Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biol Blood Marrow Transplant 24 (2018) 563–570

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Epidemiologic and Clinical Characteristics of Coronavirus and Bocavirus Respiratory Infections after Allogeneic Stem Cell Transplantation: A Prospective Single-Center Study

José Luis Piñana [1,2,3,*], Silvia Madrid [4], Ariadna Pérez [1], Juan Carlos Hernández-Boluda [1], Estela Giménez [4], María José Terol [1], Marisa Calabuig [1], David Navarro [4,5], Carlos Solano [1,6]

[1] *Department of Hematology, Hospital Clínico Universitario, Fundación INCLIVA, Valencia, Spain*
[2] *Department of Hematology, Hospital Universitari i Politècnic la Fe, Valencia, Spain*
[3] *CIBERONC, Instituto Carlos III, Madrid, Spain*
[4] *Microbiology Service, Hospital Clínico Universitario, Valencia, Spain*
[5] *Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain*
[6] *Department of Medicine, School of Medicine, University of Valencia, Valencia, Spain*

Article history:
Received 26 September 2017
Accepted 1 November 2017

Key Words:
Coronavirus
Bocavirus
Community-acquired respiratory virus
Respiratory virus infection
Allogeneic stem cell transplantation
Viral pneumonia

A B S T R A C T

Epidemiologic data about coronaviruses (CoVs) and human bocavirus (HBoV) in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT) are scarce. We conducted a prospective longitudinal study on respiratory viral infections (RVIs) in allo-HSCT recipients with respiratory symptoms from December 2013 until June 2016. Respiratory virus in upper and/or lower respiratory tract (URT and LRT) specimens were tested using Luminex xTAG RVP Fast v1 assay. Seventy-nine consecutive allo-HSCT recipients developed a total of 192 virologically documented RVI episodes over 30 months. The median follow-up after RVI was 388 days (range, 5 to 923). CoV or HBoV was detected in 27 of 192 episodes (14%); 18 of 79 recipients (23%) developed a total of 21 CoV RVI episodes, whereas 6 recipients (8%) had 1 HBoV RVI episode each. Fourteen CoV RVI episodes were limited to the URT, whereas 7 affected the LRT. Co-pathogens were detected in 8 (38%) CoV cases. Type OC43 CoV was the dominant type (48%) followed by NL63 (24%), KHU1 (19%), and 229E (9%); the CoV hospitalization rate was 19%, whereas mortality was 5% (1 patient without any other microbiologic documentation). Among the 6 recipients with HBoV (3%), only 1 had LRT involvement and no one died from respiratory failure. In 5 cases (83%) HBoV was detected along with other viral co-pathogens. CoV RVIs are common after allo-HSCT, and in a significant proportion of cases CoV progressed to LRT and showed moderate to severe clinical features. In contrast, HBoV RVIs were rare and mostly presented in the context of co-infections.

INTRODUCTION

There is an important amount of data concerning the most frequent community-acquired respiratory viruses (CARVs) such respiratory syncytial virus (RSV), human parainfluenza virus (HPiV), human influenza virus, human metapneumovirus (HMPV), or human rhinovirus in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT). These CARVs cause upper and/or lower respiratory tract disease (URTD and LRTD) after allo-HSCT, and these are associated with high morbidity and mortality [1,2]. Recently, the availability of more sophisticated diagnostic tools based on reverse transcription PCR (RT-PCR) have improved the diagnosis of CARVs and have led to the identification of new emerging respiratory viruses such as coronaviruses (CoVs) and human bocavirus (HBoV). However, little is known about the epidemiology, prevalence, and clinical features of CoVs and HBoV in immunocompromised patients [3].

To date, 6 human CoVs have been identified, namely CoV-229E, CoV-NL63, CoV-OC43, CoV-HKU1, severe acute respiratory syndrome CoV, and Middle East respiratory syndrome CoV; of these, 4 (*Alphacoronaviruses*, CoV-229E and CoV-NL63; *Betacoronaviruses*, CoV-OC43 and CoV-HKU1) are known to contribute to common-cold infections in humans [4], circulate simultaneously [5], and affect people with and without underlying conditions [6,7]. Case reports have detailed instances of severe CoV-related pneumonia in immunocompromised adult and pediatric patients treated for hematologic malignancies [5,8-10]. However, the largest series

Financial disclosure: See Acknowledgments on page 569.
* Correspondence and reprint requests: Jose Luis Piñana, MD, Division of Clinical Hematology, Hospital Universitario la Fe de Valencia, Avda Fernando Abril Martorell, 106 CP, 46026 Valencia, Spain.
E-mail address: jlpinana@gmail.com (J.L. Piñana).

https://doi.org/10.1016/j.bbmt.2017.11.001
1083-8791/

of CoVs analyzed in allo-HSCT patients published to date is a prospective observational study that detected CoV in 22 of 215 allo-HSCT recipients with an estimated incidence of 11% at 100 days after stem cell infusion [11].

HBoV, however, was originally identified by a random PCR amplification/cloning technique in pooled respiratory secretions from hospitalized children with respiratory tract infection symptoms [12]. This virus affects young children with winter seasonality [13-15]. However, scarce data are available concerning the relationship between HBoV and respiratory disease in immunocompromised patients. Preliminary evidence from case reports describes disseminated HBoV infections with involvement of the respiratory tract, blood, and stool in several patients and that is sometimes associated with graft-versus-host disease (GVHD) and prolonged fecal viral shedding [16,17]. Other studies, however, report little evidence linking this virus with pulmonary pathologies or severe respiratory disease in allo-HSCT or lung transplant recipients [18-20].

