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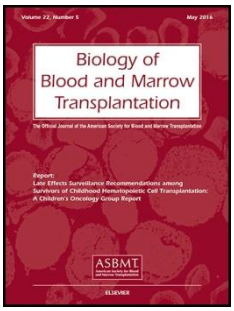

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Epidemiological and clinical characteristics of Coronavirus and Bocavirus Respiratory Infections After Allogeneic Stem Cell Transplantation: A Prospective single center study

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Highlights

- Human coronavirus are common after allogeneic stem cell transplantation, that they can progress to LRTDs, and in some cases, this leads to hospitalization and requires supportive care.
- Human bocavirus are quite rare after allogeneic stem cell transplantation and are commonly detected in conjunction with other viral co-pathogens.

ABSTRACT

Epidemiological data about coronaviruses (CoVs) and human bocavirus (HBoV) in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is scarce. **Methods:** We conducted a prospective longitudinal study on respiratory viral infections (RVIs) in allo-HSCT recipients having 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.

Results: 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 5-923). CoV or HBoV was detected in 27 of the 192 episodes (14%); 18 of the 79 recipients (23%) developed a total of 21 CoV RVI episodes, while 6 recipients (8%) had one CoV 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% while mortality was 5% (one patient without any other microbiological documentation). Among the 6 recipients with HBoV (3%), only one had LRT involvement and no one died from respiratory failure. In 5 cases (83%) HBoV was detected along with other viral co-pathogens.

Conclusion: 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.

Keywords: Coronavirus, bocavirus, community acquiered respiratory virus, respiratory virus infection, allogeneic stem cell transplantation, viral pneumonia

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 (HRhV) 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 polymerase chain reaction (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, six human CoVs have been identified, namely CoV-229E, CoV-NL63, CoV-OC43, CoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV); of these, four (*Alphacoronaviruses*: CoV-229E and CoV-NL63, and *Betacoronaviruses*: CoV-OC43 and CoV-HKU1) are kwon 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[8-11]. However, the largest series of CoVs analyzed in allo-HSCT patients published to date is in a prospective observational study which detected CoV in 22 out of 215 allo-HSCT recipients with an estimated incidence of 11% at 100 days after stem cell infusion[12].

HBoV, however, was originally identified by a random PCR amplification/cloning technique in pooled respiratory secretions from hospitalized children with respiratory tract infection symptoms[13]. This virus affects young children with winter seasonality[14-16]. However, scarce data is 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 which is sometimes associated with graft versus host disease (GVHD) and prolonged fecal viral shedding[17,18]. But other studies report little evidence linking this virus with pulmonary pathologies or severe respiratory disease in allo-HSCT or lung transplant recipients[19-21].

Thus, we conducted a prospective epidemiological study of 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 30-month period.

PATIENTS AND METHODS

Patients

This was a prospective longitudinal study of RVIs in adults (>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 care-givers explaining in detail about the risks of having respiratory virus infections in the context of immunosuppression. Specific information included a description of respiratory symptoms, that should be reported as soon as possible to the transplant team, and recommendations concerning the infectious prevention control measures for patients and health care-givers. A telephone number

(on-call 24h) 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 biological characteristics were prospectively recorded as reported in detail elsewhere[22]. Immunodeficiency scoring index (ISI) variables were recorded at the first clinical evaluation as previously described[23]. 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 (T > 38°C). We retrospectively analyzed the epidemiology of the CoV and HBoV viruses 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) as well as positive identification of a CARV by a PCR test and the absence of LRTI symptoms and/or any indication of pulmonary infiltrates in the radiology results by chest X-ray or computed tomography (CT) scan. We classified LRTDs as possible or confirmed as previously described[24]. 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 LRTIs 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 ECIL-4 recommendations[25], 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 two 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 microbiological 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 virological, bacterial and fungal evaluation. When bronchoscopy was performed a detailed microbiological evaluation including respiratory viruses, bacterial, fungal, and acid-fast bacilli cultures, Aspergillus galactomannan assay, and detection of cytomegalovirus (CMV) was performed. Patients with URTD symptoms underwent nasopharyngeal aspiration, nasopharyngeal swabs, or an induced sputum test, whereas BAL was performed in patients with a LRTD whenever possible. All clinical samples were tested by RT-PCR using the Luminex xTAG RVP Fast v1 assay, as described in detail elsewhere[26]. Briefly, all specimens were received at the laboratory within 30 minutes of collection and were conserved at 4°C until they were processed (within 18 h of receipt). Nucleic acid extraction was performed using the Qiagen EZ-1 viral

