Diplomarbeit

Approaches to differentiate between colonization and infection of cultural confirmed Nocardia spp.

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Graz, am 22.04.2015 *Robert Kaiser eh*

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Zusammenfassung

Hintergrund: Bei der Nocardiose handelt es sich um eine vor allem bei immunsupprimierten PatientInnen oft disseminierende und potentiell lebensbedrohliche Infektionskrankheit. Die antibiotische Therapie sollte ohne Zeitverzögerung begonnen und dann über Monate fortgeführt werden. Dennoch ist davon auszugehen, dass nicht jeder kulturelle Nocardien-Nachweis mit einer Infektion gleichzusetzen ist, sondern auch mit einer Kolonisation assoziiert sein kann. Da keine validierten Kriterien zur Unterscheidung zwischen harmloser Kolonisation und therapiebedürftiger Infektion existieren, besteht im klinischen Alltag das Risiko der unnötigen Behandlung einer Nocardien-Kolonisation, aber auch der Unterlassung einer notwendigen Antibiose im Falle einer vital bedrohlichen Nocardiose. Zur besseren Unterscheidung zwischen einer Nokardien-Kolonisation oder -Infektion analysierten wir Nocardien-Befunde nach mikrobiologischen, klinischen und radiologischen Aspekten.

Methoden: In der Studie wurde ein Klassifikationsschema zur klinischen Differenzierung von Kolonisation oder Infektion erstellt und an 62 Fällen mit kulturell nachgewiesenen Nocardien retrospektive angewandt. Das Schema besteht aus klinischen (Symptomatik UND Ausschluss anderer Ätiologien), radiologischen (Befund im Einklang mit Nocardiose) und mikrobiologischen Kriterien (≥2 positive Kulturen aus Sputa ODER eine positive Kultur aus einer BAL, Biopsie oder aus einem anderen sterilen Kompartiment ODER 1 positive Kultur bei immunsupprimierten Patienten), von welchen alle für eine Nocardiose erfüllt sein mussten.

Ergebnisse: 25 von 62 PatientInnen mussten aufgrund mangelnder medizinischer Daten ausgeschlossen werden. Von den verbliebenen 37 PatientInnen wurden 13 (35%) als infiziert (Infektionsgruppe) klassifiziert. Von diesen 13 PatientInnen erhielten nur 10 (77%) eine für Nocardien effektive antibiotische Therapie. Von den 3 nicht behandelten infizierten PatientInnen verstarben 2 (66%) und bei den therapierten PatientInnen 3 von 10 (30%). Von den 37 eingeschlossenen PatientInnen wurden 24 (65%) als kolonisiert klassifiziert. Von diesen erhielten nur 4 (17%) eine effektive antibiotische Therapie. In der Gruppe der kolonisierten PatientInnen gab es jedoch keine Todesfälle. Somit war die Therapie- aber auch die Sterblichkeitsrate signifikant höher in der Infektionsgruppe ($p=0.002$ bzw. $p=0.003$) und das hier vorgeschlagene

Klassifikationssystem zur Erkennung einer Nocardien-Infektion wies einen negativ prädiktiven Wert von 84% auf.

Zusammenfassung: Durch das verwendete Klassifikationsschema konnten alle lebensbedrohlichen Infektionen detektiert werden und keine/r der untherapierten kolonisierten PatientInnen entwickelte später eine Nocardiose. Beim kulturellen Nachweis von Nocardien könnte die vorgeschlagene Klassifikation Therapieentscheidungen erleichtern, dringliche Indikationen für eine antibiotische Therapie aufzeigen und unnötige Therapien vermeiden.

Abstract

Background: Nocardiosis occurs mainly in immunocompromised patients, can affect virtually any organ and requires prolonged antibiotic treatment. However, cultural detection of Nocardia does not imply inevitably infection and therefore colonisation should also be considered.

Methods: Clinical data from 62 patients with cultural Nocardia detection were reviewed and applied to a proposed classification for differentiation between colonization and infection. The classification includes clinical (symptomatic disease AND exclusion of other aetiologies), radiological (findings compatible with nocardiosis) and microbiological criteria (≥2 positive cultures from sputum OR one positive culture from BAL, biopsy or sterile compartments OR one positive culture from sputum in case of immunosuppression). Nocardial infection was presumed when all three criteria were fulfilled.

Results: 24 from 62 patients were excluded because of insufficient medical data. From the remaining 37 patients 13 (35%) were categorized as infected. Of these, 10 (77%) received a presumably effective antiinfective treatment and 3 (10%) of them died. From the three infected patients who did not receive appropriate antibiotics two (66%) died in the course of the disease. Overall 5 from 13 (38%) patients of the infection-group died. 24 from 37 (65%) patients were classified as colonized. None of these died and only 4 (17%) received appropriate antibiotics. Therefore, the proportion of effective treatment and mortality were significantly less than in the infected group ($p=0.002$ and $p=0.003$, respectively) and the proposed classifications reached a negative predictive value of 84%.

