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Review

Pulmonary nocardiosis in Western Europe—Clinical evaluation of 43 patients and population-based estimates of hospitalization rates

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ABSTRACT

Background: Pulmonary nocardiosis (PN) is an uncommon but potentially life-threatening infection. Most of our knowledge on PN is derived from case reports and small case series. Increasing incidence rates of PN have been reported recently. The aim of this study was to describe the clinical course of and risk factors for PN in four Western European countries and to estimate population-based annual hospitalization rates.
Methods: This was a retrospective evaluation (1995–2011) of the clinical course of and risk factors for PN in patients at 11 hospitals in four European countries (Germany, Austria, Switzerland, and the Netherlands). Population-based estimates of hospitalization rates for PN in Germany (2005 to 2011) were calculated using official German nationwide diagnosis-related groups (DRG) hospital statistics.
Results: Forty-three patients fulfilled stringent criteria for proven ($n = 8$) and probable ($n = 35$) PN; seven had extrapulmonary dissemination. For these 43 patients, the major risk factors for PN were immunocompromising (83.7%) and/or pulmonary (58.1%; as only comorbidity in 27.9%) comorbidities. The median duration of PN targeted therapy was 12 weeks. Distinctive patterns of resistance were observed (imipenem susceptibility: *Nocardia farcinica* 33.3%; *Nocardia asteroides* 66.7%). The overall mortality rate was 18.9% (50% in disseminated PN). Over time, annual PN hospitalization rates remained unchanged at around 0.04/100 000, with the highest rate among men aged 75–84 years (0.24/100 000).
Conclusions: PN is a rare, but potentially life-threatening disease, and mainly affects immunocompromised elderly males. Overall, annual hospitalization rates remained stable between 2005 and 2011.

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Introduction

Nocardiosis is a rare infection caused by *Nocardia* spp. *Nocardiaceae* are aerobic, Gram-positive, branching, partially acid-fast filamentary bacteria that are found worldwide in soil, decomposing vegetation, dust, and in fresh as well as salt water (Brown-Elliott et al., 2006). More than 60 different species have been identified and half of them have been described as pathogens in humans (Martinez et al., 2008). Despite their ubiquitous occurrence, *Nocardia* spp. are considered to be neither a commensal of the normal human flora nor a common laboratory contaminant (McNeil and Brown, 1994). Isolation of *Nocardia* spp. from lower respiratory tract samples in the presence of clinical signs and symptoms of a lower respiratory tract infection or compatible radiographic findings should always raise suspicion of a causative role for this pathogen. Pulmonary nocardiosis (PN) may have a potentially life-threatening course, especially in disseminated cases (Hardak et al., 2012; Beaman et al., 1976; Boiron et al., 1992; Minero et al., 2009). So far, transmission from human to human has not been documented, and dissemination usually results from a pulmonary focus (Ambrosioni et al., 2010).

Nocardiosis mainly manifests as an opportunistic infection in immunocompromised patients (Chen et al., 2013). An impairment of cell-mediated immunity is considered to be the major risk factor (Tremblay et al., 2011; Deem et al., 1983; Beaman and Beaman, 1994; Filice and Niewoehner, 1987). Taking into account the increasing use of immunosuppressive therapies, including new treatments such as biological medicines, e.g. monoclonal antibodies, the incidence of *Nocardia* infection seems to be on the rise (Minero et al., 2009; Tremblay et al., 2011). However, reports of immunocompetent patients with nocardiosis are also emerging (Wilson, 2012). In these patients, pulmonary comorbidities, mainly chronic obstructive pulmonary disease (COPD), are important risk factors for PN (Martinez et al., 2008).

The aims of this study were (1) to characterize the risk factors, microbiology, and clinical course of PN in a well-defined cohort of patients in four Western European countries, and (2) to estimate population-based age- and sex-specific, as well as age-adjusted hospitalization rates for PN in Germany.

Materials and methods

Cohort study

This retrospective multicentre study included patients from 11 hospitals in four countries (Germany ($n = 7$), Austria ($n = 2$), Switzerland ($n = 1$), the Netherlands ($n = 1$)); the patients were diagnosed with PN between January 1, 1995 and December 31, 2011. A search of the microbiological laboratory data at each institution was performed to identify eligible patients. Data were collected from the patient medical records, including demographic characteristics, clinical signs and symptoms, radiological abnormalities on chest X-ray or computed tomography (CT) scans, laboratory results, microbiological findings, comorbidities and risk factors, underlying pulmonary morbidities, immunosuppressive medications, antibiotic treatment, and outcome.

