Nocardia infections in hematopoietic cell transplant recipients: a multicenter international retrospective study of the Infectious Diseases Working Party (IDWP) of the European Society for Blood and Marrow Transplantation (EBMT)

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Summary

Nocardiosis is a late post-HCT infection usually manifesting as a pulmonary disease with frequent dissemination, brain infection and bacteremia. Brain imaging should be performed in HCT recipients with nocardiosis regardless of neurological symptoms. Overall mortality is high.

Abstract

Background. Nocardiosis is rare after hematopoietic cell transplantation (HCT). Little is known regarding its presentation, management, and outcome in this population.

Methods. In this retrospective international study, we reviewed nocardiosis episodes in HCT recipients (01.01.2000-31.12.2018; 135 transplant centers; 33 countries) and described their clinical, microbiological, radiological, and outcome characteristics.

Results. We identified 81 nocardiosis episodes in 74 allo- and 7 auto-HCT recipients. Nocardiosis occurred at a median of 8 (IQR 4–18) months post-HCT. The most frequently involved organs were lungs (70/81; 86%) and brain (30/81; 37%); 29 (36%) patients were afebrile; 46/81 (57%) had disseminated infections. The most common lung imaging findings were consolidations (33/68; 49%) or nodules (32/68; 47%); and brain imaging findings were multiple brain abscesses (19/30; 63%). 10/30 (33%) patients with brain involvement lacked neurological symptoms. 14/48 (29%) patients were bacteremic. *N. farcinica* was the most common among molecularly identified species (27%, 12/44). Highest susceptibility rates were reported to linezolid 45/45 (100%), amikacin 56/57 (98%), trimethoprim-sulfamethoxazole 57/63 (90%), and imipenem 49/57 (86%).

One-year and last follow-up (IQR: 4-42.5 months) all-cause mortality were 40% (32/81) and 52% (42/81), respectively. In the multivariable analysis, underlying disease not in complete remission (HR 2.81, 95%CI 1.32-5.95), and prior bacterial infection (HR 3.42, 95%CI 1.62-7.22) were associated with higher one-year all-cause mortality.

Conclusions. Nocardiosis is a late post-HCT infection usually manifesting as a pulmonary disease with frequent dissemination, brain infection and bacteremia. Brain imaging should be performed in HCT recipients with nocardiosis regardless of neurological symptoms. Overall mortality is high.

Keywords

Nocardiosis

Hematopoietic cell transplantation

Central nervous system infection

Mortality

Introduction

Nocardia spp. are environmental Gram-positive bacilli that may cause infection in humans. Because inhalation is the commonest *Nocardia* spp. acquisition route, the lung is the most frequently involved organ. Dissemination to the brain, skin, soft tissues and/or other body sites is common [1].

While *Nocardia* infection (nocardiosis) may occur in previously healthy people, most of those infected are immunocompromised, with solid organ transplant (SOT) recipients the most affected [2]. SOT recipients with nocardiosis have been the subject of several studies in recent years [1, 3, 4], but very little is known regarding the disease in hematopoietic cell transplant (HCT) recipients.

Available reports indicate that nocardiosis is rare after HCT, occurring in 0.3-1.7% of allogeneic and 0.2% of autologous HCT recipients [5-7]. Since 2000, one multi-center case series (10 patients with hematological malignancies) [8], seven small single-center HCT case series [5, 6, 9-13], and one retrospective analysis of 112 cases, including 16 HCT recipients [14] have been published.

The current international retrospective study aims to clarify the characteristics and outcome of nocardiosis in HCT recipients.

Materials and Methods

Study objectives

Our primary objective was to describe the clinical, radiological, and microbiological characteristics of nocardiosis in HCT recipients.

Secondary objectives were: (1) assessing all-cause mortality at one year after nocardiosis, and (2) determining factors associated with one-year all-cause mortality.

Study Design and Settings

This was an international retrospective study supported by the Infectious Diseases Working Party (IDWP) of the European Society for Blood and Marrow Transplantation (EBMT). Demographic and HCT-related data were obtained using the EBMT database. Data on nocardiosis episodes were collected using a dedicated case report form (see Appendix and Supplementary Table 1 for details including data collected and definitions used [3, 4, 15]).

Inclusion criteria

Adults (≥ 18 years of age) and children meeting the following criteria were eligible: (1) allogeneic or autologous HCT history, (2) *Nocardia* spp. isolation in a clinical sample after start of HCT conditioning, (3) suggestive clinical and/or radiological nocardiosis signs, and (4) diagnosis made between 01.01.2000 and 31.12.2018.

