Clinical Infectious Diseases Advance Access published June 13, 2016

Clinical Infectious Diseases

MAJOR ARTICLE

Nocardia Infection in Solid Organ Transplant Recipients: A Multicenter European Case-control Study

Julien Coussement,[1,a] David Lebeaux,[2,a] Christian van Delden,[3,4] Hélène Guillot,[5] Romain Freund,[6,7] Sierk Marbus,[8] Giovanna Melica,[9] Eric Van Wijngaerden,[10] Benoit Douvry,[11] Steven Van Laecke,[12] Fanny Vuotto,[13] Leïla Tricot,[14] Mario Fernández-Ruiz,[15] Jacques Dantal,[16] Cédric Hirzel,[4,17] Jean-Philippe Jais,[6,7] Veronica Rodriguez-Nava,[18] Olivier Lortholary,[2,b] and Frédérique Jacobs[1,b]; for the European Study Group for Nocardia in Solid Organ Transplantation[c]

[1]Department of Infectious Diseases, CUB-Hôpital Erasme, Université Libre de Bruxelles, Brussels; [2]Université Paris Descartes, Sorbonne Paris Cité, AP-HP, Hôpital Necker Enfants Malades, Centre d'Infectiologie Necker-Pasteur and Institut Imagine, France; [3]Transplant Infectious Diseases Unit, Hôpitaux Universitaires de Genève, and [4]Swiss Transplant Cohort Study, Switzerland; [5]Service des Maladies Infectieuses et Tropicales, Sorbonne Universités, UPMC Université Paris 06, AP-HP, Hôpital Pitié-Salpêtrière, [6]Université Paris Descartes, INSERM UMRS 1138 Team 22, and [7]Biostatistics Unit, AP-HP, Hôpital Necker Enfants Malades, Paris, France; [8]Department of Infectious Diseases, Leiden University Medical Center, The Netherlands; [9]Immunologie Clinique et Maladies Infectieuses, AP-HP, Hôpital Henri Mondor, Créteil, France; [10]Department of General Internal Medicine, University Hospitals Leuven, Belgium; [11]Service de Pneumologie et de Transplantation Pulmonaire, Hôpital Foch, Suresnes, France; [12]Renal Division, Ghent University Hospital, Belgium; [13]Infectious Diseases Unit, Huriez Hospital, CHRU Lille, and [14]Service de Néphrologie–Transplantation Rénale, Hôpital Foch, Suresnes, France; [15]Unit of Infectious Diseases, University Hospital 12 de Octubre, Instituto de Investigación Hospital "12 de Octubre" (i+12), Madrid, Spain; [16]Institut de Transplantation, d'Urologie et de Néphrologie, CHU Nantes, France; [17]Department of Infectious Diseases, Bern University Hospital, University of Bern, Switzerland; and [18]Research Group on Bacterial Opportunistic Pathogens and Environment UMR5557 Écologie Microbienne, French Observatory of Nocardiosis, Université de Lyon 1, CNRS, VetAgro Sup, France

Background. Nocardiosis is a rare, life-threatening opportunistic infection, affecting 0.04% to 3.5% of patients after solid organ transplant (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, the 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 transplant. In multivariable analysis, high calcineurin inhibitor trough levels in the month before diagnosis (odds ratio [OR], 6.11; 95% confidence interval [CI], 2.58–14.51), use of tacrolimus (OR, 2.65; 95% CI, 1.17–6.00) and corticosteroid dose (OR, 1.12; 95% CI, 1.03–1.22) at the time of diagnosis, patient age (OR, 1.04; 95% CI, 1.02–1.07), and length of stay in the intensive care unit after SOT (OR, 1.04; 95% CI, 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 5 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.

Keywords. *Nocardia*; nocardiosis; organ transplant; opportunistic infections.

Nocardia species 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 immunodeficiency [3], including that associated with corticosteroid therapy, transplantation, human immunodeficiency virus (HIV) infection [4, 5], cancer [6], chronic granulomatous disease [7], or presence of autoantibodies against granulocyte macrophage colony-stimulating factor [8], and/or in patients with chronic lung disease [9, 10].

