Seasonal human coronaviruses respiratory tract infection in recipients of allogeneic hematopoietic stem cell transplantation.

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Brief summary : Seasonal HCoV infection in allo-HSCT recipients was associated with considerable morbidity in particular in those who had radiological signs of pulmonary involvement. Three-month mortality was 7% in the whole cohort and 16% in those with pulmonary involvement.

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Abstract

Background: Little is known about characteristics of seasonal human coronavirus (HCoV) (NL63, 229E, OC43 and HKU1) after allogeneic stem cell transplantation (allo-HCT).

Patients and methods: this is a collaborative Spanish and European bone marrow transplantation groups retrospective multicentre study, which included allo-HCT recipients (adults and children) with upper and/or lower respiratory tract disease (U/LRTD) caused by seasonal HCoV diagnosed through multiplex PCR assays from January 2012 to January 2019.

Results: We included 402 allo-HCT recipients who developed 449 HCoV U/LRTD episodes. Median age of recipients was 46 years (range 0.3-73.8 years). HCoV episodes were diagnosed at a median of 222 days after transplantation. The most common HCoV subtype was OC43 (n=170, 38%). LRTD involvement occurred in 121 episodes (27%). HCoV infection frequently required hospitalization (18%), oxygen administration (13%) and intensive care unit (ICU) admission (3%). Three-month overall mortality after HCoV detection was 7% in the whole cohort and 16% in those with LRTD. We identified 3 conditions associated with higher mortality in recipients with LRTD: absolute lymphocyte count <0.1 x10^9/mL [hazard ratio (HR), 10.8], corticosteroid (HR 4.68) and ICU admission (HR 8.22) ($p<0.01$).

Conclusions: Seasonal HCoV after allo-HCT may involve the LRTD in many instances, leading to a significant morbidity.

Key Words: Seasonal human coronavirus, HCoV-NL63, HCoV-229E, HCoV-OC43, HCoV-HKU1, community-acquired respiratory virus, allogeneic hematopoietic stem cell transplantation, immunocompromised, upper and lower respiratory tract disease, immunodeficiency score index, multiplex PCR assay.

INTRODUCTION

The development of molecular technologies and the widespread use of multiplex PCR assays for community-acquired respiratory viruses (CARVs) screening allows epidemiologic and clinical characterization of seasonal human coronaviruses (HCoV) infections in immunocompromised patients[1,2,3]. Coronaviruses are a group of enveloped viruses with non-segmented, single-stranded, and positive-sense RNA genomes. Of the 4 genera of coronaviruses, Gammacoronavirus and Deltacoronavirus exclusively infect animals whereas most of the Alphacoronavirus and some of the Betacoronavirus are well recognized to infect humans[4]. Among the seven known HCoV subtypes that affect humans, HCoV-229E and HCoV-NL63 belong to Alphacoronavirus, whereas HCoV-OC43 and HCoV-HKU1 belong to lineage A, severe acute respiratory syndrome-human coronavirus (SARS-CoV) and novel coronavirus (SARS-CoV-2) to lineage B, and Middle East respiratory syndrome-HCoV (MERS-HCoV) to lineage C, both belonging to Betacoronavirus[5]. Priors and recent outbreaks of zoonotic HCoV infections such as SARS-CoV[6-8], MERS-HCoV[9] and recently SARS-CoV-2[10,11], support the idea that coronavirus could be one of the most rapidly evolving viruses owing to its high genomic nucleotide substitution rates and recombination[12]. However, seasonal HCoV (HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1) have circulated globally in the human population for decades and although they contribute to approximately one-third of common colds in humans, their severity seems not as devastating as the zoonotic coronavirus outbreaks with no fatalities in pediatric[13] or relatively low mortality rate (4%) in elderly chronic obstructive pulmonary disease (COPD) patients[14]. Nevertheless, knowledge of the consequence of seasonal HCoV respiratory infection in highly immunocompromised patients, such as allogeneic stem cell transplantation (allo-HCT) recipients, remains poorly characterized to date.

CARVs epidemiology in allo-HCT recipients parallels the epidemiology in the general population[15], although these respiratory infections are particularly threatening after allo-HCT[16-18]. Early studies showed that HCoVs were detected in lung tissues in transplant recipients developing severe

pneumonia[19-22]. Compared to other CARVs, prior reports with a small number of cases suggest that HCoV upper and/or lower respiratory tract disease (U/LRTD) were quite frequent after allo-HSCT, representing 11% to 14% of all CARVs[1,2,3]. In contrast to previous observations, recent smaller studies have shown that HCoV could involve the LRT in many instances in allo-HCT recipients (14% to 33%)[1,3]. Overall mortality of such cases ranged from 11% to 54% at 3 months after the HCoV detection which was similar to that seen in RSV, influenza and parainfluenza virus LRTD in allo-HCT recipients[1,3,23].

In this large retrospective international multicenter cohort, we aimed to characterize epidemiological and clinical features, risk factors (RFs) and outcome of seasonal HCoV infections in a severe immunocompromised population such as allo-HCT recipients.

PATIENTS AND METHODS

Study population

This is a retrospective collaborative multicenter cohort study between the Infectious Diseases Working Party (IDWP) of the European Society for Blood and Marrow Transplantation (EBMT) and the Infectious Complications Subcommittee (GRUCINI) of the Spanish Hematopoietic Stem Cell Transplantation and Cell Therapy Group (GETH), focused on allo-HCT recipients with U/LRTD symptoms caused by seasonal HCoV types (HCoV-NL63, HCoV-229E, HCoV-OC43 or HCoV-HKU1) which were detected by multiplex PCR panels. The EBMT is a scientific organization that collects data from associated centers that perform HSCT through a web-based registry called ProMISe in accordance with standards at every center for patient confidentiality and good clinical practice.