extraction kit with a EZ1 Robot (Qiagen, Valencia, CA, USA). The Luminex xTAG RVP Fast v1 assay can detect adenoviruses (ADVs); HBoV; CoV 229E, HKU1, NL63, and OC43; influenza A virus (InfA) A/H1N1, InfA/H3N2, and other InfA viruses (non-subtypificable); influenza B virus (InfB); 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 end point was to describe the clinical characteristics and outcomes of patients suffering URTDs and/or LTRDs caused by these viruses. Epidemiological, clinical, and RVI characteristics were compared using the *chi*-squared test for categorical variables and with paired Student t-tests for continuous variables; the statistical significance was set at $p < 0.05$ and where relevant, the standard deviation is shown.

RESULTS

Patient characteristics

A total of 79 out of 88 allo-HSCT recipients (89%) screened for upper and/or lower respiratory symptoms developed at least one episode of virologically documented RVIs over the study period. The clinical and biological 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 Haplo-identical family donors) and 35% had at least one 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 respiratory viral infection episode characteristics

The person-time of observation for the cohort was 140 person-year in this study. Overall, we identified at least one CARV in 192 of the 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%), ADV 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 of the 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%) 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 (n = 22 episodes, 81%) with sporadic cases between April to November (n = 5, 19%). Moreover, we observed an increase in the frequency of CoV and HBoV RVI episodes during the study period. We detected in 2014, only 15% and 7% of all the CoV / HBoV episodes and of all the CARV episodes respectively, whereas we diagnosed 41% and 44% of all the CoV and HBoV RVI episodes and 11% and 29% of all the CARV episodes in 2015 and mid-2016, respectively.

Clinical characteristics and type of coronavirus infection episodes

The clinical and biological characteristics of CoV RVIs are detailed in table 2. Overall, 18 recipients (23%) suffered at least one CoV RVI episode. Fifteen patients developed only one episode while 3 had two CoV RVI episodes. Among the 3 recipients with two

episodes, the median time elapsed from the first to the second episode was 445 days (range 296 to 686 days). The type of CoV detected in the first and the second episode was different in all 3 recipients (one 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 lastly, type 229E (9%).

Table 3 shows the clinical and biological 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, higher rates of LRTDs and higher levels of reactive-C-protein (RCP) at the time of the RVI evaluation. Co-pathogens were detected in 8 out of 21 CoV RVI episodes (38%) (see table 3). Of note, the three 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 one case with *pneumocystis jirovecci* detected by PCR. Co-infections were limited to recipients allografted from alternative donors (53% vs. 0%, $p = 0.05$). However, we did not observe any statistical difference in terms of clinical presentation, LRTI or admission rates between mono and co-infections. Overall, 3 of the 18 recipients with CoV RVIs (17%) died. One patient deceased from respiratory distress syndrome 5 days after the identification of CoV type OC43 in a nasal swab with no other microbiological documentation at any site (including blood, urine or stools 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, the mortality directly attributable to CoV RVIs was 5%. Two other recipients died at 5 and 9 months after the CoV RVI episode due to disease progression and obliterans bronchiolitis, respectively.