Solid organ transplant (SOT) 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 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 transplant (estimates between 0.8% and 3.5%) and the lowest after liver and kidney

Received 19 January 2016; accepted 7 April 2016.

[a]J. C. and D. L. contributed equally to this work.

[b]O. L. and F. J. contributed equally to this work.

[c]Individual collaborators and scientific groups that are members of the European Study Group for *Nocardia* in Solid Organ Transplantation are listed in the Supplementary Appendix.

Correspondence: J. Coussement, Department of Infectious Diseases, CUB-Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium (jcoussem@ulb.ac.be).

Clinical Infectious Diseases®

DOI: 10.1093/cid/ciw241

transplants (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 6–12 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 3 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 6 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.

MATERIALS AND 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 2 other European transplant 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 transplant databases. In France, the study was approved by the CPP Ile-de-France I Ethical board (7 March 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* species isolated in a clinical sample after transplant; (3) presence of signs and/or symptoms compatible with nocardiosis; (4) diagnosis made between January 2000 and December 2014. We selected 2 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 (eg, sputum) yielding *Nocardia* species was collected. For control patients, a corresponding date was chosen on the basis of their matched case's date of diagnosis, to obtain a similar period of time from transplant. 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 transplant, need for posttransplant dialysis or mechanical ventilation, and comorbidities (chronic obstructive pulmonary disease, diabetes). Recorded therapeutic data included immunosuppressive regimen at the time of transplant, occurrence and treatment of acute allograft rejection episodes between transplant and the date of nocardiosis diagnosis (including use of high-dose corticosteroids, ie, >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 ng/mL for tacrolimus and >300 ng/mL for cyclosporine) and receipt of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis at the time of nocardiosis diagnosis. We also recorded any prescriptions of lymphocyte-depleting and/or modulating antibodies, such as antithymocyte globulin, rituximab, or basiliximab/daclizumab, in the 12 months prior to diagnosis. Occurrence of bloodstream infections after transplant was noted. Development of CMV infection and/or disease [20] between transplant and date of diagnosis was recorded, as were CMV serostatus and white blood cell counts at 1 and 2 months after transplant 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 level, and leukocyte, neutrophil, and lymphocyte counts), species identification, and radiological findings. Dissemination was defined as infection of at least 2 noncontiguous 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]. In brief, 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;

Table 1. Clinical and Biological Characteristics of Cases and Controls Up to the Diagnosis of Nocardiosis