Case definition

Only patients with a proven or probable pulmonary *Nocardia* infection were included. Due to clinical similarities to fungal infections, proven and probable PN were defined according to the European Organisation for Research and Treatment of Cancer (EORTC) criteria for invasive aspergillosis. Patients had to fulfil one of the following conditions for study entry:

(1) Proven PN: a positive culture from samples taken from a sterile primary site, e.g. pleural effusion, blood, Cerebrospinal fluid, tissue biopsy, or abscess puncture AND (i) radiological findings on chest X-ray or CT scan of the thorax compatible with pulmonary nocardiosis OR (ii) clinical signs and symptoms of a lower respiratory tract infection (cough, sputum, dyspnoea, fever >38.3 °C).
(2) Probable PN (one of the following): (i) a positive culture from sputum, tracheobronchial aspirate, or bronchoalveolar lavage (BAL) AND (a) clinical signs and symptoms of a lower respiratory tract infection (cough, sputum, dyspnoea, fever >38.3 °C) AND (b) radiological findings on chest X-ray or CT scan of the thorax consistent with pulmonary nocardiosis; (ii) microscopic proof of Gram-positive, branching, partially acid-fast filamentary bacteria in histological samples (identified as *Nocardia* spp.) AND (a) clinical signs and symptoms of a lower respiratory tract infection (cough, sputum, dyspnoea, fever >38.3 °C) OR (b) radiological findings on chest X-ray or CT scan of the thorax consistent with pulmonary nocardiosis.

Patients with proven and probable PN were then included as a single cohort and their data summarized and analyzed. Patients under the age of 18 years were excluded from this study.

Microbiology

At all institutions, the identification of *Nocardia* spp. and susceptibility testing were performed only upon special request. Since genotypic methods for species identification as part of clinical routine (e.g., 16S rRNA gene PCR) became available only late during the study period, patients with conventional phenotypic and biochemical species identification were included, as well as patients with genotypic identification results.

Definitions

A disseminated course was defined as proven or probable PN with involvement of at least one other non-contiguous organ, or a radiological finding, e.g. abscess formation in organs other than the lungs, such as the brain or abdomen, interpreted as being compatible with *Nocardia* infection in a patient with PN.

Immunosuppressive medication was defined as chronic (>4 weeks) intake of steroids at a dosage of >10 mg prednisone or equivalent per day, or the use of another immunosuppressive agent such as a calcineurin inhibitor, mycophenolate, cytostatic agent, mTOR inhibitor, TNF-binding protein, or other biological medicine.

Estimation of the hospitalization rate in Germany

For the estimation of the annual population-based hospitalization rates for PN, the official German nationwide diagnosis-related groups (DRG) hospital statistics were analyzed (German Federal Health Monitoring System, 2013). These have included data on primary discharge diagnoses since inception and on all secondary discharge diagnoses since 2005, from all German hospitals using DRG billing of medical services. Almost all German hospitals use this system, with the exception of hospitals for prevention, rehabilitation, and mental and mood disorders, and day care units. Cases were identified by extracting all records for which the four-digit International Classification of Diseases, 10th revision (ICD-10) code A43.0 (pulmonary nocardiosis) within the three-digit category A43 (nocardiosis) was listed as a primary or secondary hospital discharge diagnosis. De-identified DRG diagnosis data were provided for the whole of Germany as absolute numbers stratified by age group, sex, and year of diagnosis.

Statistical analysis

Results are expressed as frequencies or as the mean ± standard deviation (SD) unless indicated otherwise. The two-tailed Fisher's exact test was used to compare proportions. The significance level was set to 5% for all analyses, and *p*-values are reported. All data were analyzed and processed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA) on a Windows XP operating system (Microsoft, Redmond, WA, USA).

For the estimation of PN hospitalization rates, all hospitalizations with PN as the primary or secondary hospital discharge diagnosis in Germany from 2005 through 2011 were included. Official German census age- and sex-specific population data were used as the denominator for all calculations (German Federal Health Monitoring System, 2013). Age adjustment was performed by direct method in order to control for different age distributions across Germany and to allow for comparison between different years. Age-adjusted hospitalization rates were estimated using the appropriate German Census Standard Population as the reference population. The data analysis was performed in Excel (Microsoft).

Results

Cohort study

Patients

Fifty-eight patients with microbiologically proven *Nocardia* spp. were identified. Of these, 43 patients fulfilled the criteria for proven ($n = 8$) or probable ($n = 35$) PN and were included in the study. Most patients were recruited in Germany ($n = 22$), followed by Austria ($n = 13$), Switzerland ($n = 6$), and the Netherlands ($n = 2$).

Forty patients (93.0%) presented with relevant comorbidities. A pulmonary comorbidity was the most frequent predisposing factor ($n = 25$); this was the only underlying comorbidity in 12/43 patients (27.9%). Twenty-two patients (51.2%; $n = 22/43$) were receiving immunosuppressive treatment, of whom eight were receiving corticosteroid monotherapy (see Table 1). HIV testing was performed for 20 patients and all tested negative. Twenty-four patients had received antibiotic treatment prior to admission. Details of the demographic and epidemiological characteristics are summarized in Table 1.