Microbiology

Diagnosis required *Nocardia* growth in a clinical sample. *Nocardia* identification at the genus level required one of the following methods, performed on bacterial colonies:

1) Molecular identification (*i.e.*, gene sequencing);

2) Matrix-assisted laser desorption/ionization time-of flight spectrometry (MALDI-TOF);

3) Typical bacterial colonies growth (dry chalky-white appearance, buff or pigmented, waxy cerebriform colonies) in association with suggestive microscopy (partial acid-fast stained Gram-positive branching filamentous organisms).

Identification at the species level required the amplification and sequencing of a gene fragment coding for the 16S ribosomal RNA or *hsp65* [2].

Antibiotic susceptibility testing (AST) was performed by local laboratories. The method of testing was recorded. Pathogens classified as *in vitro* "resistant" or "intermediate" were considered as "resistant". Only susceptibility to antibiotics for which CLSI breakpoints are available is reported [16].

Statistical analysis

Clinical, radiological, laboratory, treatment and outcome data were described for the first episode of nocardiosis. Continuous data are presented as median (Interquartile range, IQR). Categorical data are presented as numbers and percentage of total. One-year all-cause survival was assessed using Kaplan-Meier estimator and compared between groups using log-rank tests. To identify factors associated with one-year all-cause survival, a univariate Cox regression model was used. Variables with a p-value <0.05 on univariable analysis were included in a multivariable Cox regression model. A bilateral p-value <0.05 was considered as statistically significant. All analyses were performed using the statistical software SAS v.9.4.

Results

Participating centers and included patients

We invited 587 EBMT centers to participate, 135/587 agreed (representing 33 countries). The highest response rate was in Belgium, France, and Israel (29/81 centers [36%], compared with 106/506 centers [21%] in the 30 remaining countries; Supplementary Table 2). Among the 135 participating centres, 99/135 centers reported having no cases and 36/135 centers reported at least one case. In 19/33 countries, all 26 centers (27337 HCT performed during the study period) reported no cases. In the 14/33 other countries, 73 centers reported no cases (77178 HCT performed); and 36 centers reported 81 nocardiosis cases including 57 cases from 19 Belgian, French, and Israeli centers (36126 HCT performed); and 24 cases from 17 centers from 11 other countries (28036 HCT performed).

Supplementary Figure 1 presents distribution of nocardiosis cases according to the year of diagnosis. Four were children. Most patients were allo-HCT recipients (74/81, 91%). The most common underlying disease was acute leukemia (25/81, 31%). None of the patients was severely neutropenic at diagnosis of nocardiosis; 47% were severely lymphocytopenic. Half of the patients had comorbidities, mainly underlying pulmonary disease (17/81, 21%) and diabetes mellitus (11/81, 14%) (Table 1).

Nocardiosis presentation (Table 2)

Nocardiosis occurred at a median of eight (IQR 4-18) months following HCT (Figure 1). The most frequently involved organs were lungs (70/81, 86%) and brain (30/81, 37%). Most infections were disseminated (46/81, 57%). One third of the patients were afebrile.

Lung imaging was performed in 75/81 (93%) patients (CT-scan in 66 patients, FDG-PET-CT in one patient, and X-ray only in 8 patients). Lung lesions were observed in 68/75 (91%) cases. The most frequently reported lesions were consolidations (n=33, 49%) and nodules (n=32, 47%); among nodules, 19/32 (59%) were cavitated.

Brain imaging was performed in 59/81 patients, being CT-scan in 36/59 (61%) and MRI in 38/59 (64%) cases. In 30 cases (51%), results were abnormal. Most common brain imaging finding was multiple brain abscesses (n=19, 63%). Among 35 patients who had brain imaging despite having no neurological abnormalities on clinical examination, the presence of brain abscess(es) was detected in 10/35 cases (29%); meaning that 10/30 (33%) of patients with brain involvement were without neurological symptoms. Brain involvement generally occurred in the setting of disseminated infection (27/30, 90%).

A total number of 16/81 (20%) patients had concomitant microbiologically documented infection/s, including bacteremia (6/81), other bacterial infection (4/81), invasive fungal disease (3/81), CMV disease (1/81), or respiratory viral infection (4/81) (see details in Supplementary table 3).

Microbiological characteristics

Blood cultures were collected in 48 (59%) patients, 14 (29%) of them had *Nocardia* bacteremia; three of these 14 patients (all with central line at diagnosis of nocardiosis) had an isolated bacteremia, i.e. without any other site involved.

Other procedures most commonly performed to diagnose nocardiosis were bronchoalveolar lavage (BAL), sputum culture, brain and lung biopsy (Supplementary table 4).

