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Bone Marrow Transplantation (2021) 56:2555–2566
https://doi.org/10.1038/s41409-021-01333-7

ARTICLE

Legionellosis after hematopoietic stem cell transplantation

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Received: 20 October 2020 / Revised: 20 April 2021 / Accepted: 29 April 2021 / Published online: 22 May 2021

Abstract

Limited data are available on legionellosis after hematopoietic stem cell transplant (HSCT). The aim of this study was to report the cases of legionellosis and to identify predictors of legionellosis, legionellosis-associated death, and non-relapse mortality (NRM). All cases of post-HSCT legionellosis from the EBMT registry were included and matched with controls in a 3:1 ratio for the analyses of risk factors. In the years 1995–2016, 80 cases from 52 centers in 14 countries were identified (mainly from France, Italy, and Spain). Median time from HSCT to legionellosis was 203 days (range, 0–4099); 19 (23.8%) patients developed early legionellosis (within-day +30 post-HSCT). Patients were mainly male (70%), after allogeneic HSCT (70%), with acute leukemia (27.5%), lymphoma (23.8%), or multiple myeloma (21.3%), and the median age of 46.6 (range, 7.2–68.2). Predictors of legionellosis were allogeneic HSCT (OR = 2.27, 95%CI:1.08–4.80, $p = 0.03$) and recent other infection (OR = 2.96, 95%CI:1.34–6.52, $p = 0.007$). Twenty-seven (33.8%) patients died due to legionellosis (44% after early legionellosis), NRM was 50%. Predictors of NRM were female sex (HR = 2.19, 95%CI:1.13–4.23, $p = 0.02$), early legionellosis (HR = 2.24, 95%CI:1.13–4.46, $p = 0.02$), and south-eastern geographical region (HR = 2.16, 95% CI:1.05–4.44, $p = 0.036$). In conclusion, legionellosis is a rare complication after HSCT, mainly allogeneic, occurring frequently within 30 days after HSCT and associated with high mortality.

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Introduction

Legionella is an intracellular bacterial pathogen with worldwide distribution, transmitted through water aspiration or aerosol inhalation. It may cause self-limited febrile disease, but it is mainly known as a cause of, potentially severe, community-acquired or nosocomial pneumonia. Legionellosis was first described in 1977 following the outbreak in 1976, and a rapid and reliable diagnostic assay—ELISA urinary antigen test for serogroup 1 of *Legionella pneumophila*—has become available in mid-80' [1, 2].

The rate of Legionnaires' disease in the EU in 2016 varied from 0.01 to 0.49 to over two cases per 100.000 population, with Italy, Spain, Denmark, the Netherlands, and Slovenia reporting the highest rates [3]. Legionellosis has seasonal distribution, with 58% of cases in 2016 occurring between June and October, although outbreaks may occur any time throughout the year [3].

Although there are several species of *Legionella*, *L. pneumophila* is the most commonly identified, accounting for 79% of culture-confirmed cases [3]. Legionellae multiply within human monocytes and macrophages, and cell-mediated immunity is the primary host defense mechanism against *Legionella* infection [4, 5]. The known risk factors include advanced age, male sex, smoking, chronic cardiovascular and respiratory diseases, diabetes mellitus, alcohol abuse, and malignancy [2].

Immunocompromised patients, particularly transplant recipients, are at an increased risk of *Legionella* infection [5]. Although the epidemiology after HSCT is unknown, HSCT recipients accounted for 18–42% of all hospital cases of legionellosis in several US series [6–8]. Nevertheless, data on legionellosis after HSCT is provided mainly by single-center case series which included all different types of patients, with the highest number of 26 and 27 patients with hematological malignancy or HSCT, while the predictors and factors influencing the outcome remain largely unknown [9–11].

The aim of this study was to report the cases of legionellosis after HSCT included in the EBMT registry and to identify predictors of legionellosis, legionellosis-associated death, and non-relapse mortality (NRM) in patients with this infection.

Patients and methods

Study design

This retrospective observational study included all patients from the EBMT registry who were transplanted after 1990 and in whom legionellosis was reported. Data collection was performed by the EBMT infectious diseases working party (IDWP) data office (Leiden, the Netherlands) according to EBMT guidelines (http://www.ebmt.org/retrospectivestudies). All patients signed at transplant an informed consent form for data collection in the EBMT registry. No additional ethics committee approval was necessary.

The following variables were collected: country of HSCT; underlying disease and its status at HSCT; type of donor; year of HSCT after which legionellosis occurred; previous HSCT; age; sex; time from the last transplant to legionellosis; engraftment before legionellosis; previous (i.e., after HSCT and within 6 months preceding legionellosis) viral, bacterial and fungal infection reported to EBMT registry; GvHD within 6 months before legionellosis; relapse of the underlying disease within 2 years before legionellosis; outcome; date of the last follow up; cause of death.

For the analyses of risk factors, controls were identified in the EBMT registry in 3:1 ratio, with the following matching criteria: the same center, the same year of transplant, and the length of follow-up at least until the day of diagnosis of legionellosis in the matched case. The remaining aforementioned variables were collected and analyzed as potential predictors of legionellosis. In order to assure reliable follow-up data on controls, we excluded from case-control study the cases that occurred more than 2 years after HSCT or before the year 2000.

