Clinical Assessment of a *Nocardia* PCR-Based Assay for Diagnosis of Nocardiosis

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ABSTRACT The diagnosis of nocardiosis, a severe opportunistic infection, is challenging. We assessed the specificity and sensitivity of a 16S rRNA *Nocardia* PCR-based assay performed on clinical samples. In this multicenter study (January 2014 to April 2015), patients who were admitted to three hospitals and had an underlying condition favoring nocardiosis, clinical and radiological signs consistent with nocardiosis, and a *Nocardia* PCR assay result for a clinical sample were included. Patients were classified as negative control (NC) (negative *Nocardia* culture results and proven alternative diagnosis or improvement at 6 months without anti-*Nocardia* treatment), positive control (PC) (positive *Nocardia* culture results), or probable nocardiosis (positive *Nocardia* PCR results, negative *Nocardia* culture results, and no alternative diagnosis). Sixty-eight patients were included; 47 were classified as NC, 8 as PC, and 13 as probable nocardiosis. PCR results were negative for 35/47 NC patients (74%). For the 12 NC patients with positive PCR results, the PCR assay had been performed with respiratory samples. These NC patients had chronic bronchopulmonary disease more frequently than did the NC patients with negative PCR results (8/12 patients [67%] versus 11/35 patients [31%]; *P* = 0.044). PCR results were positive for 7/8 PC patients (88%). There were 13 cases of probable nocardiosis, diagnosed solely using the PCR results; 9 of those patients (69%) had lung involvement (consolidation or nodule). *Nocardia* PCR testing had a specificity of 74% and a sensitivity of 88% for the diagnosis of nocardiosis. *Nocardia* PCR testing may be helpful for the diagnosis of nocardiosis in immunocompromised patients but interpretation of PCR results

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from respiratory samples is difficult, because the PCR assay may also detect colonization.

Nocardiosis is a rare infection caused by *Nocardia* spp., environmental Gram-positive bacteria (1). Management of nocardiosis is challenging, with mortality rates ranging from 20 to 30% among patients with invasive infection and up to 50% for patients with central nervous system involvement (1). Clinical and radiological features are diverse and nonspecific and include pneumonia, cutaneous or subcutaneous infections, and brain or other solid organ abscesses. Nocardiosis mainly affects immunocompromised patients, including patients receiving immunosuppressive therapy, post-organ transplantation or post-stem cell transplantation patients, patients with primary immunodeficiencies, and patients with chronic bronchopulmonary disease (1–4). A positive microbiological culture from an infected site remains the diagnostic gold standard. Because of the severity of infection among immunocompromised patients and the many possible differential diagnoses, however, most patients receive antibiotic treatment prior to culture sampling for *Nocardia*, reducing the sensitivity of microbial cultures in this setting. In addition, although most cultures of *Nocardia* spp. are positive in 2 to 7 days, culture duration must be extended to 2 to 3 weeks because of slow-growing species. Therefore, alternative techniques are required to improve the diagnosis of nocardiosis.

In 2005, Couble et al. described a 16S rRNA PCR-based assay, with hybridization confirmation, that could be performed directly on human clinical samples for the rapid detection of *Nocardia* spp. (5). The method was tested with bronchoalveolar lavage (BAL) fluid, sputum, pleural fluid, pus, and biopsy specimens from 18 patients with cultures that were positive for various *Nocardia* species (*Nocardia brasiliensis*, *Nocardia farcinica*, *Nocardia nova*, *Nocardia otitidiscaviarum*, and strains formerly classified as *Nocardia asteroides*). Twenty clinical samples from patients with confirmed tuberculosis were used as negative-control (NC) samples. In that publication, the specificity and sensitivity for detection of *Nocardia* spp. were both 100%.

A few case reports using the PCR assay have been reported, but the diagnostic performance of *Nocardia* PCR has never been studied in patients with a wide range of underlying conditions (6, 7). Therefore, our main objective was to assess the specificity and sensitivity of a 16S rRNA PCR-based *Nocardia* assay performed with clinical samples in a pragmatic setting. A secondary objective was to describe the characteristics of patients with a diagnosis of probable nocardiosis based solely on PCR results, without positive culture findings.

MATERIALS AND METHODS

Study design and setting. Between 1 January 2014 and 30 April 2015, patients admitted to three French tertiary-care teaching hospitals (Hôpital Necker-Enfants Malades, Hôpital Foch, and Hôpital Avicenne) involved in the management of patients with underlying conditions favoring nocardiosis were prospectively considered for inclusion. During the study period, the three centers used comparable diagnostic evaluation protocols for opportunistic infections in immunocompromised patients, which included *Nocardia* PCR testing performed on samples from all patients with conditions favoring nocardiosis and clinical or radiological signs consistent with nocardiosis.