Clinical and radiological findings

The clinical picture was non-specific and comparable to other entities of lower respiratory tract infection (see Table 2). Due to incomplete data, information on sputum colour, pleuritic chest pain, and haemoptysis were not available. Overall, inflammatory parameters were only moderately elevated, despite wide ranges of values (Table 2). Owing to the non-specific clinical appearance of PN, the median time to final diagnosis was 14 days (range 0–54 days).

Detailed clinical and/or radiological data on extrapulmonary involvement were available for 38 patients, and seven patients (18.4%; $n = 7/38$) suffered from disseminated nocardiosis (Table 2). The central nervous system (CNS) was the most frequently affected site ($n = 5$). Two patients presented extrapulmonary foci in more than one additional organ.

Table 1
Demographic and epidemiological characteristics of 43 patients with pulmonary nocardiosis.[a]

Age (years), mean ± SD (range)	55.8 ± 14.4 (21–75)
Sex	
Male	31/43 (72.1%)
Female	12/43 (27.8%)
Admission diagnosis ($n = 38$)	
Pneumonia	21/38 (55.3%)
Lung cancer/metastasis	3/38 (7.9%)
Tuberculosis	2/38 (5.3%)
Acute exacerbation of a pre-existing lung disease (e.g. bronchiectasis, COPD)	2/38 (5.3%)
Pleural effusion/empyema	2/38 (5.3%)
Pulmonary nocardiosis	2/38 (5.3%)
Other[b]	6/38 (15.8%)
Antibiotic pretreatment	24/43 (55.8%)
Underlying disease[c]	40/43 (93.0%)
Pulmonary morbidity[c]	25/43 (58.1%)
COPD	8/43 (18.6%)
Tuberculosis	5/43 (11.6%)
Bronchiectasis	3/43 (7.0%)
Interstitial lung disease	4/43 (9.3%)
Other pulmonary disease[d]	10/43 (23.3%)
Solid organ transplantation	11/43 (25.6%)
Haematological disease	10/43 (23.3%)
Diabetes mellitus	6/43 (14.0%)
Autoimmune disease	5/43 (11.6%)
Liver disease	2/43 (4.7%)
Severe renal impairment	2/43 (4.7%)
Immunosuppressive therapy	22/43 (51.2%)
Combination therapy	14/43 (32.6%)
Monotherapy (corticosteroid)	8/43 (18.6%)

COPD, chronic obstructive pulmonary disease; CMV, cytomegalovirus; SD, standard deviation.

[a] Results are presented as the number and percentage (%), unless specified otherwise.

[b] One patient each with one of the following: haemoptysis, fever of unknown origin, meningitis, vertigo and nausea, rejection following lung transplantation, cryptogenic organizing pneumonia.

[c] Patients could have more than one underlying disease or pulmonary morbidity.

[d] One patient each with: asthma, cystic fibrosis, chronic pulmonary CMV infection, obstructive sleep apnoea, pulmonary aspergillosis, non-tuberculous mycobacteria infection, primary ciliary dyskinesia, pulmonary p-ANCA vasculitis, chronic bronchitis.

Table 2
Clinical signs and symptoms, additional extrapulmonary foci, time from admission to diagnosis, and laboratory values.

	Number	%
Clinical signs and symptoms[a] ($n = 43$)		
Cough	33/43	76.7%
Fever	27/43	62.8%
Sputum	26/43	60.5%
Dyspnoea	17/43	39.5%
Weight loss	7/43	16.3%
Disseminated course, extrapulmonary foci[a] ($n = 38$)	7/38	18.5%
Brain	5/38	13.2%
Soft tissue	2/38	5.3%
Skin	1/38	2.6%
Abdomen	1/38	2.6%
	Mean ± SD	Median (range)
Time from admission to final diagnosis (days) ($n = 29$)	16.8 ± 13.2	14.0 (0–54)
Inflammatory markers on blood examination ($n = 37$)		
CRP (mg/l)	102.1 ± 104.9	66.0 (0–429)
WBC ($\times 10^9$/l)	11.6 ± 8.5	10.5 (2.1–37.1)

CRP, C-reactive protein; SD, standard deviation; WBC, white blood cell count.
[a] Patients could have more than one.

Evaluable chest X-rays and/or CT scans were available for 39 patients. Similar to the clinical picture, radiological findings were also variable, non-specific, and heterogeneous. The most frequent abnormality was pulmonary airspace consolidation (89.7%; $n = 35/39$) with or without air bronchograms, followed by (reticulo)-nodular changes (30.1%; $n = 12/39$). Abscess-forming consolidations or cavitations were present in nine patients (23.1%; $n = 9/39$) and pleural effusion in four patients (10%; $n = 4/39$). Details of the distribution of radiological findings are presented in Figure 1.