Clinical characteristics of human bocavirus respiratory viral infections

The clinical and biological characteristics of HBoV RVI are detailed in table 2. Overall, 6 recipients (8%) suffered an episode of a HBoV RVI. Interestingly, 5 of the 6 HBoV detection cases (83%) also tested positive for other co-infective viruses (3 cases with EvRh and two 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 we will refer to respiratory detection instead of respiratory infection when we mention HBoV. We detected HBoV in only one patient with possible LRTD, which was likely caused by EvRh. Three out of 6 recipients with HBoV detection in respiratory secretions required hospital admission, one 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 one 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 RVI 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 in fifth position 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[12]. 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[8,12,27]. 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. All the hematology team, including fellows, were involved in this project and they 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 to 8 and 7 of RVI episodes per month in 2015 (n = 96) and the first 6 months of 2016 (n = 41; $p < 0.01$), 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. Lastly, we cannot exclude the possibility that there was 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 codetections were common[12]. 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 make 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 to 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 that they may occasionally cause LRTDs after allo-HSCTs, but the overall progression rate seems to be very low[12,28]. 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 due to 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, one 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 their only microbiological 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, influeza virus and HPiV[29].

Regarding the CoV types, and in contrast with Milano et al. and others[28], 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 epidemiological data for infants and adults from several other countries and continents[30,31] 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 which may protect against HHCoV-HKU1 and HHCoV-229E infections, respectively. However, this protective relationship may not be reciprocal[31]. Interestingly, the two most common CoV types (OC43 and NL63) showed more clinically-intense features, as reflected higher occurrence of fever, co-pathogens, hospitalization rates, higher rates of LRTDs and higher levels of reactive-C-protein (RCP). 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[17,32], we found that 83% of positive HBoV samples tested positive for other co-pathogens. Again, this is very difficult to interpret given that there is 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 no implies necessarily pathogenicity in the respiratory tract by itself. This study found that HBoV capsid mRNA detection could differentiate acute infections from prolonged shedding[33]. 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%[34].

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, as well as the homogenous viral diagnostic tool we used, are part of this study's strengths[26].

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.

CONFLICT OF INTERESTS

The author(s) declare that they have no conflict of interests.