Definitions

Early legionellosis was defined as occurring within 30 from transplant and late legionellosis as occurring afterward. Engraftment was defined as the first of 3 days of neutrophil count >500/mm^3 after HSCT.

Geographical regions were divided in north-western, including Austria, Belgium, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, UK; and south-eastern, including Bulgaria, Croatia, Czech Republic, Cyprus, Greece, Hungary, Israel, Italy, Lithuania, Macedonia, Poland, Portugal, Romania, Russia, Slovakia, Spain, Turkey [12].

The countries were also grouped according to the incidence of legionellosis in the general population based on the 2016 ECDC data, into those with a high incidence of legionellosis if >1.5/100.000 inhabitants were diagnosed (Denmark, France, Italy, Spain, the Netherlands), and others [3].

Death was classified as due to legionellosis if defined as such in the database, or if the following three conditions were present: occurring due to pulmonary failure, within 30 days from the onset of legionellosis, and no other possible cause provided.

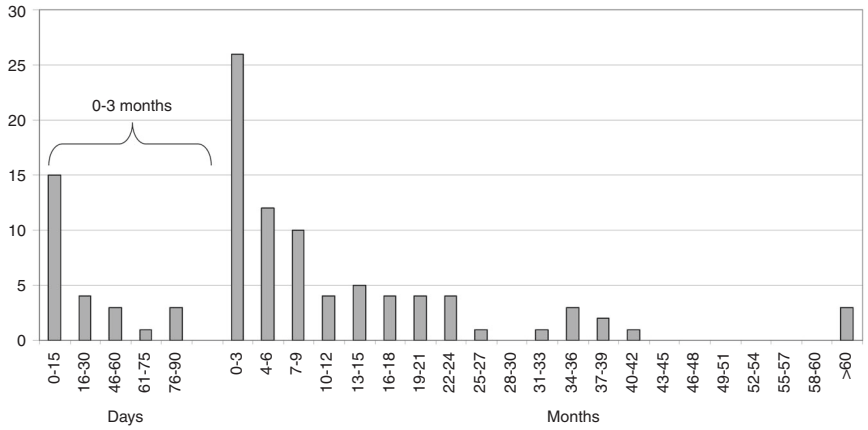

Fig. 1 Timing of legionellosis after HSCT. Number of cases of legionellosis in the months after transplant, split into 15-days periods for the first months.

Statistical analyses

The main characteristics of patients were reported by descriptive statistics of the total of the available information: absolute and percent frequencies were used in case of categorical variables, whilst median and range were used in case of continuous variables. The length of follow-up was reported as median with 95% confidence intervals (95% CI).

The cumulative incidence of NRM was estimated by the cumulative incidence method, considering the non-relapse death as an event of interest and the relapse of the underlying disease as a competing event. Moreover, the cumulative incidence of death due to legionellosis was also estimated.

The cause-specific Cox regression model was applied to investigate the impact of the risk factors on the survival outcomes. The variables associated with legionellosis in the matched case-control study part were assessed with the conditional logistic regression model. Variables with a significant p-value in the univariate analysis were included in the multivariate analysis.

A p-value <0.05 was considered statistically significant. All the analyses were performed using the statistical software SAS v 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Legionella cases

Between 1995 and 2016, 80 cases of legionellosis from 52 centers in 14 countries were identified. The countries with the highest number of reported cases were France ($n = 27$), Italy ($n = 11$), Spain ($n = 8$), UK ($n = 7$), and the Netherlands ($n = 6$). On a median, four cases per year were reported (range, 1–8). One and two centers reported, respectively, three and two cases occurring the same year. In two centers, two cases were diagnosed within 30 days, suggesting a possible cluster.

The median time from HSCT to the diagnosis of legionellosis was 203 days (range, 0–4099). Early legionellosis was identified in 19 (23.8%) patients. The distribution of legionellosis over time from transplant is reported in Fig. 1: 47.5% of patients developed legionellosis within 6 months post-HSCT, including 26 cases (32.6%) within 3 months; 17.5% between 7 and 12 months from HSCT; 21.3% between 13 and 24 months; and 13.8% >24 months after HSCT. Most of the cases of legionellosis occurred in May and June (Fig. 2).

During the study period, the centers which reported the cases of legionellosis performed 107,137 transplant procedures, thus the estimated incidence of legionellosis after any HSCT in these centers was 0.076% (80/107,137); and 0.038% (24/63,515) for autologous and 0.13% (56/43,619) for allogeneic HSCT.

The characteristics of all patients with legionellosis, and those with early and late legionellosis, are outlined in Table 1. Comparing the characteristics of patients with early vs. late legionellosis, the former were more likely to be in complete remission and have undergone HSCT in the earlier years of the study period (Table 1).

Risk factors

Eleven patients who developed legionellosis more than 2 years after the last HSCT and six who received transplant before the year 2000 were excluded from the analysis of predictors of legionellosis. Data from these 63 patients were compared to data from 189 controls (Table 2).

