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Nocardia infection in solid organ transplant recipients: a multicenter European case-control study

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40-word summary: In this European multicenter case-control study, *Nocardia* infection after organ transplantation was associated with high blood concentrations of calcineurin inhibitors, use of tacrolimus, dose of corticosteroids, patient age and length of stay in the intensive care unit after transplantation.

ABSTRACT

Background. Nocardiosis is a rare, life-threatening opportunistic infection, affecting 0.04% to 3.5% of patients after solid organ transplantation (SOT). The aim of this study was to identify risk factors for *Nocardia* infection after SOT and to describe the presentation of nocardiosis in these patients.

Methods. We performed a retrospective case-control study of adult patients diagnosed with nocardiosis after SOT between 2000 and 2014 in 36 European (France, Belgium, Switzerland, Netherlands, Spain) centers. Two control subjects per case were matched by institution, transplant date and transplanted organ. A multivariable analysis was performed using conditional logistic regression to identify risk factors for nocardiosis.

Results. One hundred and seventeen cases of nocardiosis and 234 control patients were included. Nocardiosis occurred at a median of 17.5 [range 2-244] months after transplantation. In multivariable analysis, high calcineurin inhibitor trough levels in the month before diagnosis (OR=6.11 [2.58-14.51]), use of tacrolimus (OR=2.65 [1.17-6.00]) and corticosteroid dose (OR=1.12 [1.03-1.22]) at the time of diagnosis, patient age (OR=1.04 [1.02-1.07]) and length of stay in intensive care unit after SOT (OR=1.04 [1.00-1.09]) were independently associated with development of nocardiosis; low-dose cotrimoxazole prophylaxis was not found to prevent nocardiosis. *Nocardia farcinica* was more frequently associated with brain, skin and subcutaneous tissue infections than were other *Nocardia* species. Among the 30 cases with central nervous system nocardiosis, 13 (43.3%) had no neurological symptoms.

Conclusions. We identified five risk factors for nocardiosis after SOT. Low-dose cotrimoxazole was not found to prevent *Nocardia* infection. These findings may help improve management of transplant recipients.

LIST OF ABBREVIATIONS

16S rRNA:	16S ribosomal ribonucleic acid
95% CI:	95% confidence interval
ATG:	antithymocyte globulin
CMV:	cytomegalovirus
CNS:	central nervous system
COPD:	chronic obstructive pulmonary disease
CRP:	C-reactive protein
DNA:	deoxyribonucleic acid
HIV:	human immunodeficiency virus
ICU:	intensive care unit
MALDI-TOF:	matrix-assisted laser desorption/ionization time-of-flight
MRI:	magnetic resonance imaging
NS:	not significant
OR:	odds ratio
PCR:	polymerase chain reaction
SOT:	solid organ transplantation
spp.:	species
TMP-SMX:	trimethoprim–sulfamethoxazole (cotrimoxazole)
USA:	United States of America

INTRODUCTION

Nocardia species (spp.) are ubiquitous environmental Gram-positive filamentous bacteria and can be responsible for severe opportunistic infections in humans [1]. Direct inoculation through the skin is possible [2], but most *Nocardia* infections occur via the respiratory tract, with possible subsequent dissemination to other tissues, such as brain, skin and subcutaneous tissues [1]. *Nocardia* can infect immunocompetent patients, but invasive nocardiosis is mainly observed in patients with immune deficiency [3], including that associated with corticosteroid therapy, transplantation, human immunodeficiency virus (HIV) infection [4, 5], cancer [6], chronic granulomatous disease [7] or presence of auto-antibodies against granulocyte-macrophage colony stimulating factor [8] and/or in patients with chronic lung disease [9, 10].

Solid organ transplant recipients are at risk of opportunistic events, such as *Nocardia* infections [11], and nocardiosis has been described in these patients since the early years of solid organ transplantation (SOT) in the 1960s [12]. The risk of developing nocardiosis after SOT varies with the type of organ transplanted, the highest infection rates being observed after lung transplantation (estimates between 0.8% and 3.5%) and the lowest after liver and kidney transplantations (0.04-1.2%) [13-19]. *Nocardia* infection after SOT is a severe disease associated with a mortality rate of about 20% [19]. Managing these opportunistic infections is difficult, especially because of the need for long-term treatment (usually 6 to 12 months) to avoid relapses, and the toxicity of the antibiotics, particularly when combined with immunosuppressive drugs [11].