Characteristics	Cases (n = 117)	Controls (n = 234)	OR (95% CI)	Univariate Analysis *P* Value
Clinical characteristics				
Age at diagnosis, y, mean ± SD	55.6 ± 13.5	50.7 ± 13.5	1.04 (1.02–1.06)	<.001
Male sex	74 (63.2)	150 (64.1)	0.96 (.59–1.55)	.87
No. of transplants				
1st	99 (84.6)	211 (90.2)		
2nd or 3rd	18 (14.5)	23 (7.7)	1.84 (.88–3.82)	.10
Donor age, mean ± SD (n = 332)	47.5 ± 16.9	43.3 ± 25.0	1.02 (1.00–1.04)	.02
Deceased donor (vs living) (n = 349)	107 (91.5)	217 (93.5)	1.41 (.58–3.43)	.44
Length of stay in the ICU after transplant, d, mean ± SD (n = 343)	7.9 ± 9.8	6.2 ± 8.6	1.04 (1.00–1.07)	.04
COPD after transplant (n = 350)	11 (9.4)	17 (7.3)	1.40 (.58–3.35)	.45
Dialysis posttransplant (n = 348)	23 (19.8)	40 (17.2)	1.22 (.68–2.17)	.51
Mechanical ventilation posttransplant (n = 347)	28 (24.6)	61 (26.3)	0.74 (.29–1.88)	.53
Diabetes at diagnosis (n = 350)	43 (37.7)	54 (23.2)	1.91 (1.17–2.10)	.01
Acute rejection episode in the 6 mo before diagnosis (n = 350)	25 (21.6)	29 (12.4)	2.56 (1.23–5.33)	.01
CMV infection in the 6 mo before diagnosis (n = 350)	17 (14.5)	24 (10.3)	1.70 (.78–3.73)	.18
CMV disease in the 6 mo before diagnosis (n = 350)	5 (4.27)	5 (2.15)	2.20 (.58–8.36)	.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 (.91–3.04)	.10
D^+R^-	31 (27.4)	40 (17.5)	2.65 (1.32–5.31)	.01
Bloodstream infection before diagnosis (n = 350)	25 (21.4)	28 (12.0)	2.05 (1.10–3.80)	.02
Therapeutic characteristics				
Immunosuppressive induction (n = 342)				
None	5 (4.4)	18 (7.9)		
ATG	77 (68.1)	137 (59.8)	3.39 (.72–16.00)	.12
Anti-CD25	31 (27.4)	74 (32.3)	1.83 (.35–9.57)	.47
Corticosteroid bolus at transplant, mg, mean ± SD (n = 339)	518 ± 235	541 ± 257	1.00 (1.00–1.00)	.24
Corticosteroids at M1, mg[a], mean ± SD (n = 335)	17.5 ± 11.4	17.1 ± 11.4	1.01 (.98–1.04)	.64
Corticosteroids at M2, mg[a], mean ± SD (n = 340)	13.6 ± 10.3	12.4 ± 8.3	1.04 (1.00–1.09)	.06
Corticosteroids at diagnosis, mg[a], mean ± SD (n = 342)	8.8 + 6.8	6.5 ± 5.2	1.16 (1.08–1.25)	<.001
High-dose steroids in the 6 mo before diagnosis (n = 350)	20 (17.2)	16 (6.8)	3.56 (1.58–8.01)	.002
CsA at diagnosis (n = 350)	21 (18.0)	71 (30.5)	0.38 (.19–.74)	.005
Tacrolimus at diagnosis (n = 350)	93 (79.5)	143 (61.4)	3.73 (1.88–7.41)	<.001
High CNI blood level in the month before diagnosis (n = 349)	51 (43.6)	40 (17.2)	7.29 (3.51–15.15)	<.001
AZA at diagnosis (n = 350)	16 (13.7)	27 (11.6)	1.3 (.60–2.81)	.50
MMF at diagnosis (n = 350)	79 (67.5)	175 (75.1)	0.61 (.34–1.08)	.09
Use of antiproliferative agents (AZA or MMF) at diagnosis (n = 350)	95 (81.2)	202 (86.7)	0.59 (.30–1.19)	.14
Plasma exchange in the 6 mo before diagnosis (n = 350)	5 (4.3)	0 (0)	NA	.004
Depleting antibodies (ATG or rituximab)[b] in the 6 mo before diagnosis (n = 350)	6 (5.2)	3 (1.3)	5.31 (1.06–26.77)	.04
Depleting antibodies (ATG or rituximab)[b] in the 12 mo before diagnosis (n = 350)	7 (6)	3 (1.3)	11.13 (1.34–92.45)	.03
TMP-SMX prophylaxis at diagnosis (n = 350)	21 (18.0)	57 (24.5)	0.36 (.14–.93)	.03
Biological characteristics				
WBC count at M2, ×1000/μL, mean ± SD (n = 344)	7.5 ± 4.0	7.2 ± 2.7	1.03 (.95–1.11)	.49
WBC count 1 mo before diagnosis, ×1000/μL, mean ± SD (n = 327)	8.3 ± 3.9	7.2 ± 2.5	1.13 (1.04–1.23)	.004
Lymphocyte count at M2, ×1000/μL, mean ± SD (n = 304)	0.8 ± 0.6	1.0 ± 0.7	0.47 (.29–.78)	.003
Lymphocyte count 1 mo before diagnosis, ×1000/μL, mean ± SD (n = 299)	0.7 ± 0.5	1.3 ± 0.9	0.22 (.29–.78)	<.001
Neutrophil count at M2, ×1000/μL, mean ± SD (n = 301)	5.7 ± 3.6	5.5 ± 2.5	1.02 (.92–1.12)	.74
Neutrophil count 1 mo before diagnosis, ×1000/μL, mean ± SD (n = 300)	6.5 ± 3.7	5.2 ± 2.2	1.19 (1.08–1.31)	<.001

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ATG, antithymocyte globulin; AZA, azathioprine; CI, confidence interval; 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, 1 month after transplant; M2, 2 months after transplant; 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.