Microbiological identification method was reported in 77/81 cases (95%). Specifically, molecular biology was performed in 44/81 cases (54%), allowing identification at the species level. The remaining cases were identified using either MALDI-TOF (28/81, 35%) or identification of typical colonies with suggestive microscopy (5/81, 6%), and were therefore reported as *Nocardia* spp. *N. farcinica* was the most frequent species (27%, 12/44; Figure 2). The proportion of brain (6/12; 50% vs. 7/32; 22%; p=0.1) and skin/soft tissue/muscle involvement (4/12; 33% vs. 2/32; 6%; p=0.04) was higher in infections caused by *N. farcinica* than in those due to other species.

AST (Supplementary Table 5) was performed by E-test (n=37; 46%), broth microdilution (n=9; 11%), or other method (n=3; 4%); in 23 (28%) method was not reported and in nine (11%) cases susceptibility was not reported. All 45 tested isolates were found to be susceptible to linezolid, 56/57 (98%) to amikacin, 57/63 (90%) to TMP-SMX, and 49/57 (86%) to imipenem. Thirty-three (42%) of 79 patients were receiving TMP-SMX prophylaxis at the time of nocardiosis. The proportion of TMP-SMX-resistant pathogens was 4/25 (16%) among patients who received TMP-SMX prophylaxis at the time of nocardiosis, as compared to 2/36 (6%) among those who did not.

Management of nocardiosis

Antibiotic therapy data was available in 80 patients (Supplementary table 6). One patient did not receive treatment for an ulcerative skin lesion and recovered following reduction of immune suppression. The most frequently used drugs were TMP-SMX (54/79; 68%); imipenem (36; 46%), and amikacin (35; 44%). Drug-related adverse events were recorded in 27/80 patients (34%); leading to

drug discontinuation in 18/80 patients (23%) (Supplementary table 7). Treatment duration was known for 72/80 treated patients: 49 patients completed antibiotic therapy (median duration 7 months, IQR 3.5-12 months), 20 patients died before antibiotic cessation, and 3 patients were still on antibiotics at last follow-up (range 4-10 months).

Among the three patients who had isolated *Nocardia* bacteremia, two had infections that were probably central line related, with persistence of bacteremia until line removal (at days 7 and 9). However, catheter tips cultures remained negative, differential time-to-positivity was not measured, and semiquantative/quantative cultures were not performed. One of these two patients received 4.5 months of antibiotic treatment and died 5.5 months after treatment cessation (death not attributed to nocardiosis); the second patient received 10 months of antibiotic treatment and was alive and cured at last follow-up. The third patient had two days of *Nocardia* bacteremia and was cured without line removal (unknown duration of antimicrobial therapy).

After completion of anti-*Nocardia* therapy, 14 patients received secondary TMP-SMX prophylaxis (low dose TMP-SMX in 12 patients, and 800/160 mg/day in two patients). Sixteen patients underwent a surgical procedure for treatment of nocardiosis.

Outcome of nocardiosis

One-year all-cause mortality was 40% (32/81). The median time to death in these patients was 2.5 months (IQR: 1–6 months). In 11 (14%) patients (10 following allo-HCT and one following auto-HCT) who developed nocardiosis a median of seven (range 2-35) months after HCT, death was attributed to nocardiosis.

Median duration of follow-up for the entire cohort was 18 months; IQR: 4-42.5 months. At last follow-up, all-cause mortality was 52% (42/81).

Significant or approaching significance parameters associated with one-year all-cause mortality are presented in Supplementary table 8. In univariable and multivariable analyses,

not being in continued complete remission of the underlying disease, and prior bacterial infection were significantly associated with mortality (Table 3; Figures 3 a-c). Age, gender, underlying disease and its status at HCT, presence of comorbidities, HCT number, stem cell source, HLA matching, conditioning, acute or chronic graft-versus-host disease (GVHD) at the time of nocardiosis, immune suppressive medications number, lung or brain involvement, bacteremia, disseminated disease, lymphopenia and *Nocardia* species were not associated with one-year mortality.

Among the 64 patients who completed antimicrobial therapy, three (5%) experienced nocardiosis relapse (Supplementary table 9).

Comparison of early and late nocardiosis (≤24 versus >24 months post-HCT).

We compared 66/81 (82%) early to 15/81 (18%) late post-HCT nocardiosis cases. Median time from symptoms onset to diagnosis was shorter in early than in late cases (9 days [IQR: 5-20] versus 26 days [10-45], p=0.03). Use of antilymphocyte agents (as anti-thymocyte globulin, anti-lymphocyte globulin, alemtuzumab) was more frequent in patients with early than in those with late nocardiosis (42/64 [66%] versus 5/15 [33%], p=0.02). The proportions of disseminated disease (40/66 [61%] versus 6/15 [40%]) and brain involvement (27/66 [41%] versus 3/15 [20%]) were higher in patients with early vs. late nocardiosis, but these differences were not statistically significant (p=0.1 for both comparisons). There were no other statistically significant differences in terms of clinical presentation, imaging findings, previous infections, relapse, and mortality.