In univariate analysis, cases were more likely to have received allogeneic and not autologous HSCT ($p = 0.01$), to have undergone previous HSCT ($p = 0.02$), to have developed other infections in the 6 months before developing legionellosis ($p = 0.003$) and to have experienced GvHD in the 6 months before developing legionellosis ($p = 0.02$).

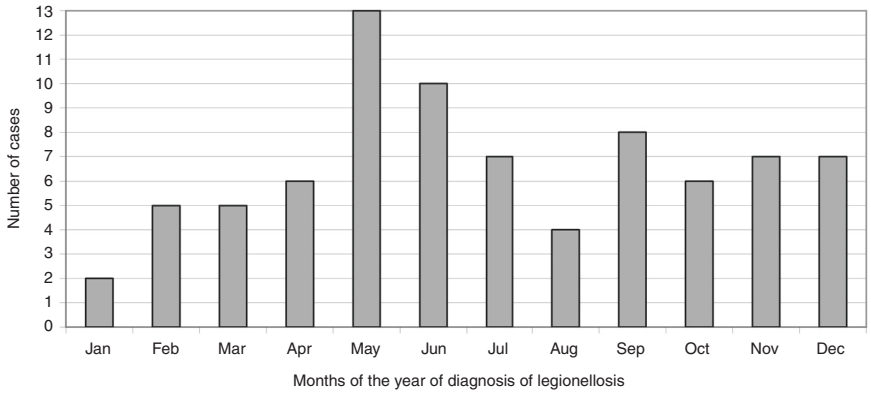

Fig. 2 The distribution of cases of legionellosis by month of diagnosis.

The multivariate analysis confirmed the role of allogeneic HSCT (OR 2.27, 95% CI 1.08–4.80, $p = 0.03$) and previous other infections (OR 2.96, 95% CI 1.34–6.52, $p = 0.007$) as predictors of legionellosis.

Outcome

Among 80 patients with legionellosis, 52 (65%) were dead at the last follow-up. The median survival time was 67.8 months (95% CI: 43.9–108.3) after HSCT and 29.0 months (95% CI: 17.1–82.6) after the onset of legionellosis. The cause of death was classified as transplant-related (non-relapse) in 40 patients (50%), and as due to legionellosis in 27 (33.8% of the whole cohort). Among 19 patients with early legionellosis, 18 died (94.7%), 12 due to infectious causes, including legionellosis as the main cause of death in 8 (44%) (Table 1).

The impact of early vs. late legionellosis on the NRM is shown in Fig. 3.

Predictors of NRM, both in univariate and multivariate analysis, were female sex (HR 2.19, 95%CI 1.13–4.23, $p = 0.02$), development of early legionellosis (HR 2.24, 95% CI 1.13–4.46, $p = 0.02$), and south-eastern geographical region (HR 2.16, 95%CI 1.05–4.44, $p = 0.036$), as shown in Table 3. The southeastern geographical region was the only independent predictor of death due to *Legionella* (HR 2.59, 95%CI 1.21–5.56, $p = 0.01$) (Table 3).

Discussion

This study, with 80 cases included, is currently the largest case series of legionellosis occurring after HSCT. The main finding from this cohort is a high number of early, probably nosocomial, cases of legionellosis which are characterized by a very high mortality rate (44% legionellosis-related). In addition, we identified risk factors for the development of legionellosis in this set which includes allogeneic transplant and recent other infections. *Legionella* infection was also associated with a very high mortality rate, both overall mortality (65%) and mortality due to legionellosis (33.8%). Few specific predictors of death were identified, suggesting that even patients without additional risk factors, such as non-engraftment, older age, or GvHD, have a high risk of mortality in case of this infection.

Legionellosis is rare, as it accounts for only 2–9% of cases of community-acquired pneumonia, but immunocompromised patients, particularly with cancer and transplant recipients, are at significantly increased risk [5, 13]. This might be due to impaired cellular immunity that contributes to reduced clearance of *Legionella* and to a higher risk of nosocomial transmission because of long hospital admissions. Indeed, in our cohort, we found that one-third of 80 cases occurred very early after transplant (within 30 days, with the peak within 14 days after HSCT). Since HSCT recipients are usually hospital-admitted several days before stem cell infusion, most of these cases could be classified as definite nosocomial legionellosis (defined as developing in a person who was in a hospital for 10 days before symptoms onset) and only a few as possible nosocomial (defined as developing in a person who was in a hospital for 1–9 of the 10 days before the onset of symptoms in a hospital not previously known to be associated with any case of Legionnaires' disease, and where no microbiological link has been established between the infection and the hospital) [14]. This rate of early legionellosis after HSCT is even higher than in solid organ transplant recipients, in whom only 21% developed legionellosis within 3 months from transplant [15]. Such a high proportion of nosocomial infections is consistent with previous reports that the nosocomial source of legionellosis is the most frequent one in transplant recipients and in oncology patients [16, 17]. For instance, it accounted for 29% of cases among patients with head and neck cancer [17]. Moreover, also some cases of late legionellosis might be of nosocomial origin, but data on hospital admission were not available in the EBMT registry. Nevertheless, the positive change is that the number of reported cases of early legionellosis has significantly

Table 1 Characteristics of all patients with legionellosis, and those with early and late legionellosis.