Thus, we conducted a prospective epidemiologic study of respiratory viral infections (RVIs) in allo-HSCT recipients who developed URTD and LRTD symptoms after allo-HSCT. Here, we report the frequency and clinical features of CoV and HBoV URTDs and LRTDs diagnosed by RT-PCR in a series of patients at a single center over a 30-month period.

METHODS

Patients

This was a prospective longitudinal study of RVIs in adult (>18 years) allo-HSCT recipients from the time of their allograft and during their follow-up at our transplant unit. For the study purpose, in late 2013 we implemented the medical information/education for recipients and caregivers explaining in detail about the risks of having RVIs in the context of immunosuppression. Specific information included a description of respiratory symptoms, which should be reported as soon as possible to the transplant team, and recommendations concerning the infectious prevention control measures for patients and healthcaregivers. A telephone number (on-call 24 hours) for emergent conditions was also provided. The current study cohort comprised all the consecutive allo-HSCT recipients with virologically documented RVIs diagnosed at the Hospital Clinic i Universitari in Valencia during the 30-month study period. All recipients with respiratory symptoms between December 23, 2013 and June 26, 2016 were prospectively screened for CARVs by real-time PCR. Clinical and biologic characteristics were prospectively recorded as reported in detail elsewhere [21]. Immunodeficiency scoring index (ISI) variables were recorded at the first clinical evaluation as previously described [22]. A detailed clinical assessment was also performed and prospectively recorded in our transplant database at the time the respiratory symptoms were noted. Clinical manifestations included rhinorrhea, cough, rales, wheezing, shortness of breath, dyspnea, sinusitis, otitis, pharyngitis, tonsillitis, and fever (temperature > 38°C). We retrospectively analyzed the epidemiology of the CoV and HBoV detected. The local ethics committee approved the study, and all subjects gave their written informed consent before participating in the study.

Definitions

URTDs were defined by the combination of upper respiratory symptoms (rhinorrhea, sinusitis, otitis, or pharyngitis) and positive identification of a CARV by a PCR test and the absence of lower respiratory tract infection symptoms and/or any indication of pulmonary infiltrates in the radiology results by chest x-ray or computed tomography scan. We classified LRTDs as possible or confirmed as previously described [23]. Possible LRTDs were defined by the detection of a CARV in a nasopharyngeal or sputum sample taken from patients showing clinical symptoms of tracheitis, bronchitis, bronchiolitis, or pneumonia (new onset of cough, rales, wheezing, cough-related chest pain, shortness of breath, dyspnea, or hypoxia) or new detection of abnormal pulmonary function in conjunction with the identification of new pulmonary infiltrates (but without confirmation of their presence in the lower respiratory tract). Confirmed lower respiratory tract infections were defined when the abovementioned clinical features were accompanied by isolation of the virus in tracheal aspirates or by bronchoalveolar lavage (BAL).

According to the Fourth European Conference on Infections in Leukaemia recommendations [24], we defined episodes as an URTD or LRTD. An infectious disease episode was considered to be resolved when complete remission of respiratory symptoms was observed. Further episodes of respiratory tract infectious diseases were documented after a symptom-free period of at least 2 consecutive weeks from the resolution of the previous episode and/or the isolation of a different virus in conjunction with the onset of new respiratory symptoms. Respiratory co-infection was defined as the identification of additional microbiologic agents, including bacterial or fungal specimens and/or other CARVs, in the same sample, either in the upper or lower respiratory tract.

Technical and Diagnostic Considerations

All recipients who developed signs and symptoms of a URTD and/or LRTD underwent a detailed virologic, bacterial, and fungal evaluation. When bronchoscopy was performed, a detailed microbiologic evaluation including respiratory viruses; bacterial, fungal, and acid-fast bacilli cultures; *Aspergillus* galactomannan assay; and detection of cytomegalovirus was performed. Patients with URTD symptoms underwent nasopharyngeal aspiration, nasopharyngeal swabs, or an induced sputum test, whereas BAL was performed in patients with an LRTD whenever possible.

All clinical samples were tested by RT-PCR using the Luminex xTAG RVP Fast v1 assay (Luminex, Austin, TX), as described in detail elsewhere [25]. Briefly, all specimens were received at the laboratory within 30 minutes of collection and were conserved at 4°C until processed (within 18 hours of receipt). Nucleic acid extraction was performed using the Qiagen EZ-1 viral extraction kit with a EZ1 Robot (Qiagen, Valencia, CA). The Luminex xTAG RVP Fast v1 assay can detect adenoviruses; HBoV; CoV 229E, HKU1, NL63, and OC43; influenza A virus/H_1N_1, influenza A virus/H_3N_2, and other influenza A viruses (non-subtypifiable); influenza B virus; HMPV A and B; HPiV 1, 2, 3, and 4A-4B; RSV A-B; and enterovirus/rhinovirus (EvRh).

Statistical Analysis

Our primary objective was to describe the epidemiology of CoV and HBoV RVIs among all the circulating CARVs in the allo-HSCT setting. The secondary endpoint was to describe the clinical characteristics and outcomes of patients suffering URTDs and/or LTRDs caused by these viruses. Epidemiologic, clinical, and RVI characteristics were compared using the chi-square test for categorical variables and with paired Student *t*-tests for continuous variables; the statistical significance was set at $P < .05$, and, where relevant, the standard deviation is shown.