Inclusion criteria and data preparation

The EBMT participating centers were requested to include all consecutive allo-HCT recipients (children and adults) with laboratory-documented seasonal HCoV respiratory infection during a period comprised from January 1st 2012 to January 30th 2019. All consecutive HCoV respiratory infection episodes per recipient that occurred from the day of conditioning regimen to the last follow-up during the aforementioned period were included. The inclusion of HCoV cases that were detected during conditioning but before stem cell infusion is justified by the potential negative impact of pre-transplant CARV detection[24]. The exclusion criterion was baseline disease relapse or progression before the HCoV detection.

During the study period, all allo-HCT procedures were registered in ProMISe by completing the essential medical data form. This form is mandatory for all centers belonging to the EBMT network. Data that is more detailed was collected using a second transplant form that contained specific information regarding a description of respiratory symptoms, HCoV-related hospital admission, oxygen requirement and ICU admission. Variables such as immunosuppressant drugs, corticosteroids, the presence of signs or symptoms of acute or chronic graft-versus-host disease (GvHD), prior development of bronchiolitis obliterans syndrome (BOS) and variables for immunodeficiency scoring index (ISI) computation[25] (i.e. lymphocyte count, neutrophils count, myeloablative conditioning regimen, age, corticosteroids therapy and graft-versus-host disease) were requested at the time of CARV PCR screening.

Definitions

URTD was defined as the combination of upper respiratory symptoms (rhinorrhea, sinusitis, otitis, or pharyngitis), identification of seasonal HCoVs by PCR assay and the absence of LRTD symptoms and/or any pulmonary infiltrates on chest X-ray or computed tomography (CT) scan of the lung. We classified LRTD as possible, probable or confirmed, as previously described[26]. There were no

probable episodes because bronchoscopies were not performed in patients without radiological proof of pulmonary involvement. We defined episodes as an URTD or LRTD according to ECIL-4 recommendations[27]. An infectious disease episode was considered to be resolved when complete remission of respiratory symptoms was observed. A further episode of a respiratory tract infectious disease required the presence of a symptom-free period of at least two consecutive weeks from the resolution of the previous episode and/or the isolation of a different subtype of HCoV in conjunction with the onset of new respiratory symptoms. Acute and chronic GvHD, including BOS were diagnosed according to standard criteria[28].

A co-infection was defined as a significant co-pathogen detected in concurrent nasopharyngeal, bronchoalveolar lavage, or in a blood sample obtained during the course of HCoV infection.

Technical and diagnostic considerations

CARV testing in respiratory samples were performed with different multiplex PCR platforms. Details regarding the CARV type's performance for each PCR test is provided in Table 1. Briefly, 5 of 9 commercial multiplex PCR assays and other unspecified PCR platforms were able to detect and discriminate all four common HCoV subtypes, whereas one commercial PCR assay only detected three out of four HCoV (NL63, 229E, OC43), one in-house PCR assay only detected 2 HCoV (229E and OC43) and one commercial PCR assay only detected HCoV-229E subtype. A commercial multiplex PCR assay detected the four strains of HCoV but was not able to discriminate among them. Finally, an unknown PCR platform was able to detect all four HCoV without information on HCoV subtype and then HCoVs in these cases were classified as non-subtypable.

Endpoints and statistical analysis

The primary objective of the study was to describe epidemiological and clinical characteristics of U/LRTD in allo-HCT recipients with seasonal HCoV infection. We also analyzed differences in clinical manifestations among HCoV subtypes as well as RFs for HCoV-related hospital admission, oxygen requirement, LRTD involvement, and all-cause mortality by day 90 after HCoV detection, the later in recipients with LRTD. We selected day 90 as a cut-off for mortality analysis to capture HCoV-related late events since CARV shedding could be longer than 12 weeks in allo-HCT recipients[17].

The main characteristics of patients were reported by descriptive statistics on the total of the available information, median and range were used for continuous variables, whilst absolute and percentage frequency were used for categorical variables. Differences between groups were tested using linear or logistic regression models, using the generalized estimating equation methods to take into account the dependence of observations, nested by patient. Variables with a p-value < 0.1 in the univariate model were included in the multivariate analysis. In recipients with LRTD, the survival analysis was performed by using the Cox regression model. A p-value <0.05 was considered statistically significant. All p-values were two-sided. All the analyses were performed using the statistical software SAS v. 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Patient characteristics

Overall, we included 402 pediatric and adult allo-HCT recipients with a median age of 46 years (range 0.3-73.8) who developed 449 U/LRTD episodes of HCoV between January 2012 and January 2019 reported from 31 EBMT transplant centers in 13 countries around the world (including Europe, Asia, Australia and South America). Clinical and transplant characteristics of the series are detailed in Table 2. The study population comprised a high-risk cohort, since 57% of the recipients were allografted from alternative donors [unrelated adult donor, cord blood units (CBU) or haplo-identical

family donors]. There were 364 allo-HCT recipients with one HCoV episode and 38 (9.5%) recipients with two or more HCoV episodes.

Epidemiological and clinical characteristics according to HCoV subtypes

Median time from allo-HCT to first HCoV episode was 222 days (range -9 days to 20 years). Seven cases (1.5%) were diagnosed before stem cell infusion whereas most of cases occurred within the first year of stem cell infusion (n= 262, 58%). There were 434 episodes with only one HCoV subtype whereas in 15 episodes (3%) we observed two or more HCoV subtypes in the same respiratory sample. In this series the most common HCoV was OC43 (n= 170, 38%) followed by 229E (n= 97, 22%), NL63 (n= 64, 14%) and KHU1 (n=54, 12%). This order was maintained when we analyzed the HCoV subtypes diagnosed through multiplex PCR assays capable of detecting and differentiating all four HCoV strains (n= 306, 68%): OC43 (n=134, 43.5%) followed by 229E (n=64, 20.8%), and by NL63 (n=54, 17.5%) and HKU1 (n=54, 17.5%). There were 79 episodes (17.5%) with non-subtypable HCoVs.