Despite these therapeutic challenges and poor outcomes, little is known about the risk factors for nocardiosis after SOT. Conducting prospective studies is difficult because of the low incidence of this infection. In 2007, Peleg and colleagues reported a case-control study of nocardiosis after SOT [15]. In this study, 35 cases and 70 controls were included and three factors were significantly associated with an increased risk of *Nocardia* infection: use of high-dose steroids, a high median calcineurin inhibitor level in the month prior to infection and cytomegalovirus (CMV) disease

in the preceding six months. This study provided considerable insight into our understanding of nocardiosis after SOT, but was conducted in a single center with a limited number of cases. To increase the statistical power to detect risk factors, we therefore conducted a retrospective case-control study of *Nocardia* infections in a large number of SOT centers in Western Europe.

Our main objective was to identify risk factors for *Nocardia* infections in SOT recipients. A secondary aim was to describe the clinical, biological and radiological presentation of nocardiosis in this population.

MATERIAL & METHODS

Study design, setting and participants

This was an international nested case-control study. All Belgian, French and Swiss hospitals with an SOT program were asked to participate in the study and two other European transplantation centers also took part: Leiden University Medical Center (Leiden, The Netherlands) and University Hospital 12 de Octubre (Madrid, Spain). To avoid selection bias, cases were identified in each institution using a systematic and comprehensive screening of local microbiological, pathology and transplantation databases. In France, the study was approved by the CPP Ile-de-France I Ethical board (March 7, 2014). In other countries, the participating centers obtained approval from their respective Ethics Committees before joining the study.

Inclusion criteria

Patients meeting all the following criteria were included in the study: (1) SOT recipient; (2) *Nocardia* spp. isolated in a clinical sample after transplantation; (3) presence of signs and/or symptoms compatible with nocardiosis; (4) diagnosis made between January 2000 and December 2014. We selected two matched controls for each case. Matched controls were SOT recipients who: (1) had received the same type of transplanted organ in the same institution as the case; (2) had no evidence

of *Nocardia* infection up to the date of inclusion; (3) had received their transplant at about the same time as the case; (4) had survived as long as the case had prior to the diagnosis of *Nocardia* infection.

Clinical data and definitions

The date of diagnosis of nocardiosis was defined as the day on which the first clinical sample (e.g., sputum) yielding *Nocardia* spp. was collected. For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, in order to obtain a similar period of time from transplantation. We collected demographic and transplant data with a specific focus on possible nocardiosis risk factors, such as type of organ donation, length of stay in the intensive care unit (ICU) after transplantation, need for post-transplantation dialysis or mechanical ventilation and co-morbidities (chronic obstructive pulmonary disease [COPD], diabetes). Recorded therapeutic data included immunosuppressive regimen at the time of transplantation, occurrence and treatment of acute allograft rejection episodes between transplantation and the date of nocardiosis diagnosis (including use of high-dose corticosteroids [>20 mg/day of prednisone for at least 1 month or >2 pulses of 500 mg of intravenous methylprednisolone [15]] and/or plasma exchange), presence of a high calcineurin inhibitor trough level in the month prior to diagnosis (defined as >10 µg/mL for tacrolimus and >300 ng/mL for cyclosporine) and receipt of trimethoprim-sulfamethoxazole (TMP-SMX, cotrimoxazole) prophylaxis at the time of nocardiosis diagnosis. We also recorded any prescriptions of lymphocyte-depleting and/or modulating antibodies, such as antithymocyte globulin (ATG), rituximab or basiliximab/daclizumab, in the 12 months prior to diagnosis. Occurrence of bloodstream infections after transplantation was noted. Development of CMV infection and/or disease [20] between transplantation and date of diagnosis was recorded as were CMV serostatus and white blood cell counts at 1 and 2 months after transplantation and at 1 month before the diagnosis of nocardiosis. We also recorded clinical signs of nocardiosis, sites of infection, biological findings at the time of *Nocardia* infection (kidney function, C-reactive protein [CRP] level, leukocyte, neutrophil and lymphocyte counts), species identification and radiological findings. Dissemination

was defined as infection of at least two non-contiguous organs. Outcome was assessed by all-cause mortality 12 months after the diagnosis of nocardiosis.