RESULTS

Patient Characteristics

Of 88 allo-HSCT recipients, 79 (89%) screened for upper and/or lower respiratory tract symptoms developed at least 1 episode of virologically documented RVIs over the study period. The clinical and biologic characteristics of the subjects are shown in Table 1. Of note, this series comprised a high-risk cohort with a profound immunosuppression status because 66% of the recipients included were allografted from alternative donors (unrelated donor and haploidentical family donors) and 35% had at least 1 antigen mismatch with the donor in the HLA-A, -B, -C, or -DR alleles, as determined by high-resolution genotyping. Additionally, the number of recipients with acute or chronic GVHD was also high, representing 57% and 87% of the 79 allo-HSCT recipients, respectively. Although the frequency of hospitalization directly attributable to RVIs was high (47%), the overall mortality was relatively low (18%) in the entire cohort.

Epidemiology, Etiology, and RVI Episode Characteristics

The person-time of observation for the cohort was 140 person-years in this study. Overall, we identified at least 1 CARV in 192 of 232 screened episodes (82%) in the 79 recipients. Of the 192 microbiologically documented RVIs, we identified RSV in 32 episodes (17%), HPiV in 34 (18%), EvRh in 88 (46%), HiV in 29 (15%), HMPV in 22 (12%), adenoviruses in 7 (4%), CoV in 21 (11%), and HBoV in 6 (3%). Co-infective viruses were documented in 51 RVI episodes (27%). As shown in Figure 1, most CARV RVI episodes occurred from October to June (autumn, winter, and spring). In summer only EvRh, CoV, and HPiVs were still circulating. We diagnosed 55 (29%)

Table 1
Patient Characteristics and Transplant Outcomes

Characteristics	All Recipients (N = 79)	Recipients with CoV or HBoV (n = 21)
Median age, yr (range)	52 (20-72)	52 (24-73)
Male sex	48 (61)	21 (71)
Baseline disease		
AL/MDS/MPN	17 (21)/8 (10)/6 (8)	5 (24)/1 (5)/3 (14)
NHL/HL/CLL/MM	26 (33)/6 (8)/10 (12)/6 (8)	7 (33)/1 (5)/2 (10)/2 (19)
Disease status at transplant		
CR	49 (62)	10 (48)
PR	20 (26)	8 (38)
Refractory/active disease	10 (13)	3 (14)
Prior ASCT	22 (28)	4 (20)
Conditioning regimen		
RIC (Flu-Mel/Flu-Bu/Thio-Flu-Bu/CFM-Flu-Bu)	44 (56)/5 (6)/5 (6)/17 (16)	13 (62)/1 (5)/2 (10)/4 (18)
Myeloablative	8 (10)	1 (5)
Type of donor		
HLA-identical sibling donor	27 (34)	8 (38)
Unrelated donor	35 (44)	9 (43)
Haploidentical family donor	17 (22)	4 (19)
HLA fully matched	51 (65)	13 (62)
ATG as a part of the conditioning	11 (15)	2 (10)
Recipient and/or donor cytomegalovirus seropositive	71 (91)	19 (90)
GVHD prophylaxis		
Sir-Tac	29 (37)	8 (38)
CsA + MTX	20 (25)	7 (33)
PTCy	17 (22)	4 (19)
Others	13 (16)	2 (10)
Year of allo-HSCT		
2010-2013	25 (32)	6 (29)
2014-2016	54 (68)	15 (71)
Post-transplant outcome		
Acute GVHD	45 (57)	12 (57)
Overall chronic GVHD/extensive (72 assessable patients)	62 (87)/31 (43)	17 (80)/8 (38)
Relapse	14 (18)	3 (14)
Overall mortality	14 (18)	3 (14)
Median time from allo-HSCT to first RVI, days (range)	225 (6-1575)	238 (6-1575)
Number of RVI episodes		
1 episode	28 (35)	15 (71)
2 episodes	24 (30)	6 (29)
3 episodes	11 (14)	0
4 or more episodes	16 (21)	0
Admission rate due to any RVI (192 RVI episodes)	37 (19)	5 (24)
Overall survival	65 (82)	18 (85)
Median follow-up after RVI, days (range)	388 (5-923)	252 (5-886)

Values are number of cases with percents in parentheses, unless otherwise defined. AL indicates acute leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; CR, complete remission; PR, partial remission; ASCT, autologous stem cell transplantation; RIC, reduced-intensity conditioning; Flu, fludarabine; Mel, melphalan; Bu, busulfan; Thio, thiotepa; CFM, cyclophosphamide; ATG, antithymocyte globulin; Siro, sirolimus; Tac, tacrolimus; CsA, cyclosporine A; MTX, methotrexate; PTCy, post-transplant cyclophosphamide.

of the RVI episodes in 2014, 96 (50%) in 2015, and 41 (21%) in the 6 first months of 2016.

As shown in Figures 1 and 2, CoVs and HBoVs predominated in the winter months from December to March (22 episodes, 81%) with sporadic cases between April to November (5, 19%). Moreover, we observed an increase in the frequency of CoV and HBoV RVI episodes during the study period. In 2014 we detected only 15% and 7% of all CoV/HBoV episodes and of all CARV episodes, respectively, whereas we diagnosed 41% and 44% of all CoV and HBoV RVI episodes and 11% and 29% of all CARV episodes in 2015 and mid-2016, respectively.