Conclusion: The evaluated algorithm detected all live-threatening infections and none of the untreated presumably colonized patients developed nocardiosis. Therefore, the proposed classification might prevent under- and overtreatment in case of Nocardia positive cultures.

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Abbreviation

AECOPD	Acute Exacerbated Chronic Obstructive Pulmonary Disease
AIDS	Acquired Immunodeficiency Syndrome
AS	Aortic Valve Sclerosis
BAL	Bronchoalveolar Lavage
BMI	Body Mass Index
BMT	Bone Marrow Transplantation
CDC	Center Of Disease Control
CF	Cystic Fibrosis
CHD	Chronic Heart Disease
CKD	Chronic Kidney Disease
CMV	Cytomegalovirus
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
CT	Computed Tomography
DM	Diabetes Mellitus
E-Test	Epsilometer Test
FUO	Fever of Unknown Origin
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
Hsp	Heat Shock Protein
IDDM	Insulin Dependent Diabetes Mellitus
i.v.	Intravenous
LTOT	Long Term Oxygen Therapy
MIC	Minimal Inhibitory Concentration
MLSA	Multilocus Sequence Analysis
MRI	Magnetic Resonance Imaging
MTX	Methotrexat
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NSCLC	Non-Small-Cell Lung Carcinoma
N.	Nocardia
p.o.	Per Os
PCR	Polymerase Chain Reaction

RNA	Ribonucleic Acid
SLE	Systemic Lupus Erythematosus
SMX	Sulfamethoxazole
Spp.	Species Pluralis
TBC	Tuberculosis
TMP	Trimethoprim
Tx	Transplantation

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1 Introduction

1.1 Microbiology

The genus Nocardia consists of gram-positive, aerobically growing bacteria and counts - with the Rhodococci- to the family Nocardiaceae, which belongs to the Corynebacteriaceae, a suborder of Actinomycetales. Other relevant human pathogenic representatives of this suborder are the Mycobacteriae and Corynebacteriaceae among others (1,2).

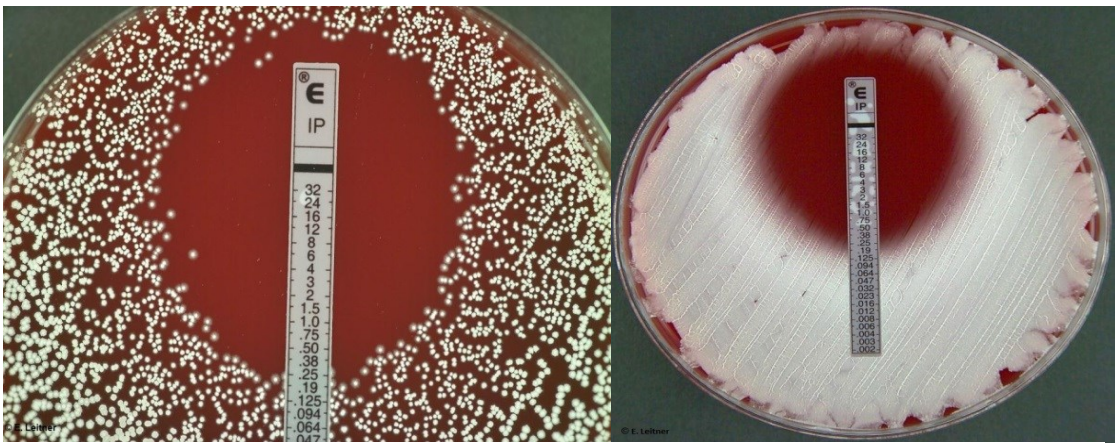

Figure 1: Epsilometertest performed on a blood agar with several chalky white growing Nocardia colonies. (IP: imipenem). Courtesy of Dr. Eva Leitner

Nocardia spp. can be fragmented into rod shaped or coccoid forms and have a filamentous branched and hyphal like growing pattern. Thus they were wrongly distinguished as fungi for a long time, until molecular methods eventually suggested that Nocardiae are in fact bacteria (3). The mentioned branching can go both horizontally and vertically, which is why they appear as chalky cotton balls macroscopically; depending on the extent of their filamentous growing.

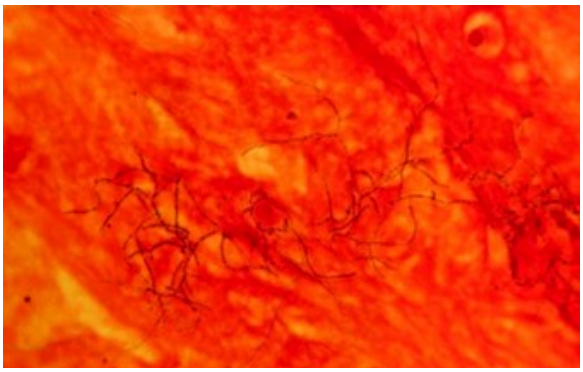

Figure 2: Filamenteous growing pattern of Nocardia seen through an optical microsope (*N. asteroides* in a x1000 magnification) (56).