Microbiology

To identify the species of each *Nocardia* strain, amplification and sequencing of a fragment of the gene coding for the 16S ribosomal RNA (16S rRNA) or *hsp65* were mandatory [21]. Briefly, around 500 base pairs of the 16S rRNA gene were sequenced using polymerase chain reaction (PCR), as described previously [22]. Sequences were compared with those stored in GenBank using blast alignment software (http://www.ncbi.nlm.nih.gov/blast) and BIBI (Bio Informatic Bacteria Identification tool; http://pbil.univ-lyon1.fr/bibi) [3]. Identification at the species level required 99% sequence similarity with the type strain of a single species. If required, the *hsp65* gene was amplified and sequenced to allow adequate identification [23]. For strains that were not analyzed using molecular methods, species identification was not considered reliable. However, the *Nocardia* genus could be identified using a validated non-PCR method, such as matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry or phenotypic testing showing aerobic filamentous and branching Gram-positive rods, lysozyme-resistant with aerial hypha [1, 24]. When needed, stored samples were sent *a posteriori* to the French expert laboratory for nocardiosis (Observatoire Français des Nocardioses, Lyon, France) to perform missing analyses and obtain molecular identification.

Statistical analysis

Final analysis was conducted after all data had been recorded and verified. Clinical, biological and radiological data of cases at the time of diagnosis are described. Continuous variables are presented as means (± standard deviation) or medians (range). Categorical variables are presented as numbers and frequencies. Associations between clinical and biological determinants and *Nocardia* infection were analyzed using univariate conditional logistic regression. A two-sided p-value < 0.05 was

considered as statistically significant. Clinical and therapeutic determinants with a p-value < 0.05 on univariate analysis were included in the final multivariable conditional logistic regression analysis. Because of the large amount of missing data, biological variables were not included in the final multivariable analysis. A systematic search for interaction between determinants with a p-value < 0.05 on univariate analysis was performed. All statistical analyses were performed using R Statistical software (version 3.2.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Characteristics of the patients

We included a total of 117 cases of nocardiosis from 23 French (n=74), 7 Belgian (n=28), 4 Swiss (n=5), 1 Dutch (n=7) and 1 Spanish (n=3) transplant centers, and 234 matched controls. Only one of these cases has been reported previously [25]. The patients' characteristics are shown in **Table 1**. The kidney was the most frequently transplanted organ (n=69, 59%), followed by the heart (n=23, 19.7%), lung (n=16, 13.7%), pancreas (n=4, 3.4%) and liver (n=4, 3.4%). A single patient (0.9%) received combined organs at transplantation. Nocardiosis occurred at a median of 17.5 months [range 2-244] after SOT. Forty-eight of the cases of nocardiosis (41%) were diagnosed within the first year after transplantation while 37 (31.6%) occurred at least 3 years after transplantation (**Figure 1**). The length of time between transplantation and diagnosis of nocardiosis was statistically different for the different organs (heart: median 10 months [3-198]; lung: 17 months [2-106]; and kidney: 20 months [2-244]; p=0.035, Kruskal-Wallis test).

Characteristics and outcome of post-transplant nocardiosis

The clinical, biological and radiological characteristics of the nocardiosis cases are shown in **Table 2**. The most frequent clinical presentation was pulmonary disease (101/117, 86.3%) and in 55 (54.5%) of these cases the lung was the only site of infection. Among the patients with lung involvement, a lung computed tomography (CT)-scan was performed for initial workup in 91 cases (90.1%) and

showed nodule(s) as the most commonly observed feature (68/91, 74.7%), 32.3% of which were cavitated. Brain imaging (magnetic resonance imaging [MRI] and/or CT-scan) was performed in 90 of the patients (76.9%). Among the 30 patients in whom central nervous system (CNS) nocardiosis was demonstrated on imaging, 13 (43.3%) had no neurological abnormalities on clinical examination.

Reliable species identification was obtained using molecular biology tools in 105 of the 117 cases (89.7%). *N. farcinica*, *N. nova* complex and *N. cyriacigeorgica* were the most frequently identified species, responsible for 35% (41/117), 24% (28/117), and 7% (8/117) of the cases, respectively (**Figure 2**). Nocardiosis caused by *N. farcinica* more frequently involved the brain (16/41 [39%] versus 14/76 [18%]) and the skin and soft tissues (20/41 [49%] versus 17/76 [22%]), than did nocardiosis caused by other species ($p<0.05$, Chi-square test) (**Table S1 - Appendix**).