Clinical Characteristics and Type of CoV Infection Episodes

The clinical and biologic characteristics of CoV RVIs are detailed in Table 2. Overall, 18 recipients (23%) suffered at least 1 CoV RVI episode. Fifteen patients developed only 1 episode, whereas 3 had 2 CoV RVI episodes. Among the 3 recipients with 2 episodes, the median time elapsed from the first to the second episode was 445 days (range, 296 to 686). The type of CoV detected in the first and the second episode was different in all 3 recipients (1 with CoV type OC43 and type 229E, another with KHU1 and OC43, and the third with NL63 and OC43). Figure 3 shows the distribution of CoV-type RVIs. The most frequent CoV type was OC43 (48%), followed by NL63 (24%), KHU1 (19%), and type 229E (9%).

Table 3 shows the clinical and biologic characteristics of the RVIs according to the CoV type. Types OC43 and NL63 were apparently more clinically intense, as reflected by a higher occurrence of fever, co-pathogens, hospitalization rates, LRTDs and C-reactive protein at the time of the RVI evaluation. Co-pathogens were detected in 8 of 21 CoV RVI episodes (38%) (Table 3). Of note, the 3 cases with proven CoV LRTD also had bacterial or fungal co-infection detected in the BAL, 2 cases with *Stenotrophomonas maltophilia* and *Mycobacterium tuberculosis*, respectively, and 1 with *Pneumocystis jirovecci* detected by PCR. Co-infections were limited to recipients allografted from alternative donors (53% versus 0%, $P = .05$). However, we did not observe any statistical difference in terms of clinical presentation, lower respiratory tract

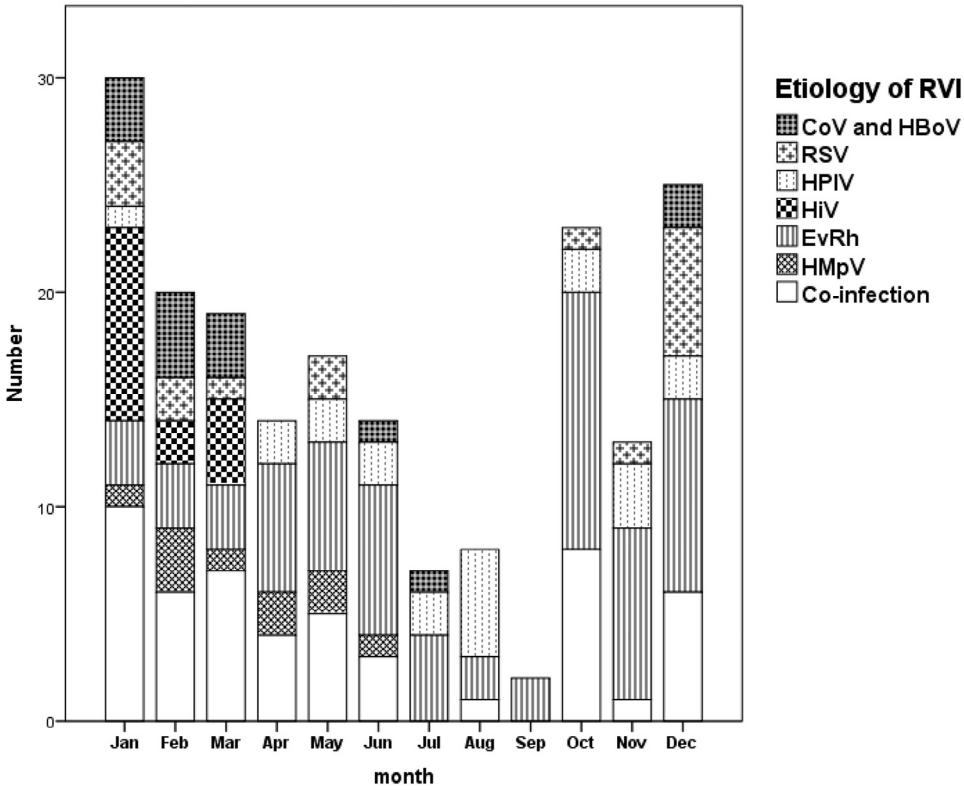

Figure 1. Type of community-acquired respiratory virus according to the month of detection.