Microbiological findings

Data on species identification were available for 28 patients. A total of 37 different isolates were identified (see Figure 2). A single *Nocardia* spp. ($n = 6$) was identified in most patients; four patients had two different species, one patient had three different species, and one patient had four different species. *Nocardia farcinica* ($n = 8$), *Nocardia asteroides* ($n = 7$), and *Nocardia cyriacigeorgica* ($n = 3$) accounted for almost 50% of all infections with identification beyond just genus recognition. Data on the duration of growth in culture were available for 24 patients, and the median time to growth was 16 days (mean 18.2 ± 14.7 days; range 2–58 days).

Specimens obtained by bronchoscopy such as BAL, transbronchial lung biopsy, and tracheobronchial aspirate were diagnostic in 27 patients, indicating that endoscopic procedures were performed in approximately two-thirds of all patients (Table 3). Nineteen additional microorganisms were recovered from 16 patients (37.2%; $n = 16/43$) (Table 3).

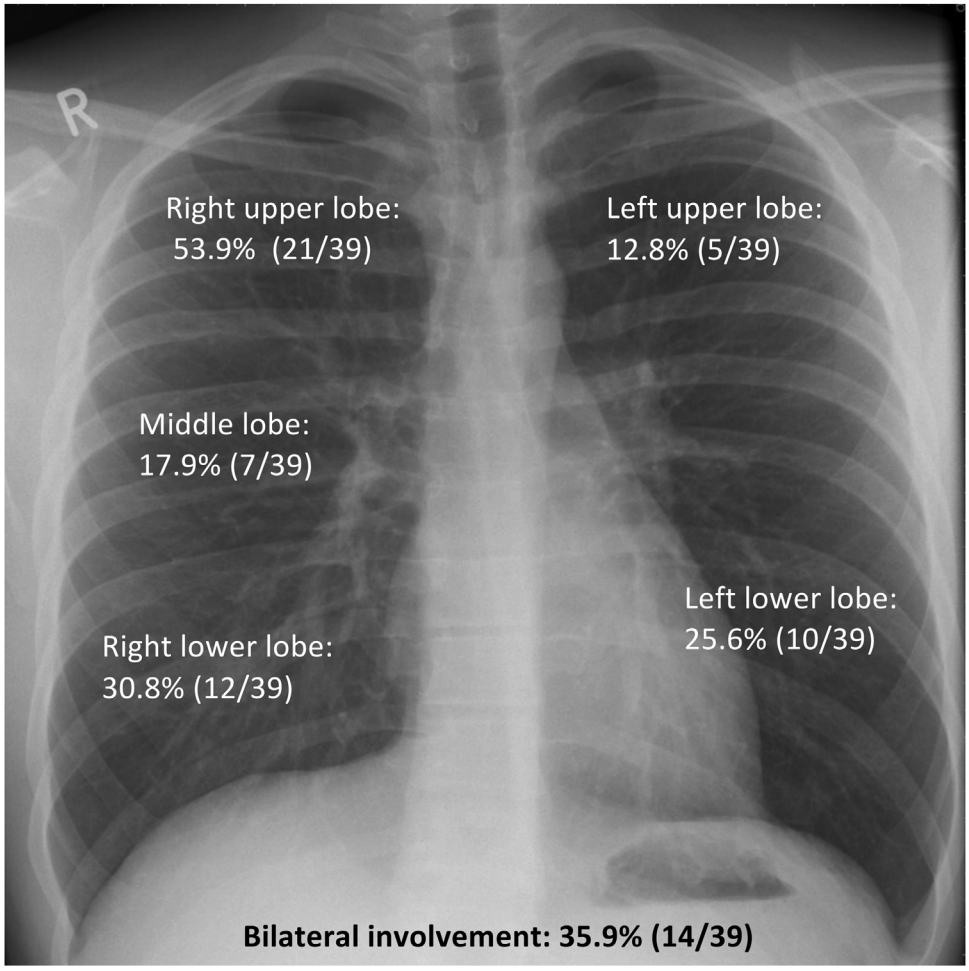

Figure 1. Radiological distribution of abnormalities in 39 patients with pulmonary nocardiosis.

