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Community-acquired respiratory virus lower respiratory tract disease in allogeneic stem cell transplantation recipient: Risk factors and mortality from pulmonary virus-bacterial mixed infections

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Abstract

Risk factors (RFs) and mortality data of community-acquired respiratory virus (CARVs) lower respiratory tract disease (LRTD) with concurrent pulmonary co-infections in the setting of allogeneic hematopoietic stem cell transplantation (allo-HSCT) is scarce. From January 2011 to December 2017, we retrospectively compared the outcome of allo-HSCT recipients diagnosed of CARVs LRTD mono-infection (n = 52, group 1), to those with viral, bacterial, or fungal pulmonary CARVs LRTD co-infections (n = 15, group 2; n = 20, group 3, and n = 11, group 4, respectively), and with those having bacterial pneumonia mono-infection (n = 19, group 5). Overall survival (OS) at day 60 after bronchoalveolar lavage (BAL) was significantly higher in group 1, 2, and 4 compared to group 3 (77%, 67%, and 73% vs 35%, respectively, P = .012). Recipients of group 5 showed a trend to better OS compared to those of group 3 (62% vs 35%, P = .1). Multivariate analyses showed bacterial co-infection as a RF for mortality (hazard ratio[HR] 2.65, 95% C.I. 1.2-6.9, P = .017). We identified other 3 RFs for mortality: lymphocyte count <0.5 × 10^9/L (HR 2.6, 95% 1.1-6.2, P = .026), the occurrence of and CMV DNAemia requiring antiviral therapy (CMV-DNAemia-RAT) at the time of BAL (HR 2.32, 95% C.I. 1.1-4.9, P = .03), and the need of oxygen support (HR 8.3, 95% C.I. 2.9-35.3, P = .004). CARV LRTD co-infections are frequent and may have a negative effect in the outcome, in particular in the context of bacterial co-infections.

KEYWORDS
allogeneic hematopoietic stem cell transplantation, CMV DNAemia, community acquired respiratory virus, immunodeficiency score index, respiratory virus co-infections, virus-bacterial mixed infections

1 | INTRODUCTION

Community-acquired respiratory viruses (CARVs) are a common cause of upper and/or lower respiratory tract infection (URTI and LRTD) after allogeneic stem cell transplantation (allo-HSCT) resulting in high morbidity and mortality, especially when the lower respiratory tract is involved.[1-6] Recently, the introduction in daily clinical practice of more sophisticated diagnostic tools based on reverse transcription polymerase chain reaction (RT-PCR), such as those testing for CARVs and other pathogens, has permitted to expand microbiological findings in bronchoalveolar lavage (BAL) of allo-HSCT recipients harboring lower respiratory tract (LRT) complications. This fact has likely increased the ability to diagnose infectious pneumonia and probably has led to the identification of a high number of cases with co-pathogens as compared to conventional microbiological studies (ie, viral culture or antigen testing only available for some CARVs). Currently, there is a lack of studies analyzing in detail the incidence, characteristics, and consequences of co-infective pathogens in the LRT in the setting of CARVs LRTD after allo-HSCT. Some studies have reported that the presence of co-infective agents at the time of CARVs LRTD may increase overall mortality.[5,7-10] However, it is still unknown to what extent such increase in mortality could only be attributed to the co-infection by itself or by the aggressiveness of the concurrent co-infective pathogens. To elucidate this issue, clinical outcome comparisons between CARVs LRTD co-infection, CARVs LRTD mono-infection and other mono-infections (ie, bacterial pneumonia mono-infection) are suitable to better establish the putative effect of each microbiological co-pathogen in the outcome.

To this purpose, this study analyzes the clinical implications of co-infections (viral, fungal, and bacterial CARVs LRTD co-infections) detected in BAL samples in a cohort of allo-HSCT recipients with a first-proven CARVs LRTD episode and compared the outcome to mono-infections (CARV and bacterial LRT monoinfections). Additionally, we analyzed risk factors (RFs) and the value of the immunodeficiency score index (ISI)[11] to predict morbidity and mortality in allo-HSCT recipients with CARVs LRTD in the RT-PCR era.

2 | PATIENTS AND METHODS

2.1 | Study population

This observational retrospective study included all consecutive allo-HSCT recipients (n = 153) who developed infectious lower respiratory tract complications (episodes=174) and who underwent BAL sampling for microbiological studies between January 2011 and December 2017 at two Spanish transplant centers. The cases selection is detailed in Figure 1 and focused on those recipients free of base-line disease at time of BAL and whose BAL samples were screened for CARVs by RT-PCR (n = 133). Overall, 117 allo-HSCT recipients were included. Forty-seven form Hospital Clínico Universitario—HCUV—and 70 from Hospital Universitario y Politécnico La Fe—HLF—. Patients were divided into 5 groups: group 1, allo-HSCT recipients with a first proven CARV LRTD mono-infection diagnosed by RT-PCR in BAL specimens (n = 52, 44%) without any other microbiological agent detected in the BAL; group 2, allo-HSCT recipients with a first proven CARV LRTD with 2 or more detected viruses (n = 15, 13%); group 3,
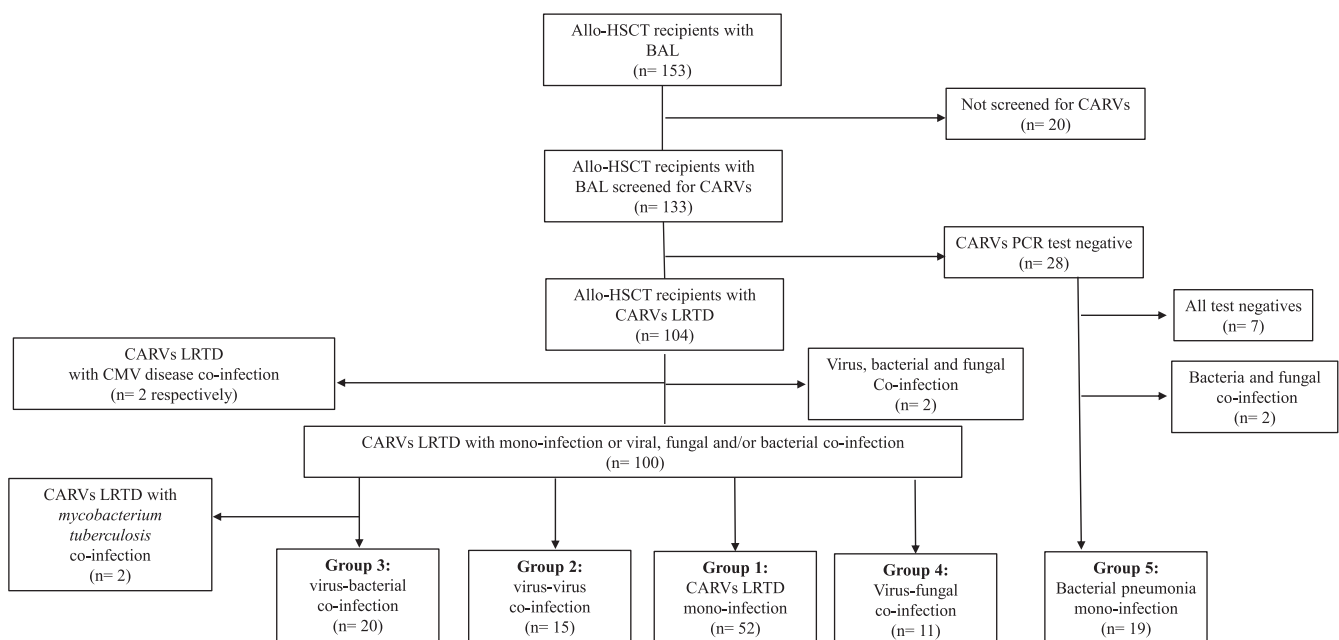