Inclusion criteria and classification. Patients were included if they met all the following criteria: (i) at least one underlying condition favoring nocardiosis, (ii) clinical and/or radiological signs consistent with a diagnosis of nocardiosis, and (iii) a clinical sample sent for *Nocardia* PCR testing. Underlying conditions considered to favor nocardiosis were solid organ or hematopoietic stem cell transplantation, primary or secondary (human immunodeficiency virus [HIV] infection or immunosuppressive therapy) immunodeficiency, chronic bronchopulmonary disease, and asthma. Clinical and radiological signs considered consistent with nocardiosis were lung consolidation or nodule (with or without cavitation), cerebral abscess, meningitis, muscle abscess, cutaneous or subcutaneous abscess or nodule, dermohypodermitis or mycetoma, osteoarthritis, solid organ abscess, and adenitis.

Two senior authors (C.R. and D.L.) reviewed the medical charts of the included patients and retrospectively classified patients into one of the three following groups (Fig. 1). The NC group had negative microscopic examination results, including Gram staining and culture, for *Nocardia* and a
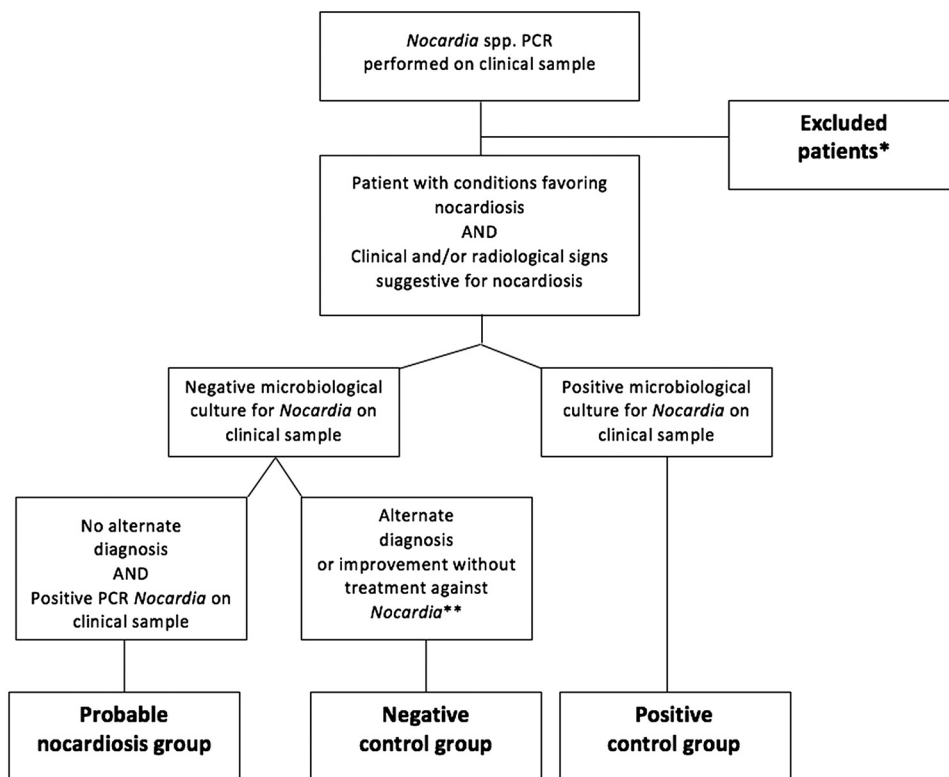
FIG 1 Retrospective classification of the study patients. *, patients were excluded (*n* = 25) on the basis of no underlying condition favoring nocardiosis (*n* = 8), no proven alternative diagnosis or improvement at 6 months without anti-*Nocardia* treatment (*n* = 9), no clinical or radiological signs consistent with nocardiosis (*n* = 4), or positive microbiological cultures for *Nocardia* without PCR testing being performed (*n* = 4). **, antibiotic treatment for ≤14 days was allowed.

proven alternative diagnosis or improvement within 6 months without anti-*Nocardia* treatment (≤14 days of antibiotic administration was not considered treatment). The positive-control (PC) group had positive culture results for *Nocardia*. During the study period, 3 patients were diagnosed as having confirmed nocardiosis based on bacterial culture results and 1 additional patient was considered colonized without clinical and/or radiological signs. Unfortunately, none of these patients had a clinical sample sent for *Nocardia* PCR testing and so none could be included in our study (see Table S1 in the supplemental material). Therefore, to obtain positive controls, we selected *Nocardia* culture-positive patients who had had a clinical sample sent to the French Observatory of Nocardiosis (Lyon, France) for both culture and PCR testing between 2010 and 2014. The probable nocardiosis group had positive *Nocardia* PCR results for a clinical sample, no alternative diagnosis (following a comprehensive microbiological evaluation; see below), and negative culture results for *Nocardia*. Patients with coinfections were discussed further, to confirm a diagnosis of nocardiosis or to exclude the case. Patients who fit into none of the aforementioned groups were excluded (Table S1).

Clinical data and definitions. We recorded demographic data (age and sex), underlying conditions favoring nocardiosis, comorbidities (chronic obstructive pulmonary disease and diabetes mellitus), and prophylaxis with sulfamethoxazole-trimethoprim (SXT) at diagnosis. The type of clinical sample with which the *Nocardia* PCR assay was performed and the antibiotic regimen prior to sampling were noted. We recorded clinical and radiological signs (involved organ, dissemination, and radiological description), histological and microbiological investigations (bacteriological, mycological, and virological studies) and indirect tests (e.g., antigen- or PCR-based diagnostic tools), and biological findings (neutrophil count and C-reactive protein concentration) at the time of diagnosis.