Twelve months after the diagnosis of nocardiosis or the equivalent period for the controls, the all-cause mortality rate was 16.2% (19/117) among cases and 1.3% (3/233) among controls ($p<0.001$ Fisher's exact test). Among the 98 nocardiosis cases alive at 12 months, the median follow-up was 51 months [12-151]; six patients [6.1%] had a relapse during follow-up.

Risk factors for nocardiosis

In univariate analysis (**Table 1**), recipient age at diagnosis, donor age, length of stay in the ICU after transplantation, comorbid diabetes, history of bloodstream infection between transplantation and nocardiosis and acute rejection in the six months before nocardiosis were significantly associated with the development of nocardiosis. High trough blood concentrations of calcineurin inhibitors in the month before *Nocardia* infection, use of tacrolimus at the time of diagnosis, a high dose of corticosteroids at the time of diagnosis, high dose corticosteroids in the six months before nocardiosis and use of plasma exchange or depleting antibodies in the six months prior to infection were also significantly associated with *Nocardia* infection. Regarding CMV, D+R- serostatus was significantly associated with the occurrence of nocardiosis in univariate analysis. Patients with

nocardiosis had significantly lower lymphocyte counts two months after transplantation and one month before diagnosis than did the control patients.

In the multivariable analysis, high blood calcineurin inhibitor trough levels in the month before diagnosis, use of tacrolimus at the time of diagnosis, corticosteroid dose at the time of diagnosis, patient age and length of stay in the ICU after SOT were significantly associated with the development of nocardiosis (**Table 3**). Nocardiosis did not occur earlier in patients who had a short length of stay in the ICU after SOT (<8 days), when compared with those with a long length of stay (≥8 days): 17 [1-65] vs. 20 [3-195] months after transplantation, respectively (p=NS, Kruskal-Wallis test).

Effect of anti-*Pneumocystis* prophylaxis with cotrimoxazole on the risk of nocardiosis

Twenty-one of the nocardiosis cases (18%) were receiving anti-*Pneumocystis* prophylaxis with TMP-SMX at the time of diagnosis compared with 57 (24.5%) of the control patients (OR, 0.36; 95% CI, 0.14-0.93; p=0.03). In multivariable analysis, use of TMP-SMX prophylaxis was not found to be protective against occurrence of nocardiosis. The mean weekly dose of SMX was 1819 (±668) mg in cases vs. 2161 (±957) mg in control patients (p=NS). Among the 351 patients, only two of the control patients (0.6%) were receiving high-dose prophylaxis (i.e., 160/800 mg of TMP-SMX daily). Regarding the 21 episodes of nocardiosis breaking through TMP-SMX prophylaxis, antimicrobial testing was performed on 19 of these cases (90.5%) and 15/19 isolates (78.9%) were susceptible to TMP-SMX.

DISCUSSION

We report the results from the first multicenter case-control study on nocardiosis after SOT. We describe the clinical, biological and radiological presentations of *Nocardia* infection and identified 5 variables that were significantly associated with the occurrence of this opportunistic event in multivariable analysis: a high blood trough level of calcineurin inhibitor in the month before

diagnosis, use of tacrolimus at the time of diagnosis, corticosteroid dose at the time of diagnosis, patient age and length of stay in the ICU after transplantation.

Although nocardiosis is an opportunistic infection that has been described since the very early years of SOT, there are a number of unsolved issues that still need to be addressed. Indeed, most of the publications on this topic have been case reports or small, uncontrolled series that did not allow evaluation of the risk factors that may lead to post-transplant nocardiosis. However, identifying risk factors in this population is important because *Nocardia* infection is a rare but life-threatening event and patients could benefit from prevention, early diagnosis and appropriate treatment. In a case-control study in a single center in Pittsburgh (USA), Peleg and colleagues identified three risk factors for nocardiosis: use of high-dose steroids, a high calcineurin inhibitor level in the month prior to diagnosis and CMV disease in the 6 months prior to diagnosis [15].