[a] All the corticosteroid doses are expressed in milligrams (mg) of methylprednisolone equivalent per day.

[b] In the 12 months before diagnosis of *Nocardia* infection, none of our patients received other types of lymphocyte-depleting or modulating antibodies.

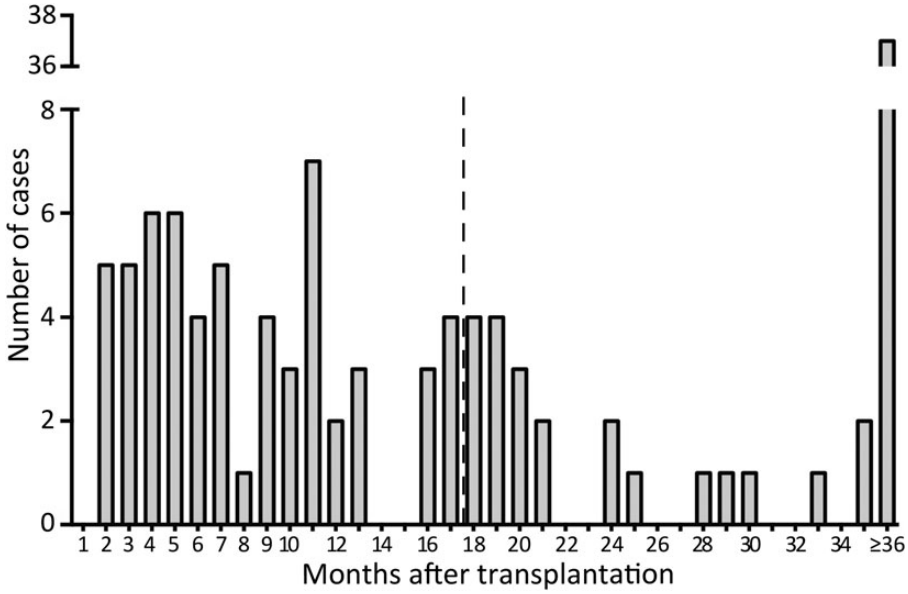

Figure 1. Distribution of the time (in months) between solid organ transplant and the occurrence of nocardiosis in the 117 cases. Dashed line represents the median time point (17.5 months).

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 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 the mean (± standard deviation) or median (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 2-sided *P* value <.05 was considered as statistically significant. Clinical and therapeutic determinants with a *P* value <.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 <.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 patient's 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 transplant. Nocardiosis occurred at a median of 17.5 (range, 2–244) months after SOT. Forty-eight of the cases of nocardiosis (41%) were diagnosed within the first year after transplant while 37 (31.6%) occurred at least 3 years after transplant (Figure 1). The median length of time between transplant and diagnosis of nocardiosis was statistically different for the different organs (heart, 10 [range, 3–198] months; lung, 17 [range, 2–106] months; and kidney, 20 [range, 2–244] months; *P* = .035, Kruskal–Wallis test).

Characteristics and Outcome of Posttransplant 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

Table 2. Description of Cases of Posttransplant Nocardiosis at Diagnosis

Characteristics	N = 117
Clinical characteristics	
Time from onset of symptoms to diagnosis, d, 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[a] (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, ×1000/μL, mean ± SD (n = 115)	11.5 ± 6.5
Neutrophil count, ×1000/μL, mean ± SD (n = 105)	9.7 ± 6.5
Lymphocyte count, ×1000/μL, mean ± SD (n = 106)	1.3 ± 0.8
Glomerular filtration rate[b], mL/min/1.73 m^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*[c]	
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 1 positive respiratory, lung, or pleural sample	79 (67.5)
Positive direct examination	59 (50.4)

Table 2 continued.

Data are No. (%) unless otherwise indicated.

Abbreviation: SD, standard deviation.

[a] If mechanical ventilation was required.

[b] As estimated by Modification of Diet in Renal Disease (MDRD) Study Group formula.

[c] Each patient could have several positive samples. n = number of data analyzed (when <117).