FIGURE 1 Cases selection algorithm. Horinzontal arrows represent primarily excluded cases. Vertical arrows represent the final selection. Allo-HSCT means allogeneic stem cells transplantation; BAL, bronchoalveolar lavage; CARVs, community acquire respiratory virus; LRTD, lower respiratory tract disease

TABLE 1 Patient characteristics and transplant outcomes

Characteristics	LRTD RV (n = 98)	Bacterial pneumonia (n = 19)	P
Age (y), median (range)	0	48 (35-70)	.7
Male sex, n (%)	55 (56)	13 (68)	.4
Baseline disease, n (%)			
AL/MDS/MPN/AA	43 (44)/5 (5)/6 (6)/1 (1)	7 (37)/1 (5)/2 (10)/1 (5)	.8
NHL/HL/CLL/MM	20 (20)/5 (5)/15 (15)/3 (4)	5 (26)/1 (5)/2 (10)/0	
Disease status at transplant, n (%)			
CR/Untreated	61 (62)/6 (6)	11 (58)/4 (20)	.8
PR	19 (19)	2 (10)	
Refractory/active disease	11 (11)	3 (16)	
Prior ASCT, n (%)	24 (24)	5 (26)	.2
Conditioning regimen, n (%)			
RIC	55 (56)	11 (58)	.9
Myeloablative	43 (44)	8 (42)	
Type of donor, n (%)			
HLA-identical sibling donor	26 (26)	9 (47)	.2
Unrelated donor	24 (24)	4 (21)	
Umbilical cord blood	28 (28)	1 (5)	
Haploidentical family donor	20 (20)	5 (26)	
HLA fully matched, n (%)	38 (38)	10 (53)	.9
ATG as a part of the conditioning, n (%)	40 (40)	4 (21)	.7
Recipient and/or donor CMV seropositive, n (%)	86 (88)	17 (90)	.9
GvHD prophylaxis, n (%)			
Sir-Tac	17 (17)	5 (26)	.7
CsA + MTX	23 (23)	8 (42)	
Post-CyPh	25 (30)	5 (26)	
CsA + PDN/Others	27 (27)/6 (6)	1 (5)	
Post-transplant outcome			
GvHD at the time of BAL, n (%)	59 (59)	10 (53)	.6
Acute grade II-IV	29 (29)	5 (26)	
Chronic	30 (30)	6 (32)	
Overall mortality by day 60 after BAL, n (%)	33 (33%)	7 (37)	.5
Median time from allo-HSCT to LRTD, days (range)	145 (0-1568)	174 (5-6700)	.4

(Continues)

TABLE 1 (Continued)

Characteristics	LRTD RV (n = 98)	Bacterial pneumonia (n = 19)	P
LRTD type, n (%)			
CARV mono-infection	52 (52)		
Viral co-infection	15 (15)		
Bacterial co-infection	20 (20)		
Fungal co-infection	11 (11)		
Bacterial mono-infection		23 (100)	
Admission ICU, n (%)	26 (26)	10 (53)	.1
Median F-Up after BAL for survivors, days (range)	267 (62-2230)	207 (60-1387)	.6

AA, aplastic anemia; AL, acute leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; ASCT, autologous stem cell transplantation; ATG, anti thymocytic globulin; BAL, bronchoalveolar lavage; CARV, community acquired respiratory virus; CLL, chronic lymphocytic leukemia; CR, complete remission; CsA, cyclosporine A; F-up, follow-up; GvHD, graft versus host disease; HLA, human leukocyte antigen; HL, Hodgkin lymphoma; ICU, intensive care unit; LRTD RV, lower respiratory tract disease by respiratory viruses; MDS, myelodysplastic syndrome; MM, multiple myeloma; MPN, myeloproliferative neoplasm; MTX, methotrexate; NHL, non-Hodgkin's lymphoma; PDN, prednisone; Post-CyPh, post-transplant cyclophosphamide; PR, partial remission; RIC, reduced intensity conditioning; Sir, sirolimus; Tac, tacrolimus.

allo-HSCT recipients with a first-proven CARV LRTD with viral-bacterial co-infection (n = 20, 17%); group 4, allo-HSCT recipients with a first proven CARV LRTD with proven/probable pulmonary invasive aspergillosis (IA) co-infection (n = 2 and 9, respectively, 9%) and group 5, allo-HSCT recipients with a first episode of bacterial pneumonia mono-infection (n = 19, 16%). Excluded cases are summarized in Figure 1.