Although HCoV circulated all year long, most of the episodes (n= 375, 83%) were diagnosed during cold months (Figure 1A). We did not observe significant differences in HCoV subtype distribution between countries and continents. However, according to the year of HCoV detection, we observed a gradual increase of reported HCoV episodes over the years (from 28 HCoV episodes reported in 2012 to 97 in 2018). Alphacoronavirus genus (HCoV-229E and HCoV-NL63) predominated in 2012 and 2013 whereas Betacoronavirus genus (HCoV-OC43 and HCoV-HKU1) predominated from 2014 to 2018. The genera behaviors mainly correlate with HCoV-OC43 and HCoV 229E prevalence each year (Figure 1B). Clinical characteristics according to HCoV subtype are summarized in Table 3.

Clinical and laboratory characteristics according to the U/LRTD involvement

Clinical and laboratory differences according to URTD or LRTD involvement are summarized in Table 4. Overall, 446 out of 449 HCoV episodes (99%) involved the URTD (328 of them, 73%, limited to URTD) whereas 121 (27%) had LRTD involvement (106 possible and 15 proven). A third of episodes (n=153, 35%) had fever at the time of HCoV detection leading to hospital admission in 80 cases (18%), oxygen requirement in 56 cases (13%) and ICU admission in 13 cases (3%). As expected, the group developing HCoV LRTD had significantly higher rates of severe immunosuppression-related factors. ISI variables (lymphopenia, active GVHD, corticosteroid therapy) as well as bacterial, fungal and other CARV co-infections were significantly over-represented in the HCoV LRTD group ($p \leq 0.05$ for all comparisons). Characteristics of significant co-pathogens including CARV, bacterial and fungal agents are detailed in table 5. As expected, HCoV LRTD showed higher rates of fever, hospital admission, oxygen requirement and intensive care unit admission ($p< 0.001$ for all comparisons).

Risk factors for hospital admission, oxygen requirements, lower respiratory tract involvement and mortality

Logistic regression and Cox regression multivariate analyses of conditions associated with hospital admission, oxygen requirements, HCoV LRTD and all-cause day 90 mortality in those with LRTD involvement are shown in Table 6.

We identified five conditions associated with hospital admission; HCoV LRTD [Odds ratio (OR) 5.46], corticosteroids use (OR 2.98), fever (OR 2.3), myeloablative conditioning regimen (OR 0.46) and HCoV infection occurring after the first year of transplant (OR 2.15).

For oxygen requirement, we identified 4 independent RFs; HCoV LRTD (OR 11.86), corticosteroids therapy (OR 6.46), fever (OR 3.31) and immunoglobulin replacement within 2 months before HCoV detection (OR 3.47).

Regarding the risk of LRTD we identified four conditions associated with this event; ALC < 0.5 × 109/L (OR 2.4), active GvHD (OR 1.79), HCoV infection occurring after the first year of transplant (OR 2.1) and fever (OR 3.56).

Finally, the conditions associated with increased mortality in recipients developing HCoV LRTD were ALC < 0.1 × 10^9/L [hazard ratio (HR) 10.82], corticosteroids therapy (HR 4.68) and ICU admission (HR 8.22). Mortality of patients with LRTD increased according to the presence of these RFs. Those with none or 1 RF had a mortality rate of 11% compared to those with 2-3 RFs (57%) (p <0.0001).

We did not found differences in outcomes among pediatric (< 18 years) and adults (≥18 years). The rate of LRTD, hospital admission, oxygen support and overall mortality of pediatric recipients compared to adults were 31% vs 26% (p= 0.6), 7% vs 18% (p= 0.15), 18% vs 12% (p= 0.3) and 2% vs 7% (p= 0.3), respectively.

Mortality and cause of death

All-cause mortality rate at three months after HCoV detection was 7% (n= 31) for the entire group. Mortality of recipients with HCoV limited to URTD was 3.5% (n= 11) whereas it was 16% (n= 20) (p <0.0001) in those with LRTD. According to the coronavirus genera, 3-month overall mortality was 3% in the Alphacoronavirus group vs 7% in the Betacoronavirus group (p= 0.28) in both U/LRD, whereas it was 3% vs 10% for those with LRTD, respectively (p= 0.25).

Fifteen recipients died by day 30 after HCoV diagnosis. Ten additional recipients died at day 60 and six more recipients died by day 90 after HCoV diagnosis. In total, 11 and 20 recipients with URTD or LRTD died, respectively. The numbers of death by day 30, day 60 and day 90 were 6, 3 and 1 for URTD and 9, 6 and 5 for LRTD.

Cause of death in recipients who died by day 30 after HCoV detection were relapse (n=5), GvHD (n=4), infectious respiratory failure (n=3), and other complications (n=3: VOD, systemic infection and unknown cause). The additional 10 deaths occurring by day 60, were due to disease relapse (n=5), infectious respiratory failure (n=2), and other causes (n=3; graft failure, bleeding disorder and unknown cause). For the remaining six patients who died by day 90 after HCoV detection the causes of death were disease relapse (n=2), GvHD (n=1), infectious respiratory failure (n=2), and other causes (n=1, or GvHD and bleeding disorder). Overall, 10 patients (3%) died from infectious respiratory failure.

DISCUSSION

This study shows that HCoV episodes in the setting of allo-HCT predominate during cold months, with HCoV-OC43 (38%) being the most common HCoV subtype. The detection of seasonal HCoV was associated with considerable morbidity after allo-HCT and it was frequently accompanied by co-pathogens in the LRT leading to hospitalization, oxygen requirement and ICU admission in a not irrelevant proportion of cases. Three-month overall mortality after HCoV detection was 7% in the whole cohort and 16% in those with LRTD. We identified several RFs for different outcomes that could be of value for close clinical monitoring and/or risk-stratification for future clinical trials.