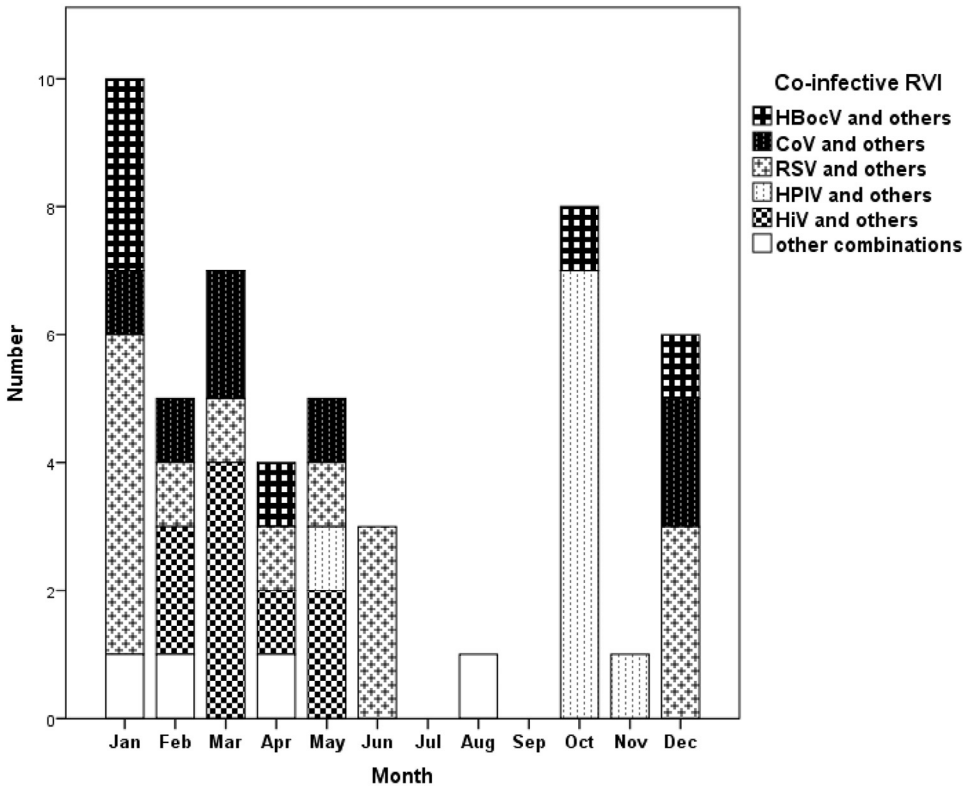

Figure 2. Type of co-infections according to the month of detection.

Table 2
Characteristics of CoV and HBoV RVI Episodes

	CoV RVI* (n = 21 episodes)	HBoV RVI* (n = 6 episodes)
Number of recipients	18	6
ECIL-4†		
Lymphopenia < $.2 \times 10^9$/L	1	1
Older age (>65 yr)	3	0
Mismatched/unrelated donor	8/10	1/4
Allo-HSCT < 1 mo	2	1
Neutropenia < $.5 \times 10^9$/L	0	0
Pre-engraftment	0	1
ISI†		
ANC < $.5 \times 10^9$/L (3 patients)	0	1
ALC < $.2 \times 10^9$/L (3 patients)	1	1
Age ≥ 40 yr (2 patients)	13	6
Myeloablative conditioning regimen (1 patient)	2	0
GVHD (acute or chronic; 1 patient)	13	4
Corticosteroids (1 patient)	2	4
Recent or pre-engraftment allo-HSCT (1 patient)	2	1
Risk index		
Low risk (0-2)	8	1
Moderate risk (3-6)	12	2
High risk (7-12)	1	3
Other characteristics†		
Median IgG levels, mg/dL (range)	674 (207-1480)	427 (215-1798)
On immunosuppression therapy	14 (66)	4
ALC, $\times 10^9$/L	1.62 (.6-8.4)	.34 (.19-1.67)
Co-infective virus	8 (38)	5 (83)
EvRh	2	3
HMPV	1	2
HPiV or RSV	3	
ADV	1	
HiV	1	
URTD	14 (67)	5 (83)
LRTD	7 (33)	1 (17)
Possible‡	4	1
Proven	3	0
Empiric antibiotics	16 (76)	6 (100)
Elevated CRP§	16 (76)	5 (83)
Immunoglobulin support	4 (19)	1 (17)
Fever	15 (71)	2 (33)
Admission rate	4 (19)	3 (50)
Median time from allo-HSCT to RVI, days (range)	241 (27-1040)	135 (6-1575)
Median symptoms length, days (range)	12 (5-60)	20 (3-31)
Mortality rate	1 (5)	0

Values in parentheses are ranges or percents. ECIL-4 indicates Fourth European Conference on Infections in Leukaemia; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; ADV, adenovirus; HiV, human influenza virus; CRP, C-reactive protein.

* Three patients had an episode of CoV and another had an episode of HBoV respiratory infection.

† All variables were assessed at the time of RVI diagnosis.

‡ All of our possible LRTD cases showed a radiology pattern suggesting a viral etiology, and the only microbiologic agent isolated at any site in such cases was CoV or HBoV in the upper respiratory tract.

§ Considered when it was higher than 10 mg/L.

infection, or admission rates between mono- and co-infections. Overall, 3 of the 18 recipients with CoV RVIs (17%) died. One patient died from respiratory distress syndrome 5 days after the identification of CoV type OC43 in a nasal swab with no other microbiologic documentation at any site (including blood, urine, or stool cultures). Their ISI score was high (9 points), and a possible CoV-related LRTD was radiologically documented on day +31 after stem cell infusion. Thus,

Table 3
Clinical Characteristics of CoV RVI According to the Viral Strain

Type of CoV	Fever* n (%)	Median PCR (range) (mg/L)	ISI (Low/Mod/High)	Co-pathogens n (%)	Alternative Donors n (%)	LRTD n (%)	Hospitalization n (%)	Duration (range) (days)
OC43 (n = 10)	7 (70)	26 (4-144)	5/4/1	4 (40)†	7 (70)	4 (40)	3 (30)	14 (5-35)
NL63 (n = 5)	4 (80)	79 (6-158)	2/3/0	3 (60)‡	4 (80)	3 (60)	1 (20)	16 (6-60)
KHU1 (n = 4)	2 (50)	11 (4-14)	0/4/0	0	3 (75)	0	0	11 (8-58)
229E (n = 2)	0	4/9	1/1/0	1 (50)§	1 (50)	0	0	14/21

* Considered when higher than 38°C.