Microbiology. Microbiological analyses included prolonged bacteriological cultures (on buffered charcoal-yeast extract [BCYE] agar plates), direct examination (with Gram staining), and cultures for fungi (2 weeks) and mycobacteria (8). Virological investigations (PCR assays for cytomegalovirus and respiratory viruses) were performed with BAL fluid samples in cases with pulmonary involvement. The results of β-D-glucan, galactomannan antigen, and cryptococcal antigen detection, *Pneumocystis jirovecii* immunofluorescence assays, and PCR assays were recorded if such tests were performed. Cytological or histological analysis was performed for all liquid or tissue samples.

The *Nocardia* genus PCR was based on amplification and detection of the gene encoding 16S rRNA and was performed at the French Observatory of Nocardiosis. Biological samples were transported at room temperature. *Nocardia* DNA was extracted with a *Mycobacteria tuberculosis* respiratory specimen preparation kit (Roche, Meylan, France). NG1 (5′-ACCGACCACAAGGGGG-3′) and NG2 (5′-GGTTGTAAAC

CTCTTTCGA-3′) primers were used to amplify a *Nocardia* genus-specific 590-bp fragment of 16S rRNA. In order to amplify a 590-bp fragment of the 16S rRNA gene, amplification was carried out using packaged PCR tubes (Ready-to-Go PCR beads; Amersham Biosciences, Orsay, France). Ten microliters of extracted DNA and primers NG1 (5′-ACCGACCACAAGGGG-3′) and NG2 (5′-GGTTGTAACCTCTTCGA-3′), at a final concentration of 1 μM for each primer, were added for a final volume of 25 μl of PCR mixture. First, we performed an initial denaturation at 94°C for 11 min. After 40 cycles of denaturation at 94°C for 60 s, primer annealing at 55°C for 20 s, and extension at 72°C for 60 s, followed by postamplification extension at 72°C for 10 min, PCR products were purified with the E.Z.N.A. gel extraction kit (Omega Bio-Tek, Vaulx-en-Velin, France). Because the hybridization step is time-consuming and thus difficult to perform in routine practice, we replaced this method with a DNA dilution step, as reported previously (9). Therefore, nondiluted and diluted (1:10, 1:25, 1:100, and 1:250) DNA samples were used to reduce the concentrations of inhibitors. β-Globin PCR was used as a control to monitor specimen processing and DNA extraction. *Nocardia* species-specific PCR was performed for *Nocardia farcinica*, targeting the *nfa* 29510 gene, according to the method described by Hasegawa et al. (10).

Statistical analysis. The final analysis was conducted after all data had been recorded and verified. Clinical, biological, and radiological data recorded at the time of diagnosis are described. Continuous variables are presented as medians and ranges. Categorical variables are presented as numbers and percentages. The specificity of the PCR for a diagnosis of nocardiosis was calculated as the proportion of negative *Nocardia* PCR results in the NC group. *Nocardia* PCR sensitivity was calculated as the proportion of positive *Nocardia* PCR results in the PC group. Bivariate analyses were performed using Fisher's exact test to compare categorical variables and Student's *t* test to compare continuous variables.

Ethics. This study was approved by the Comité de Protection des Personnes Ile-de-France 1 (CCTIRS approval no. 15.1016) and the Commission Nationale de l'Informatique et des Libertés.

RESULTS

Characteristics of the study population and excluded patients. During the study period, 85 patients were considered for inclusion, and 25 were excluded (see Table S1 in the supplemental material). With the additional 8 PC patients, we therefore studied a total of 68 patients; their characteristics are presented in Table 1. Most of the patients had more than one underlying condition for nocardiosis (43/68 patients [63%]). Twenty-five patients (37%) were receiving prophylaxis with SXT at the time of diagnosis (700 to 5,600 mg of sulfamethoxazole/week). The organs most frequently involved were the lungs (61/68 patients [90%]), and infection was disseminated in 15 of the patients (22%). *Nocardia* PCR was most often performed with respiratory samples (58/68 patients [85%]).

Nocardia PCR specificity. Forty-seven patients (30 men and 17 women; median age, 52 years [range, 5 to 83 years]) were classified as NC. PCR was performed with respiratory samples for 41 of those patients (32 BAL fluid samples, 6 bronchial aspiration samples obtained during bronchial endoscopy, 2 sputum samples, and 1 lung biopsy specimen), with pleural fluid samples for 2 patients, with cutaneous biopsy specimens for 2 patients, with a cerebrospinal fluid sample for 1 patient, and with a lymph node tissue specimen for 1 patient. Forty-three patients had another diagnosis (pneumonia with bacterial identification, $n = 15$; pneumonia without microbial identification, $n = 9$; noninfectious pneumonia, $n = 3$; fungal infection, $n = 8$; mycobacterial infection, $n = 3$; viral infection, $n = 3$; hematological malignancy, $n = 3$; other diseases, $n = 3$); some patients had several diagnoses. The microbiological evaluations for the NC group are detailed in Table S2 in the supplemental material.