Our study confirms that seasonal HCoV predominate during cold months[29] also in allo-HCT recipients. In line with several reports in the general population[29-31] the most common seasonal HCoV in our series was OC43, belonging to betacoronavirus genus. Although we observed subtle differences over the years in HCoV subtypes' prevalence, it is noteworthy to mention that betacoronavirus genus (OC43 and HKU1) predominated from 2014 and onwards. This fact, along with the recent pandemic caused by another betacoronavirus (SARS-COV-2), suggests that differential characteristics of betacoronavirus genus may provide a biological advantage to survive

and spread among humans as compared to alphacoronavirus genus. From 2012 we observed a continuous increase in the number of reported seasonal HCoV episodes which is in line with a prior report[1]. This observation is likely related to an increase of awareness in the importance of monitoring viral infections in allo-HCT recipients and to an increased widespread use of multiplex PCR assay as a first-line test, progressively incorporating the 4 HCoV subtypes, for CARV screening in clinical practice over years[32].

Seasonal HCoV usually causes mild respiratory illnesses in the general population. Although prior studies and reviews suggested that seasonal HCoV may occasionally cause LRTDs after allo-HSCT[2,33], our study showed that 27% of allo-HSCT recipients with HCoV may developed LRTD. This is in line with prior reports where LRTD occurred in 14% to 33% of cases[1,3]. Although we report a relevant rate of HCoV LRTD, attributing LRTD to HCoV is challenging due to the frequent presence of co-pathogens. In addition, it should be note that the only way to establish the true effect of HCoV in the lungs is through the demonstration of HCoV's antigens and/or RNA in lung tissues. The observation that 18% of HCoV cases required hospital admission, 13% oxygen support and 3% ICU admission, indicates that seasonal HCoV could be related with a severe course in these highly immunosuppressed patients. Although recipients with isolated URTD had a relatively low overall mortality rate at 3-months after HCoV detection (3.5%), those who developed pulmonary complications showed a significant higher mortality rate (16%). This observation was also true when we looked at day +30 mortality which could be a more accurate time point to evaluate direct effects of HCoV (1.8% vs 7.4%, p= 0.01, respectively). These facts support recent findings from a retrospective study where the detection of HCoV in the LRT was significantly related with higher rates of respiratory support and mortality in immunocompromised hosts, similar to that of established respiratory pathogens including respiratory syncytial virus, influenza virus and human parainfluenza virus[23]. Importantly, we did not observe significant differences in terms of severity and mortality among HCoV subtypes.

The large number of cases included herein allowed us to identify several RFs which influenced outcomes. We differentiated 2 types of RFs: first, variables considered as surrogate markers of a profound immunosuppression status (corticosteroids, ALC <0.5 or <0.1 x10^9/mL, conditioning regimen intensity, immunoglobulin replacement and the presence of active GvHD): second, those related to the HCoV clinical behavior (LRTD, fever, HCoV after the first year of allo-HCT and ICU admission). Of note, we did not find any differences among children and adult allo-HCT. Most of the RFs we identified have been previously recognized as RFs of poor outcome in other CARV studies in the allo-HCT setting, such as corticosteroids use, GvHD, ALC, LRTD, ICU admission and conditioning regimen intensity[34]. However, for the first time we identified immunoglobulin replacement and HCoV infection beyond the first year of allo-HCT as RFs for severity. Immunoglobulin replacement may discriminate patients with severe post-transplant immunoparesis and may thereby identify an increased risk of severe infections. In addition, immunoglobulin G levels have previously been recognized as a RF of poor outcome in other CARV in allo-HCT recipients[35,36]. In contrast, the development of HCoV infection beyond 1 year after transplant is a somewhat unexpected finding since prior studies indicated that early CARV infections had a worse outcome[37]. However, transplant physicians use to phase out clinical monitoring in allo-HCT recipients beyond the first year after transplantation. Therefore, there might be a selection bias in that only long-term survivors with severe HCoV infection have looked for medical attention or testing.

Our multivariate analyses have depicted the relevance of each type of RFs according to the severity of the outcome analyzed. In this sense, hospital admission was mainly triggered by clinical factors (LRTD, fever and allo-HCT >12 months) whereas the need for oxygen support and pulmonary involvement were both influenced by clinical and immunosuppression conditions (fever, LRTD, HCoV after the first year of allo-HCT and corticosteroids, immunoglobulin replacement, ALC <0.5 x10^9/L, the presence of active GvHD, respectively). Lastly, mortality in recipients with LRTD was mainly influenced by immunosuppression factors (ALC <0.1 x10^9/mL and corticosteroids). These

observations emphasize the significant role of the immune system (humoral and cellular) in minimizing the severity of HCoV infections in this scenario.

We acknowledge that this study has some limitations, such as the retrospective nature of the analyses, the low proportion of BAL performed, the absence of lung tissues analyses to establish the real role of HCoV and the use of several different PCR methods differing in their analytical performance for detection and identification of HCoV subtypes. In spite of this, our study has strengths that merit consideration. We included a large multi-center cohort of HCoV cases with detailed clinical and laboratory data in the molecular testing era.

In conclusion, we provide insights of seasonal HCoV infections after allo-HCT in terms of epidemiology and clinical outcome. Our study supports that these infections can have moderate to severe direct and indirect consequences in a significant proportion of cases and that testing for seasonal HCoV should be included in the CARV screening test in the allo-HCT setting.

CONFLICT OF INTERESTS

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

NOTES

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Legend

Figure 1. Seasonality of human coronavirus (HCoV) infections in recipients of allogeneic hematopoietic stem cell transplant. A) HCoV serotype according to the month of detection, **B)** HCoV serotype according to seasons.

Table 1. Multiplex PCR platforms according to the community acquired respiratory viruses' type performance.