Discussion

This multinational retrospective study of nocardiosis is the largest performed in HCT patients, to date. We present clinical, radiological, microbiological, treatment and outcome data for 81 HCT recipients with nocardiosis. Nocardiosis after HCT is rare although severe

infection, with 40% mortality at one year after diagnosis, directly attributed to the infection in 11 patients (14%).

The seven single-center case series reported since 2000 each comprise 5 to 15 nocardiosis cases among adults following allo-HCT [5, 6, 9-13]. A review of 50 cases in adults after allo-HCT — these case series and others — has recently been published [10]. In addition, a retrospective study recently compared nocardiosis between 67 immunocompromised (including 16 HCT recipients) and 45 immunocompetent patients [14].

We found nocardiosis to be a late post-HCT complication, with a median time of 8 months after transplant. This is a shorter interval than for SOT recipients, in whom nocardiosis is generally diagnosed after the first post-transplant year [3, 12]. In line with this finding, and as observed elsewhere, none of the patients in our study was neutropenic at the time of nocardiosis [14]. Supporting the importance of T-cell immunity in preventing nocardiosis [2, 13], half our patients were lymphocytopenic, 73% received steroids, 60% had acute or chronic GVHD at the time of infection, and 41% had received antilymphocyte agent within one year before nocardiosis. Nocardiosis appears to be mainly a concern for allo-HCT patients, with only a few following autologous HCT reported in our study, as well as in the literature [17].

As seen in other populations [1, 14], lung nocardiosis was the most common type of infection in HCT recipients. In our study, lung consolidations and nodules were almost equally reported in about half the patients, with most nodules cavitated. Diagnostically, this association between nocardiosis and lung nodules is important because such nodules in allo-HCT recipients often suggest invasive mold infection. Another important observation is that a third of our patients were afebrile at presentation. Thus, even in afebrile patients, presence of late post-HCT lung consolidations or nodules should prompt investigation for nocardiosis.

BAL was frequently performed with high yield — but sputum examination was positive in

43% of cases, indicating that this simple examination should be performed as a complementary test whenever possible or when BAL is contraindicated.

Brain involvement was frequent in our study. It was found in almost 40% of patients, a rate higher than in previous reports looking at SOT recipients and other immunocompromised hosts [3, 14]. Previously published studies were often too small (and/or included non-HCT patients) to provide a precise estimate of the brain involvement frequency in HCT patients with nocardiosis. Notably, asymptomatic brain involvement was frequent in our cohort, with detection of brain abscess(es) in 29% of patients who underwent brain imaging despite having no neurological manifestations. The frequency of brain involvement may thus probably have been underestimated because not all patients had brain imaging. Asymptomatic brain involvement, previously described in a non-HCT setting [3], emphasizes the importance of systematic brain imaging in immunocompromised patients with nocardiosis.

Disseminated disease was more frequent in our study (57%) than in SOT patients (42.7%) [3] or in a heterogenous immunocompromised patients cohort (27%) [14]. Forty-two percent of allo-HCT patients were receiving TMP-SMX anti-*Pneumocystis* prophylaxis at time of nocardiosis. This rate is higher than in previous HCT studies (23% in a recent literature review [10]), and appears to substantiate that low-dose TMP-SMX does not effectively prevent nocardiosis after HCT. Whereas some researchers have suggested that TMP-SMX prophylaxis may reduce nocardiosis risk after HCT [11], evidence from case-control studies suggests that low-dose TMP-SMX (≤ 800/160 mg, three times a week) does not prevent nocardiosis in SOT recipients [3, 18]. Its effect on nocardiosis risk in HCT recipients requires further study.

Optimal management of nocardiosis has not been determined for HCT recipients. Its treatment is challenging due to antibiotic toxicity (e.g, myelotoxicity), resistance and

unknown efficacy of different agents. As in other studies, TMP-SMX was frequently part of the treatment protocol, followed by imipenem and amikacin; and TMP-SMX resistance was infrequent [10, 11, 14]. A third of patients developed side effects attributed to antibiotics, some of which led to treatment discontinuation — a finding consistent with limited tolerance reported in other series [6, 12].

Available data suggest that patients with nocardiosis have a greater mortality risk than control HCT patients [7]. Case series found an overall mortality rate of 40-70%, with nocardiosis being the cause of death in 0-33% of infected patients [5, 6, 9-12, 14]. Similarly, in our study, 40% of patients died within a year of nocardiosis diagnosis; in 14%, death was attributed to nocardiosis. Identifying variables associated with poor outcome may be of major interest in this population. In our study, one-year mortality was associated with not being in continued complete remission of the underlying disease and prior bacterial infection.