Although our two studies differ in location (USA vs. Europe), study period (1995-2005 vs. 2000-2014) and design (single center vs. multicenter), both indicate that a high degree of immune suppression (i.e., high exposure to calcineurin inhibitors and corticosteroids) plays a key role in the development of nocardiosis in SOT recipients. Furthermore, we showed that use of tacrolimus (a variable that was not recorded by Peleg and coworkers) was independently associated with development of nocardiosis, as has been previously suggested [26, 27]. Interestingly, previous studies have reported a protective role of tacrolimus against the development of opportunistic fungal infections, including *Pneumocystis jirovecii* pneumonia and mucormycosis [28, 29]. This apparent discrepancy may be explained by a direct anti-fungal effect of tacrolimus or by the inhibition of a specific anti-*Nocardia* T-cell or macrophage function [30]. Alternatively, tacrolimus use may reflect a greater state of overall immunosuppression as this drug is often used in patients at high risk of graft rejection [31, 32].

In contrast to Peleg and colleagues who identified CMV disease as a risk factor for nocardiosis, CMV serostatus and CMV disease or infection were not significantly associated with

development of *Nocardia* infection in our study. This observation may be due to differences in the definitions of and use of prevention strategies for CMV infection and disease between our studies.

We also identified older patient age and longer length of stay in the ICU after transplantation as risk factors for nocardiosis. Interestingly, we observed no significant difference when comparing the time between transplantation and the occurrence of nocardiosis between patients who had a short length of stay in the ICU after SOT (<8 days) and those with a long length of stay (≥8 days). Therefore, these factors may reflect a general frailty of the recipient, making them more likely to develop complications after transplantation.

No intervention has yet been shown to prevent nocardiosis in transplant recipients. Interestingly, administration of TMP-SMX (the most common treatment for nocardiosis) as prophylaxis against pneumocystosis was not found to effectively prevent nocardiosis in our study, or in the study by Peleg et al. [15], although our study design does not allow definitive conclusions to be drawn regarding the use of TMP-SMX for this purpose. As described elsewhere, we have observed that occurrence of TMP-SMX-susceptible *Nocardia* infections was common in subjects receiving this agent as prophylaxis against pneumocystosis, suggesting that resistance to TMP-SMX was not responsible for the breakthrough nocardiosis [3, 14, 17]. A possible explanation for this lack of prophylactic effect is that the relatively low dose of TMP-SMX used to prevent pneumocystosis may be insufficient to prevent *Nocardia* infection. Indeed, HIV-infected patients, who usually receive a higher weekly dose of TMP-SMX, have a lower incidence of nocardiosis compared to SOT recipients [33]. Although the mean weekly dose of TMP-SMX was similar in cases and controls, it is difficult to interpret these data, because renal function, body weight and compliance also need to be taken into account.

No clinical, biological or radiological signs were specific for nocardiosis, but pulmonary nocardiosis (the most common presentation in our cohort) was associated with nodule(s) in 68/91 (74.7%) patients. Therefore, a nodular pneumonia occurring after SOT should raise suspicion of possible nocardiosis. Furthermore, our study demonstrated that CNS nocardiosis is common

(detected in 30/117 patients, 25.6%) and that in many of the patients with CNS nocardiosis, neurological clinical examination is normal. The latter observation suggests that brain imaging is justified in any SOT recipient with nocardiosis [11].

Once nocardiosis is suspected and confirmed in the microbiology laboratory, identification of *Nocardia* at the species level requires molecular tools, such as amplification and sequencing of the gene coding for the 16S rRNA or *hsp65*. Using such approaches, it has been shown that *N. asteroides* –the most frequently identified species until the end of the 1990s- is an uncommon cause of nocardiosis [19]. Because each species has its own antimicrobial susceptibility profile, accurate identification can help guide choice of empirical antibiotic therapy [1]. Previous studies of post-transplant nocardiosis rarely used sequencing as an identification tool, resulting in the frequent and incorrect identification of *N. asteroides.* In our study, partial sequencing was performed in 105/117 of our strains (89.7%). *N. farcinica* and *N. nova* complex were the two most frequently isolated species, identified in 35% (41/117) and 24% (28/117), respectively, of our European cases of nocardiosis after SOT. We identified no cases of *N. asteroides.*

Our retrospective study has several limitations. First, it was not possible to evaluate the role of potential risk factors that were not recorded in patients' medical records (e.g., environmental exposure to soil or decaying vegetation). Second, there were some missing data (Table 1), so we were unable to analyze the role of biological variables in our multivariable analysis. However, conducting a prospective cohort study was considered impractical because of the rarity of post-SOT nocardiosis.