Colonies can be colored in yellow, orange, red or brown, with the intensity depending on the growing and the culture conditions, respectively. The color is determined by the strain their produced pigments (4). Similar to the Mycobacteriae, Nocardiae are partially acid fastened, which is due to a mycolic acid in their cell wall (1,2).

1.2 Taxonomy

Originally Nocardia were solely classified upon their exhibiting biochemical features and cell wall components (4). Newer methods like PCR of the 16S rRNA, hsp or multilocus sequence analysis (MLSA) made clinical detection not only less cumbersome, but also much faster. As a side effect, various different strains got discovered and made the Nocardia cluster a lot more complex and eventually taxonomy had to be revised several times (1,2,5).

Up until today 101 different species have been discovered and about 30 of them count as human pathogen and the quantity is on the raise (6,7).
The species which causes most of the infections in humans is the *N. asteroides complex*. This complex has been reorganized into six different species, based on different drug susceptibilities within the complex:
N. abscessus (I), N. brevicatena/paucivorans complex (II), N. nova complex (III), N. transvalensis complex (IV) N. farcinica (V) and *N. asteroids (VI)* (1,2)

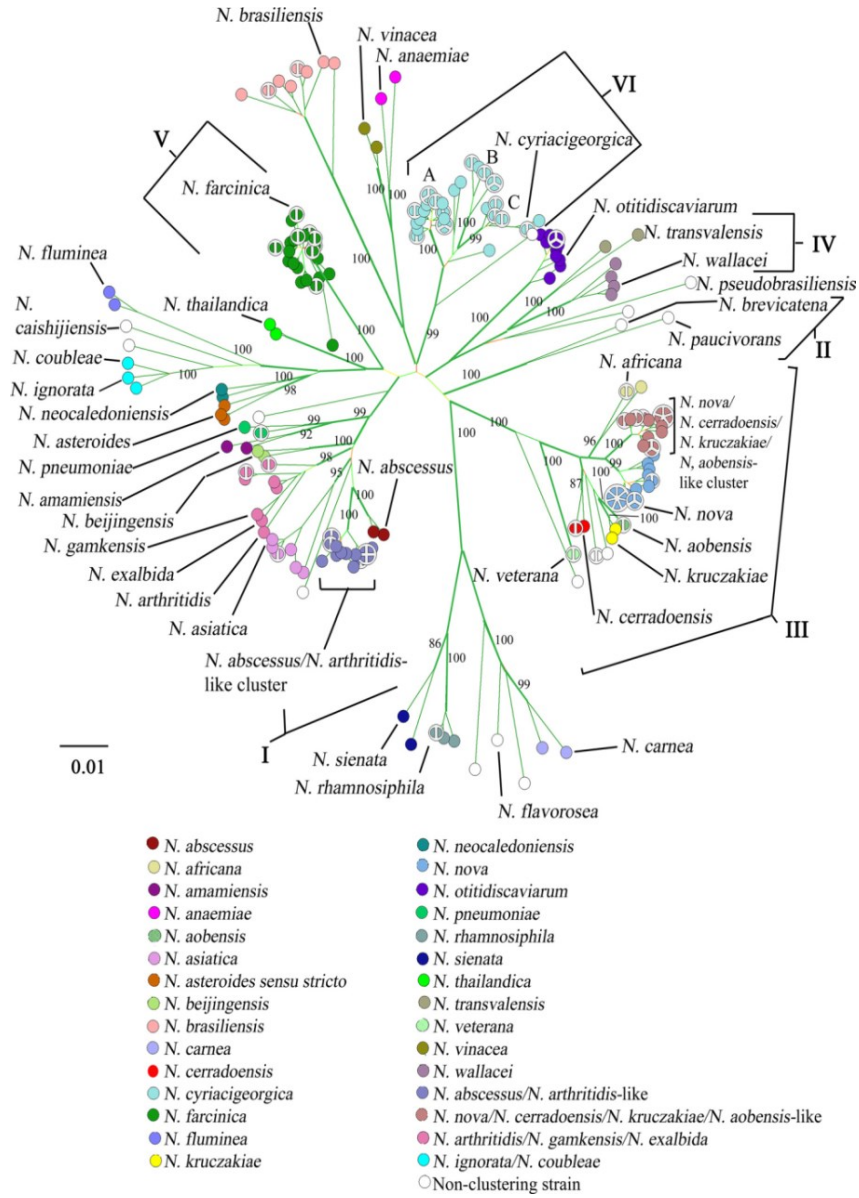

Figure 3: NJ tree constructed of different Nocardia species, showing the complexity of the genus. The roman numbers I VI refer to the six drug-susceptibility patterns of Nocardiae (12).