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

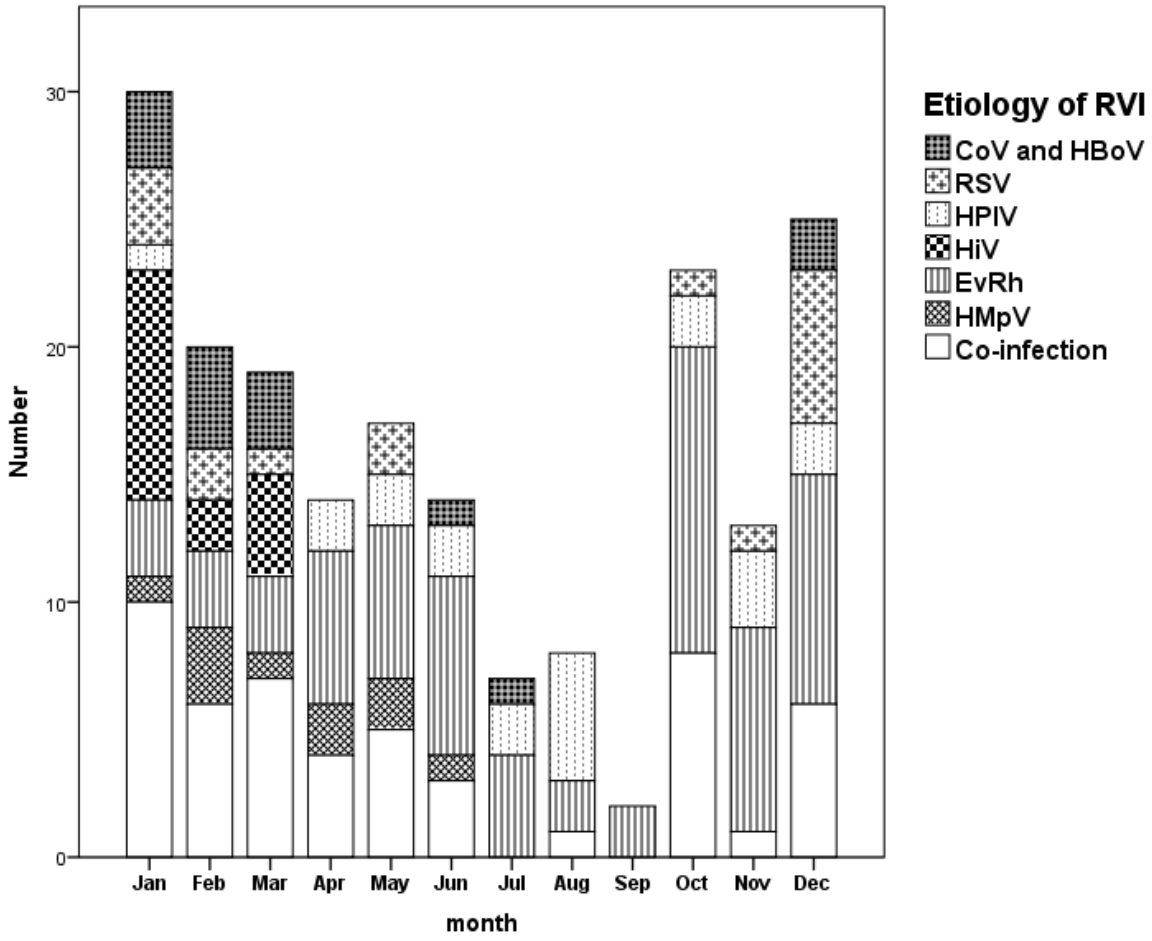

Figure 2. Type of co-infections according to the month of detection.

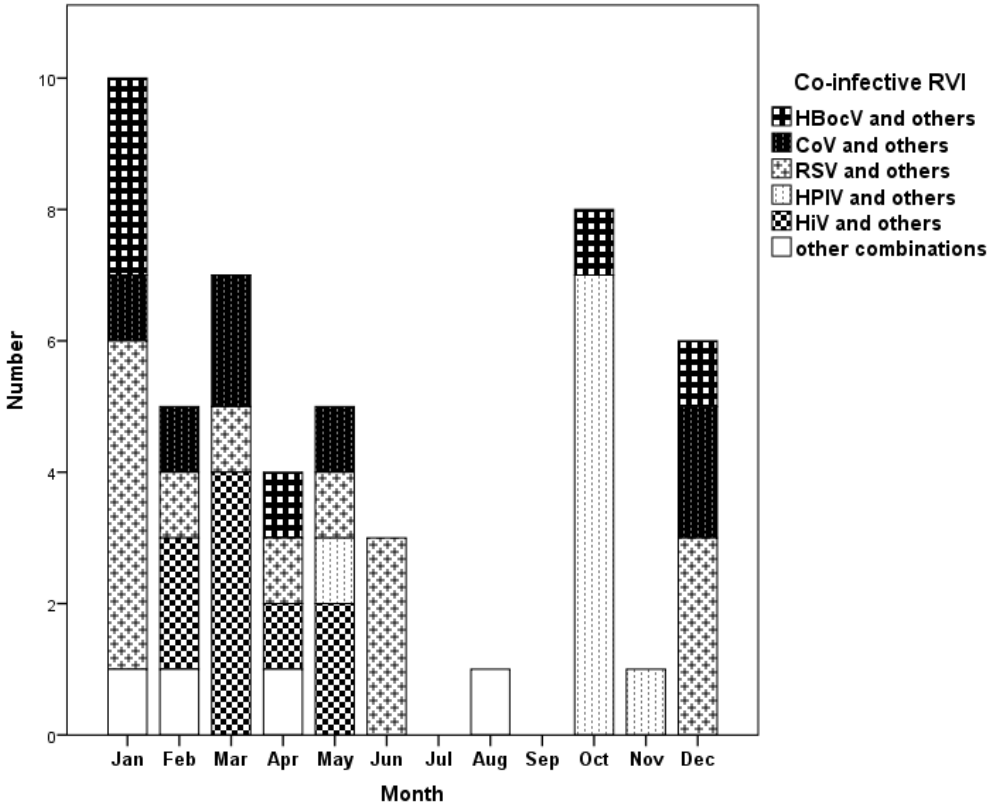

Figure 3. Distribution of CoV viral strains.