Nocardia PCR test results were negative for 35 of the 47 NC patients and positive for 12, with 6 species-specific PCR assays being positive for *N. farcinica*. The specificity of the *Nocardia* PCR assay was thus 74% (35/47 patients). The positive PCR test results for the NC patients were all from respiratory samples; patients in this group were more likely to have chronic bronchopulmonary disease than were patients with negative PCR results (8/12 patients [67%] versus 11/35 patients [31%]; $P = 0.044$) (Table 2). An alternative diagnosis of fungal, bacterial, or viral infection was identified for one-half of the patients with positive PCR results (6/12 patients).

Nocardia PCR sensitivity. The 8 PC patients included 3 men and 5 women, with a median age of 54 years (range, 19 to 89 years). One patient was receiving prophylaxis with SXT at diagnosis and 5 patients had received antibiotic treatment before sampling for PCR testing and culture (β-lactams for 4 patients, SXT for 2 patients, and aminoglycosides, fluoroquinolone, and linezolid for 1 patient each). All patients had lung involvement (4 had nodules, 2 had excavations, 1 had nodules and excavations, 1 had

TABLE 1 Main characteristics of the study population ($n = 68$)

Characteristic[a]	Finding
Age at diagnosis (median [range]) (yr)	54 (5–89)
Male (n [%])	42 (61.7)
Underlying conditions for nocardiosis (n [%])[b]	
Corticosteroid	27 (39.7)
Immunosuppressive treatment	27 (39.7)
Chronic bronchopulmonary disease or asthma	27 (39.7)
Solid organ transplantation	14 (20.5)
Allogeneic HSCT	14 (20.5)
Primary immunodeficiency[c]	9 (13.2)
HIV infection	7 (10.2)
Autologous HSCT	4 (5.8)
Comorbidities (n [%])	
Diabetes mellitus (n = 66)	10 (15.1)
Neutropenia (n = 64)[d]	2 (3.1)
SXT prophylaxis (n [%])	25 (36.7)
Type of clinical sample for *Nocardia* PCR testing (n [%])	
Respiratory samples	
BAL fluid sample	40 (58.8)
Bronchial sampling specimen	7 (10.2)
Sputum sample	6 (8.8)
Lung biopsy specimen	2 (2.9)
Other specimens	
Abscess specimen	5 (7.3)
Cutaneous biopsy specimen	3 (4.4)
Pleural fluid sample	2 (2.9)
CSF sample	2 (2.9)
Lymph node specimen	1 (1.4)
PCR result (n [%])	
Positive	32
Positive for *N. farcinica*	14 (43.7)
Clinical/radiological presentation (n [%])	
Pulmonary	61 (89.7)
Disseminated disease	15 (22)
Cutaneous or muscular	10 (14.7)
Cerebral	6 (8.8)
Deep-seated abscess	4 (5.8)
Other[e]	8 (11.7)
Duration of symptoms before sampling (median [range]) (days) (n = 57)	30 (1–730)
Outcome of death at 6 mo (n [%]) (n = 67)	10 (14.7)

[a]BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transplantation; SXT, trimethoprim-sulfamethoxazole.
[b]Each patient could have several underlying conditions.
[c]Primary immunodeficiencies included chronic granulomatous disease ($n = 3$), defect of the interleukin-12 (IL-12) axis ($n = 2$), severe combined immunodeficiency ($n = 2$), lymphohistiocytosis ($n = 1$), and X-linked agammaglobulinemia ($n = 1$).
[d]Defined as polymorphonuclear cell counts of <500 cells/mm^3.
[e]Other includes lymph node ($n = 4$), bone ($n = 2$), blood ($n = 1$), eye ($n = 1$), and liver ($n = 1$) involvement.

lung consolidation, and 1 had interstitial pneumonia), and 2 had disseminated nocardiosis (1 had cerebral and ocular abscesses and 1 had cerebral and other abscesses with lymph node involvement). Bacterial or fungal coinfection was identified for 5 patients. PCR testing was performed with respiratory samples (2 BAL fluid samples, 1 bronchial sampling specimen, and 4 sputum samples) and a muscle abscess sampling specimen ($n = 1$). The *Nocardia* species identified were *Nocardia abscessus* ($n = 3$), *Nocardia cyriacigeorgica* ($n = 1$), *N. farcinica* ($n = 1$), *N. nova* ($n = 1$), *Nocardia paucivorans* ($n = 1$), and *Nocardia wallacei* ($n = 1$). After 6 months, 2 patients had died; 1 death was

TABLE 2 Characteristics of the 47 patients in the negative-control group, according to *Nocardia* PCR results