1.3 Epidemiology

1.3.1 Distribution

Nocardia can be found worldwide in salt- and freshwater, decomposing organic matter, soil, and dust. Thus inhalation of contaminated particles or direct inoculation (e.g. into a skin lesion), are the major way of admission (1,2). Of direct transmissions from person-to-person are not known yet (3,8). The prevalence of a particular species depends probably on both the climate and geographic aspects (6,9-11).

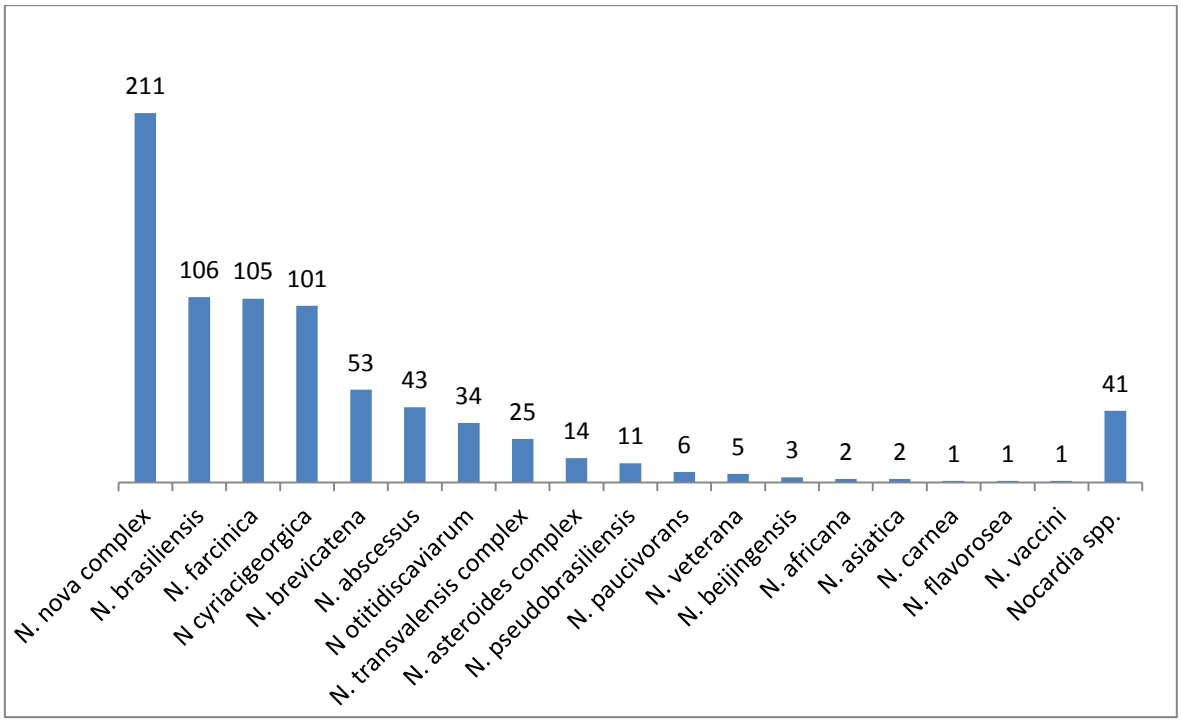

Figure 4: Distribution of Nocardia in the USA compiled of Nocardia isolates submitted to the Center of Disease Control between 1995¬ 2004 (n=765) (9); Nocardia spp. = unnamed and undefined species

For example, the most frequent species in Western Europe seems to be *N. farcinica* (3,12). This has been shown in a study from Belgium in 2005 - among others -, in which 44,1% out of 86 cases were caused by *N. farcinica* (13). The most frequent strain in the United States, on the other hand, seems to be the *N. nova complex*, which is also shown in figure 4 (9).

1.3.2 Incidence

Although nocardiosis is a rare disease, the incidence had been increased over the past years. The most probable reason for that is number of immunocompromised patients, which is also increasing (8).

The estimated incidence of Nocardia infections reaches from 0.31 per 100.000 inhabitants to 0.55 per 100.000 inhabitants, whereas the latter value descends from recently performed studies and is therefore probably more relevant (6,11).
However, the rise of incidence might also be due to the newer and also better detection procedures that have been developed over the last decades.

1.4 Risk factors

Nocardiosis is considered to be an opportunistic infection, which occurs mostly in immunocompromised patients. Nevertheless, about 15% to 33% of all patients with nocardiosis are immunocompetent and have no known risk factors (1,4,14).
These patients are also usually displaying a different clinical picture, depending on their immune status. While immune competent patients seem to have a tendency to develop a chronic nocardiosis limited to a single organ, patients with immune compromised conditions show higher rate of disseminations in multiple organs (8).