Our study has several limitations. First, it is retrospective, and some data are missing. We were unable to determine the effects of interventions (for example, therapy duration, drugs used, combination vs. monotherapy) on one-year survival because of the retrospective study design, treatment protocols heterogeneity, significant missing data regarding isolate susceptibility and the therapy timing. Second, species distribution data were limited, with molecular identification performed in only 44 isolates. Third, AST was not performed in a substantial proportion of isolates. Moreover, agents for which CLSI breakpoints are not determined were frequently used. This underscores the importance of further studies to define the best treatment strategies and enable distinct standardized treatment recommendations. Lastly, although considerable effort was invested in exhaustive reporting of nocardiosis in participating centers, no conclusions concerning overall nocardiosis incidence in HCT recipients can be drawn, since a significant proportion of contacted centers did not respond.

The number of nocardiosis cases and response rate were higher in Belgium, France, and Israel compared to other countries. Significant heterogeneity in response rates (with more respondents coming from the countries where the study coordinators are from) is common in multicenter European studies [3,19]. In the future, similar studies should ideally have a more balanced representation and coordinators in each participating country. Other potential explanations for the differences observed between countries in terms of numbers of cases include differences in patients` characteristics, immune suppression, post-transplant TMP-SMX prophylaxis, and methods used to diagnose nocardiosis.

Our study, nevertheless, has important strengths. Comprising the largest cohort of HCT recipients with nocardiosis during the past 20 years, it enabled a summary of microbiological, clinical, imaging and outcome data, and highlighted specific features useful for managing this infrequent complication. In particular, we demonstrated the importance of brain imaging in all HCT patients with nocardiosis, irrespective of neurological signs or symptoms. This is the first study that enabled multivariable analysis of factors associated with one-year mortality following nocardiosis.

Conclusions

In this large retrospective international cohort, we found that nocardiosis is a late post-HCT infection that usually manifests as a pulmonary disease with frequent dissemination and brain involvement. Overall mortality is high. Underlying disease status and concurrent infections appear to play key roles in prognosis.

The partial results of this study were presented orally at the 47th virtual Annual EBMT Conference, 2021.

NOTES

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Table 1. Patient characteristics

Patients characteristics	**Total, n (%)**
Number of patients	81 (100)
Male	58 (72)
Age at HCT (years) median (range)	50.2 (1.2 –71.1)
Children (<18 years)	4 (5)
Underlying disease	
Acute myeloid leukemia	18 (22)
Acute lymphoblastic leukemia	7 (9)
Myelodysplastic/myeloproliferative + chronic myeloid leukemia	9 (24)
Lymphoma	16 (20)
Plasma cell disorders	11 (14)
Chronic lymphocytic leukemia	7 (9)
Others	3 (4)
Type of HCT	

Allogeneic	74 (91)
Autologous	7 (9)
Chronological number of the most recent HCT for this patient	
First	57 (70)
Second or more	24 (30)
Source of stem cells	
Bone marrow/cord blood	15/1 (20)
Peripheral blood	65 (80)
HLA match type (n=74)	
Matched related	31 (42)
Matched unrelated	25 (34)
Mismatched	18 (24)
Conditioning (n=80): myeloablative (vs. non-myeloablative)	44 (55)
Acute GVHD at the time of Nocardiosis n=69	12 (17)
Chronic GvHD at the time of Nocardiosis n = 72	31 (43)
Antilymphocyte agents any time before nocardiosis (as anti-thymocyte globulin, anti-lymphocyte globulin, alemtusumab)	48 (59)
Within 12 months before nocardiosis	33 (41)

Immune suppressive medications at the time of nocardiosis n=80	69 (86)
Steroids	58 (73)
<1 mg/kg/day	42 (53)
≥1 mg/kg/day	10 (12)
dose unknown	6 (8)
Tacrolimus	23 (29)
Cyclosporine	22 (28)
Mycophenolate mofetil	12 (15)
Others	8 (10)
Two or more immune suppressive drugs	42 (52)
Underlying disease status after HCT at the time of Nocardiosis n = 78	
Continued complete remission	59 (76)
Never in complete remission / relapse/progression	19 (24)
Comorbidities at the time of nocardiosis	41 (51)
Pulmonary disease	17 (21)
Diabetes mellitus	11 (14)
Renal disease	6 (7)
Solid tumor	4 (5)

Other	22 (27)
Trimethoprim-sulfamethoxazole prophylaxis at nocardiosis diagnosis n=79	33 (42)