† Co-pathogens included HMPV and *Stenotrophomonas maltophilia* in the BAL of 1 patient; in the other 3 patients we identified RSV type A, EvRh, and HPiV in nasopharyngeal swabs, respectively.

‡ Co-pathogens included *Pneumocystis jirovecii* DNA and RSV A detected by PCR in the BAL of 1 patient and EvRh and *Mycobacterium tuberculosis* in the BAL of another patient. The remaining patient showed the presence of ADV in a nasopharyngeal swab.

§ Patient with HiV A/H_1N^1 in a nasopharyngeal swab.

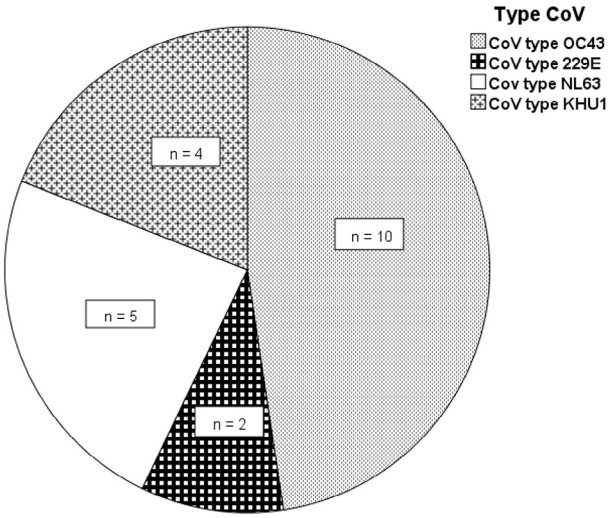

Figure 3. Distribution of CoV viral strains.

the mortality directly attributable to CoV RVIs was 5%. Two other recipients died at 5 and 9 months after the CoV RVI episode because of disease progression and obliterans bronchiolitis, respectively.

Clinical Characteristics of HBoV RVIs

The clinical and biologic characteristics of HBoV RVIs are detailed in Table 2. Overall, 6 recipients (8%) suffered an episode of HBoV RVI. Interestingly, 5 of the 6 HBoV detection cases (83%) also tested positive for other co-infective viruses (3 cases with EvRh and 2 with HPMV). Given the high frequency of co-infections in cases with HBoV detection in this series, we questioned the putative pathogenic effect of HBoV by itself in the respiratory tract of our patients. So, from now on we will refer to respiratory detection instead of respiratory infection when we mention HBoV. We detected HBoV in only 1 patient with possible LRTD, which was likely caused by EvRh. Three of 6 recipients with HBoV detection in respiratory secretions required hospital admission, and 1 of them had possible LRTD. None of the patients with HBoV respiratory detection died during the study period.

DISCUSSION

This prospective longitudinal RVI survey study provides insights into the epidemiology, type of CoVs, and clinical features of CoV RVIs and characteristics of HBoV respiratory detections in the allo-HSCT setting. CoV and/or HBoV were detected in 26% of the allo-HSCT recipients who developed at least 1 episode of a virologically documented URTD and/or LRTD over a period of 30 months. Together, both these CARVs represented 14% of all the documented RVI episodes over the observation period. We observed that a significant proportion of CoV RVIs require hospitalization, and some progressed to LRT. In contrast, HBoV detection was rare and commonly associated with co-pathogens.

In this series the frequency of CoVs ranked fifth after EvRhs, HPiVs, RSVs, and influenza viruses, respectively. Other recently published prospective data indicated that after hRhV, CoV was the second most commonly detected virus in allo-HSCT recipients 100 days after stem cell infusion [11]. Both data sets suggest that CoV RVIs are common in the allo-HSCT setting and should be included in the screening test when respiratory symptoms are present so that CARV RVI diagnoses can be expanded in this scenario.

In line with previous reports we also found that most CoV RVIs exhibited winter seasonality, even though in our series there were still many cases up until May [5,11,26]. Interestingly, although the number of allo-HSCTs remained stable over the study period (40 allo-HSCTs per year), we noticed an increase in number and frequency in CoV detections, and CARVs in general, over the years the study was conducted. We only diagnosed 15% of the total number of CoV RVIs during 2014 (representing 7% of all RVIs that year), whereas during 2015 and the first half of 2016 the number and frequency increased to 41% and 44% of CoV RVI episodes and 11% and 29% of the total RVI episodes, respectively. These observations merit attention. First, it is likely that there was a learning curve in efficiently identifying recipients with respiratory symptoms and asking for the appropriate screening tests. The entire hematology team, including fellows, were involved in this project and became progressively more aware of the importance of monitoring viral infections in allo-HSCT recipients, especially during out-of-hours periods (nights and weekends). This fact could partly explain the significant difference in the rate of documented CARV RVIs in 2014 (n = 55; number of RVIs per month, 4) compared with 8 and 7 of RVI episodes per month in 2015 (n = 96) and the first 6 months of 2016 (n = 41; $P < .01$), respectively. Although this fact could be regarded as a limitation, it likely occurs in several sites when novel strategies/protocols are implemented. Second, although the study period did not extend over 3 complete respiratory virus seasons, we cannot rule out the possibility that the seasonal changes commonly seen in the prevalence of CARVs may have influenced the different CoV RVI rates observed in 2014, 2015, and the first half of 2016. Finally, we cannot exclude the possibility of a peak in the prevalence of CoV RVIs in our community in 2016.