2.2 | Clinical and biological factors

Variables for immunodeficiency scoring index (ISI)[11] and Basel Immunodeficiency grading computation[12,13], CMV DNAemia requiring antiviral therapy (CMV DNAemia-RAT) during CARV LRTD, radiological pulmonary patterns, oxygen support requirement to maintain oxygen saturation >92%, immunosuppressant drugs, corticosteroids doses, the presence of signs or symptoms of acute or chronic graft versus host disease (GvHD) and requirement of intensive care unit (ICU) admission were captured from patients' chart the day of hospital admission and/or before the BAL was performed. Since immunoglobulin G levels were not available at the time of BAL in most of our patients we adapted the Basel Immunodeficiency grading score as follows; moderate, severe, and very severe immunodeficiency status according to the presence of none, one or ≥2 of the following

TABLE 2 Characteristics of CARVs lower respiratory tract disease according to the type of co-infection

	Mono-infection (n = 52)	Viral co-infection (n = 15)
Immunodeficiency scoring index, n (%)[a]		
ANC < 0.5×10^9/L (3pts)	13 (25)	1 (7)
ALC< 0.2×10^9/L (3pts)	17 (33)	4 (27)
Age ≥ 40 y (2pts)	37 (71)	11 (73)
Myeloablative conditioning regimen (1pt)	24 (46)	4 (27)
GvHD (acute or chronic; 1pt)	25 (48)	10 (67)
Corticosteroids (1pt)	21 (40)	8 (53)
Recent or pre-engraftment allo-HSCT (1pt)	17 (33)	2 (13)
ISI, n (%)		
Low risk (0-2)	14 (27)	7 (47)
Moderate risk (3-6)	28 (54)	7 (47)
High risk (7-12)	10 (19)	1 (6)
Basel Immunodeficiency grading score [c], n (%)[a]		
Allo-HSCT ≤ 6 mo	32 (62)	8 (53)
T-cell or B-cell depletion ≤3 mo	8 (15)	4 (27)
GVHD grade ≥2 or extensive chronic	22 (42)	8 (53)
ANC < 0.5×10^9/L	13 (25)	1 (7)
ALC< 0.1×10^9/L	13 (25)	3 (20)
Adapted Basel IG, n (%)		
Moderate	12 (23)	4 (27)
Severe	12 (23)	6 (40)
Very severe	28 (54)	5 (33)
Other characteristics[a]		
CMV DNAemia-RAT	16 (31)	3 (20)
CMV serostatus D neg/R pos	19 (36)	3 (20)
CMV DNA in BAL		
Positive	12 (23)	3 (30)
Negative	30 (58)	10 (66)
Not performed	10 (19)	2 (13)
CMV DNA load in BAL >500 UI/mL	7	2
On IS, n (%)	47 (90)	14 (93)
ALC < 0.5×10^9/L, n (%)	31 (60)	6 (40)
Steroids 1 mg/kg/d, n (%)	13 (25)	3 (20)
ICU admission, n (%)	10 (19)	5 (33)
Oxygen support[b], n (%)	31 (60)	10 (67)
Type of donor, n (%)		
HLA-identical sibling donor	17 (33)	3 (20)
Unrelated donor	12 (23)	4 (27)
Umbilical cord blood	12 (23)	3 (20)
Haploidentical family donor	11 (21)	5 (33)
Median days from allo-HSCT to LRTI, median (range)	101 (0-1568)	181 (18-1043)
Mortality rate, n (%)	12 (23)	5 (33)

Fungal co-infection (n = 11)	Bacterial co-infection (n = 20)	Bacterial mono-infection (n = 19)	P value
4 (36)	4 (20)	5 (26)	.7
4 (36)	8 (40)	6 (32)	.8
9 (82)	18 (90)	14 (73)	.3
7 (64)	8 (40)	8 (42)	.3
6 (55)	14 (70)	11 (58)	.2
8 (72)	16 (80)	11 (58)	.1
2 (18)	2 (10)	7 (37)	.06
2 (18)	5 (25)	5 (26)	.8
6 (55)	11 (55)	9 (47)	
3 (27)	4 (20)	5 (26)	
7 (63)	11 (55)	9 (47)	.8
4 (36)	3 (15)	3 (16)	.4
4 (36)	14 (70)	8 (42)	.2
4 (36)	4 (20)	5 (26)	.7
3 (27)	7 (35)	5 (26)	.8
2 (18)	2 (10)	4 (21)	.8
3 (27)	6 (30)	5 (26)	
6 (55)	12 (60)	10 (53)	
5 (45)	13 (65)	7 (37)	.04
4 (36)	9 (45)	5 (26)	.2
1 (9)	6 (30)	6 (32)	.3
8 (72)	11 (55)	13 (68)	
2 (18)	3 (15)	0	
1	4	3	.7
9 (82)	19 (95)	15 (79)	.4
5 (45)	15 (75)	11 (58)	.6
5 (45)	10 (50)	7 (37)	.5
3 (27)	8 (40)	10 (53)	.04
10 (91)	16 (80)	12 (63)	.4
3 (27)	3 (15)	9 (47)	.6
2 (18)	6 (30)	4 (21)	
5 (45)	8 (40)	1 (5)	
1 (9)	3 (15)	5 (26)	
136 (8-865)	166 (3-1113)	217 (5-6700)	.6
3 (27)	13 (65)	7 (37)	.026

(Continues)

TABLE 2 (Continued)

	Mono-infection (n = 52)	Viral co-infection (n = 15)
Median follow-Up after BAL in days, median (range)	150 (3-2233)	453 (3-1597)
Microbiological findings in the BAL		
Bacterial agents		
Mixed flora, n (%)		
Pseudomonas aeruginosa/putida		
Klebsiella pneumoniae		
Streptococcus pneumoniae		
Stenotrophomonas maltophilia		
Proteus mirabilis		
Raoultella ornithinolytica		
Staphylococcus aureus		
Rothia mucilaginosa		
Citrobacter freundii		
Escherichia coli		
Haemophilus influenzae		
Nocardia asteroides		
Legionella feeli		
Serratia marcescens		
Enterobacter cloacae		
Respiratory virus		
More than one RV, n (%)	0	15 (100)
EvRh	18	8
RSV	9	10
HPiV	11	4
HMPV	5	2
CoV	1	3
Infl	7	2
ADV	1	4