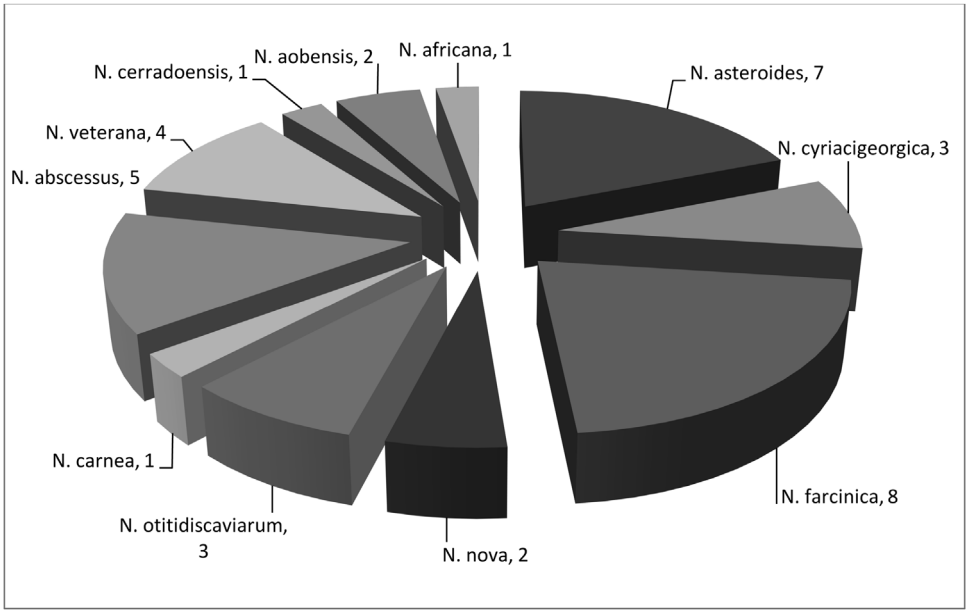

Figure 2. Distribution of *Nocardia* spp. ($n = 37$).

Evaluable data on antimicrobial sensitivity were limited to 20 isolates from six different species (Table 4).

Treatment and outcome

All patients received antibiotic therapy, and a variety of antibacterial agents were given (see Table 5). Orally and intravenously applied trimethoprim–sulfamethoxazole (TMP–SMX; 15 mg/kg of the trimethoprim component per day) was the most frequently prescribed treatment, either as initial therapy or as sequential oral treatment following parenteral 'induction' therapy with imipenem with or without amikacin. Most patients received antibiotic therapy for a prolonged period of time (median 12 weeks; range 1–52 weeks).

Six patients were lost to follow-up, most frequently due to transfer to other medical institutions. The overall mortality rate was 18.9% ($n = 7/37$) and a trend towards higher mortality in patients with disseminated nocardiosis was observed (50% vs. 15.4%; $p = 0.101$), with the highest mortality rate in patients with CNS involvement (75%); all patients with a fatal disseminated infection had CNS involvement.

Table 3
Microbiological results: source of clinical specimen and presence of co-infections ($n = 43$).

	Number	%
Type of diagnostic specimen		
BAL	19/43	44.2%
Sputum	9/43	20.9%
Tracheobronchial aspirate	5/43	11.6%
Lung biopsy	3/43	7.0%
Brain abscess	3/43	7.0%
Skin and soft tissue abscess	2/43	4.7%
Pleural effusion	2/43	4.7%
Pulmonary co-infection[a]	16/43	37.2%
CMV	3/16	18.8%
Staphylococcus aureus	2/16	12.5%
Pseudomonas aeruginosa	2/16	12.5%
MTB	2/16	12.5%
NTM	2/16	12.5%
Aspergillus spp.	2/16	12.5%
Other[b]	6/16	37.5%

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; MTB, *Mycobacterium tuberculosis*; NTM, non-tuberculous mycobacteria; SD, standard deviation.

[a] Patients could have more than one.

[b] One patient each with one of the following: *Moraxella* spp., *Escherichia coli*, *Stenotrophomonas maltophilia*, *Haemophilus parainfluenzae*, *Pneumocystis jirovecii*, *Ralstonia pickettii*.

Population-based estimates of annual rates of hospitalized PN in Germany between 2005 and 2011

From 2005 to 2011, the average annual German population was 82.1 million (range 81.1–82.4 million). During this period, an average 1673 hospitals (95% confidence interval (CI) 1633–1719) were subject to DRG billing of medical services and the average overall number of hospitals in Germany was 2087 (95% CI 2067–2108). Therefore, 80% of all hospitals were covered by the study analysis. A total of 125.2 million hospitalizations were analyzed for the period 2005–2011 (an average 17.9 (95% CI 17.4–18.3) million hospitalizations per year) over an observation period of 574.4 million person-years. Overall, 266 hospitalizations with PN as either the primary or secondary diagnosis were identified. The overall average annual age-adjusted hospitalization rate was 0.04 per 100 000 inhabitants: 0.05 per 100 000 in males and 0.03 per 100 000 in females. However, there was considerable variation with age, with the highest age-specific hospitalization rate of 0.24/100 000 inhabitants among men aged 75–84 years (Figure 3A). During the study period, the annual number of PN-associated hospitalizations remained relatively unchanged between 2005 and 2011 for both males and females (Figure 3B).

Discussion

This study investigated a large cohort of PN patients in European countries, applying a stringent definition of PN. It therefore provides important insights into the epidemiology and clinical appearance and course of this rare infection in high-income countries.