Another important observation was that in 38% of cases CoVs were detected in association with other co-pathogens, especially viruses, thus supporting prior findings where co-detections were common [11]. This raises interesting questions concerning the role of co-pathogenesis in disease in allo-HSCT recipients. The high frequency of co-infections in this series makes it difficult to interpret the clinical significance of CoVs on their own because the clinical effects cannot be attributed to their presence alone. The limited number of cases of viral co-infections reported in the medical literature limits our knowledge of the clinical relevance of such co-infections in the allo-HSCT setting. Thus, analysis of the putative clinical effect of CoVs detected as co-pathogens compared with RVIs caused by a single viral agent would be a useful line of future investigation.

Although the clinical significance of CoVs is poorly understood, prospective studies and reviews have suggested they may occasionally cause LRTDs after allo-HSCTs, but the overall progression rate seems to be very low [11,27]. However, our data indicate that at least 14% of CoV RVIs progressed to proven LRTDs, reaching 33% when possible LRTDs were considered. Again, it remains unknown if the presence of co-pathogens favors CoV progression to LRTD. Additionally, CoV RVIs led to hospital admissions because of fever, dyspnea, and/or clinical instability in 19% of cases. This suggests that CoV RVIs could be moderate to severe in allo-HSCT recipients and that additional supportive care is a common requirement. In relation to this, 1 of our study patients (representing 5% of the total CoV cases) with a possible LRTD and a high ISI died from respiratory failure soon after transplant, and his or her

only microbiologic documentation at any site sampled was a CoV type OC43 in a nasal swab, 5 days before death. These findings are in line with a recent retrospective study where the presence of CoV in BAL samples in immunocompromised hosts was significantly associated with high rates of respiratory support and mortality, similar to that of established respiratory pathogens including RSV, influenza virus, and HPiV [28].

Regarding the CoV types, and in contrast with Milano et al. [11] and others [27], we observed that the most common circulating CoV in our recipients was type OC43 (48%) followed by NL63 (24%), KHU1 (19%), and the 229E subtype (9%). This order agrees with epidemiologic data for infants and adults from several other countries and continents [29,30] and may be valuable for vaccine development purposes. Some authors suggest that this order might be the consequence of the generation of cross-reacting antibodies after CoV-OC43 and CoV-NL63 infections that may protect against HHCoV-HKU1 and HHCoV-229E infections, respectively. However, this protective relationship may not be reciprocal [30]. Interestingly, the 2 most common CoV types (OC43 and NL63) showed more clinically ntense features, as reflected higher occurrence of fever, co-pathogens, hospitalization rates, LRTDs, and C-reactive protein. How these facts might relate to patient immunogenicity and epidemiology is intriguing and merits further study.

In contrast with CoV RVIs, the clinical impact of HBoV infections in allo-HSCT is more ambiguous. Similar to many other reports that observed significant HBoV co-pathogenesis [16,31], we found that 83% of positive HBoV samples tested positive for other co-pathogens. Again, this is very difficult to interpret given the scarce clinical evidence for the pathogenesis of HBoV alone in allo-HSCT recipients. A recent case-control study shown similar rates of HBoV genomic DNA detection in symptomatic (10.4%) and asymptomatic children (7.5%), suggesting that its detection did not imply necessarily pathogenicity in the respiratory tract by itself. This study found that HBoV capsid mRNA detection could differentiate acute infections from prolonged shedding [32]. Still, in our series HBoV detection was a rare phenomenon, representing 3% of all CARVs. This finding agreed with preliminary allo-HSCT data where the cumulative incidence of HBoV detection in the first 100 days was 2.1% [33].

Finally, we acknowledge that our study has some limitations, including its relatively small cohort size and our decision to classify LRTDs as possible or proven, which may have led us to overestimate the true LRTD rate. However, the prospective nature of this study and the homogenous viral diagnostic tool we used are part of this study's strengths [25].

In summary, our data confirm that CoV RIVs are common after allo-HSCTs, that they can progress to LRTDs, and, in some cases, this leads to hospitalization and requires supportive care. In contrast, HBoVs are quite rare and are commonly detected in conjunction with other viral co-pathogens. However, this fact currently limits us from drawing firm conclusions concerning the clinical significance of HBoV detection in the pathogenicity of RVIs after allo-HSCTs.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