ADV, adenovirus; ALC, absolute lymphocyte count; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; ANC, absolute neutrophil count; BAL, bronchoalveolar lavage; Basel IG, Basel Immunodeficiency grading; CoV, human coronavirus; CMV DNAemia-RAT, cytomegalovirus DNAemia requiring antiviral therapy; D, donor; EvRh, enterovirus/rhinovirus; F-up, follow-up RV, respiratory virus; GvHD, graft versus host disease; HMPV, human metapneumovirus; HPiV, human parainfluenza virus; ICU, intensive care unit; Infl, human influenza virus; IS, immunosuppressants; ISI, Immunodeficiency Scoring Index; LRTI, lower respiratory tract infection; neg, negative; pos, positive; R, recipient; RSV, respiratory syncytial virus.
[a]All variables were captured at the time of BAL.
[b]Oxygen support was considered when in air room oxygen saturation was below 92%.
[c]The original Basel Immunodeficiency grading score also included immunoglobulin G levels (IgG < 4 g/L). We adapted the Basel IG since immunoglobulin G levels were not available at the time of BAL in most of our patients.

variables, respectively: HSCT ≤ 6 months, T-cell or B-cell depletion ≤3 months, graft versus-host disease (GVHD) grade 2 or extensive disease, neutropenia ≤ 0.5×10^9/L, and lymphopenia ≤ 0.1×10^9/L.

All microbiological findings from BAL samples and radiology patterns were also collected and critically reviewed. The local ethics committee approved the study and when available subjects gave their written informed consent before participating in the study.

2.3 | Technical and diagnostic considerations

2.3.1 | Respiratory virus

Bronchoscopy was performed using standard procedures according to international consensus guidelines.[14] CARVs testing in BAL samples was performed with 2 RT-PCR multiplex platforms. At the HCUV, samples were tested by RT-PCR using the Luminex xTAG RVP Fast v1 assay (Luminex Molecular Diagnostics, Toronto, ON,

	Fungal co-infection (n = 11)	Bacterial co-infection (n = 20)	Bacterial mono-infection (n = 19)	P value
	133 (25-596)	44 (3-1835)	84 (1-1387)	.01
		4 (20)	3 (16)	
		10/0	8/1	
		4	2	
		0	1	
		8	2	
		1	0	
		1	0	
		0	1	
		1	0	
		1	0	
		1	2	
		0	1	
		0	1	
		0	1	
		0	1	
		1	1	
	0	5 (25)		
	1	5		
	3	5		
	3	3		
	2	4		
	0	2		
	1	6		
	1	0		

Canada), whereas at HLF the CLART® PneumoVir DNA array assay (Genomica, Coslada, Spain) was performed and interpreted following the manufacturer's recommendations. Technical methodologies have been previously reported in detail elsewhere.[15] The Luminex xTAG RVP Fast v1 assay can detect adenoviruses (ADVs); human bocavirus (HBoV); human coronavirus (CoV) types 229E, HKU1, NL63, and OC43; influenza A virus (InfA) A/H1N1, InfA/H3N2, and other InfA viruses (non-subtypificable); influenza B virus (InfB); human metapneumovirus (HMPV) A and B; human parainfluenza virus (HPiV) 1, 2, 3, and 4A-4B; respiratory syncytial virus (RSV) A-B; and enterovirus/rhinovirus (EvRh). The CLART® PneumoVir DNA array assay differs from the Luminex xTAG RVP Fast assay in that it detects influenza C virus but does not allow the detection of the alphacoronavirus NL63 virus and the betacoronaviruses HKU1 and OC43. The CLART® PneumoVir is able to discriminate between rhinovirus and enterovirus genus, and it permits the identification of the new influenza A/H1N1v. Overall, both technics showed comparable sensitivity for the detection of CARVs.[15]

2.4 | Bacterial microbiological studies

Quantitative cultures of BAL specimens for bacterial isolation were performed on conventional media as recommended[16]; in agreement with the generally accepted thresholds,[16] bacterial loads >10^4 CFU/mL were deemed to be clinically relevant. BAL specimens were cultured on BCYE-alpha agar, BD (becston Dickinson) MGIT® (Mycobacteria growth indicator tube)/Lowenstein-Jensen agar slants and Sabouraud agar for recovery of *Legionella pneumophila*, *Mycobacterium* spp., and fungal organisms, respectively. The Platelia™ Aspergillus Ag Kit (Bio-Rad, Hercules, CA, USA) was used for quantitation of *Aspergillus spp. galactomannan*. Calcofluor white, blue toluidine, or direct immunofluorescence staining procedures were used for detection of *Pneumocystis jiroveci*.

2.5 | CMV monitoring and management

CMV DNA in plasma was quantified using the RealT*ime* CMV PCR assay (Abbott Molecular, Des Plaines, IL, USA), which exhibits a limit of detection of approximately 31 IU/mL at the HCUV, as previously described.[17] At the HLF, the CMV R-GENE® (Biomerieux, L'Etoile, Paris, France), which displays a limit of detection of 150 IU/mL, was performed.[18] Surveillance for CMV DNAemia quantitation was conducted at least once a week within the first 100 days after allo-HSCT and at each outpatient visit while on immunosuppression at both centers. A preemptive antiviral therapy approach was used at HCUV to prevent CMV end-organ disease.[19] Patients were preemptively treated with oral valganciclovir, i.v. ganciclovir, or i.v. foscarnet upon detection of CMV DNA levels exceeding 1500 IU/mL or a CMV DNA doubling time ≤2 days, as previously reported.[19,20] In turn, a universal prophylaxis strategy was used at HLF until December 2016.[21] Briefly, HLA-matched related allo-HSCT recipients were given oral valganciclovir (900 mg/d, three times a week) through day 90 after transplantation. Unrelated allo-HSCT recipients were treated with oral valganciclovir (900 mg/d) through day 180 after transplantation. Detection of any level of CMV DNA in plasma prompted the administration of antiviral therapy with (val)ganciclovir or foscarnet at the doses specified above. From January 2017 a preemptive strategy was carried out upon detection of CMV DNAemia at any level.