Interestingly, the all-cause mortality rate was 16.2% (19/117) in our cohort, a finding that is compatible with previous studies in which the mortality rate was about 20% [19], suggesting that our cohort is representative of the population of solid organ transplant recipients with nocardiosis in western countries.

In conclusion, a high calcineurin inhibitor trough level in the month prior to diagnosis, use of tacrolimus at the time of diagnosis, corticosteroid dose at the time of diagnosis, patient age and

length of stay in the ICU after transplantation were independent risk factors for nocardiosis after SOT. At the doses used in our cohort, cotrimoxazole was not found to prevent development of nocardiosis. Further studies are needed to assess the benefits and disadvantages of higher doses of TMP-SMX in the prevention of *Nocardia* infection in high-risk patients and to evaluate the outcome of patients with post-transplant nocardiosis.

NOTES

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Figure 1. **Distribution of the time (in months) between solid organ transplantation and the occurrence of nocardiosis in the 117 cases. Dashed line represents the median time point (17.5 months)**

Figure 2. **Distribution of the various *Nocardia* species identified using molecular biology in the 117 cases of post-transplantation nocardiosis. ***Other species include *N. otitidiscaviarum* (n=1), *N. brevicatena/paucivorans* complex (n=1), *N. cerradoensis* (n=1), *N. pseudobrasiliensis* (n=1), *N. anaemiae* (n=1), *N. takedensis* (n=1).

Table 1. **Clinical and biological characteristics of cases and controls up to the diagnosis of nocardiosis**

Characteristics	Cases (n=117)	Controls (n=234)	OR [IC95%]	Univariate analysis p-value
Clinical characteristics				
Age at diagnosis (years) (mean ± SD)	55.6 ± 13.5	50.7 ± 13.5	1.04 [1.02-1.06]	<0.001
Male	74 (63.2)	150 (64.1)	0.96 [0.59-1.55]	0.87
Number of transplants				
1st	99 (84.6)	211 (90.2)		
2nd or 3rd	18 (14.5)	23 (7.7)	1.84 [0.88-3.82]	0.10
Donor age (mean ± SD) n=332	47.5 ± 16.9	43.3 ± 25.0	1.02 [1.00-1.04]	0.02
Deceased donor (*vs.* living) n=349	107 (91.5)	217 (93.5)	1.41 [0.58-3.43]	0.44
Length of stay in the ICU after transplantation (days) (mean ± SD) n= 343	7.9 ± 9.8	6.2 ± 8.6	1.04 [1.00-1.07]	0.04
COPD after transplant n=350	11 (9.4)	17 (7.3)	1.40 [0.58-3.35]	0.45
Dialysis post-transplant n=348	23 (19.8)	40 (17.2)	1.22 [0.68-2.17]	0.51
Mechanical ventilation post-transplant n=347	28 (24.6)	61 (26.3)	0.74 [0.29-1.88]	0.53
Diabetes at diagnosis n=350	43 (37.7)	54 (23.2)	1.91 [1.17-2.10]	0.01

Acute rejection episode in the 6 months before diagnosis n=350	25 (21.6)	29 (12.4)	2.56 [1.23-5.33]	0.01
CMV infection in the 6 months before diagnosis n=350	17 (14.5)	24 (10.3)	1.70 [0.78-3.73]	0.18
CMV disease in the 6 months before diagnosis n=350	5 (4.27)	5 (2.15)	2.20 [0.58-8.36]	0.25
CMV Serostatus n=340				
D-R-	22 (19.5)	68 (29.8)		
D-R+ or D+R+	60 (51.3)	120 (51.3)	1.66 [0.91-3.04]	0.10
D+R-	31 (27.4)	40 (17.5)	2.65 [1.32-5.31]	0.01
Bloodstream infection before diagnosis n=350	25 (21.4)	28 (12.0)	2.05 [1.10-3.80]	0.02
Therapeutic characteristics				
Immunosuppressive induction n=342				
None	5 (4.4)	18 (7.9)		
ATG	77 (68.1)	137 (59.8)	3.39 [0.72-16.00]	0.12
Anti-CD25	31 (27.4)	74 (32.3)	1.83 [0.35-9.57]	0.47
Corticosteroid bolus at transplant (mg) (mean ± SD) n=339	518 ± 235	541 ± 257	1.00 [1.00-1.00]	0.24
Corticosteroids at M1 (mg†) (mean ± SD) n=335	17.5 ± 11.4	17.1 ± 11.4	1.01 [0.98-1.04]	0.64
Corticosteroids at M2 (mg†) (mean ± SD) n=340	13.6 ± 10.3	12.4 ± 8.3	1.04 [1.00-1.09]	0.06
Corticosteroids at diagnosis (mg†) (mean ± SD) n=342	8.8 + 6.8	6.5 ± 5.2	1.16 [1.08-1.25]	<0.001
High-dose steroids in the 6 months before diagnosis n=350	20 (17.2)	16 (6.8)	3.56 [1.58-8.01]	0.002