Characteristic[a]	Negative *Nocardia* PCR result (n = 35)	Positive *Nocardia* PCR result (n = 12)	P (bivariate analysis)
Age at diagnosis (median [range]) (yr)	53 (5–83)	49 (17–70)	0.7
Male (n [%])	24 (68.5)	6 (50)	0.3
Sample origin (n [%])			
Pulmonary	29 (82.8)	12 (100)	0.3
Other[b]	6 (17.1)	0	
Underlying conditions (n [%])			
Corticosteroid	13 (37.1)	4 (33.3)	1
Immunosuppressive treatment	13 (37.1)	7 (58.3)	0.3
Hematological malignancy	12 (34.2)	6 (50)	0.5
Chronic bronchopulmonary disease	11 (31.4)	8 (66.6)	0.044
HIV	6 (17.1)	0	0.31
PID	5 (14.2)	2 (16.6)	1
SOT	9 (25.7)	3 (25)	1
SXT prophylaxis (n [%])	17 (48.5)	3 (25)	0.19
Antibiotic treatment before sampling (n [%]) (n = 46)	18 (51.4)	4 (36.3)	0.33

[a]HIV, human immunodeficiency virus; PID, primary immunodeficiency; SOT, solid organ transplantation; SXT, trimethoprim-sulfamethoxazole.
[b]Other includes cutaneous biopsy (n = 2), pleural effusion (n = 2), cerebrospinal fluid (n = 1), and abscess (n = 1).

related to nocardiosis (10 days after diagnosis), and 1 death was related to the patient's underlying condition. Outcomes were favorable for the other 6 patients.

Nocardia PCR test results were positive for 7 of the 8 PC patients, giving a sensitivity of 88%. The infection not detected by PCR was caused by *N. paucivorans*. After identification of this strain in culture, the strain was tested using the *Nocardia* genus PCR, which yielded positive results. Microscopic examination results were positive for 6/7 PC patients (data were not available for 1 patient), including the aforementioned patient with negative PCR results.

Probable nocardiosis. Thirteen cases of probable nocardiosis were diagnosed based on positive *Nocardia* PCR results despite negative culture and Gram staining results (Table 3). Nine of the patients were men (69.2%), and the median age was 54 years (range, 22 to 82 years). PCR was mostly performed with respiratory samples. Antibiotics were prescribed before microbiological sampling in 5 cases, and 4 patients were receiving prophylaxis with SXT. Coinfections were diagnosed for 5 patients (Table 3). Among the pulmonary infections, we observed lung consolidation in 5 cases, nodules in 5 (1 excavated nodule), and alveolointerstitial pneumonia in 2. Clinical and radiological features are shown in Fig. 2. Two patients required surgical or radiological drainage. Cure was observed for 8 patients, 1 patient experienced relapse, 1 patient was lost to follow-up monitoring after 2 months of antibiotic treatment, and 3 patients died; no deaths were attributed to nocardiosis.