2.6 | Definitions

CMV DNAemia-RAT was defined as described above. Acute graft versus host disease (aGvHD) was diagnosed and graded according to standard criteria.[22] Confirmed CARV LRTDs were defined according to the recent consensus criteria.[23] LRT co-infection was considered when additional clinically significant microbiological agents, including bacterial, fungal, and/or other CARVs specimens, were also detected in the same BAL sample. Except for *Staphylococcus aureus*, *Staphylococcus spp* were not deemed to be co-infective agents. As well, *Streptococcus spp* (ie, *Streptococcus viridans*) and *Enterococcus spp* (ie, *Enterococcus faecalis or E. faecium*) were not considered as a putative causation of bacterial pneumonia. When more than 2 pathogenetically

significant bacteria were identified in the same BAL sample, the term "mixed flora" was used. Yeast belonging to *Candida spp* were not considered as co-infective agents. In contrast, probable/proven pulmonary IA diagnosed at the time of CARV LRTD was considered as a fungal co-infection. The detection of CMV DNA in the BAL by RT-PCR was not considered as a co-pathogen entity in this study since it is a quite common phenomenon and its interpretation is still uncertain.[24] All allo-HSCT recipients received broad-spectrum antibiotics (carbapenems or cefepime or piperacilline-tazobactam). In cases of hemodynamic instability, akimacin was added. At the time of microbiological results, antimicrobial therapy was adapted accordingly.

2.7 | End points and statistical analysis

The primary objective of the study was to describe the clinical and microbiological characteristics of CARVs LRTD and co-infections as well as to evaluate the effect of co-infection subtypes on the clinical outcome of CARVs LRTD as compared to respiratory virus and bacterial mono-infection. Secondary end points included the identification of RFs for bacterial co-infection and for all causes mortality at day 60 after BAL sampling in recipients with CARVs LRTD.

Frequencies were compared using the χ^2 test (Fisher exact test) for categorical variables. Differences between medians were compared using the Mann-Whitney *U* test. Univariate and multivariate analyses of the association of clinical and microbiological RFs with bacterial co-infection and overall mortality were calculated using Cox regression models including time-dependent covariates when appropriate. For multivariate analysis, only variables with parameter estimates showing a *P* value ≤.10 in the univariate analysis were finally included. Two-sided exact *P* values are reported and *P* values ≤.05 were considered statistically significant. The probability of mortality after CARVs LRTD was estimated by cumulative incidence curves, treating base-line disease relapse as a competing event. The probability of OS and cumulative incidence plots of mortality were estimated from the time of BAL using Kaplan-Meier curves[25] and univariate comparisons were done with the log-rank test.[26,27] The data were analyzed with the SPSS (version 20.0) statistical package and R v2.12.2 (The CRAN project) with the survival v2.36-10, Design v2.3-0, prodlim v1.2.1, and cmprsk v2.2-221packages.

3 | RESULTS

3.1 | Patient characteristics

Detailed clinical characteristics of the subjects with CARVs LRTD w/o co-infection and with bacterial pneumonia mono-infection are shown in Table 1. Patients were allografted between February 2007 and July 2017. Of note, most patients were at high-risk with a profound immunosuppression status because 70% of the recipients included were allografted from alternative donors (adult unrelated donor, cord blood, and haplo-identical family donors) and 58% had at least one antigen mismatch with the donor in the HLA A, B, C, or DR alleles, as determined by high-resolution genotyping.

3.2 | CARVs LRTD characteristics

The clinical and biologic characteristics of CARVs LRTD are shown in Table 2. The most common type of CARVs detected was EvRh in 32 (33%) cases, followed by RSV in 27 (28%), HPiV in 21 (22%), influenza in 16 (17%), HMPV in 13 (14%), ADV in 6 (6%), and CoV in 6 cases (6%). Seventy-four (75%) of the CARVs LRTD occurred within the first year after allo-HSCT. Twenty-two (23%) cases occurred before day +30 after stem cells infusion while 17 (18%) developed LRTD from day +30 to day +100 and 35 (36%) from day +100 until a year. We did not observe significant clinical and/or biological differences among groups 1, 2, 3, and 4 except for a lower rate of CMV DNAemia-RAT in the group 2 (P = .04).

3.3 | Risk factors for bacterial co-infection and for day 60 all causes mortality

Univariate and multivariate analyses for RFs of CARVs LRTD bacterial-virus co-infection and for day 60 all causes mortality were shown in Table 3.

Multivariate analysis identified 2 independent variables associated with increased risk of bacterial co-infection: Corticosteroids ≥1 mg/kg/d (hazard ratio [HR] 4.1, 95% confidence interval [C.I.] 1.6-10.3, P = .003) and CMV DNAemia-RAT at the time of BAL (HR 3.4, 95% C.I. 1.2-9.4, P = .02).

Regarding the RFs for day 60 all causes mortality, multivariate model identified 4 variables associated with increased mortality: lymphocyte count < 0.5 × 10^9/L (HR 2.6, 95% 1.1-6.2, P = .026), the occurrence of CMV DNAemia-RAT at the time of BAL (HR 2.32, 95% C.I. 1.1-4.9, P = .03), bacterial co-infection (HR 2.65, 95% C.I. 1.2-6.9, P = .017), and the need of oxygen support at the time of BAL (HR 8.3, 95% C.I. 2.9-35.3, P = .004). Based on 3 of these RFs (lymphocyte count, CMV DNAemia-RAT, and oxygen support) we elaborated a risk score according to the presence of 0 to 1, 2, or 3 RFs (Figure 2). This risk model was predictive (c-statistics 0.69) and differentiated 3 groups with different mortality rates. We identified a subgroup of patients with a low risk of mortality (<7%) irrespective of CARVs LRTD mono- or co-infections (Figure 2).