PCR platform¥	Transplant centers / episodes n (%)‡	HCoV non-subtypable	NL63	OC43	HKU1	229E	Influenza A/B	HMPV	HPiV 1-4	RSV A/B	EvRh	HBoV	ADV
Allplex™ Respiratory Panel 1-2-3 / Anyplex™ RV16	4 (11.4) / 52 (11.4)		D	D	N	D	D	D	D	D	D	D	D
Argene® Respiratory	6 (17.1) / 52 (11.4)	D	I	I	I	I	D	D	D	D	D	D	D
BioFire® FilmArray® Respiratory	3 (8.6) / 42 (9.2)		D	D	D	D	D	D	D	D	D	N	D
FTD® respiratory pathogens 33	1 (2.9) / 7 (1.5)		D	D	D	D	D	D	D	D	D	D	D
Multiplex RT-nested PCR assay*	1 (2.9) / 12 (2.6)		N	D	N	D	N	N	D	N	D	N	N
NxTAG® Respiratory Pathogen Panel	3 (8.6) / 15 (3.3)		D	D	D	D	D	D	D	D	D	D	D
CLART R PNEUMOVIR 1®	1 (2.9) / 1 (0.2)		N	N	N	D	D	D	D	D	D	D	D
RespiFinder®	7 (20.0) / 156 (34.3)		D	D	D	D	D	D	D	D	D	D	D
xTAG® Respiratory Viral Panel	1 (2.9) / 30 (6.6)		D	D	D	D	D	D	D	D	D	D	D
Unknown w/ subtype identification†	11 (31.4) / 58 (12.7)		D	D	D	D	D	D	D	D	D	D / N	D
Unknown w/o subtype identification‡	5 (14.3) / 24 (5.3)	D	U	U	U	U	D	D	D	D	D	D / N	D

¥ None of the multiplex PCR platform was able to detect MERS-HCoV and/or SARS-CoV.

*In-house platform: M.T. Coiras, J.C. Aguilar, M.L. Garcia, et al. (2004) Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested PCR assays. J. Med. Virol. 72(3): 484-495. doi: 10.1002/jmv.20008

[†]Unknown PCR platforms: Outsourcing diagnostic services to independent institutes rendered the PCR platform used to detect HCoV unidentifiable. Depending on the degree of detail from the virology reports, unknown PCR platforms were distinguished according to HCoV subtype identification. Unknown PCR platforms were confirmed to detect the four conventional HCoV subtypes (NL63, OC43, HKU1, 229E) but not MERS or SARS.

[‡]Total of 31 participating transplant centers: some transplant centers reported use of different PCR panels over the course of the study.

Abbreviations: HCoV non-subtypable, human coronavirus (without subtyping); HMPV, human metapneumovirus; HPiV, human parainfluenza virus; RSV, respiratory syncytial virus; EvRh, enterovirus/rhinovirus; HBoV, human bocavirus; ADV, adenovirus; D, detectable by the PCR platform; N, not detectable by the PCR platform; I, detectable but indistinguishable from other HCoV subtypes by the PCR platform; U, unknown whether PCR platform is able to distinguish between HCoV subtypes.

Table 2. Patient and transplant characteristics.

Characteristics	(n = 402)
Age at allo-HCT (years), median (range)	46.6 (0.3 - 73.8)
• < 18 years, n (%)	40 (10)
• ≥18 year, n (%)	362 (90)
Male, n (%)	245 (60.9)
Baseline disease, n (%)	
• AL/MDS/MPD	241 (60)
• Chronic leukaemia	32 (8)
• Lymphoid disorders	86 (21.4)
• Others	39 (9.7)
• Missing data	4 (1)
Disease status at transplant, n (%)	
• CR	219 (54.5)
• PR	43 (10.7)
• Active disease at transplant	64 (15.9)
• Other	76 (18.9)
Prior Autologous-HSCT, n (%)	21 (5.2)
Period of transplant, n (%)	
• 2017-2018	105 (26.1)
• 2015- 2016	122 (30.3)
• 2013-2014	88 (21.9)
• Before 2013	87 (21.6)
Conditioning regimen, n (%)	
• No conditioning	3 (0.7)
• RIC	198 (49.3)
• MAC	192 (47.8)
• Missing data	9 (2.2)
Type of donor, n (%)	
• HLA-identical sibling donor	157 (39.1)
• Unrelated donor	169 (42)
• Unrelated umbilical cord blood	17 (4.2)
• Haploidentical family donor	45 (11.2)
• Other	11 (2.7)
• Missing	3 (0.7)
PB stem cell source, n (%)	332 (82.6)
HLA fully-matched, n (%)	242/307 (78.8)

ATG as a part of conditioning regimen, n (%)	147/398 (36.9)
GvHD prophylaxis, n (%)	
• Sir-Tac	20 (5)
• Tacrolimus or CsA+MTX	204 (50.7)
• Post-Cy	51 (12.7)
• CsA + PDN and Others	116 (28.9)
• No prophylaxis regimen	4 (1.0)
• Missing data	7 (1.7)
Number HCoV episodes, n (%)	
• 1	364 (90.5)
• >1	38 (9.5)
Median time from allo-HCT to 1st episode of HCoV, days (range)	222 (-12 – 20.7 years)
Time from allo-HCT to 1st episode of HCoV (category)	
• until day +180	173 (43)
• 181 – 1 year	89 (22.1)
• 1 – 2 year	76 (18.9)
• after 2 years	64 (15.9)
Median F/U after last episode of HCoV, years (95% CI)	2.32 (2.09 – 2.52)

Abbreviations. allo-HSCT, allogeneic hematopoietic stem cell transplantation; AL, acute leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disease; CR, complete remission; PR, partial remission; ASCT, autologous stem cell transplantation; RIC, reduced intensity conditioning; MAC, myeloablative conditioning; PB, peripheral blood; HLA, human leucocyte antigen system; ATG, anti-thymocyte globuline; GvHD, graft versus host disease; Sir, sirolimus; Tac, tacrolimus; CsA, cyclosporine A; MTX, methotrexate; Post-Cy, post-transplant cyclophosphamide; PDN, prednisone; SCT, stem cell transplantation; HCoV human coronavirus; F/U, follow-up; CI, confidence interval.