The pivotal role of immunocompromising comorbidities and treatments as major risk factors for PN has been described previously (Martinez et al., 2008; Minero et al., 2009; Ambrosioni et al., 2010; Chen et al., 2013; Poonyagariyagorn et al., 2008; Takiguchi et al., 2017). In the present study cohort, 93% of all patients were suffering from a relevant comorbidity and 51.2%

Table 4
Results of susceptibility testing.[a]

	Susceptibility				
	TMP–SMX	Amikacin	Imipenem	Benzylpenicillin	Piperacillin
Nocardia spp. ($n=4$)	100% (4)	100% (4)	100% (4)	75% (3)	100% (4)
Nocardia abscessus ($n=4$)	75% (3)	100% (4)	50% (2)	75% (3)	75% (3)
Nocardia asteroides ($n=3$)	100% (3)	100% (3)	66.7% (2)	100% (3)	100% (3)
Nocardia farcinica ($n=3$)	100% (3)	100% (3)	33.3% (1)	100% (3)	100% (3)
Nocardia cyriacigeorgica ($n=2$)	100% (2)	100% (2)	100% (2)	100% (2)	100% (2)
Nocardia veterana ($n=3$)	100% (3)	100% (3)	100% (3)	66.7% (2)	100% (3)
Nocardia otitidiscaviarum ($n=1$)	100% (1)	100% (1)	100% (1)	100% (1)	100% (1)
Total ($n=20$)	95% (19)	100% (20)	75% (15)	85% (17)	95% (19)

TMP–SMX: trimethoprim–sulfamethoxazole.
[a] Breakpoints for resistance are those of the Clinical and Laboratory Standards Institute (CLSI), version M24 A2 (2011).

Table 5
Antibiotic treatment of 43 patients with pulmonary nocardiosis.

	Number	%
Antibiotics[a]		
TMP–SMX	22	51.2%
Carbapenem	18	41.9%
Amikacin	12	27.9%
Cephalosporin	8	18.6%
Penicillin	8	18.6%
Quinolone	5	11.6%
Tetracycline	4	9.3%
Linezolid	4	9.3%
Macrolide	3	7.0%
Other[b]	8	18.6%
	Mean ± SD	Median (range)
Duration of treatment (weeks) ($n=37$)	15.6 ± 14.9	12 (1–52)

SD, standard deviation; TMP–SMX, trimethoprim–sulfamethoxazole.
[a] Patients could have more than one.
[b] Standard anti-tuberculous treatment ($n=4$), glycopeptide ($n=2$), sulfadiazine ($n=1$), clindamycin ($n=1$).

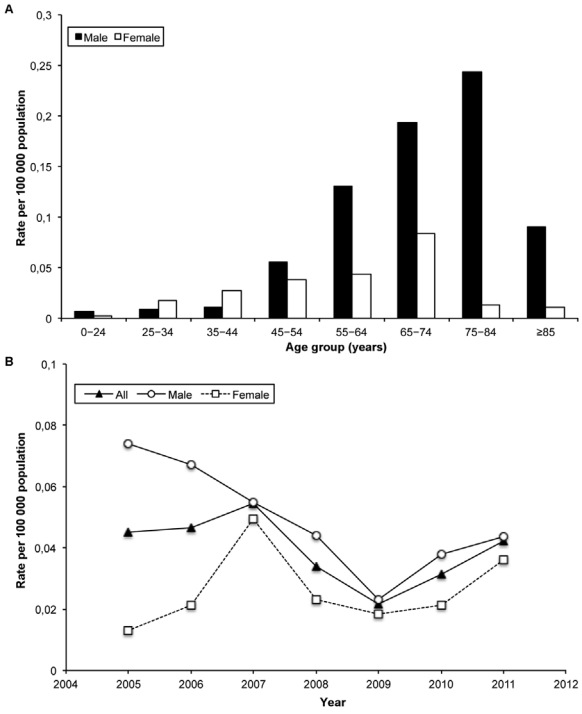

Figure 3. Population-based estimates of annual incidence rates of hospitalized cases with pulmonary nocardiosis: (A) average annual age-specific hospitalization rate; (B) annual age-adjusted incidence rates of hospitalized pulmonary nocardiosis in Germany from 2005 to 2011.

were being treated with an immunosuppressive medication. Most patients were receiving a combination of various immunosuppressive agents, although eight patients were being treated with only systemic corticosteroid monotherapy. Single organ transplantation (SOT) was the most common risk factor in this cohort. This confirms the data from most recent reports, showing the occurrence of PN in 1.3% to 3.5% of all SOT recipients (Poonyagariyagorn et al., 2008; Peleg et al., 2007; Hemmersbach-Miller et al., 2018; Majeed et al., 2018).

PN may also occur in immunocompetent patients, mainly those with a pulmonary comorbidity, especially COPD (Chen et al., 2013; Hui et al., 2003; Chen et al., 2014; Kurahara et al., 2014; Martinez Tomas et al., 2007). Impaired ciliary motility and epithelial damage lead to impaired local immune defence, and systemic corticosteroids may promote nocardial infection (Elenkov, 2004). Over half of the patients (58.1%) had an underlying pulmonary comorbidity, most commonly COPD (this was the sole comorbidity in 12 patients). Pulmonary comorbidity, particularly COPD and bronchiectasis, has been described as a risk factor for PN in non-immunosuppressed patients (Woodworth et al., 2017; Steinbrink et al., 2018).