3.4 | Causes of mortality and overall survival by day 60 after BAL

Overall, 33 recipients with CARVs LRTD (34%) died at median of 29 days after BAL (range 0-59 days). Causes of mortality were; respiratory failure attributable to the LRTD in 20 cases, while infection and GvHD accounted for 9 cases, 1 due to GVHD, 1 sinusoidal obstruction syndrome, and 2 hematological relapses. Regarding mortality according to CARV type, we observed 11/32 death cases with EvRh (34%), 7/27 with RSV (26%), 8/21 with HPiV (38%), 7/16 (43%) with influenza, 6/13 (46%) with HMPV, 3/6 (50%) cases of ADV, and finally 0/4 cases with CoV. Day 60 overall survival for groups 1, 2, 3, and 4 were 77%, 67%, 35%, and 73%, respectively (P = .012), (Figure 3A).

3.5 | Bacterial pneumonia co-infection and mono-infection characteristics and mortality

Table 2 summarizes clinical and microbiological characteristics of CARVs LRTD with bacterial co-infection (group 3) and bacterial pneumonia mono-infection (group 5). There were no clinical and/or biological significant differences among both groups in terms of well-known RFs (those included in the ISI) and other relevant clinical characteristics such as oxygen support or ICU admission.

In 20 cases from group 3 (100%) we identified gram-negative bacteria in the BAL, whereas in group 5 there were 15 cases (79%) with gram-negative bacteria (P = .05). In cases 3 and 4, respectively, we found mixed flora in the BAL. The ICU admission rates were 40% and 53% (P = .7) while mortality rates were 65% and 38%, for group 3 and 5, respectively (P = .1). Day 60 OS was higher for group 5 when compared to group 3, although significance was not reached (Figure 3). When we limited the analysis to those recipients with gram-negative bacteria we observed that 13 out of 20 recipients (65%) in group 3 die at day 60 after BAL compared to 4 of 15 (34%) in group 4 (P = .1).

4 | DISCUSSION

The study herein shows that CARVs LRTD co-infections are common after allo-HSCT. Bacterial pneumonia co-infection in recipients with CARVs LRTD was associated with increased mortality. We also identified 3 other RFs (lymphopenia <0.5 × 10^9/mL, CMV DNAemia-RAT, and the need of oxygen support at the time of BAL) that led to the stratification of 3 risk groups with significantly different mortality rates. Notably, patients at low risk (no or only 1 RF) had a very low mortality rate (≤7%) irrespective of the presence of co-infective agents.

With nucleic acid amplification testing, we report a high rate (47%) of CARV LRTD co-infections considered as respiratory virus, IA, and significant bacterial co-infection. Prior studies, before the RT-PCR era, reported lower co-infection rates (<30%) in BAL samples.[5,9,28,29] This fact has limited the knowledge regarding the role of co-infections in the clinical outcome of CARVs LRTD.

Some studies have reported that pooled co-infections (bacteremia, fungal infections, CMV reactivations, herpes simplex virus, human herpesvirus 6, and Epstein-Barr virus) significantly increased mortality of allo-HSCT recipients in several CARV types,[5,7-10] but others failed to demonstrate such a negative effect.[29] In such reports, there is a lack of comparisons with mono-infections counterpart. In our study we provided evidence that CARVs LRTD with bacterial co-infections displayed a negative effect in mortality in multivariate analysis. Interestingly, we observed a trend to poorer outcome of recipients with CARVs LRTD and bacterial co-infections compared to those with bacterial pneumonia mono-infection. These findings suggest that the negative effect of bacterial co-infection does not seem to be independently explained by the predominant influence of the bacterial agent but rather by the co-infection status.

TABLE 3 Univariate and multivariate analysis of risk factors for bacterial co-infection and LRTD RV mortality at 60 days after bronchoalveolar Lavage

| | COX Regr. bacterial co-infection | | | | COX Regr. day 60 mortality | | | |
| | Univariate analysis | | Multivariate analysis | | Univariate analysis | | Multivariate analysis | |
Variables	HR (95% C.I)	P	HR (95% C.I)	P	HR (95% C.I.)	P	HR (95% C.I.)	P
Type of donor, n (%)								
HLA-identical sibling donor	1				1			
Unrelated donor	2.5 (0.6-10.1)	.19			1.98 (0.64-6.06)	.23		
Umbilical cord blood	3.05 (0.78-11)	.1			2.8 (0.95-8.5)	.06	ns	
Haploidentical family donor	2.6 (0.5-13.1)	.23			2.4 (0.84-6.7)	.1		
ATG as a part of the conditioning	1.99 (0.8-5.1)	.14			1.29 (0.6-2.85)	.52		
R and/or D CMV seropositive	1.78 (0.54-3.397)	.46			1.18 (0.38-2.68)	.71		
GvHD at the time of BAL[a]	1.25 (0.43-3.58)	.67			1.4 (0.74-2.9)	.3		
On IS	3.6 (0.48-27.28)	.29			3.8 (0.5-28.2)	.18		
ALC < 0.5 × 109/L, n (%)	4.1 (1.4-11.2)	.007	ns		3.2 (1.4-7.4)	.006	2.6 (1.1-6.2)	.026
ALC< 0.2 × 109/L	1.87 (0.76-4.60)	.2			1.8 (0.93-3.63)	.08	NT	
ANC < 0.5 × 109/L	2.55 (0.85-7.9)	.1	ns		1.9 (0.9-3.9)	.08	NT	
Age ≥ 40 y	2.8 (0.65-12.2)	.16			1.5 (0.63-3.7)	.3		
Myeloablative	0.93 (0.37-12.28)	.87			0.87 (0.45-1.8)	.8		
Corticosteroids at any dose	2.6 (0.9-7.8)	.08	NT		1.9 (0.9-3.8)	.09	NT	
Corticosteroids ≥1 mg/kg/d	4.6 (1.8-11.5)	.001	4.1 (1.6-10.3)	.003	2.37 (1.14-4.5)	.019	ns	
Recent or pre-engraftment	1.11 (0.5-2.49)	.78			1.2 (0.55-2.5)	.6		
ISI								
Low risk (0-2)	1				1			
Moderate risk (3-6)	1.26 (0.42-3.42)	.62			3.1 (1.06-9.07)	.039	ns	
High risk (7-12)	2.39 (0.63-8.9)	.18			4.27 (1.3-13.9)	.016		
Basel IG (adapted)								
Moderate	1				1			
Severe	1.26 (0.25-6.36)	.8	ns		3.15 (0.9-11.3)	.078	ns	
Very severe	7.1 (1.54-33.1)	.012			4.25 (1.3-13.9)	.019		
BAL findings								
Mono-infection	NT				1			
RV co-infection					1.4 (0.57-4)	.5	ns	
IA co-infection					1.2 (0.33-4.1)	.8		
Bacterial co-infection					3.7 (1.7-8.2)	.001	2.65 (1.2-6.9)	.017
CMV DNAemia-RAT[a]	3.83 (1.4-7.10.6)	.009	3.4 (1.2-9.4)	.02	3.3 (1.6-6.9)	.001	2.32 (1.1-4.9)	.03
Oxygen support	1.73 (0.58-5.2)	.32			9.57 (12.3-40)	.002	8.3 (1.9-35.3)	.004
Risk score[b]								
0-1 RF	1				1		NT	
2 RFs	3.76 (0.99-14.3)	.051			11.9 (2.7-51.9)	.001		
3 RFs	7.81 (2.1-28.9)	.002			22.1 (5.03-97.2)	<.001		