CsA at diagnosis n=350	21 (18.0)	71 (30.5)	0.38 [0.19-0.74]	0.005
Tacrolimus at diagnosis n=350	93 (79.5)	143 (61.4)	3.73 [1.88-7.41]	<0.001
High CNI blood level in the month before diagnosis n=349	51 (43.6)	40 (17.2)	7.29 [3.51-15.15]	<0.001
AZA at diagnosis n=350	16 (13.7)	27 (11.6)	1.3 [0.60-2.81]	0.50
MMF at diagnosis n=350	79 (67.5)	175 (75.1)	0.61 [0.34-1.08]	0.09
Use of antiproliferative agents (AZA or MMF) at diagnosis n=350	95 (81.2)	202 (86.7)	0.59 [0.30-1.19]	0.14
Plasma exchange in the 6 months before diagnosis n=350	5 (4.3)	0 (0)	NC	0.004
Depleting antibodies (ATG or Rituximab)* in the 6 months before diagnosis n=350	6 (5.2)	3 (1.3)	5.31 [1.06-26.77]	0.04
Depleting antibodies (ATG or Rituximab)* in the 12 months before diagnosis n=350	7 (6)	3 (1.3)	11.13 [1.34-92.45]	0.03
TMP-SMX prophylaxis at diagnosis n=350	21 (18.0)	57 (24.5)	0.36 [0.14-0.93]	0.03
Biological characteristics				
WBC count at M2 (x1000/mm^3) (mean ± SD) n=344	7.5 ± 4.0	7.2 ± 2.7	1.03 [0.95-1.11]	0.49
WBC count 1 month before diagnosis (x1000/mm^3) (mean ± SD) n=327	8.3 ± 3.9	7.2 ± 2.5	1.13 [1.04-1.23]	0.004
Lymphocyte count at M2 (x1000/mm^3) (mean ± SD) n=304	0.8 ± 0.6	1.0 ± 0.7	0.47 [0.29-0.78]	0.003
Lymphocyte count 1 month before diagnosis (x1000/mm3) (mean ± SD) n=299	0.7 ± 0.5	1.3 ± 0.9	0.22 [0.29-0.78]	<0.001

Neutrophil count at M2 (x1000/mm^3) (mean ± SD) n=301	5.7 ± 3.6	5.5 ± 2.5	1.02 [0.92-1.12]	0.74
Neutrophil count 1 month before diagnosis (x1000/mm^3) (mean ± SD) n=300	6.5 + 3.7	5.2 ±2.2	1.19 [1.08-1.31]	<0.001

NOTE. ATG: antithymocyte globulin; AZA: azathioprine; CMV: cytomegalovirus; CNI: calcineurin inhibitor; COPD: chronic obstructive pulmonary disease; CsA: ciclosporin A; diagnosis: date of the diagnosis of nocardiosis; ICU: intensive care unit; M1: one month after transplantation; M2: two months after transplantation; MMF: mycophenolate mofetil; n: number of data analyzed (when <351); NA: not analyzed; OR: Odds ratio; SD: standard deviation; TMP-SMX: trimethoprim–sulfamethoxazole; WBC: white blood cell.

Data are n (%) unless otherwise indicated. † All the corticosteroid doses are expressed in milligrams (mg) of methylprednisolone equivalent per day. *In the 12 months before diagnosis of *Nocardia* infection, none of our patients received other types of lymphocyte-depleting or modulating antibodies.

