.

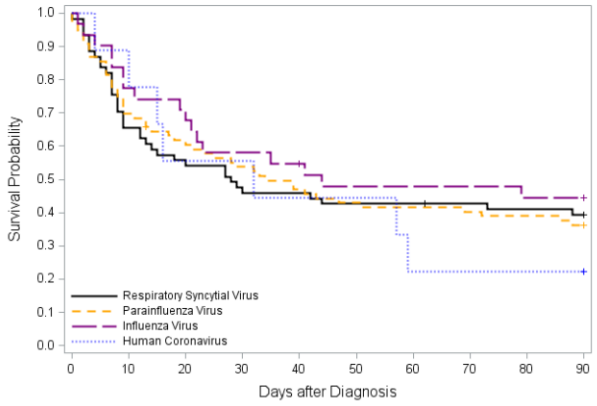

Figure 3D.