HCT hematopoietic cell transplantation

GVHD Graft versus host disease

Table 2. **Nocardiosis episodes characteristics**

Episodes characteristics	**Total (%)**	**Allo-HCT (%)**	**Auto-HCT (%)**
Number of patients	81 (100)	74 (100)	7 (100)
Time in days from onset of symptoms to the day of diagnosis: median (IQR) n = 70	12 (5.00 - 23.25)	12.0 (5.0 - 22.0) (n=65)	7.0 (7.0- 45.5) (n=5)
Time in months from HCT to onset of symptoms or (if unavailable) to the day of diagnosis: median (IQR)	8.0 (4-18)	8.0 (4-17.25)	7.0 (1-39.0)
Involved organs			
Localized disease	**35 (43)**	**32 (43)**	**3 (43)**
Lung	27 (33)	24 (32)	3 (43)
Brain	3 (4)	3 (4)	0
Skin/soft tissue/muscle	3 (4)	3 (4)	0
Joint or bone	2 (3)	2 (3)	0
Disseminated disease	**46 (57)**	**42 (57)**	**4 (57)**
Lung	43 (53)	41 (55)	2 (29)
Brain	27 (33)	25 (34)	2 (29)

Skin/soft tissue/muscle	9 (11)	9 (12)	0
Joint or bone	3 (4)	3 (4)	0
Others[a]	8 (10)	8 (11)	0
Bacteremia	14/48 (29)	12/44 (27)	2/4 (50)
Clinical signs			
Fever >38°	52 (64)	48 (65)	4 (57)
Respiratory signs	**54 (67)**	**50 (68)**	**4 (57)**
Cough/sputum production	38 (47)	35 (47)	3 (43)
Dyspnea	23 (28)	21 (28)	2 (29)
Chest pain	17 (21)	15 (20)	2 (29)
Others [b]	9 (11)	9 (12)	0
Neurological signs	**24 (29)**	**21 (28)**	**3 (43)**
Headache	12 (15)	9 (12)	3 (43)
Confusion	5 (6)	5 (7)	0
Seizures	5 (6)	5 (7)	0
Other neurological signs[c]	12 (15)	12 (16)	0
Other signs			
Asthenia	17 (21)	16 (22)	1 (14)

Skin/soft tissue/muscle lesion	12 (15)	12 (16)	0
Others [d]	23 (28)	22 (28)	1 (14)
Hospitalization in the intensive care unit due to nocardiosis	20 (25)	19 (26)	1 (14)
Imaging			
Type of lung involvement n = 68			
Lung consolidation	33 (49)	31/63 (49)	2/5 (40)
Nodules	32 (47)	30/63 (48)	2/5 (40)
Nodules with cavitation	19 (28)	18/63 (29)	1/5 (20)
Pleural effusion	10 (15)	9/63 (14)	1/5 (20)
Interstitial syndrome	5 (7)	4/63 (6)	1/5 (20)
Other types [e]	6 (9)	6 /63 (10)	0
Lobes involved n = 58			
One lobe involved	20 (35)	19/54 (35)	1/4 (25)
Multilobar involvement	38 (65)	35/54 (65)	3/4 (75)
Bilateral involvement	31 (54)	28/54 (52)	3/4 (75)
Type of brain abscess n = 30			
Single lesion	11 (37)	10/28 (36)	1/2 (50)

Multiple lesions	19 (63)	18/28 (64)	1/2 (50)
Bilateral involvement	9 (30)	8/28 (29)	1/2 (50)
Infections that occurred concomitant or before nocardiosis[f]			
Bacterial infection	31 (38)	27 (37)	4 (57)
Bacteremia concomitant to nocardiosis	6 (7)	5 (7)	1 (14)
Bacteremia before nocardiosis	14 (17)	13 (18)	1 (14)
Other bacterial infections concomitant with nocardiosis	4 (5)	4 (5)	0 (0)
Other bacterial infections before nocardiosis	11 (14)	9 (12)	2 (29)
Invasive fungal disease	18 (22)	16 (22)	2 (28)
During nocardiosis	3 (4)	2 (3)	1 (14)
Before nocardiosis	15 (18)	14 (19)	1 (14)
Cytomegalovirus reactivation within 6 months before nocardiosis n = 77	29 (38)	29/71 (41)	0/6 (0)
Epstein-Barr virus reactivation within 6 months before nocardiosis n= 63	16 (25)	15 (25)	1 (25)
OUTCOME			
Relapse n = 64	3 (5)	3/57 (5)	0/7

Mortality one year after nocardiosis	32 (40)	31 (42)	1 (14)
Mortality at the last follow up	42 (52)	39 (53)	3 (43)

HCT hematopoietic cell transplantation

GVHD Graft versus host disease

[a]other organs included (n=8 patients): liver (n=4); spleen (n=2), and one each of sinuses; lymph nodes; oral lesion; spinal cord; pancreas; kidneys.