Table 3. Type of HCoV and mortality according to HCoV type, timing of infection and upper or lower respiratory tract disease.

	All HCoV cases	Non-subtypable	OC43*	NL63*	HKU1*	229E*	P value*
Number of Episodes, n (%)¥	449	79	170	64	54	97	
URTD, n (%)	446/449 (99.3)	77 (97.5)	170 (100)	63 (98.4)	54 (100)	97 (100)	0.2
LRTD, n (%)	121 (26.9)	29 (36.7)	41 (24.1)	19 (29.7)	16 (29.6)	20 (20.6)	0.1
• **possible**	106 (23.6)	23 (29.1)	39 (22.9)	16 (25)	14 (25.9)	18 (18.6)	0.5
• **proven**	15 (3.3)	6 (7.6)	2 (1.2)	3 (4.7)	2 (3.7)	2 (2.1)	
Fever, n (%)	153/442 (34.6)	31/79 (39.2)	58/168 (34.5)	24/62 (38.7)	19/52 (36.5)	25/95 (26.3)	0.1
CRP in mg/dL, median (range)	12 (0 - 560)	13.7 (0.1 - 560)	12.0 (0 - 346.6)	17.5 (0 - 296)	13.9 (0 - 347)	8 (0 - 358)	0.4
ISI (Low/Mod/High), n (%)	152 (37.1)/ 221 (53.9)/ 37 (9)	26 (35.1)/ 40 (54.1) 8 (10.8)	65 (42.8)/ 75 (49.3)/ 12 (7.9)	17 (30.4)/ 34 (60.7)/ 5 (8.9)	19 (35.8)/ 27 (50.9)/ 7 (13.2)	30 (33.7)/ 53 (59.6)/ 6 (6.7)	0.6
Empirical antibiotic, n (%)	276/443 (62.3)	48/79 (60.8)	106/167 (63.5)	40/63 (63.5)	28/53 (52.8)	60/95 (63.2)	0.9
Immunoglobulin support, n(%)	92/435 (21.1)	16/73 (21.9)	29/169 (17.2)	18/63 (28.6)	16/54 (29.6)	22/91 (24.2)	0.2
Hospitalization, n (%)	80/442 (18.1)	17/79 (21.5)	26/167 (15.6)	13/64 (20.3)	9/53 (17.0)	18/94 (19.1)	1
Oxygen support, n (%)	56/441 (12.7)	18/79 (22.8)	19/168 (11.3)	5/59 (8.5)	9/54 (16.7)	9/96 (9.4)	0.06
ICU, n (%)	13/448 (2.9)	6/79 (7.6)	3/170 (1.8)	1/64 (1.6)	1/54 (1.9)	2/96 (2.1)	0.1
U/L RTD 30-day OM, n	15/449	6/79 (7.6)	3/170	2/64	2/54	2/97	0.4

(%)	(3.3)		(1.8)	(3.1)	(3.7)	(2.1)	
LRTD 30-day OM, n (%)	9/121 (7.4)	6/29 (20.7)	2/41 (4.9)	0/19 (0.0)	0/16 (0.0)	1/20 (5)	0.06
U/L RTD 90-day OM, n (%)	31/449 (6.9)	13/79 (16.5)	10/170 (5.9)	3/64 (4.7)	4/54 (7.4)	2/97 (2.1)	0.02
LRTD 90-day OM, n (%)	20/121 (16.5)	13/29 (44.8)	4/41 (9.8)	1/19 (5.3)	1/16 (6.3)	1/20 (5)	0.015

Abbreviations: URTD, upper respiratory tract disease; LRTD, lower respiratory tract disease; CRP, C-reactive protein; ISI, immunodeficiency score index, CARV, community acquired respiratory virus; ICU, intensive care unit; OM, overall mortality.

¥ The sum of the episodes does not match the overall number of episodes (n = 449) since multiple CARVs were detected in the same respiratory sample in 15 (3%) CARV episodes. 90-days all-cause mortality after CARV co-viral infection was 10% (17 out of 165). Sixty-one co-virus infectious episodes occurred within the first 6 months after stem cell infusion and mortality was 15% (9 out of 61). Sixteen (10%) and 14 (24%) out of 164 and 59 patients with URTD and LRTD CARV co-viral infection died, respectively. Finally, 7 (37%) out of 19 patients with LRTD CARV co-viral infection died within the first 6 months after stem cell infusion.

Table 4. Clinical and biological characteristics of HCoV infection episodes in allo-HCT recipients according to the upper or lower respiratory tract involvement.

	HCoV URTD (n = 328)	*HCoV LRTD (n = 121)*	*P value*
Transplant characteristics			
Age			
• <18 years	29 (8.8)	13 (10.7)	0.6
• ≥18 years	299 (91.2)	108 (89.3)	
ATG as part of conditioning, n (%)	123/326 (37.7)	46/119 (38.7)	0.8
GvHD prophylaxis, n (%)			
• No prophylaxis regimen	2 (0.6)	2 (1.7)	
• Sir-Tac	16 (4.9)	5 (4.1)	
• Tacrolimus or CsA + MTX	169 (51.5)	59 (48.8)	
• Post-Cy	45 (13.7)	9 (7.4)	
• CsA + PDN and Others	92 (28.0)	43 (35.5)	
• Missing	4 (1.2)	3 (2.5)	
HLA mismatch, n (%)	53/255 (20.8)	24/93 (25.8)	0.4
Type of donor, n (%)			
• HLA-identical sibling donor	127 (38.7)	45 (37.2)	
• Unrelated donor	136 (41.5)	57 (47.1)	
• Unrelated umbilical cord blood	15 (4.6)	3 (2.5)	
• Haplo-identical family donor	39 (11.9)	11 (9.1)	
• Other	9 (2.7)	4 (3.3)	
• Missing	2 (0.6)	1 (0.8)	
Immunodeficiency Scoring Index, n (%) ‡			
ANC < 0.5 × 10^9/L	26/298 (8.7)	12/116 (10.3)	0.4
• **Missing data**	30	5	
ALC< 0.2 × 10^9/L	28 (9.6)	21 (18.1)	0.01
• **Missing data**	35	5	
Age at HCoV (years), median (range)	48.8 (0.5 - 74.3)	51.4 (0.4 - 72.8)	0.9
Age ≥ 40 y	210 (64.0)	81 (66.9)	0.7