DISCUSSION

In this first pilot study assessing the 16S rRNA PCR-based *Nocardia* assay in clinical practice, we observed a specificity of 74% and a sensitivity of 88% for diagnosis of *Nocardia* infection. In our study population, positive *Nocardia* PCR results in the NC group were observed only with respiratory samples, suggesting that the PCR may detect airway colonization (1, 11). This hypothesis is supported by the fact that bronchopulmonary disease was more frequent in NC patients with positive *Nocardia* PCR results (67%) than in those with negative PCR results. A high frequency of upper respiratory tract *Nocardia* colonization among asymptomatic healthy adults (45/101 subjects [44.5%]) was recently detected using PCR and electrospray ionization-time of
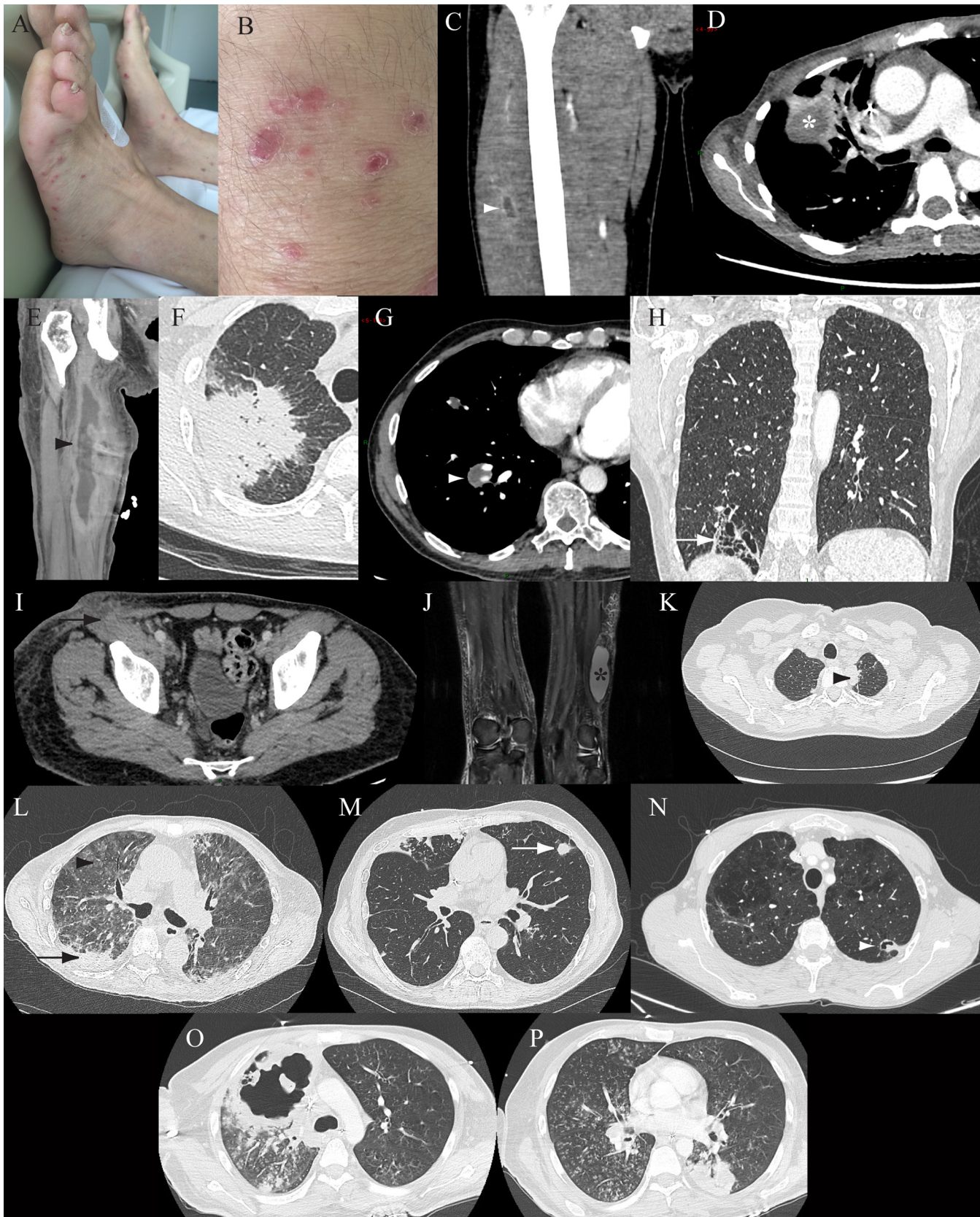
FIG 2 Clinical and radiological features in a series of 13 patients with probable nocardiosis. (A and B) Patient 1, multiple superficial and purple nodular skin lesions on both legs. (C) Patient 2, coronal enhanced computed tomography (CT) of the right leg, showing a muscle abscess of the thigh (white arrowhead, central low attenuation with peripheral enhancement). (D and E) Patient 3, axial enhanced lung CT (D), showing a right pulmonary mass (white star, central

flight mass spectrometry (12). Use of a quantitative PCR threshold may help to distinguish airway colonization from infection, as suggested for *Pneumocystis* (13, 14). To reduce possible misdiagnosis and excess antibiotic treatment, we suggest that *Nocardia* PCR testing should be used only for patients with a high index of suspicion of nocardiosis, i.e., with an underlying condition favoring nocardiosis, signs consistent with a diagnosis of nocardiosis, and no other obvious diagnosis. Use of antibiotic prophylaxis with SXT in immunocompromised patients with airway colonization may warrant further evaluation.

The sensitivity of the *Nocardia* PCR assay was not 100%, indicating that a negative *Nocardia* PCR result does not necessarily exclude a diagnosis of nocardiosis (5). Although the small sample size of our PC group prevents us from drawing any definitive conclusions, our results suggest that no test can completely exclude a diagnosis of nocardiosis in an immunocompromised patient with a clinical picture consistent with this diagnosis.

We described the clinical, biological, and radiological presentations of patients with probable nocardiosis diagnosed solely using positive PCR results, without positive culture results. Because bacteriological cultures are thought to have low sensitivity for detection of *Nocardia* (although this has never been assessed), especially among antibiotic-treated patients, choosing a diagnostic gold standard is difficult. Our classification criteria, defining probable nocardiosis based on a comprehensive negative evaluation for alternative diagnoses, reflect a real-life approach. For a few patients, improvement with anti-*Nocardia* treatment was an additional argument in favor of this diagnosis. We decided to include coinfected patients because this situation is frequently (10 to 30%) observed during nocardiosis, mainly among solid organ recipients or HIV-infected patients. The most frequently identified copathogens are *Aspergillus fumigatus*, *Pneumocystis jirovecii*, *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and cytomegalovirus (1, 11, 15).

For one-half of our patients with probable nocardiosis, identification at the species level enabled the antibiotic regimen to be adapted. Among the different *Nocardia* species, *N. farcinica* has one of the highest degrees of resistance to available antibiotics. Although imipenem and amoxicillin-clavulanic acid are the β-lactam antibiotics that are most frequently active against this pathogen (33 to 100% susceptibility for imipenem and 73 to 100% for amoxicillin-clavulanic acid) (1, 11, 16–19), they should be prescribed in association with amikacin, because of the high frequency of resistant strains (18). In contrast, *N. farcinica* strains have a low rate of resistance to SXT and no resistance to linezolid. If *N. farcinica*-specific PCR results are negative, then a broad-spectrum antibiotic, such as SXT, linezolid, or the combination of a β-lactam and amikacin, is required. Among our nocardiosis cases, 4/13 patients with probable nocardiosis and 1/8 patients with proven nocardiosis were receiving SXT prophylaxis, confirming that a diagnosis of nocardiosis should not be ruled out simply because prophylaxis has been given.