[b] other respiratory complaints: acute respiratory distress (n=5), hemoptysis (n=4)

[c] other neurological signs included: paresis (n=4); ataxia (n=3), facial nerve paralysis (n=2), behavioral changes (n=2), vomiting (n=1) and one each of blurred vision, drowsiness, speech disturbances, gate disturbances, tremor, and anosmia

[d] other signs included: weight loss (n=6), arthritis (n=4), chills (n=3), abdominal pain (n=3), limb pain and/or swelling (n=3), hypoxia (n=2), and one each of neck swelling, back pain, diarrhea, lymphadenopathy local, septic shock, sweating, syncope, ageusia, hypotension, dysphagia, renal failure

[e] others: abscess – n = 3, one each of atelectasis, lymph nodes mediastinum, tree in bud infiltrations

[f] Additional details on infections that occurred concomitant or before nocardiosis are provided in Supplementary Table 3

Table 3. Factors significantly associated with one-year all-cause mortality

Patients' characteristics	Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value
Underlying disease status after HCT at the time of nocardiosis n = 78		0.005		0.01
Continued complete remission	1.00			
Never in complete remission / relapse/progression	2.91 (1.38-6.13)		2.81 (1.32-5.95)	
Presence of bacterial infection (within 3 months prior to nocardiosis or during nocardiosis)		0.003		0.001
No	1.00			
Yes	2.91 (1.43-5.90)		3.42 (1.62-7.22)	

HCT hematopoietic cell transplantation

Figure 1. Distribution of the time (in months) between hematopoietic cell transplantation and the occurrence of nocardiosis.

Dashed line represents the median time point (8 months).

Figure 2 Distribution of *Nocardia* species (for 44/81 isolates identified by molecular method)

Footnotes: Others: one each of *N. paucivorans, N. veterana, N. mexicana* and *N. shimofuesnsis/ shigoensis*.

Figure 3 Survival curves one year after nocardiosis diagnosis. The Kaplan-Meier curves are presented up to one year after nocardiosis diagnosis. Comparison between the groups in Figure 3b and 3c was performed using log-rank test

Figure 3a Survival curve in all patients

Figure 3b Survival curves in patients in continued complete remission and in those not in continued complete remission of the underlying disease

Figure 3c Survival curves in patients with and without bacterial infections before or during nocardiosis