Recent reports addressing radiological abnormalities in PN agree that the alterations found in PN are diverse, not pathognomonic, and may mimic a multitude of pulmonary diseases (Chen et al., 2014; Kurahara et al., 2014; Blackmon et al., 2011; Tsujimoto et al., 2012). Despite the non-discriminative and non-specific radiographic picture of PN, this study is novel in demonstrating that PN predominantly affects the right upper lobe, even after the exclusion of co-infected patients from the analyses. However, this contradicts the findings of previous studies, which have shown no particular distribution of radiographic abnormalities or even a lower lobe predominance (Chen et al., 2014; Blackmon et al., 2011; Tsujimoto et al., 2012). A possible explanation could be the stringent definition of PN applied in the present study, ensuring the inclusion of 'true' PN only. Furthermore, the aerobic nature of *Nocardia* spp. and the fact that the right upper lobe is regarded as one of the best ventilated areas of the lungs may potentially explain the greater upper lobe involvement.

Due to the non-specific clinical and radiological presentation, a delay between the onset of symptoms and a definitive PN diagnosis is common, and PN is initially often misdiagnosed. The diagnostic delay of 14 days in the study cohort is in line with the recent literature, reporting mean diagnostic intervals of 13.6 to 42 days (Poonyagariyagorn et al., 2008; Martinez Tomas et al., 2007). The diagnostic delay may also be attributed to the delayed growth in culture, which may take up to 3 weeks (Ambrosioni et al., 2010; Ashdown, 1990). Therefore, the microbiology laboratory should always be contacted when a nocardial infection is suspected to ensure that the corresponding specimens are incubated for a

prolonged period of time and to enable early application of molecular techniques for the detection of the organism (Ambrosioni et al., 2010).

In this cohort, 18.4% of all evaluable patients had a disseminated infection, mainly with CNS involvement. However, evaluable cerebral CT scans were available for only 12 patients; therefore, the true incidence of this disseminated course is possibly underestimated. An active search for dissemination, including cerebral CT scan or magnetic resonance imaging and ophthalmological investigations (uveitis), is of particular importance, because dissemination is directly linked to the outcome and the selection of definitive antibiotic therapy (CNS penetration) (Ambrosioni et al., 2010; Corti and Villafane-Fioti, 2003).

The most commonly used antibiotics were TMP–SMX, imipenem, amikacin, and ceftriaxone, which are all generally recommended as initial treatment for PN (Brown-Elliott et al., 2006; Wilson, 2012). In most patients, a treatment duration of 6 months is considered sufficient, whereas in cases with CNS involvement or in severely immunosuppressed cases, even more prolonged treatment may be necessary (Ambrosioni et al., 2010; Corti and Villafane-Fioti, 2003). However, some authors have recommended shorter treatment courses (Tripodi et al., 2011). The results of the present study, with a high clinical cure rate (overall >80% and almost 90% in non-disseminated cases) and a median treatment duration of 12 weeks may provide additional support for future shortening of antibiotic therapy in selected patients.

Some species presented clinically relevant resistance that may lead to inadequate treatment and treatment failure. *N. farcinica* and *N. asteroides*, the two most frequent species in this cohort, were susceptible to imipenem in only 33.3% and 66.7% of cases, respectively, whereas susceptibility to all other tested antibiotics was 100%. Of note, amikacin was the only tested drug with proven in vitro efficacy against all *Nocardia abscessus* isolates in this cohort. The study findings add to the available data on susceptibility testing and demonstrate increasing resistance to commonly recommended antibiotics (Minero et al., 2009; Uhde et al., 2010; Lebeaux et al., 2018; Schlaberg et al., 2014; Valdezate et al., 2017; McTaggart et al., 2015). In addition, the data are in line with those of a recent study from France, showing that *N. farcinica* is the most common species in human infection in Western Europe, with a distinct pattern of susceptibility (Lebeaux et al., 2018). Early species identification using 16sRNA PCR sequencing or multilocus sequencing typing and susceptibility testing may help to improve antibiotic treatment, although the clinical impact of the observed resistance remains unclear.

In contrast to earlier case series and recent epidemiological studies based on laboratory testing only, a major strength of the present study is the clear and restrictive definition of PN, making the unintended inclusion of cases with colonization improbable. Although *Nocardia* spp. are neither commensals of the normal human flora nor common laboratory contaminants, the sole detection of *Nocardia* spp. in respiratory samples, especially sputum, in the absence of clinical or radiological signs and symptoms, does not necessarily represent an infection in progress. Another strength of this study is the fact that patients from an unselected cohort were included (all patients from the participating institutions) rather than searching for cases in selected populations such as HIV-positive patients or SOT and bone marrow transplant recipients. The data therefore reflect the complete clinical, microbiological, and radiographic spectrum of PN that may be encountered in general medical departments and pulmonology divisions.