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ATG, anti-thymocytic globuline; BAL, bronchoalveolar lavage; Basel IG, Basel Immunodeficiency grading; C.I., confidence interval; COX. Regr, Cox regression Hazard model; CMV DNAemia-RAT, cytomegalovirus DNAemia requiring antiviral therapy; D, donor; GvHD, graft versus host disease; HR, hazard ratio; IA, invasive aspergillosis; ISI, immunodeficiency score index; Log Regr, logistic regression; ns, not significant; NT, not tested; OR, odds ratio; R, recipient; RV, respiratory virus; RFs, risk factors.
[a]Analyzed as time-dependent covariates.
[b]Risk score was based on the presence of the following RFs: lymphopenia <0.5 × 109/L, CMV DNAemia-RAT and oxygen support.
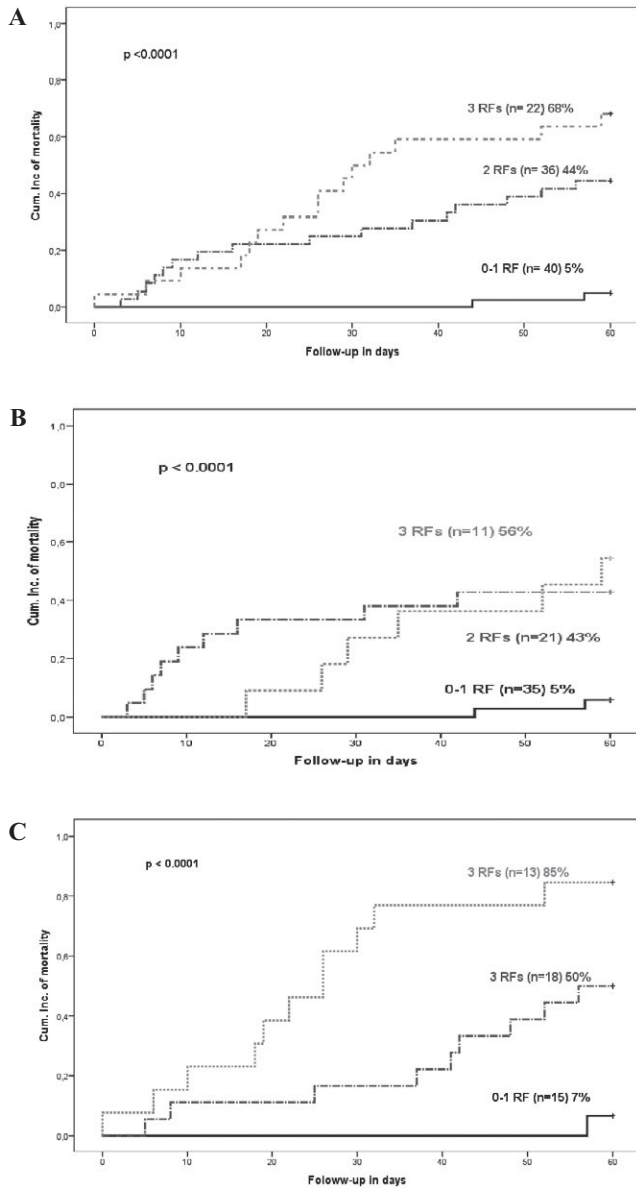
FIGURE 2 Probability of mortality at day 60 after of bronchoalveolar lavage according to the absence or presence of risk factors (CMV DNAemia requiring antiviral therapy, lymphopenia <0.5 × 109/L and/or oxygen support). All 3 variables captured at the time of BAL. A, All recipients with respiratory virus lower respiratory tract disease, B, recipients with respiratory virus (mono- and RV co-infection), C, recipients with co-infections (viral, fungal, and/or bacterial co-infection)

When we limited the analysis to gram-negative bacilli, we also observed higher mortality of CARV LRTD with gram-negative bacterial co-infection (65%) compared to gram-negative bacterial pneumonia mono-infection (26%), although significance was not reached probably due to the low number of recipients included.