Nocardiosis shows strong associations with diseases and/or medication, causing a suppression of the cell mediated immunity. Thus patients at the highest risk are those with malignancy (i.e. hematologic malignancies like lymphoma), history of organ transplantation and long-term glucocorticoid therapy.
Most infections in organ-transplanted patients occur within the first year after the transplantation, which is probably due to the higher dosed immunosuppressive therapy against early rejection reactions (1,4,15).

Table 1: Overview about the risk of Nocardia infections after particular organ transplantation (15)

Type of transplant	Number of transplant recipients (n=5126)	Number of nocardial infections
Lung	521	18 (3.5%)
Heart	392	10 (2.5%)
Small bowel/multivisceral	155	2 (1.3%)
Kidney	1717	3 (0.2%)
Liver	1840	2 (0.1%)
Pancreas	180	0
Kidney and Pancreas	297	0
Heart and Lung	24	0

Studies have also shown that the risk for nocardiosis seems to differ between certain transplants. As it can be seen in table 1, patients after heart or lung transplantation seem to develop rather a nocardiosis than other transplant recipients.
Table 2 below lists relevant concomitant factors in transplant recipients, which may lead to an increase in risk for a nocardiosis.

Table 2: Significant risk factors of Nocardia infection in organ recipients, adapted from (15). [a] defined as ≥20mg of prednisone ≥1 month(s) or ≥2 pulses of i.v. methylprednisolone [b] defined as >15 µg/ml for tacrolimus or >300ng/ml for cyclosporine

Variable	Case patients (n=35)	Control patients (n=70)	Odds Ratio (CI 95%)	Significance
Receipt of antifungal prophylaxis	15 (43)	18 (26)	3 (1.01–8.88)	p= .047
Allograft rejection in preceding 6 months	20 (57)	14 (20)	6.7 (2.2–20.2)	p= .001
Receipt of high-dose prednisone in preceding 6 months[a]	23 (66)	16 (23)	26 (3.4–195.1)	p= .002
Prednisone dose at the time of event, median mg (range)	10 (0–30)	7.5 (0–37.5)	1.13 (1.03–1.23)	p= .007
Elevated median calcineurin inhibitor level in preceding 30 days[b]	18 (51)	15 (21)	4.1 (1.6–10.7)	p= .004
Receipt of lymphocyte-depleting antibody in preceding 12 months	17 (49)	16 (23)	6.3 (1.8–22.9)	p= .005
Lymphocyte count in preceding 30 days, median cells/mm³ (range)	420 (0–1400)	800 (40–2800)	0.998 (0.996–0.999)	p= .003
Neutrophil count in preceding 30 days, median cells/mm³ (range)	6 (0.92–32)	3.8 (1.2–12.7)	1.3 (1.1–1.6)	p= .006

Although HIV does also often rank among the main risk factors for Nocardia infections, there are relatively few reports of nocardiosis in HIV patients, which may be due to the TMP-SMX prophylaxis against *Pneumocystis jirovecii*. Most reported cases of nocardiosis in Patients with an underlying HIV infection concerned already severe immunocompromised patients with a median $CD4^+$ count of 35 cells/ µl, being already in the AIDS stage of their disease (16,17).

Apparently males have a 2 to 3 times higher risk than female, but the reason for it is still unclear, but may be related protective effect of estrogens (4).

Other associated factors are diabetes mellitus, CMV disease, calcineurin inhibitor therapy, pregnancy, alcoholism, chronic granulomatous diseases, COPD, SLE, tuberculosis and vasculitis (1,4,14,15,17).

1.5 Clinical presentation

A rather problematic fact about Nocardiae is their observed tendency to disseminate into virtually any organ from pulmonary or cutaneous infection sides. Another complicating feature is their ability to spontaneously relapse years after an actual sufficient antimicrobial therapy (1,2,11).

Table 3:Distribution of Nocardiosis in comparison between different organs (4)
Most of these cases were caused by N. asteroids (n=831)

Infection	Number of cases (n=1050)	Percent
Pulmonary	412	39.2
Systemic and CNS	334	31.8
CNS only	91	8.7
Cutaneous and lymphocutaneous	82	7.8
Eyes	29	2.7
Bone	26	2.5
Other	76	7.2

1.5.1 Pulmonary Nocardiosis

Since the inhalation of airborne Nocardia carrying particles represents the main route for acquisition, almost two out of three nocardioses affect primarily the lung (15,18,19). The infection can progress rapidly as acute disease or slowly progressive disease with a more sub-acute or chronic pattern. Since there are no specific symptoms, indicating a Nocardia infection, a definite diagnosis is often made delayed (3). The spectrum of symptoms includes productive or unproductive cough, hemoptysis, pleuritic chest pain, shortness of breath, fever, night sweat, weight loss, abdominal pain and fatigue (1,4,17,20).

One study indicates, that approximately 50 percent of all pulmonary nocardioses disseminate outside the lung into other organs or tissues, with a notable tendency to the central nervous system (19).