Table 2. Description of the 117 cases of post-transplantation nocardiosis at diagnosis

Characteristics	117 cases
Clinical characteristics	
Time from onset of symptoms to diagnosis (days) (median, range)	19.5 [1-139]
Involved organs	
Lung	101 (86.3)
Skin and soft-tissue	37 (31.6)
Skin and soft-tissue as the only site of infection	8 (6.8)
Brain	30 (25.6)
Joint(s) or bone(s)	3 (2.5)
Disseminated infection	50 (42.7)
Clinical signs	
Fever > 38°C	71 (60.7)
Chills	25 (21.4)
Weight loss (n=115)	40 (34.8)
Asthenia (n=116)	74 (63.8)
Dyspnea (n=116)	48 (41.4)
Chest pain (n=116)	28 (24.1)
Cough (n=116)	65 (56)
Sputum production (n=115)	43 (37.4)
Acute respiratory distress† (n=116)	5 (4.3)
Headache (n=116)	15 (12.9)
Coma (n=116)	3 (2.6)
Seizures (n=116)	9 (7.8)
Focal neurological signs (n=116)	11 (9.5)
Cutaneous lesions (n=116)	37 (31.9)

Arthritis (n=116)	1 (1)
Biological characteristics on the day of diagnosis	
White blood cell count (x1000/mm^3) (mean ± SD) (n=115)	11.5 ± 6.5
Neutrophil count (x1000/mm^3) (mean ± SD) (n=105)	9.7 ± 6.5
Lymphocyte count (x1000/mm^3) (mean ± SD) (n=106)	1.3 ± 0.8
Glomerular filtration rate ¶ (ml/min/1.73m^2) (mean ± SD) (n=116)	48.6 ± 27.2
C-reactive protein (mg/l) (median, range) (n=109)	104 [1-469]
Radiological characteristics	
Type of lung involvement (n=91)	
Nodules	68 (74.7)
Among nodules, cavitation (n=68)	22 (32.3)
Lung consolidation	37 (40.6)
Pleural effusion	24 (26.4)
Interstitial syndrome	11 (12.1)
No other lesions than interstitial syndrome	2 (2.2)
Multilobar involvement	51 (56)
Bilateral involvement	46 (50.5)
Type of brain abscess (n=30)	
Multiple lesions	24 (80)
Bihemispheric	16 (53.3)
Supratentorial	28 (93.3)
Infratentorial	9 (30)
Microbiological characteristics	
Source of the culture that grew *Nocardia**	
Bronchoalveolar lavage	51 (43.6)
Sputum	25 (21.4)
Bronchial aspirate	23 (19.6)
Pleural fluid	8 (6.8)

Transbronchial biopsy	6 (5.1)
Surgical or percutaneous lung biopsy	4 (3.4)
Abscess fluid	29 (24.8)
Cutaneous biopsy	11 (9.4)
Blood culture	9 (7.7)
Cerebrospinal fluid	2 (1.7)
At least one positive respiratory, lung or pleural sample	79 (67.5)
Positive direct examination	59 (50.4)

NOTE. Data are n (%) unless otherwise indicated.

† If mechanical ventilation was required, ¶ as estimated by MDRD formula, *Each patient could have several positive samples. n: number of data analyzed (when <117)

Table 3. Risk factors for nocardiosis in 117 cases compared to 234 controls after multivariable analysis by conditional logistic regression

Characteristic	OR [95%IC]	-value
High calcineurin inhibitor level in the month before nocardiosis	6.11 [2.58-14.51]	0.001
Use of tacrolimus at diagnosis	2.65 [1.17-6.00]	015
Corticosteroid dose at diagnosis (per mg†)	1.12 [1.03-1.22]	002
Age at diagnosis (per year)	1.04 [1.02-1.07]	0.001
Length of first ICU stay after transplantation (per day)	1.04 [1.00-1.09]	049

NOTE. Diagnosis: date of the diagnosis of nocardiosis; ICU: intensive care unit; OR: Odds ratio

† Expressed in milligrams (mg) of methylprednisolone equivalent per day

High calcineurin inhibitor level was defined as a trough blood level > 10 µL/mL for tacrolimus and > 300 ng/mL for cyclosporine

Because of the large number of missing data, biological variables were not included in the multivariable analysis