	Total, $n = 80$ (%)	Early legionellosis (until day +30), $n = 19$ (%)	Late legionellosis (from day +31), $n = 61$ (%)	P^*
Age at legionellosis, median, range	50.1 (7.2–73.0)	49.4 (7.2–73.0)	50.2 (12.5–68.2)	0.9
Sex, male	56 (70.0)	13 (68.4)	43 (70.5)	0.9
Diagnosis				0.2
Myeloid	34 (42.5)	6 (31.6)	28 (45.9)	
AML	16	4	12	
CML	8	1	7	
Myelodysplastic/myeloproliferative disorders	10	1	9	
MDS	6	1	5	
MPN	4	0	4	
Lymphoid	44 (55.0)	13 (68.4)	31 (50.8)	
ALL	7	3	4	
CLL	1	0	1	
Lymphoma	19	7	12	
Plasma cell disorders (MM)	17	3	14	
Bone marrow failure[a]	2 (2.5)	0 (0.0)	2 (3.3)	
Disease status at latest HSCT[b]				0.03
Complete remission	38 (48.1)	14 (73.7)	24 (40.0)	
Partial remission/chronic phase/stable disease/BM failure	29 (36.7)	3 (15.8)	26 (43.3)	
Active disease	12 (15.2)	2 (10.5)	10 (16.7)	
The decade of HSCT in which legionellosis occurred				
1995–1999	6 (7.5)	1 (5.3)	5 (8.2)	
2000–2004	20 (25.0)	10 (52.6)	10 (16.4)	
2005–2009	22 (27.5)	3 (15.8)	19 (31.1)	
2010–2016	32 (40.0)	5 (26.3)	27 (44.3)	
Period of HSCT in which legionellosis occurred				0.01
1995–2004	26 (32.5)	11 (57.9)	15 (24.6)	
2005–2016	54 (67.5)	8 (42.1)	46 (75.4)	
Season of the diagnosis of legionellosis				0.8
Warm: April–September	48 (60.0)	11 (57.9)	37 (60.7)	
Cold: October–March	32 (40.0)	8 (42.1)	24 (39.3)	
Latest HSCT before legionellosis[b]				0.4
First	60 (75.0)	15 (78.9)	45 (73.8)	
Second or more	19 (23.8)	3 (15.8)	16 (26.2)	
Type of HSCT donor				
Autologous	24 (30.0)	8 (42.1)	16 (26.2)	
Identical sibling	18 (22.5)	2 (10.5)	16 (26.2)	
Matched unrelated	30 (37.5)	5 (26.3)	25 (41.0)	
Mismatched related	4 (5.0)	1 (5.3)	3 (4.9)	
Mismatched unrelated	4 (5.0)	3 (15.8)	1 (1.6)	
Type of HSCT donor				0.2
Autologous	24 (30.0)	8 (42.1)	16 (26.2)	
Allogeneic	56 (70.0)	11 (57.9)	45 (73.8)	
Source				
PB	58 (72.5)	14 (73.7)	44 (72.1)	0.9
BM or CB	22 (27.5)	5 (26.3)	17 (27.9)	
BM	17	1	16	
CBT	3	3	0	
BM + PB	2	1	1	
Conditioning regimen				
Myeloablative	58 (72.5)	15 (78.9)	43 (70.5)	0.5
Reduced intensity	22 (27.5)	4 (21.1)	18 (29.5)	

Table 1 (continued)

	Total, $n = 80$ (%)	Early legionellosis (until day +30), $n = 19$ (%)	Late legionellosis (from day +31), $n = 61$ (%)	P^*
T-cell depletion				
Yes	34 (42.5)	8 (42.1)	26 (42.6)	1
In vivo only	27	5	22	
Ex vivo only	5	1	4	
In vivo and ex vivo	2	2	0	
No	46 (57.5)	11 (57.9)	35 (57.4)	
ATG use				
Yes	19 (25.0)	3 (16.7)	16 (27.6)	0.5
No	57 (75.0)	15 (83.3)	42 (72.4)	
Engraftment before legionellosis				<0.0001
Yes	64 (81.0)	5 (26.3)	59 (98.3)	
No	15 (19.0)	14 (73.7)	1 (1.7)	
Disease status in the two years before legionellosis				0.3
Relapse	12 (15.0)	1 (5.3)	11 (18.0)	
No relapse	68 (85.0)	18 (94.7)	50 (82.0)	
Infection (viral, bacterial, or fungal) in the 6 months before legionellosis				0.06
Yes	27 (33.8)	3 (15.8)	24 (39.3)	
No	53 (66.3)	16 (84.2)	37 (60.7)	
Acute or chronic GvHD in the 6 months before legionellosis				<0.0001
Yes	30 (37.5)	0 (0.0)	30 (49.2)	
No	26 (32.5)	11 (57.9)	15 (24.6)	
Not applicable (auto HSCT)[a]	24 (30.0)	8 (42.1)	16 (26.2)	
Acute or chronic GvHD starting within 6 months before legionellosis				0.049
Yes	14 (17.5)	0 (0.0)	14 (23.0)	
No	42 (52.5)	11 (57.9)	31 (50.8)	
Not applicable (auto HSCT)[a]	24 (30.0)	8 (42.1)	16 (26.2)	
Status				
Alive	28 (35.0)	1 (5.3)	27 (44.3)	
Dead	52 (65.0)	18 (94.7)	34 (55.7)	
Dead due to legionella	27/52 (51.9)	8/18 (44.4)	19/34 (55.9)	
Dead due to relapse/progression	12/52 (23.1)	5/18 (27.8)	7/34 (20.6)	
Other infection	6/52 (11.5)	4/18 (22.2)	2/34 (5.9)	
GvHD	2/52	0	2/34 (5.9)	
Other	5/52	1/18	4/34 (11.8)	

[*] P for the difference between early and late legionellosis.