Complications can also be caused by continuous spread of inflammation into surrounding tissues. Pleuritis, pericarditis and the superior vena cava syndrome are some exemplary complications among others (21,22).

Radiographic findings are diverse and show a high variety between different cases. Most of the patients exhibit multifocal consolidation patterns in chest radiography.

According to the literature, cavernous lesions can be found in up to a third of all pulmonary nocardioses. Other possible findings can be single or - more frequently - multiple nodules in various sizes, common reticulonodular or interstitial infiltrates.

Pleural involvement presents itself on imaging as a pleural thickening, with or without pleural effusion. Yoon et al. (23) have found that hiliar and mediastinal lymphadenopathy seems to be unusual in pulmonary nocardiosis.

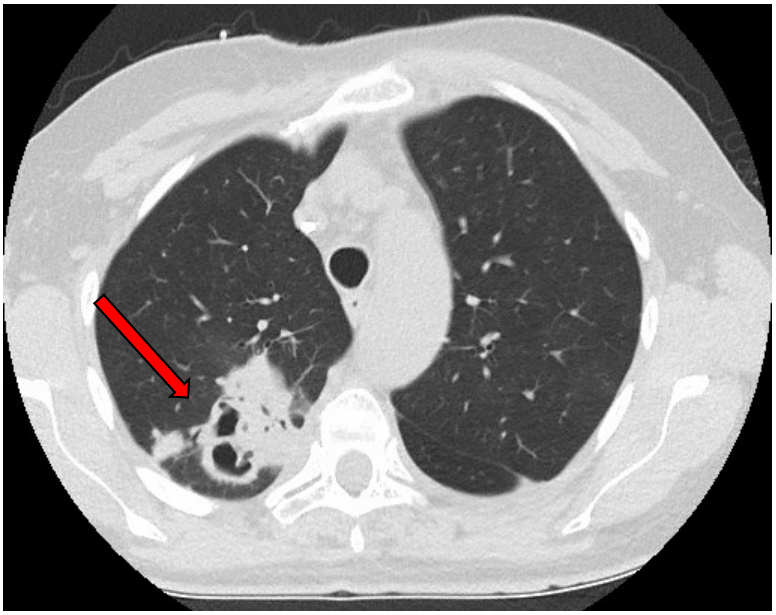

Figure 5: CT findings in a patient with a manifest pulmonary Nocardiosis at the Medical University of Graz. The red arrow points at a carvernous lesion. A nodular formation is located right by the cavern.

By using imaging, nocardiosis can be easily misdiagnosed as fungal infection, mycobacterial infection other type of bacterial infections. An excerpt of relevant differential diagnoses is listed in table 4. It is of importance, that the isolation of Nocardia from the bronchopulmonary system does not necessarily imply an infection, since colonization seems to be frequent, overall in patients with chronic or destructive bronchial diseases (e.g. COPD, bronchiolitis, TBC, aspergillosis, etc.) (4,17,24).

1.5.2 Central nervous system

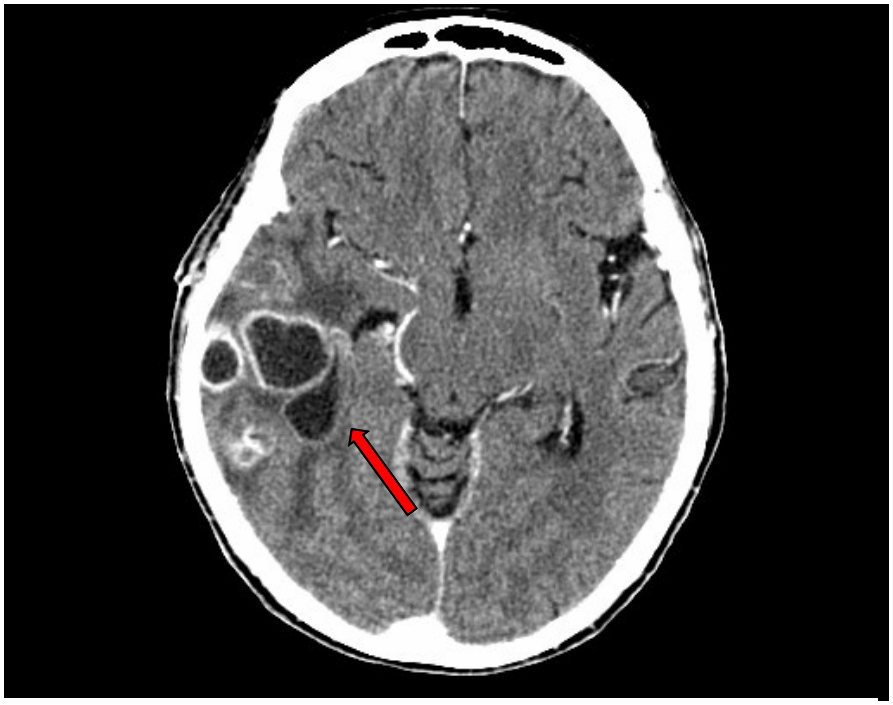

Figure 6: CT of a patient with a manifest CNS-nocardiosis at the Medical University of Graz. The red arrow points at abscess formations in the right hemisphere, which are partially septated.