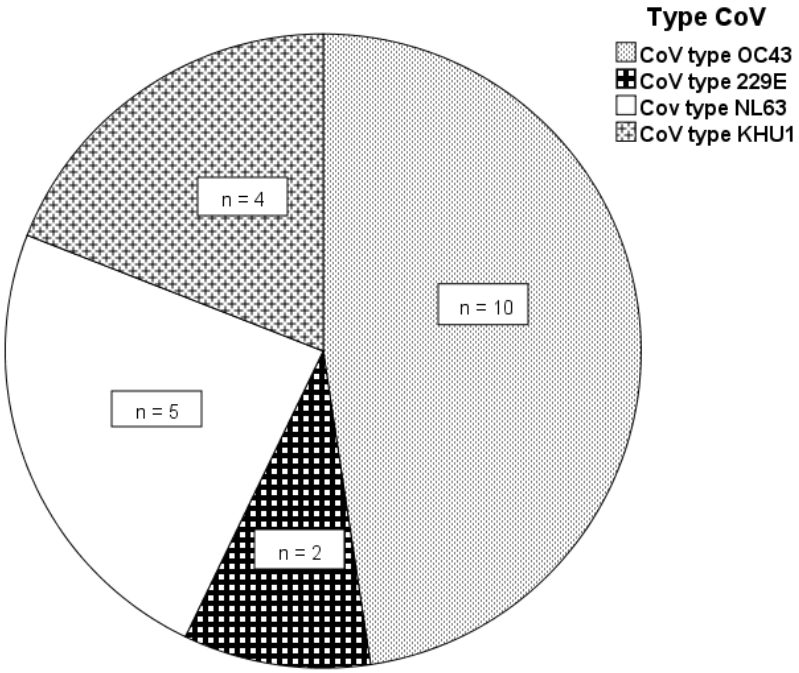

Table 1. Patient characteristics and transplant outcomes

Characteristics	All recipients (n = 79) (n, %)	Recipients with CoV or HBoV (n = 21) (n, %)
Age (years), median (range)	52 (20-72)	52 (24-73)
Male sex, n (%)	48 (61)	21 (71)
Baseline disease, n (%)		
• 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, n (%)		
• CR	49 (62)	10 (48)
• PR	20 (26)	8 (38)
• Refractory / active disease	10 (13)	3 (14)
Prior ASCT, n (%)	22 (28)	4 (20)
Conditioning regimen, n (%)		
• 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, n (%)		
• HLA-identical sibling donor	27 (34)	8 (38)
• Unrelated donor	35 (44)	9 (43)
• Haploidentical family donor	17 (22)	4 (19)
HLA fully-matched, n (%)	51 (65)	13 (62)
ATG as a part of the conditioning, n (%)	11 (15)	2 (10)
Recipient and/or donor CMV seropositive, n (%)	71 (91)	19 (90)
GvHD prophylaxis, n (%)		
• Sir-Tac	29 (37)	8 (38)
• CsA + MTX	20 (25)	7 (33)
• Post-CyPh	17 (22)	4 (19)
• Others	13 (16)	2 (10)
Year of Allo-HSCT, n (%)		
• 2010-2013	25 (32)	6 (29)
• 2014-2016	54 (68)	15 (71)
Post-transplant outcome		
Acute GvHD, n (%)	45 (57)	12 (57)
Overall chronic GvHD / extensive, n (%)/ 72 evaluable patients	62 (87) / 31 (43)	17 (80) / 8 (38)
Relapse, n (%)	14 (18)	3 (14)
Overall mortality, n (%)	14 (18)	3 (14)
Median time from allo-HSCT to first RVI, days (range)	225 (6-1575)	238 (6-1575)
Number of RVI episodes, n (%)		
• 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, n (%)/ 192 RVI episodes	37 (19)	5 (24)
Overall survival, n (%)	65 (82)	18 (85)
Median F-Up after RVI, days (range)	388 (5-923)	252 (5-886)

Abbreviations. AL, 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; Nº, number; ASCT, autologous stem cell transplantation; RIC, reduced intensity conditioning; Siro, sirolimus; Tac, tacrolimus; CsA, cyclosporine A; MTX, methotrexate; Post-CyPh, post-transplant cyclophosphamide; allo-HSCT, allogeneic hematopoietic stem cell transplantation; GvHD, graft versus host disease; RVI, respiratory virus infection; F-up, follow-up.