[a]Excluded from the univariate analysis.

[b]Data missing for one patient.

declined over the study period (from 57.8% in 1995–2004 to 24.6% in 2005–2016), possibly in association with better control of nosocomial sources.

As far as prevention of nosocomial infections is concerned, routine microbial analyses of tap water and use of point-of-use filtration might protect the vulnerable patients against nosocomial transmission of legionellosis [18]. Specific recommendations for HSCT units have been provided since 2000 and updated subsequently [19, 20]. In addition, HSCT centers should also follow national and international recommendations on the prevention of nosocomial legionellosis [2, 20–22]. However, policies and their effective application might vary between the countries and hospital, together with differences in awareness among clinicians, diagnostic capability or capacity, and reporting, as clearly shown for community-acquired legionellosis [23]. In this cohort, only two centers reported two patients developing legionellosis within 30 days from each other, which might be considered as possible clusters. Although this would account for only 2% of centers, the possibility of an outbreak should be always considered, as they have been reported in the HSCT setting [24, 25].

Table 2 Risk factors for legionellosis identified in the case-control study.

	Total	Group		Univariate		Multivariate	
	$N = 252$ *N* (%)	Cases, $N = 63$ *N* (%)	Controls, $N = 189$ *N* (%)	OR (95%CI)	*p*	OR (95%CI)	*p*
Age at HSCT (years)							
Median	49.5	49.8	49.5	1.08 (0.89–1.31)	0.435		
Range	0.6–102.5	7.2–67.7	0.6–102.5				
Mean (SD)	45.00 (17.26)	46.27 (14.69)	44.58 (18.05)				
Sex							
Male	149 (59.1)	42 (28.2)	107 (71.8)	1.00			
Female	103 (40.9)	21 (20.4)	82 (79.6)	0.66 (0.36–1.19)	0.17		
Diagnosis							
Myeloid	99 (39.3)	31 (31.3)	68 (68.7)	1.00	0.08		
Lymphoid	137 (54.4)	31 (22.6)	106 (77.4)	0.59 (0.31–1.13)			
Other	16 (6.3)	1 (6.3)	15 (93.8)	0.15 (0.02–1.16)			
Status							
Complete remission	126 (51.2)	33 (26.2)	93 (73.8)	1.00	0.35		
Partial remission/chronic phase/stable disease/BM failure	78 (31.7)	22 (28.2)	56 (71.8)	1.07 (0.57–2.02)	0.25		
Active disease	42 (17.1)	7 (16.7)	35 (83.3)	0.52 (0.19–1.38)	0.15		
Number of HSCT					0.0211		
First	218 (86.5)	49 (22.5)	169 (77.5)	1.00			
Second or third	34 (13.5)	14 (41.2)	20 (58.8)	2.50 (1.15–5.45)			
Type of HSCT					0.0145		0.03
Allogeneic	163 (64.7)	48 (29.4)	115 (70.6)	2.48 (1.20–5.13)		2.27 (1.08–4.80)	
Autologous	89 (35.3)	15 (16.9)	74 (83.1)	1.00		1.00	
Source					0.43		
PB	192 (76.2)	46 (24.0)	146 (76.0)	1.00			
BM or CB	60 (23.8)	17 (28.3)	43 (71.7)	1.35 (0.64–2.86)			
Conditioning regimen					0.49		
Myeloablative	183 (72.9)	44 (24.0)	139 (76.0)	1.00			
Reduced-intensity	68 (27.1)	19 (27.9)	49 (72.1)	1.28 (0.64–2.56)			
T-cell depletion					0.37		
Yes	110 (44.7)	31 (28.2)	79 (71.8)	1.34 (0.71–2.56)			
No	136 (55.3)	32 (23.5)	104 (76.5)	1.00			
ATG use					0.83		
Yes	74 (31.0)	17 (23.0)	57 (77.0)	0.93 (0.46–1.89)			
No	165 (69.0)	43 (26.1)	122 (73.9)	1.00			
Disease status in 2 years before legionellosis					1.00		
No relapse	216 (85.7)	54 (25.0)	162 (75.0)	1.00			
Relapse	36 (14.3)	9 (25.0)	27 (75.0)	1.00 (0.42–2.40)			
Engraftment before legionellosis					0.55		
Yes	194 (78.2)	49 (25.3)	145 (74.7)	1.00			
No	54 (21.8)	13 (24.1)	41 (75.9)	0.61 (0.12–3.06)			
Infection (viral, bacterial, or fungal) in 6 months before legionellosis					0.0032		
No infections	187 (74.2)	39 (20.9)	148 (79.1)	1.00		1.00	
Yes	65 (25.8)	24 (36.9)	41 (63.1)	3.22 (1.48–7.00)		2.96 (1.342–6.52)	0.007
Acute or chronic GvHD before legionellosis (Allo-HSCT only)					0.0242		
No	105 (64.8)	25 (23.8)	80 (76.2)	1.00			
Yes	57 (35.2)	23 (40.4)	34 (59.6)	2.78 (1.14–6.77)			