The exhibition of a special tropism for the endothelial cells in specific brain regions is peculiar to Nocardia. This characteristic has also been experimentally verified in murine and mouse models (3). Therefore, secondary CNS infections are a common complication in disseminated nocardial infections. The overall risk for a CNS involvement during a disseminated nocardiosis is at about 40 percent and highest in association with a pulmonary nocardiosis. Therefore, it is suggested to perform cranial CT or MRI in every patient with pulmonary and disseminated nocardiosis, respectively (3).

Patients with CNS nocardiosis frequently exhibit singular or multiple localized abscesses in the brain. Forms of meningitis caused by Nocardia have indeed been found, but are rare. Depending on underlying concomitant diseases or immune deficiency, it may take years to develop significant symptoms in a patient, which are common due to a raise in the intracranial pressure. Frequent symptoms are headache, vomiting, seizures, focal deficits or meningism signs (4).

In some cases CNS nocardiosis may be misdiagnosed for degenerative or neoplastic processes in the CNS (25), due to a possible combination of caused neurologic symptoms

but with a lack of typical infectious signs (e.g. fever, laboratory shifts etc.) (4). Yamada et al. for instance, published a case report in 2005 with a 58-year old afebrile patient, who was admitted with progressive right hemiparesis and aphasia. This patient provided only little evidence for bacterial infection in laboratory data. Also findings in scintigraphy and MRI imaging, respectively, showed a lesion resembling a malignant glioma. Thus a total resection of the "brain tumor" was performed, actually revealing an abscess formation caused by a *Nocardia spp.*. After immediately initiated TMP-SMX therapy the patient was cured with no permanent neurological deficiencies (26).

1.5.3 Cutaneous

Cutaneous nocardiosis can be caused by almost every species of Nocardia, but is commonly caused by *Nocardia brasiliensis* (17). Unlike in the other manifestations of nocardiosis, the cutaneous variant can be primarily seen in immune competent patients. The main way of acquisition is the direct inoculation via the skin, e.g. during farming, gardening or traumatic skin lacerations.
The clinical picture of the cutaneous nocardiosis varies from patient to patient, depending on specific endogenous host factors. Therefore this form can be subdivided into four different variants, depending on the pathogenesis and clinical picture, respectively: Superficial lesions, lymphocutanous disease, mycetoma or cutaneous lesions in association with a primary disseminated nocardiosis. The most common location for these infections are the extremities and the face in children, respectively (2,17).

The superficial form is mildest form of cutaneous nocardiosis and usually affects immune competent patients after a mean incubation time of 3 weeks. Typical skin lesions are nodules, pustules, ulcerations or cellulitis. Depending on the depth of the infection involvement of bones, joints, bursae etc. are also possible, but very rare (2,27,28).
The lymphocutanous form is characerized by a lymphatic spread with lymphadenitis and is also been known under the name "Sporotrichoid Nocardiosis", because of its clinical similarity to *Sporothrix schenkii* infections (3,29).

A chronic but rarely occurring variant of cutaneous nocardiosis is the mycetoma, which is defined as an indolent, localized infection of the dermis, sub-cutis and the bone. They are most common localized on the feet and are frequently present for six months before

diagnosis is made. Thus, countries with inadequate medical access show the highest prevalence (2,30).

A secondary dissemination in - mostly immune-compromised - patients, with a primary skin nocardosis is a dreaded complication, which should always be kept in mind. And vice versa, a seemingly localized primary cutaneous nocardiosis could always be a secondary nocardiosis during dissemination from another primary focus (2).