Table 2. Characteristics of CoV and HBoV respiratory viral infection episodes.

	CoV RVI ¥ (n = 21 episodes)	*HBoV RVI ¥ (n = 6 episodes)*
Number of recipients, n	***18***	***6***
ECIL-4, n (%)‡		
Lymphopenia < 0.2 × 10^9/L	**1**	**1**
Older age (> 65 years)	**3**	**0**
Mismatched / unrelated donor	**8 / 10**	**1 / 4**
Allo-HSCT < 1 month	**2**	**1**
Neutropenia < 0.5 × 10^9/L	**0**	**0**
Pre-engraftment	**0**	**1**
Immunodeficiency Scoring Index, n (%)‡		
ANC < 0.5 × 10^9/L (3pts)	**0**	**1**
ALC< 0.2 × 10^9/L (3pts)	**1**	**1**
Age ≥ 40 y (2pts)	**13**	**6**
Myeloablative conditioning regimen (1pt)	**2**	**0**
GVHD (acute or chronic; 1pt)	**13**	**4**
Corticosteroids (1pt)	**2**	**4**
Recent or pre-engraftment allo-HSCT (1pt)	**2**	**1**
Risk index		
• **Low risk (0-2)**	**8**	**1**
• **Moderate risk (3-6)**	**12**	**2**
• **High risk (7-12)**	**1**	**3**
Other characteristics‡		
IgG Immunoglobulin levels (mg/dl), median (range).	**674 (207-1480)**	**427 (215-1798)**
On IS, n (%)	**14 (66)**	**4**
ALC (×10^9/L)	**1.62 (0.6-8.4)**	**0.34 (0.19-1.67)**
Co-infective virus, n (%)	**8 (38)**	**5 (83)**
• **EvRh**	**2**	**3**
• **HMPV**	**1**	**2**
• **HPiV or RSV**	**3**	
• **ADV**	**1**	
• **HiV**	**1**	
URTD, n (%)	**14 (67)**	**5 (83)**
LRTD, n (%)	**7 (33)**	**1 (17)**
• **Possible ±**	**4**	**1**
• **Proven**	**3**	**0**
Empiric antibiotics, n (%)	**16 (76)**	**6 (100)**
Elevated RCP, n (%)*	**16 (76)**	**5 (83)**
Immunoglobulin support, n (%)	**4 (19)**	**1 (17)**
Fever, n (%)	**15 (71)**	**2 (33)**
Admission rate, n (%)	**4 (19)**	**3 (50)**
Median time from allo-HSCT to RVI	**241 (27-1040)**	**135 (6-1575)**
Symptoms length, days (range)	**12 (5-60)**	**20 (3-31)**
Mortality rate, n (%)	**1 (5)**	**0**

Abbreviations. URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; RVI, respiratory virus infection; ALC, absolute lymphocyte count; GvHD, graft versus host disease; IS, immunosuppressants; EvRh, Enterovirus/rhinovirus; ADV, adenovirus; RSV, respiratory syncytial virus; HPiV, human parainfluenza virus; HiV, human influenza virus; RCP, reactive C protein.

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

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

¥ 3 patients had an episode of CoV and another had an episode of HBoV respiratory infection.

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

Table 3. Clinical characteristics of CoV RVI according to the viral strain.

Type of CoV	Fever* n (%)	PCR‡ Median (range)	ISI (Low/Mod/High) n	Co-pathogens n (%)	Alternative donors n (%)	LRTD n (%)	Hospitalization n (%)	Duration days (range)
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

Abbreviations, CoV, coronavirus; n, number; ISI, immunodeficiency score index; URTD, upper respiratory tract disease; LRTD, lower respiratory tract disease.
*Considered when higher than 38°C.
‡ In mg/L
# Co-pathogens included HMPV and *Stenotrophomonas Maltophilia* in the BAL of one patient, and in the other 3 patients we identified RSV type A, EvRh and HPiV in nasopharyngeal swab, respectively.
¥ Co-pathogens included pneumocystis jirovecii DNA and RSV A detected by PCR in the BAL of one 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/H1N1 in a nasopharyngeal swab.