Myeloablative conditioning regimen	152/320 (47.5)	55/117 (47.0)	1
• **Missing data**	7	2	
• **No conditioning**	1	2	
GvHD (acute or chronic)	113/328 (34.5)	59/120 (49.2)	0.01
• **Missing data**	0	1	
Corticosteroids	124/328 (37.8)	68/119 (57.1)	0.001
• **Missing data**	0	2	
Recent or pre-engraftment allo-HCT	14/328 (4.3)	8/121 (6.6)	0.3
ISI, n (%)			
• Low risk (0-2)	123 (37.5)	29 (24.0)	0.003
• Moderate risk (3-6)	150 (45.7)	71 (58.7)	
• High risk (7-12)	21 (6.4)	16 (13.2)	
• Missing data	34 (10.4)	5 (4.1)	
Other characteristics ‡			
On IS, n (%)	222/328 (67.7)	80/120 (66.7)	1
ALC< 0.1 × 10^9/L , n (%)	20/293 (6.8)	17/116 (14.7)	0.01
ALC < 0.5 × 10^9/L, n (%)	72/293 (24.6)	46/116 (39.7)	0.002
RVI characteristics and clinical consequences			
CARV LRTD, n (%)			
• **Possible**	-	106/121 (87.6)	
• **Proven**	-	15/121 (12.4)	
HCoV subtype, n (%)			
• **OC43**	129/266 (48.5)	41/93 (44.1)	
• **NL63**	45/265 (17.0)	19/93 (20.4)	
• **KHU1**	38/211 (18.0)	16/85 (18.8)	
• **229E**	77/279 (27.6)	20/95 (21.1)	
• **Non-subtypable**	50/328 (15.2)	29/121 (24.0)	
Hospital admission, n (%)	28/325 (8.6)	52/117 (44.4)	<0.0001

ICU admission, n (%)	1/328 (0.3)	12/120 (10.0)	0.001
Fever during CARV, n (%)	86/323 (26.6)	67/119 (56.3)	<0.0001
Prior BOS, n (%)	26/327 (8.0)	13/120 (10.8)	0.3
Ig G levels (mg/dl) median (range)	669 (3.4-16300)	540 (4.5-5470)	0.2
Antibiotic use, n (%)	172/325 (52.9)	104/118 (88.1)	<0.0001
Immunoglobuline support, n (%)	57/320 (17.8)	35/115 (30.4)	0.01
Median time of dx after SC infusion, days (range)	243.5 (-12 – 20.7 years)	291.0 (-12 – 17.3)	0.9
Day + 30 overall mortality rate, n (%)	6/328 (1.8)	9/121 (7.4)	0.01
Day + 90 overall mortality rate, n (%)	11/328 (3.4)	20/121 (16.5)	<0.0001
Median time to death, years (95% CI)	2.38 (2.14-2.66)	2.85 (2.11-3.20)	

Abbreviations. CARV, community-acquired respiratory virus; IFD, invasive pulmonary infectious fungal disease; ATG, anti-thymocyte globuline; GvHD, graft-versus-host disease; Sir, sirolimus; Tac, tacrolimus; CsA, cyclosporine A; MTX, methotrexate; Post-Cy, post-transplant cyclophosphamide; PDN, prednisone; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; IS, immunosuppressants; URTD, upper respiratory tract disease; LRTD, lower respiratory tract disease; ICU, intensive care unit; BOS, bronchiolitis obliterans syndrome; Ig G, immunoglobuline G; SC, stem cells.

‡ All variables were captured at the time of CARV diagnosis.

Table 5. Co-infections characteristics according to to the upper or lower respiratory tract involvement.

Co-infections	*HCoV URTD (n = 328)*	*HCoV LRTD (n = 121)*	*P value*
CARV co-infections, n (%)	102 (34)	50 (41)	0.04
• HCoV	11 (3)	4 (3)	0.9
• EvRh	14 (4)	8 (7)	0.3
• RSV	36 (11)	13 (11)	0.9
• HMPV	6 (2)	5 (4)	0.17
• HPiV	13 (4)	10 (8)	0.09
• ADV	4 (1)	4 (3)	0.3
• HiV	26 (8)	7 (6)	0.5
• HBoV	7 (2)	1 (1)	0.7
Bacterial co-infection, n (%)	18 (5)	29 (24)	<0.0001
• *Pseudomonas spp*	4 (1)	5 (4)	0.2
• *Streptococcus pneumoniae*	1 (0.3)	2 (1.5)	0.3
• *Moraxella catarrhalis*	4 (1)	0	0.2
• *Haemophylius influenza*	2 (0.5)	3 (2)	0.3
• *E. coli*	1 (0.3)	3 (2)	0.2
• *Klebsiella pneumoniae*	2 (0.5)	2 (1.5)	0.3
• *Mycobacterium tuberculosis*	0	2 (1.5)	0.2
• *Stenotrophomonas maltophilia*	1 (0.3)	1 (1)	0.5
• *Mycoplasma pneumoniae*	1 (0.3)	1 (1)	0.5
• *Legionella pneumophila*	0	2 (1.5)	0.15
• *Staphylococcus aureus*	1 (0.3)	2 (1.5)	0.5
• *Enterococcus spp*	0	3 (2)	0.2
• Others	1 (0.3)	3 (2)	0.2

Fungal co-infection, n (%)	0	18 (15)	<0.0001
• Probable invasive pulmonary aspergillosis		11 (9)	
• Pneumocitis jirovecci		6 (5)	
• Mucormycosis		1 (1)	

Abbreviations, URTD, upper respiratory tract disease; LRTD, lower respiratory tract disease; EvRh, Enterovirus/rhinovirus; ADV, adenovirus; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; HPiV, human parainfluenza virus; HiV, human influenza virus; HBoV, human bocavirus; CI, confidence interval.