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 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%). *Nocardia farcinica*, *Nocardia nova* complex, and *Nocardia 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*, compared with that caused by other species, more frequently involved the brain (16/41 [39%] vs 14/76 [18%]) and the skin and soft tissues (20/41 [49%] vs 17/76 [22%]) ($P < .05$, χ^2 test) (Supplementary Appendix Table 1).

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 ($P < .001$, Fisher exact test). Among the 98 nocardiosis cases alive at 12 months, the median follow-up was 51 (range, 12–151) months; 6 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 transplant, comorbid diabetes, history of bloodstream infection between transplant and nocardiosis, and acute rejection in the 6 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 6 months before nocardiosis, and use of plasma exchange or depleting antibodies in the 6 months prior to infection were also significantly associated with *Nocardia* infection. Regarding CMV, D^+R^- serostatus was

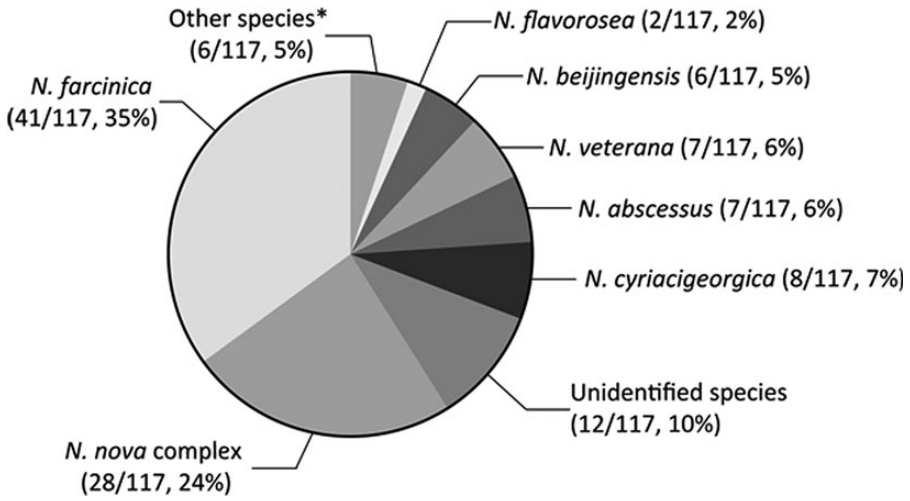

Figure 2. Distribution of the various *Nocardia* species identified using molecular biology in the 117 cases of posttransplant nocardiosis. *Other species include *N. otitidiscaviarum* (n = 1), *N. brevicatena/paucivorans* complex (n = 1), *N. cerradoensis* (n = 1), *N. pseudobrasiliensis* (n = 1), *N.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

significantly associated with the occurrence of nocardiosis in univariate analysis. Patients with nocardiosis had significantly lower lymphocyte counts 2 months after transplant and 1 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), compared with those with a long length of stay (≥8 days): median time between SOT and nocardiosis of 17 (range, 1–65) vs 20 (range, 3–195) months after transplant, respectively (P = not significant [NS], Kruskal–Wallis test).

Table 3. Risk Factors for Nocardiosis in 117 Cases Compared With 234 Controls After Multivariable Analysis by Conditional Logistic Regression

Characteristic	OR (95% CI)	*P* Value
High calcineurin inhibitor level in the month before nocardiosis	6.11 (2.58–14.51)	<.001
Use of tacrolimus at diagnosis	2.65 (1.17–6.00)	.015
Corticosteroid dose at diagnosis (per mg[a])	1.12 (1.03–1.22)	.002
Age at diagnosis (per year)	1.04 (1.02–1.07)	<.001
Length of first ICU stay after transplant (per day)	1.04 (1.00–1.09)	.049

High calcineurin inhibitor level was defined as a trough blood level >10 ng/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.

Abbreviations: CI, confidence interval; diagnosis, date of the diagnosis of nocardiosis; ICU, intensive care unit; OR, odds ratio.

[a] Expressed in milligrams (mg) of methylprednisolone equivalent per day.

Effect of Anti-*Pneumocystis* Prophylaxis With TMP-SMX 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 (odds ratio, 0.36; 95% confidence interval, .14–.93; P = .03). In multivariable analysis, use of TMP-SMX prophylaxis was not found to be protective against occurrence of nocardiosis. The mean weekly dose of sulfamethoxazole was 1819 (±668) mg in cases vs 2161 (±957) mg in control patients (P = NS). Among the 351 patients, only 2 of the control patients (0.6%) were receiving high-dose prophylaxis (ie, 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 of 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 presentation 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 transplant.