Furthermore, HSCT recipients are known to receive frequently antibiotics active against *Legionella*, such as fluoroquinolones, azithromycin, or even trimethoprim/sulfametoxazole, both as treatment and as prophylaxis. This might also contribute to the fact that we found only 80 cases of legionellosis in 20 years of transplant activity of all EBMT centers, resulting in an incidence over 6 time lower (0.0755%) than the one reported in Spain in solid organ transplant recipients (0.5%) [15]. When considering the data on incidence in multicenter studies, the bias to be

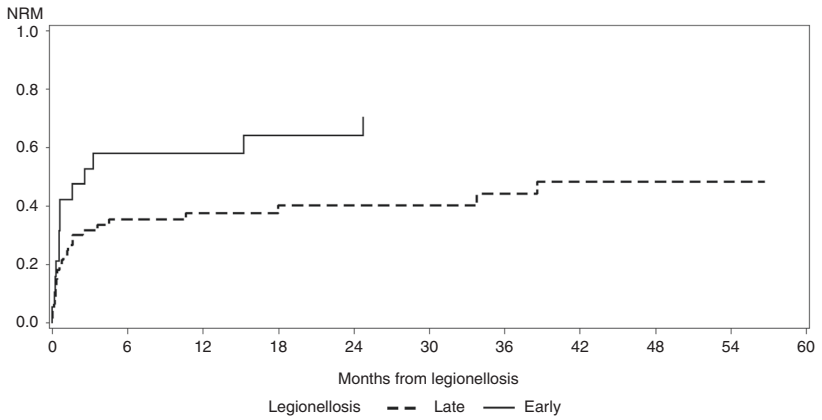

Fig. 3 Non-relapse mortality (NRM) in patients with early and late legionellosis.

Legionellosis	Patients	Events	1 year NRM	2 years NRM	p
Early	19	13	57.9 (32.1-76.9)	64.0 (35.8-82.4)	0.038
Late	61	26	37.5 (25.1-49.9)	40.3 (27.1-53.2)	

acknowledged is, on one hand, the possible risk of overestimation since the centers not reporting cases of legionellosis were excluded from calculating the rate, and on the other, underestimation of cases due to suboptimal diagnosis even in the centers in which some cases have been reported.

Regarding the general distribution of cases of legionellosis throughout the year in our cohort, it followed the same pattern as in the general population, with 60% of the cases occurring during summer and early autumn [5, 10, 13].

In previous studies, several risk factors for legionellosis were established, such as chronic lung disease and smoking, older age, immunocompromised state, anti-TNF-alfa therapy, and other biological treatments [26–28], steroid treatment [29], and hairy cell leukemia [5, 13]. However, none of the studies specifically focused on the HSCT setting, and only a few of them included HSCT recipients [11]. In our case-control study, allogeneic transplant, previous HSCT, recent other infection, or GvHD was associated with a 2.5–3 fold increased risk of legionellosis. Although we did not assess the role of single immunosuppressive treatments separately, the aforementioned characteristics are closely associated with a significantly impaired immunity and may help to identify the patients at the highest risk. However, it is noteworthy that the underlying disease, its phase, or increasing age were not found to be predictors of legionellosis. Indeed, the lack of association between legionellosis and the relapse of the underlying disease observed in our cohort is in contrast with another experience in cancer patients, in whom 80% had an active malignancy [30].

The high mortality in legionellosis is another important finding emerging from this study. The overall (65%) and *Legionella*-associated (33.8%) fatality rate in this cohort is significantly higher than in other reports, such as 14–17% fatality rate reported in SOT [15], 18% in cancer patients, including those with hematological malignancies and HSCT [11], 19% in the immunocompromised patients treated with biological therapies [27], vs. 2.9–6.1% in the cohorts with community-acquired pneumonia due to *Legionella* [31, 32]. High mortality in legionellosis might stem from late diagnosis and treatment or from a severely immunocompromised state. The delay and difficulty in diagnosing legionellosis might be improved with the wide use of urinary antigens, as suggested by more than doubling of the cases in the second compared to the first decade of the study. Indeed, a dramatic shift in the diagnosis of legionellosis has occurred with the introduction of urinary antigens, which led to the diagnosis of approximately 10% of cases in 1990 and 70% of cases in 1998 [33]. This improvement in rapid diagnosis might have contributed to a reported decrease in mortality in legionellosis in the general population [33], and in our cohort, with a 50% *Legionella*-associated fatality rate in 1995–1999, and 28% in 2010–2016.