During the study period, only 3 patients had a culture-based diagnosis of nocardiosis in our centers, providing a striking contrast with the 13 PCR-based diagnoses. We cannot exclude the possibility that some of our probable cases were the result of colonization rather than true infection; nevertheless, we suspect that the incidence of nocardiosis may be underestimated when diagnosis relies on positive culture results.

FIG 2 Legend (Continued)
attenuation), and coronal enhanced CT of the right leg (E), showing a muscle abscess of the thigh (black arrowhead, central attenuation with peripheral enhancement). (F) Patient 4, axial nonenhanced lung CT, showing a right spiculated lung consolidation. (G) Patient 5, axial enhanced lung CT, showing a right pulmonary mass (white arrowhead, low density). (H) Patient 6, coronal enhanced lung CT, showing localized bronchiectasis with thin surrounding lung consolidation (white arrow). (I) Patient 7, axial enhanced abdominal CT, showing a subcutaneous abscess and lymph node involvement (black arrow). (J) Patient 8, coronal T2 fat-saturated magnetic resonance imaging of the legs, showing a subcutaneous abscess (black star, hyperintensity). (K) Patient 9, axial nonenhanced lung CT, showing a left apical lung mass (black arrowhead). (L) Patient 10, axial enhanced lung CT, showing a lung consolidation (black arrow) and diffuse ground-glass opacities (black arrowhead). (M) Patient 11, axial nonenhanced lung CT, showing a lung nodule (white arrow). (N) Patient 12, axial nonenhanced lung CT, showing a cavitated lung nodule (white arrowhead). (O and P) Patient 13, axial nonenhanced lung CT, showing bilateral involvement with excavation of 10 cm, micronodules, and lung consolidation.

TABLE 3 Main characteristics of the 13 patients with probable nocardiosis diagnosed solely using PCR results

Patient no.	Age (yr)	Sex[a]	Clinical or radiological involvement	Sample(s) for *Nocardia* PCR	Other tests and findings	Concurrent pathogen	Nocardiosis risk factors	Positive species PCR	SXT prophylaxis (weekly dose of SMT [mg])	Prior antibiotic treatment	Treatment (overall treatment duration)	Outcome at 6 mo
1	68	M	Cutaneous	Cutaneous biopsy specimen (PCR positive)	Cutaneous biopsy (negative bacterial, mycological, and mycobacterial cultures, polymorphous inflammatory infiltrate on histological exam, PAS, Ziehl, and Grocott staining negative); Crypto Ag, CMV PCR, and BDG negative	No	Hematological malignancy	No	Yes (2,400)	No	IPM-AMK then CRO-AMK then IPM-CIP then TZP-LZD then CXM-SXT (6 mo)	Cure
2	24	M	Muscular abscess	Muscular abscess specimen (PCR positive) and blood sample (PCR positive)	Abscess puncture (negative *Nocardia* and mycobacterial cultures, polymorphonuclear cells on histological exam); BDG, GM, *A. fumigatus* PCR, and *Toxoplasma gondii* PCR negative	Yes (*Staphylococcus aureus*)	Hematopoietic stem cell transplantation	Yes (*N. farcinica*)	No	β-Lactams	LZD then AMC then AMK then AMC (4 mo)	Cure
3	63	F	Disseminated (lung infection and muscular abscess)	Muscular abscess specimen (PCR positive) and BAL fluid sample (PCR positive)	BAL (negative bacterial, mycological, and mycobacterial cultures and GM test); abscess puncture (negative bacterial and mycobacterial cultures and broad-range 16S rRNA PCR); BDG, GM, and *A. fumigatus* PCR negative	No	Hematopoietic stem cell transplantation and IS	No	No	NA	IPM-AMK then LZD then CRO-AMK then SXT-AMK (7 mo)	Cure
4	34	M	Lung infection	BAL fluid sample (PCR positive) and pulmonary biopsy specimen (PCR negative)	BAL (negative bacterial, mycological, and mycobacterial cultures, histological exam, and GM test); pulmonary biopsy (negative bacterial, mycological, and mycobacterial cultures, epithelioid granuloma on histological exam); BDG positive	No	SOT, CGD, and IS	Yes (*N. farcinica*)	Yes (5,600)	No	IPM-SXT then SXT (6 mo)	Cure
5	54	M	Lung infection	BAL fluid sample (PCR positive)	BAL (negative bacterial, mycological, and mycobacterial cultures, GM test, and histological exam); pulmonary biopsy (negative bacterial, mycological, and mycobacterial cultures, eosinophilic necrosis); BDG, GM, *A. fumigatus* PCR, Crypto Ag, and CMV PCR negative	No	Hematological malignancy and chronic bronchopulmonary disease	No	Yes (2,400)	No	IPM, AMK, SXT (NA)	NA
6	60	F	Lung infection	BAL fluid sample (PCR positive)	BAL (negative bacterial, mycological, and mycobacterial cultures and GM test, filamentous form on histological exam, Ziehl staining negative)	No	Chronic bronchopulmonary disease	Yes (*N. farcinica*)	No	No	AMC (1 mo), LZD (5 mo)	Relapse 7 mo later
7	22	M	Disseminated (subcutaneous abscess and lymph node)	Lymph node biopsy specimen (PCR positive) and cutaneous abscess specimen (PCR positive)	Lymph node biopsy and cutaneous abscess puncture (negative *Nocardia*, mycological, and mycobacterium cultures); CMV PCR negative	Yes (*S. aureus* and *Staphylococcus epidermidis*)	Autologous stem cell transplantation and corticosteroids	Yes (*N. farcinica*)	Yes (2,400)	β-Lactams, aminoglycosides	IPM-SXT-AMK then SXT then LZD (4 mo)	Death