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 posttransplant 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 (United States), Peleg and colleagues identified 3 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 2 studies differ in location (United States vs Europe), study period (1995–2005 vs 2000–2014) and design (single center vs multicenter), both indicate that a high degree of immunosuppression (ie, 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 antifungal 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 transplant as risk factors for nocardiosis. Interestingly, we observed no significant difference when comparing the time between transplant 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 transplant.

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 with 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 of 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 of 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 *Nocardia 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 empiric antibiotic therapy [1]. Previous studies of posttransplant 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 of 117 of our strains (89.7%). *Nocardia farcinica* and *N. nova* complex were the 2 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 (eg, 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 SOT 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 transplant were independent risk factors for nocardiosis after SOT. At the doses used in our cohort, TMP-SMX 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 posttransplant nocardiosis.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Acknowledgments. The authors thank Prissile Bakouboula and Caroline Elie (Unité de Recherche Clinique/Centre d'Investigation Clinique Paris Descartes Necker Cochin, Assistance Publique-Hôpitaux de Paris, Hôpital Necker Enfants Malades, Paris, France) for their help in the preparation of the study protocol and Dr Karen Pickett for her editorial suggestions.

Financial support. This work was supported by 2 grants: Bourse Junior 2015–Société de Pathologie Infectieuse de Langue Française (David Lebeaux) and Prix Fonds Carine Vyghen pour le don d'organes 2014 (Julien Coussement). The Swiss Transplant Cohort Study was supported by the Swiss National Science Foundation and the Swiss University Hospitals (G15) and transplant centers.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev **2006**; 19:259–82.
2. Yu X, Han F, Wu J, et al. *Nocardia* infection in kidney transplant recipients: case report and analysis of 66 published cases. Transpl Infect Dis **2011**; 13:385–91.
3. Minero MV, Marin M, Cercenado E, Rabadan PM, Bouza E, Munoz P. Nocardiosis at the turn of the century. Medicine (Baltimore) **2009**; 88:250–61.
4. Castro JG, Espinoza L. *Nocardia* species infections in a large county hospital in Miami: 6 years experience. J Infect **2007**; 54:358–61.
5. Biscione F, Cecchini D, Ambrosioni J, Bianchi M, Corti M, Benetucci J. Nocardiosis in patients with human immunodeficiency virus infection. Enferm Infecc Microbiol Clin **2005**; 23:419–23.
6. Wang HL, Seo YH, LaSala PR, Tarrand JJ, Han XY. Nocardiosis in 132 patients with cancer: microbiological and clinical analyses. Am J Clin Pathol **2014**; 142:513–23.
7. Marciano BE, Spalding C, Fitzgerald A, et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis **2015**; 60:1176–83.
8. Rosen LB, Rocha Pereira N, Figueiredo C, et al. *Nocardia*-induced granulocyte macrophage colony-stimulating factor is neutralized by autoantibodies in disseminated/extrapulmonary nocardiosis. Clin Infect Dis **2014**; 60:1017–25.
9. Rodriguez-Nava V, Durupt S, Chyderiotis S, et al. A French multicentric study and review of pulmonary *Nocardia* spp. in cystic fibrosis patients. Med Microbiol Immunol **2014**; 203:493–504.
10. Riviere F, Billhot M, Soler C, Vaylet F, Margery J. Pulmonary nocardiosis in immunocompetent patients: can COPD be the only risk factor? Eur Respir Rev **2011**; 20:210–2.