REFERENCES

1. Renaud C, Campbell AP. Changing epidemiology of respiratory viral infections in hematopoietic cell transplant recipients and solid organ transplant recipients. *Curr Opin Infect Dis*. 2011;24:333-343.
2. Shah DP, Ghantoji SS, Mulanovich VE, Ariza-Heredia EJ, Chemaly RF. Management of respiratory viral infections in hematopoietic cell transplant recipients. *Am J Blood Res*. 2012;2:203-218.
3. McIntosh K. Coronaviruses. In: Richman D, Whitley RJ, Hayden FG, eds. *Clinical Virology*. New York, NY: Churchill Livingstone; 1997:1123-1132.
4. Van der Hoek L. Human coronaviruses: what do they cause? *Antivir Ther*. 2007;12:651-658.
5. Kuypers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA. Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics*. 2007;119:e70-e76.
6. Heugel J, Martin ET, Kuypers J, Englund JA. Coronavirusassociated pneumonia in previously healthy children. *Pediatr Infect Dis J*. 2007;26:753-755.
7. Lee J, Storch GA. Characterization of human coronavirus OC43 and human coronavirus NL63 infections among hospitalized children <5 years of age. *Pediatr Infect Dis J*. 2014;33:814-820.
8. Pene F, Merlat A, Vabret A, et al. Coronavirus 229E-related pneumonia in immunocompromised patients. *Clin Infect Dis*. 2003;37:929-932.
9. Folz RJ, Elkordy MA. Coronavirus pneumonia following autologous bone marrow transplantation for breast cancer. *Chest*. 1999;115:901-905.
10. Oosterhof L, Christensen CB, Sengelov H. Fatal lower respiratory tract disease with human corona virus NL63 in an adult haematopoietic cell transplant recipient. *Bone Marrow Transplant*. 2010;45:1115-1116.
11. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood*. 2010;115:2088-2094.
12. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. From the hHCoVer: cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA*. 2005;102:12891-12896.
13. Bastien N, Brandt K, Dust K, Ward D, Li Y. Human bocavirus infection, Canada. *Emerg Infect Dis*. 2006;12:848-850.
14. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis*. 2010;201:1625-1632.
15. Martin ET, Kuypers J, McRoberts JP, Englund JA, Zerr DM. Human bocavirus 1 primary infection and shedding in infants. *J Infect Dis*. 2015;212:516-524.
16. Schenk T, Strahm B, Kontny U, Hufnagel M, Neumann-Haefelin D, Falcone V. Disseminated bocavirus infection after stem cell transplant. *Emerg Infect Dis*. 2007;13:1425-1427.
17. Schenk, Maier T, Hufnagel B, et al. Persistence of human bocavirus DNA in immunocompromised children. *Pediatr Infect Dis J*. 2011;30:82-84.
18. Waggoner J, Deresinski S. Rare and emerging viral infection in the transplant population. In: Safdar A, ed. *Principles and Practice of Transplant Infectious Diseases*. Berlin, Germany: Springer Medizin; 2013.
19. Schildgen O, Muller A, Allander T, et al. Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev*. 2008;21:291-304, table of contents.
20. Miyakis S, van Hal SJ, Barratt J, Stark D, Marriott D, Harkness J. Absence of human bocavirus in bronchoalveolar lavage fluid of lung transplant patients. *J Clin Virol*. 2009;44:179-180.
21. Piñana JL, Hernández-Boluda JC, Calabuig M, et al. A risk-adapted approach to treating respiratory syncytial virus and human parainfluenza virus in allogeneic stem cell transplantation recipients with oral ribavirin therapy: a pilot study. *Transpl Infect Dis*. 2017;19.
22. Shah DP, Ghantoji SS, Ariza-Heredia EJ, et al. Immunodeficiency scoring index to predict poor outcomes in hematopoietic cell transplant recipients with RSV infections. *Blood*. 2014;123:3263-3268.
23. Seo S, Xie H, Campbell AP, et al. Parainfluenza virus lower respiratory tract disease after hematopoietic cell transplant: viral detection in the lung predicts outcome. *Clin Infect Dis*. 2014;58:1357-1368.
24. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. *Clin Infect Dis*. 2013;56:258-266.
25. Costa E, Rodríguez-Domínguez M, Clari MÁ, Giménez E, Galán JC, Navarro D. Comparison of the performance of 2 commercial multiplex PCR platforms for detection of respiratory viruses in upper and lower tract respiratory specimens. *Diagn Microbiol Infect Dis*. 2015;82:40-43.
26. Leung TF, Li CY, Lam WY, et al. Epidemiology and clinical presentations of human coronavirus NL63 infections in Hong Kong children. *J Clin Microbiol*. 2009;47:3486-3492.
27. Hakki M, Rattray RM, Press RD. The clinical impact of coronavirus infection in patients with hematologic malignancies

and hematopoietic stem cell transplant recipients. *J Clin Virol*. 2015;68:1-5.
28. Ogimi C, Waghmare AA, Kuypers JM, et al. Clinical significance of human coronavirus in bronchoalveolar lavage samples from hematopoietic cell transplant recipients and patients with hematologic malignancies. *Clin Infect Dis*. 2017;64:1532-1539.
29. Sipulwa LA, Ongus JR, Coldren RL, Bulimo WD. Molecular characterization of human coronaviruses and their circulation dynamics in Kenya, 2009-2012. *Virol J*. 2016;13:18.
30. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. *J Clin Virol*. 2012;53:135-139.
31. Schenk T, Maier B, Hufnagel M, et al. Persistence of human bocavirus DNA in immunocompromised children. *Pediatr Infect Dis J*. 2011;30:82-84.
32. Schlaberg R, Ampofo K, Tardif KD, et al. Human bocavirus capsid messenger RNA detection in children with pneumonia. *J Infect Dis*. 2017;216:688-696.
33. Peck Campbell A, Kuypers J, Nguyen P, et al. Human bocavirus (BoV) detection in nasal washes of hematopoietic cell transplantation recipients. Presented at the 48th annual meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy and the 46th annual meeting of the Infectious Diseases Society of America, October 25–28, Washington DC, 2008, abstract V-3777.