Footnotes: OS – overall survival; CCR - continued complete remission

Figure 1

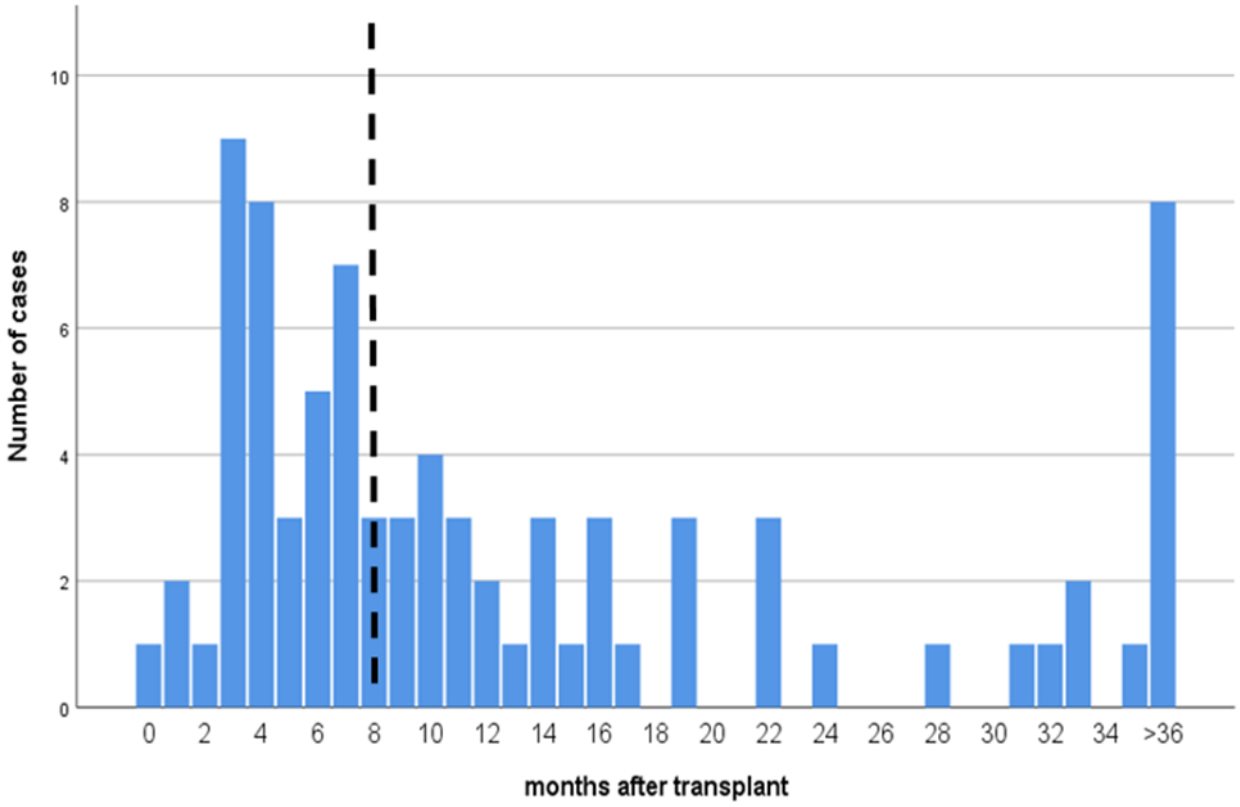


Figure 2

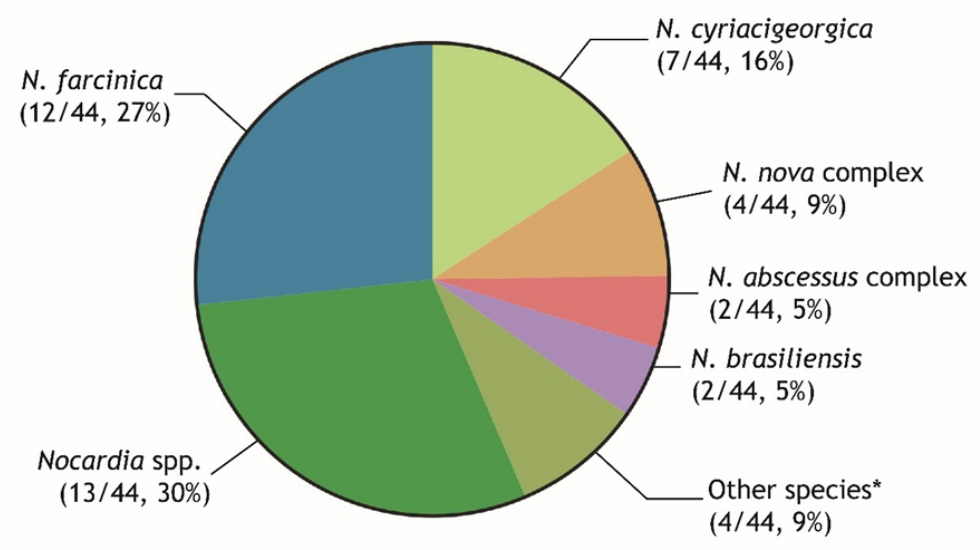
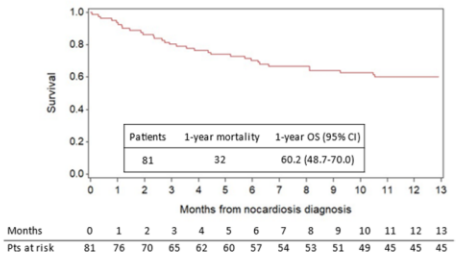
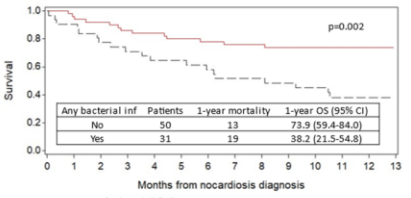


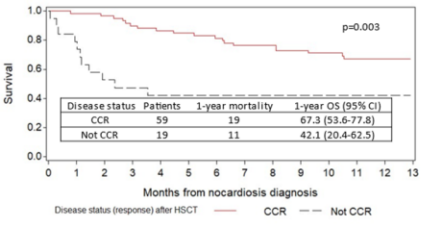
Figure 3



Months	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Pts at risk	81	76	70	65	62	60	57	54	53	51	49	45	45	45



Months	Any bacterial inf	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Pts at risk	No	50	48	46	43	42	40	39	38	37	36	35	34	34	34
	Yes	31	28	24	22	20	20	18	16	16	15	14	11	11	11



Months	Disease status	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Pts at risk	CCR	59	58	57	53	51	50	48	45	44	42	40	36	36	36
	Not CCR	19	15	10	9	8	8	8	8	8	8	8	8	8	8