Compared to other populations, there are particular challenges in the management of legionellosis in the setting of HSCT. First, a high number of other infectious and non-infectious conditions may cause similar interstitial lung infiltrates. Second, legionellosis might also present with other types of lung lesions, such as nodules with a halo or cavitations, which are typical for other pathogens (e.g., aspergillosis) in the HSCT setting [10, 13, 34, 35]. Third, in this population, there is a high prevalence of species or serogroups other than *Legionella pneumophila* serogroup 1, which cannot be detected by urinary antigen, but require culture or molecular techniques for the diagnosis [5, 10, 13].

Table 3 Risk factor for non-relapse mortality (NRM) and for death due to legionellosis.

	Total ($N = 80$)	NRM					Death due to legionellosis				
			Univariate		Multivariate			Univariate		Multivariate	
	N (%)	Events	HR (95% CI)	*p*	HR (95% CI)	*p*	Events	HR (95% CI)	*p*	HR (95% CI)	*p*
Patient age at legionellosis											
10-year increase			0.88 (0.71–1.09)	0.23				0.89 (0.70–1.14)	0.35		
Sex				0.03		0.02			0.12		
Male	56 (70.0)	23/56 (41.1)	1.00		1.00		15/56 (26.8)	1.00			
Female	24 (30.0)	16/24 (66.7)	2.04 (1.06–3.93)		2.19 (1.13–4.23)		12/24 (50.0)	1.83 (0.86–3.92)			
Underlying disease				0.70					0.51		
Myeloid	34 (42.5)	19/34 (55.9)	1.00				11/34 (32.4)	1.00			
Lymphoid	44 (55.0)	20/44 (45.5)	0.88 (0.47–1.66)				16/44 (36.4)	0.62 (0.15–2.60)			
Other[a]	2 (2.5)	0/2 (0.0)					0/2 (0.0)				
Disease status at latest HSCT[b]				0.66					0.47		
Complete remission	38 (48.1)	21/38 (55.3)	1.00				12/38 (31.6)	1.00			
Partial remission/chronic phase/stable disease/BM failure	29 (36.7)	12/29 (41.4)	0.72 (0.35–1.47)				9/29 (31.0)	1.03 (0.43–2.44)			
Active disease	12 (15.2)	6/12 (50.0)	0.91 (0.37–2.28)				6/12 (50.0)	1.79 (0.67–4.78)			
The decade of HSCT after which legionellosis occurred											
1995–1999	6 (7.5)	3/6 (50.0)					3/6 (50.0)				
2000–2004	20 (25.0)	11/20 (55.0)					8/20 (40.0)				
2005–2009	22 (27.5)	12/22 (54.5)					7/22 (31.8)				
2010–2016	32 (40.0)	13/32 (40.6)					9/32 (28.1)				
Period of HSCT after which legionellosis occurred				0.38					0.24		
1995–2004	26 (32.5)	14/26 (53.8)	1.00				11/26 (42.3)	1.00			
2005–2016	54 (67.5)	25/54 (46.3)	0.75 (0.39–1.44)				16/54 (29.6)	0.63 (0.29–1.35)			
Season				0.52					0.48		
Warm: April–September	48 (60.0)	14/48 (29.2)	1.00				22/48 (45.8)	1.00			
Cold: October–March	32 (40.0)	13/32 (40.6)	1.23 (0.65–2.33)				17/32 (53.1)	1.32 (0.62–2.80)			
HSCT number transplant				0.20					0.35		
First	60 (75.9)	32/60 (53.3)	1.00				22/60 (36.7)	1.00			
Second or more	19 (24.1)	7/19 (36.8)	0.58 (0.26–1.32)				5/19 (26.3)	0.63 (0.24–1.66)			
HSCT transplant				0.64					0.56		
Allogeneic	56 (70.0)	30/56 (53.6)	1.19 (0.57–2.52)				18/56 (32.1)	0.79 (0.36–1.76)			
Autologous	24 (30.0)	9/24 (37.5)	1.00				9/24 (37.5)	1.00			
Source				0.53					0.81		
PB	58 (72.5)	27/58 (46.6)	1.00				20/58 (34.5)	1.00			
BM or CB	22 (27.5)	12/22 (54.5)	1.24 (0.63–2.45)				7/22 (31.8)	0.90 (0.38–2.13)			

Table 3 (continued)