This was a retrospective evaluation with all the limitations that come with a retrospective study design, especially the problem of missing data. The identification of *Nocardia* spp. and susceptibility testing were performed only upon special request. Therefore, the species distribution may not reflect the real importance of each species in PN. The numbers of patients per country varied considerably, making comparison between countries impossible. However, since all four countries provide comparable standards of medical care in terms of diagnostic facilities, availability of therapies and medical procedures, and prevalence of comorbidities considered as typical risk factors for nocardiosis, it is believed that the pooled data may be representative of Western European countries, although not generally transferable to other countries.

The population-based estimate of the hospitalization rate (0.04/100 000 inhabitants) may serve as a surrogate for annual incidence and is considerably lower than rates reported previously: 0.39–0.55/100 000 inhabitants in Spain (0.20 if extrapolated to the whole population of Spain) and 0.33–0.87/100 000 inhabitants in Canada (Minero et al., 2009; Tremblay et al., 2011). There may be important reasons for this difference. The present study focused on pulmonary nocardiosis only, whereas the other cohorts included nocardiosis independent of the site of infection. Furthermore, in both previous studies, cases were identified by microbiological proof of *Nocardia* spp., without differentiating between infection and colonization. In contrast, the present study estimates are based on the coding of hospital discharge diagnoses for billing (ICD-10 codes), making colonization unlikely, because diagnostic and therapeutic efforts have to be justified for reimbursement and medical charts are frequently reviewed by health insurance companies. However, they are considered to have high specificity, but only moderate sensitivity, thus being prone to an underestimation of disease prevalence (O'Malley et al., 2005).

The study estimates are the first to be based on a representative nationwide population-based analysis, including 80% of all hospitals across Germany and >125 million hospitalizations over a 7-year period. Therefore, a possible bias due to differences in PN-associated hospitalizations in rural and urban areas, mainly due to potential regional differences in health care utilization, can be excluded. However, it was not possible to account for readmissions. Finally, the results apply to hospitalized populations only. PN is often a chronic infection and usually requires long-term follow-up care in the outpatient setting, where disease prevalence is presumably different. Therefore, the data are likely to underestimate the overall burden of PN. However, as available data are limited, the results are the best currently available surrogate for the epidemiological trends of PN across a large Western European country (Germany).

In contrast to the Canadian and the Spanish cohorts, no trend towards an increasing incidence over time was observed in this study. This previously described trend was explained by an increasing number of immunocompromised and immunosenescent patients in developed countries, improved laboratory detection techniques, and an increased awareness of PN in patients at risk during the observation periods (Minero et al., 2009; Tremblay et al., 2011; Agterof et al., 2007). The discrepancy between the present study and previous studies might be explained by differences in the observation period. Minero et al. evaluated incidences between 1995 and 2006 and Tremblay et al. between 1988 and 2008, whereas this study analyzed the period between 2005 and 2011 (Minero et al., 2009; Tremblay et al., 2011). Therefore, immunosuppressive treatment with biological medicines, which was introduced in the early 2000s, as well as genotypic methods for species identification were already in widespread use during the study period and, furthermore, the number of SOT performed annually did not increase significantly during the study period, in contrast to the

previous studies (https://www.organdonor.gov/statistics-stories/statistics/data.html).

The epidemiological data confirmed the highest incidence rate of PN in elderly males, which has previously been assumed from case series and case reports (Figure 1A) (Minero et al., 2009; Kurahara et al., 2014). For the first time, a discrete female predominance was observed among younger adults. This might be attributable to the female predominance in most autoimmune connective tissue disorders with subsequent immunosuppressive treatment. However, the data are not sufficient to draw definitive conclusions and further studies are needed to establish this as a distinct phenotype of PN patients.

In conclusion, this study shows that PN remains a rare pulmonary infection that primarily affects immunocompromised patients. Between 2005 and 2011, the PN-associated hospitalization rates remained unchanged. The clinical appearance is non-specific; heightened awareness and clinical suspicion are of particular importance in patients at risk. Dissemination to extrapulmonary organs, especially to the CNS, is frequent and associated with a worse outcome. Cerebral imaging should be prompted in all patients once the diagnosis of pulmonary nocardiosis is settled. Different *Nocardia* spp. may exhibit distinctive patterns of resistance, making identification of *Nocardia* spp. beyond just recognition of the genus indispensable. Therefore, timely prescription of empiric combination therapy may be beneficial until the results of susceptibility testing are available.

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Ethical approval

The study was approved by the ethics committees at the participating sites. Informed consent was judged not to be necessary due to the retrospective nature of the study.

Conflict of interest

None of the authors has a conflict of interest to disclose.

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