Table 6. Multivariate analyses for different outcomes.

Outcome	Variables	OR/HR (95% C.I)	P value
Hospital admission (n= 442)*		**Log. Regr.**	
	HCoV LRTD	5.46 (2.85-10.49)	<0.0001
	Corticosteroids	2.98 (1.59-5.59)	0.001
	Fever at the time of HCoV	2.30 (1.20-4.39)	0.01
	Myeloablative*	0.46 (0.24-0.88)	0.02
	Allo-HCT≥ 12 months	2.15 (1.15-4.01)	0.02
Oxygen support (n= 441)*		**Log. Regr.**	
	HCoV LRTD	11.86 (5.73-24.52)	<0.0001
	Corticosteroids	6.46 (3.22-12.98)	<0.0001
	Fever at the time of HCoV	3.31 (1.57-6.98)	0.002
	Immunoglobulin replacement	3.47 (2.06-5.84)	<0.0001
LRTD (n= 449)*		**Log. Regr.**	
	ALC < 0.5 × 109/L, n (%)	2.40 (1.32-4.35)	0.004
	Active GvHD at the time RVI*	1.79 (1.05-3.06)	0.03
	Allo-HCT≥ 12 months	2.13 (1.20-3.79)	0.01
	Fever at the time of HCoV	3.56 (2.07-6.12)	<0.0001
LRTD overall mortality (n= 121)*		**Cox Regr**	
	ALC < 0.1 × 109/L, n (%)	10.82 (3.78-31.01)	<0.0001
	Corticosteroids	4.68 (1.62-13.54)	0.0045
	ICU admission	8.22 (2.55-26.50)	0.0004

Abbreviations: C.I., confidence interval; OR, Odds Ratio; HR, hazard ratio; Log. Regr, Logistic regression model; Cox Regr, cox regression model; HCoV, human coronavirus; Allo-HSCT; allogeneic stem cell transplantation; ALC, absolute lymphocyte count; GvHD, graft-versus-host disease; ICU, intensive care unit; LRTD.

*Variables included in univariate analyses include: type of donor, recipient age, donor/receptor human leucocyte antigen (HLA) mismatch, conditioning regimen-based antithymocytic globulin, GvHD prophylaxis, absolute neutrophile count (ANC) < 0.5 × 109/L, ALC <0.2 × 10^9/L, ALC <0.1 × 10^9/L, immunossupressant drugs at the time of HCoV detection, periengraftment period, allo-HCT <100 days, allo-HCT <180 days, allo-HCT <2 years, HCoV subtypes (OC43, 229E, NL63, HKU1 and non-subtypable), corticosteroids therapy >30 mg/day at the time of HCoV detection, oxygen support, mono vs co-infections (respiratory virus, bacteria and fungus), seasons (spring, summer, autumn, winter), prior bronchiolitis obliterans syndrome, immunoglobulin G levels <400 mg/dl and immunodeficiency score index (ISI).

Figure 1A

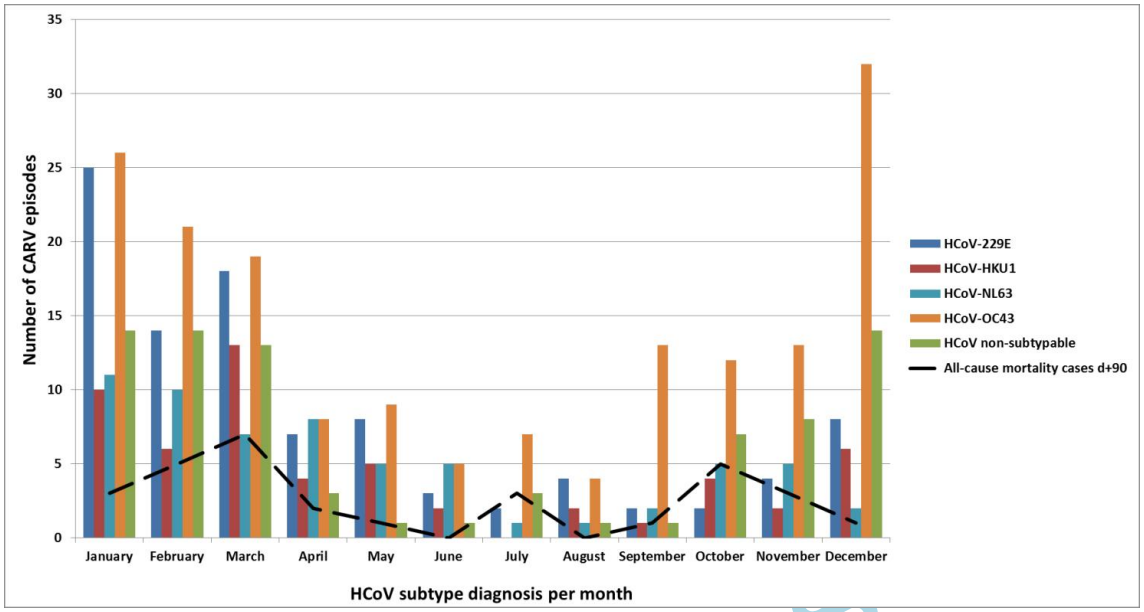

Figure 1B