(Continued on next page)

TABLE 3 (Continued)

Patient no.	Age (yr)	Sex[a]	Clinical or radiological involvement	Sample(s) for *Nocardia* PCR	Other tests and findings	Concurrent pathogen	Nocardiosis risk factors	Positive species PCR	SXT prophylaxis (weekly dose of SMT [mg])	Prior antibiotic treatment	Treatment (overall treatment duration)	Outcome at 6 mo
8	82	F	Muscular abscess	Muscular abscess specimen (PCR positive)	Abscess puncture (negative bacterial, mycological, and mycobacterial cultures, polymorphonuclear cells on histological exam); BDG positive, GM and *A. fumigatus* PCR negative	No	Corticosteroids and IS	Yes (*N. farcinica*)	No	No	IPM-AMK (0.2 mo)	Death
9	54	F	Disseminated (lung and bone infection)	BAL fluid sample (PCR positive)	BAL (negative bacterial, mycological, and mycobacterial cultures and histological exam); pulmonary biopsy (negative mycological and mycobacterial cultures, giant cell granuloma with epithelioid cells without necrosis on histological exam)	No	Hematological malignancy	Yes (*N. farcinica*)	No	No	IPM-SXT then AMK-SXT then SXT (12 mo)	Cure
10	64	M	Lung infection	BAL fluid sample (PCR positive) and lung biopsy specimen (PCR positive)	BAL (negative bacterial, mycological, and mycobacterial cultures, GM test and virus PCR negative); pulmonary biopsy (negative bacterial and mycobacterial cultures); BDG positive, GM negative	Yes (*P. jirovecii* and *A. fumigatus*)	SOT, corticosteroids, and IS	No	No	Sulfonamides	SXT then SXT-IPM (NA)	Death
11	51	M	Lung infection	BAL fluid sample (PCR positive)	BAL (negative *Nocardia*, mycological, and mycobacterial cultures); BDG and GM negative	Yes (*Moraxella* and *Actinomyces naeslundii*)	Hematopoietic stem cell transplantation	Yes (*N. farcinica*)	No	β-Lactams	CXM (6.4 mo)	Cure
12	48	M	Disseminated (meningitis and lung infection)	CSF sample (PCR positive) and bronchial sampling specimen (PCR positive)	BAL and CSF sampling (negative bacterial, mycological, and mycobacterial cultures); Crypto Ag negative	No	HIV	No	No	No	IPM-AMK then IPM-SXT then SXT-MXF (6 mo)	Cure
13	40	M	Lung infection	BAL fluid sample (PCR negative) and lung biopsy specimen (PCR positive)	BAL and pulmonary biopsy (negative *Nocardia*, mycological, and mycobacterial cultures)	No	Asthma	No	No	β-Lactams, sulfonamides, aminoglycosides	IMP-SXT-AMK then IMP-SXT then SXT (6 mo)	Cure

[a]M, male; F, female; AMC, amoxicillin-clavulanic acid; AMK, amikacin; BAL, bronchoalveolar lavage; BDG, β-D-glucan; CGD, chronic granulomatous disease; CIP, ciprofloxacin; CMV, cytomegalovirus; CRO, ceftriaxone; Crypto Ag, *Cryptococcus* antigen; CSF, cerebrospinal fluid; CXM, cefuroxime; GM, galactomannan antigen; HIV, human immunodeficiency virus; IPM, imipenem; IS, immunosuppressive treatment; LZD, linezolid; MXF, moxifloxacin; NA, not available; PAS, periodic acid-Schiff; SMT, sulfamethoxazole; SOT, solid organ transplantation; TZP, piperacillin-tazobactam; SXT, sulfamethoxazole-trimethoprim.

In conclusion, *Nocardia* PCR testing performed directly on a biological sample is a valuable additional assay for the diagnostic evaluation of infections in immunocompromised patients. The interpretation of PCR results may be challenging, however, because of possible detection of airway colonization.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM .00002-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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