	Total ($N = 80$)	NRM					Death due to legionellosis				
			Univariate		Multivariate			Univariate		Multivariate	
	N (%)	Events	HR (95% CI)	*p*	HR (95% CI)	*p*	Events	HR (95% CI)	*p*	HR (95% CI)	*p*
Conditioning regimen				0.35					0.49		
Myeloablative	58 (72.5)	30/58 (51.7)	1.00				21/58 (36.2)	1.00			
Reduced-intensity	22 (27.5)	9/22 (40.9)	0.70 (0.33–1.48)				6/22 (27.3)	0.73 (0.29–1.80)			
T-cell depletion				0.07					0.78		
Yes	34 (42.5)	21/34 (61.8)	1.81 (0.96–3.40)				12/34 (35.3)	1.11 (0.52–2.38)			
No	46 (57.5)	18/46 (39.1)	1.00				15/46 (32.6)	1.00			
ATG use				0.57					0.58		
Yes	19 (25.0)	10/19 (52.6)	1.24 (0.59–2.57)				5/19 (26.3)	0.76 (0.28–2.03)			
No	57 (75.0)	26/57 (45.6)	1.00				19/57 (33.3)				
Disease status in the two years before legionella				0.35					0.44		
Relapse	12 (15.0)	4/12 (33.3)	0.61 (0.22–1.73)				3/12 (25.0)	0.63 (0.19–2.08)			
No relapse	68 (85.0)	35/68 (51.5)	1.00				24/68 (35.3)	1.00			
Engraftment before legionellosis				0.076					1		
Yes	64 (81.0)	28/64 (43.8)	1.00				21/64 (32.8)	1.00			
No	15 (19.0)	10/15 (66.7)	1.95 (0.93–4.09)				5/15 (33.3)	1.00 (0.38–2.66)			
Infection (viral, bacterial, or fungal) in the 6 months pre legionellosis				0.31					0.46		
No	53 (66.3)	24/53 (45.3)	1.00				16/53 (30.2)	1.00			
Yes	27 (33.8)	15/27 (55.6)	1.40 (0.73–2.70)				11/27 (40.7)	1.34 (0.62–2.89)			
Acute or chronic GvHD before legionellosis (Allo-HSCT only)				0.90					0.17		
No	26 (46.4)	14/26 (53.8)	1.00				6/26 (23.1)	1.00			
Yes	30 (53.6)	16/30 (53.3)	1.05 (0.51–2.15)				12/30 (40.0)	1.97 (0.74–5.26)			
The geographical region of the country				0.04		0.036			0.01		0.01
North-western	58 (72.5)	27/58 (46.6)	1.00		1.00		15/58 (25.9)	1.00		1.00	
South-eastern	22 (27.5)	12/22 (54.5)	2.09 (1.02–4.29)		2.16 (1.05–4.44)		12/22 (54.5)	2.59 (1.21–5.56)		2.59 (1.21–5.56)	
High legionellosis incidence countries				0.20					0.33		
No (other)	27 (33.8)	12/27 (44.4)	1.00				7/27 (25.9)	1.00			
Yes (Italy, Spain, France, the Netherlands, Denmark)	53 (66.3)	27/53 (50.9)	1.58 (0.78–3.17)				20/53 (37.7)	1.54 (0.65–3.64)			
Early legionellosis (within 30 days)				0.02		0.021			0.37		
No	61 (76.3)	26/61 (42.6)	1.00		1.00		19/61 (31.1)	1.00			
Yes	19 (23.8)	13/19 (68.4)	2.27 (1.14–4.50)		2.24 (1.13–4.46)		8/13 (42.1)	1.46 (0.64–3.34)			

ATG antithymocyte globulin, *BM* bone marrow, *CB* cord blood, *HR* hazard ratio, *PB* peripheral blood.

[a]Excluded from the univariate and multivariate analyses.

[b]Data missing for one patient.

In fact, in recent experiences from two cancer centers, only 10 (31%) of 32 typed strains were *L. pneumophila* serogroup 1 [11], and species other than *L. pneumophila* predominated in patients with hematological malignancies or HSCT recipients [10, 11]. Indeed, in these two cohorts, patients with hematological malignancies accounted for 58% (18/31) and 72% (13/18) of cases due to *L. pneumophila*, and for 89% (8/9) and 93% (14/15) of cases due to other *Legionella* species [10, 11]. Finally, recurrent or persistent legionellosis has been reported in HSCT recipients, which in certain cases might require surgical treatment [36–38]. All these issues might possibly contribute to a very high fatality rate reported in this HSCT cohort.

The limitations of our study stem from a retrospective registry-based design, which lacked detailed information on clinical presentation or microbiological details. In particular, data on testing strategies were not available and might lead to underdiagnosis of *Legionella* cases, particularly of species other than serotype-1 *L. pneumophila*, which are not detected by urinary antigen. In general, the difficulties in culture-based diagnosis might have contributed to the underdiagnosis of this infection, as reported both in general and in the HSCT population. Thus, it is particularly important to keep in mind our result of the high rate of nosocomial cases and improve diagnostic strategies in this population. In addition, there were no data on the isolated species, disease severity other than data on attributable mortality, and treatment. However, such a design allowed us to report data from the highest number of HSCT recipients and to search for predictors of this infection in a case-control study design. Finally, taking into consideration the number of cases reported from single centers (up to 12 HSCT cases in 15 years), we acknowledge the possibility of underreporting of cases, both because this information was not mandatory to report and because late community-acquired cases might be missed if occurring late after HSCT [10, 11, 39]. To at least partially correct this error, we calculated the incidence of legionellosis only taking into consideration the centers that did report the cases.

In conclusion, legionellosis in HSCT is rare, but frequently of nosocomial origin, and it is associated with significant mortality. Adequate prevention measures and rapid diagnosis are mandatory, with diagnostic methods suitable also for infections due to species other than *Legionella pneumophila*. Further studies should focus on the updated epidemiology, and prompt diagnostic and treatment protocols.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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