Another relevant finding of this study was the identification of 3 RFs for mortality easily identifiable at the time of CARVs LRTD. These RFs have already been identified as prognostic markers for progression to LRTD (ie, lymphopenia) in several studies among different respiratory virus or as RFs for mortality (ie, lymphopenia <0.5 × 109/L,

oxygen requirement, and CMV DNAemia-RAT).[5,6,9-11,29-31] With these 3 RFs we built a risk score that was able to discriminate 3 groups with different risk of mortality irrespective of the co-infective status that merit to be validated in further studies. In contrast, we were not able to provide evidence of the clinical usefulness of neither, the ISI score nor the adapted Basel Immunodeficiency grading score, in predicting mortality in multivariate analysis in our pooled CARVs LRTD cohort. Although the ISI, originally designed for RSV, has been validated in influenza virus,[32] it is likely that co-infections, not included in the ISI, have hampered its ability to predict outcome in our series. In fact, none of the variables included in the ISI were statistically significant in our uni- and multivariate model reflecting the weakness of this model in our cohort. This fact suggests that validation of the ISI in other CARVs is warranted before its routine application for therapeutic decision-making. Regarding the Basel Immunodefciency grading score, the fact that we did not include immunoglobulin level-sin the score may have hampered our ability to assess its true value and further validation would be required.[12,13]

Regarding the analysis of RFs for co-infections during CARVs LRTD our decision of limiting this analysis to bacterial co-infection was justified by 3 main reasons. First, our study showed that bacterial co-infection had a significant negative effect on clinical outcome in contrast to respiratory virus and IA co-infections. Second, there is an increased evidence in the mechanism whereby viral infections enhance and aggravate bacterial co-infection, the former favoring the growth of bacterial agents by multiple factors, including local destruction of antibacterial barriers at epithelial surfaces, suppression of antibacterial immunity, and induction of apoptosis in immune cells.[10,33-35] Third, RFs for respiratory virus co-infection are expected to be different from those affecting bacterial co-infection since respiratory virus transmission depend upon epidemiologic situation such as the recipients house-hold contacts, contact with children, vaccination status, which has not been captured in our data base. Thus, we identified 2 conditions related with higher incidence of bacterial co-infection; corticosteroids ≥1 mg/kg/d, and CMV DNAemia-RAT. While corticosteroids are a well-recognized RF of profound immunosuppression and then may contribute to bacterial infection in allo-HSCT, this is the first time that CMV DNAemia-RAT was identified as a risk factor for bacterial pneumonia co-infection during CARVs LRTD. CMV is a highly pro-inflammatory and immunosuppressive virus[36] and as such it may act synergistically with respiratory virus favoring bacterial growth in the respiratory tract. In addition, CMV readily infects macrophages in vivo, impairing their ability to recognize and eliminate bacteria by phagocytosis.[37] Another contributing factor for such observation could be the development of neutropenia-related anti-CMV therapy. Further studies are warranted to confirm such findings since the use of antibiotics in allo-HSCT recipients with CARVs at risk of progression to the LRT with concurrent CMV DNAemia-RAT and/or under corticosteroids therapy may be clinically useful to prevent bacterial pneumonia. Last but not least, we reported that fungal co-infection did not show a negative effect on survival in our cohort. It is likely that the introduction of effective anti-mold drugs from 2007 has overcome the
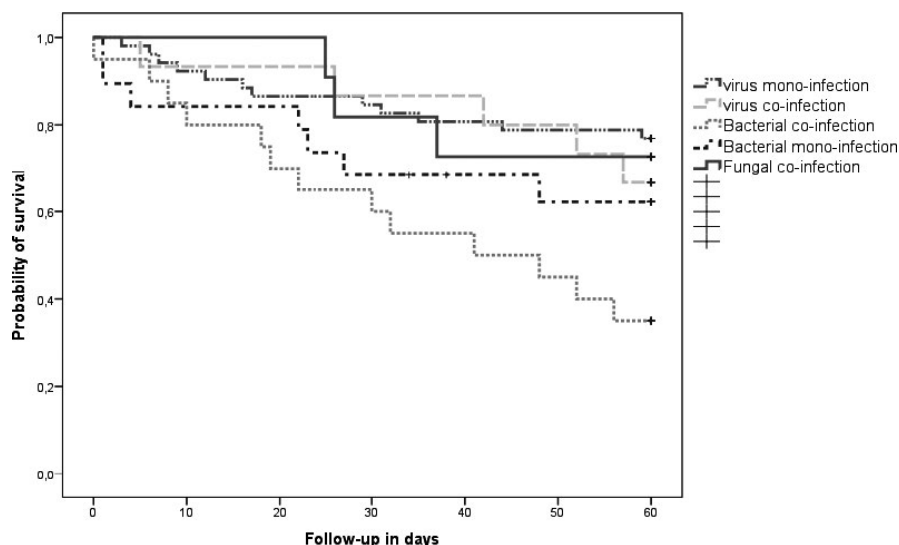
FIGURE 3 Overall survival at 60 days after bronchoalveolar lavage of respiratory virus lower respiratory tract disease according to infection type. OS for virus mono-infection was 77%, virus co-infection 67%, fungal co-infection 73%, and bacterial co-infection 35%, P = .012. Overall survival of bacterial co-infection versus bacterial pneumonia mono-infection was 35% versus 62%, P = .15

historical bad prognosis of such a complication even in the context of CARV LRTD co-infection.[38]

We acknowledge that our study has some limitations including the relatively low number of patients, the use of 2 different multiplex PCR platforms for CARV, the inclusion of several CARV types, as well as its retrospective nature. In addition, when multiple viruses were detected we were not able to differentiate between infection, shedding, or resolved infection with continued detection. To overcome this limitation, we critically reviewed the radiological patterns at the time of BAL as well as the presence of upper and lower respiratory symptoms ensuring a high degree of CARVs-related cases. Moreover, all BAL samples were homogenously and prospectively tested for CARVs, fungal, and bacterial agents, avoiding the inclusion of cases with PCR retrospectively tested in frozen BAL samples, and this fact should be considered as strength. Regarding the inclusion of several types of CARVs LRTD, that may differ in their pathogenicity, several comparative studies analyzing mortality among different CARVs LRTD showed similar mortality rates[7,8,39] and could justify our pooled cohort analysis for mortality. In fact, we did not observe significant differences on mortality among the different respiratory viruses in our series, either with or without co-infections. In addition, our data shows an overall mortality rate (35%) comparable among CARVs and in line with several reports,[1-8,39] emphasizing that any CARVs LRTD are still a common life-threatening complication after allo-HSCT.

In conclusion, CARV LRTD co-infections are frequent and may have a negative effect in the outcome, in particular in the context of bacterial co-infections. Our risk score based on easily identifiable RFs (lymphopenia <0.5 × 109/L, oxygen requirement, and CMV DNAemia-RAT) merit further validation in other cohorts whereas the ISI and the Basel immunodeficiency grading require further validation in prospective studies.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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