11. Clark NM, Reid GE, AST Infectious Diseases Community of Practice. *Nocardia* infections in solid organ transplantation. Am J Transplant **2013**; 13(suppl 4): 83–92.
12. Hill RB Jr, Rowlands DT Jr, Rifkind D. Infectious pulmonary disease in patients receiving immunosuppressive therapy for organ transplantation. N Engl J Med **1964**; 271:1021–7.
13. Nampoory MR, Khan ZU, Johny KV, et al. Nocardiosis in renal transplant recipients in Kuwait. Nephrol Dial Transplant **1996**; 11:1134–8.
14. Santos M, Gil-Brusola A, Morales P. Infection by *Nocardia* in solid organ transplantation: thirty years of experience. Transplant Proc **2011**; 43:2141–4.
15. Peleg AY, Husain S, Qureshi ZA, et al. Risk factors, clinical characteristics, and outcome of *Nocardia* infection in organ transplant recipients: a matched case-control study. Clin Infect Dis **2007**; 44:1307–14.
16. Husain S, McCurry K, Dauber J, Singh N, Kusne S. *Nocardia* infection in lung transplant recipients. J Heart Lung Transplant **2002**; 21:354–9.
17. Khan BA, Duncan M, Reynolds J, Wilkes DS. *Nocardia* infection in lung transplant recipients. Clin Transplant **2008**; 22:562–6.
18. Wiesmayr S, Stelzmueller I, Tabarelli W, et al. Nocardiosis following solid organ transplantation: a single-centre experience. Transpl Int **2005**; 18:1048–53.
19. Lebeaux D, Morelon E, Suarez F, et al. Nocardiosis in transplant recipients. Eur J Clin Microbiol Infect Dis **2014**; 33:689–702.
20. Kotton CN, Kumar D, Caliendo AM, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation **2013**; 96:333–60.
21. Wauters G, Avesani V, Charlier J, Janssens M, Vaneechoutte M, Delmee M. Distribution of *Nocardia* species in clinical samples and their routine rapid identification in the laboratory. J Clin Microbiol **2005**; 43:2624–8.
22. Cloud JL, Conville PS, Croft A, Harmsen D, Witebsky FG, Carroll KC. Evaluation of partial 16S ribosomal DNA sequencing for identification of *Nocardia* species by using the MicroSeq 500 system with an expanded database. J Clin Microbiol **2004**; 42:578–84.
23. Rodriguez-Nava V, Couble A, Devulder G, Flandrois JP, Boiron P, Laurent F. Use of PCR-restriction enzyme pattern analysis and sequencing database for hsp65 gene-based identification of *Nocardia* species. J Clin Microbiol **2006**; 44:536–46.
24. Farfour E, Leto J, Barritault M, et al. Evaluation of the Andromas matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of aerobically growing gram-positive bacilli. J Clin Microbiol **2012**; 50:2702–7.
25. Harent S, Vuotto F, Wallet F, et al. *Nocardia* pseudobrasiliensis pneumonia in a heart transplant recipient. Med Mal Infect **2013**; 43:85–7.
26. Canet S, Garrigue V, Bismuth J, et al. Nocardiosis—is it frequently observed after the introduction of new immunosuppressive agents in renal transplantation? Nephrologie **2004**; 25:43–8.
27. Vigil KJ, Pasumarthy A, Johnson LB, Sheppard T, El-Ghoroury M, Del Busto R. Nocardiosis in renal transplant patients: role of current immunosuppressant agents. Infect Dis Clin Pract **2007**; 15:171–3.
28. Singh N, Aguado JM, Bonatti H, et al. Zygomycosis in solid organ transplant recipients: a prospective, matched case-control study to assess risks for disease and outcome. J Infect Dis **2009**; 200:1002–11.
29. Iriart X, Challan Belval T, Fillaux J, et al. Risk factors of *Pneumocystis* pneumonia in solid organ recipients in the era of the common use of posttransplantation prophylaxis. Am J Transplant **2015**; 15:190–9.
30. Lamoth F, Alexander BD, Juvvadi PR, Steinbach WJ. Antifungal activity of compounds targeting the Hsp90-calcineurin pathway against various mould species. J Antimicrob Chemother **2015**; 70:1408–11.
31. Knoll GA, Bell RC. Tacrolimus versus cyclosporin for immunosuppression in renal transplantation: meta-analysis of randomised trials. BMJ **1999**; 318:1104–7.
32. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. Am J Transplant **2009**; 9:1876–85.
33. Filice GA. Nocardiosis in persons with human immunodeficiency virus infection, transplant recipients, and large, geographically defined populations. J Lab Clin Med **2005**; 145:156–62.