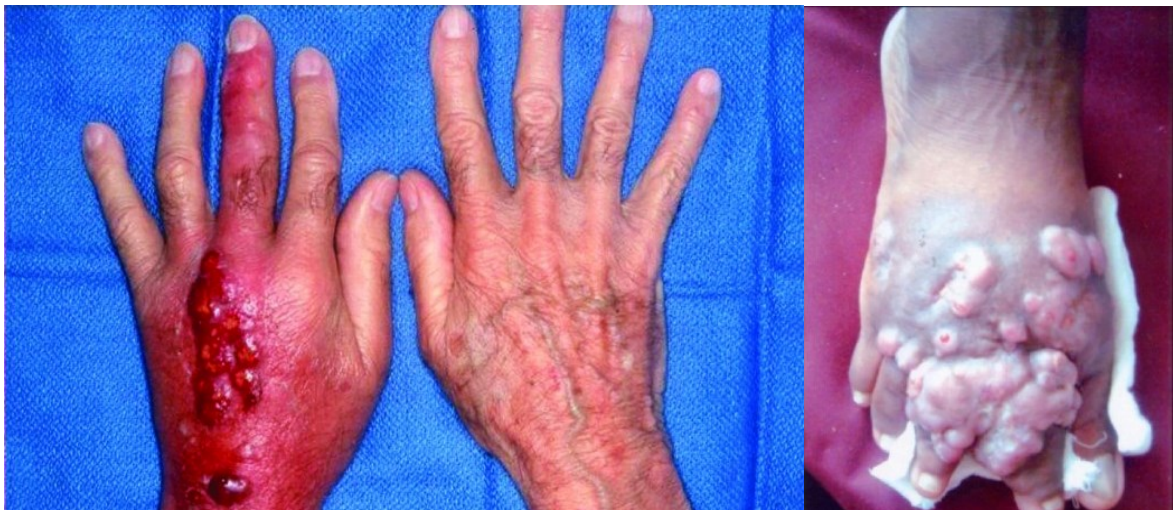

Figure 7: Examples for the cutaneous variant of nocardiosis:

Left: Sporotrichoid lymphocutaneous nocardiosis on the left hand, after traumatic inoculation in the middle finger, caused by *N. brasiliensis* (56)

Right: Mycetoma of the right foot of a 50 years old man (30)

1.5.4 Other sites

Although Nocardiae can be obtained via direct contact with contaminated material (e.g. water), primary nocardial infections occur only rarely in the eye (31). On the other hand, secondary infections have been reported more frequent in case reports. This originates from the previously mentioned fact that Nocardia might disseminate in any tissue, either per continuetatem or by hematogenic spread (2).

Involvement of bones, heart valves, joints, testicles, adrenal gland, bursae, pancreas, thyroid gland, mediastinum, pericardium and the liver among others have been described in the literature. Abscess formations in other locations, e.g. the psoas, are mentioned in the literature as well (2,4,22,28,32-40).

1.6 Diagnosis

Symptoms of a nocardiosis are non-specific and therefore differential diagnoses can be diverse. As nocardiosis is a rare disease, misdiagnoses occur frequently, especially since the affected patients usually have more than one risk factor and concomitant diseases. An excerpt of important differential diagnoses is listed in table 4 below.

Table 4: Extract of differential diagnoses of nocardiosis (1)

Pulmonary disease	• Fungal infections, including (depending on the host) aspergillosis, mucormycosis, histoplasmosis, blastomycosis, cryptococcosis • Actinomycosis, *Rhodococcus equi*, and other bacterial infections • Mycobacterial infections, including *Mycobacterium tuberculosis* and nontuberculosis mycobacterial infections • Lung malignancy (primary or secondary)
Cutaneous disease	• Lymphocutaneous disease: sporotrichosis, *Mycobacterium marinum* infection • Superficial cellulitis: group A streptococcus, *Staphylococcus aureus*, Erysipelothrix species, and *Francisella tularensis* infections • Mycetoma (late stage): actinomycosis, fungal infections (*Pseudoallescheria* and other molds) • Other: cutaneous leishmaniasis, cryptococcosis, infections with rapidly mycobacteria (eg, *Mycobacterium fortuitum, Mycobacterium chelonae*)
CNS disease	• Malignancy (primary or secondary) • Bacterial abscess (single or multiple) • Vascular infarction • Other (depending on host): toxoplasmosis, *M. tuberculosis*, fungal infection (including cryptococcosis, aspergillosis, mucormycosis), cysticercosis

Another problem arises from difficulties in the cultural detection of *Nocardia spp.* from clinical gained samples, as *Nocardia spp*. tend to have a slow growing pattern of several weeks. Therefore *Nocardia spp.* are frequently concealed on agar plates by more rapid growing bacteria and fungi, respectively. This may result to an early disposal of specimen, within 48 to 72 hours, which is an inadequate cultivation time for *Nocardia spp.* (2).
Therefore it is advisable to inform the corresponding laboratory promptly if nocardiosis is suspected. Laboratories should use special culture mediums to reduce the "overgrowth"

and should take other precautionary measures, like avoidance of some decontamination solutions, to which *Nocardia spp.* are susceptible (3).

Appropriate specimen can be acquired either under non-sterile procedures (e.g. sputum, bronchial lavage or transbronchial biopsies, etc.), or under sterile conditions (e.g. blood cultures, biopsies of abscesses or pleural effusions, etc.). Early indices can be acquired by acid-staining, due to the partial acid-fastness of Nocardia. But it must be kept in mind, that these methods may be too aggressive for some strains (2). Blood cultures tend to have a poor sensitivity for nocardemia, even though it is assumed that Nocardia usually disseminate hematogenicly (17).

Species differentiation of cultivated Nocardia tends to be a delicate matter, but is important due to differences in virulence and drug susceptibility of different species (41). Methods like biochemical verification procedures are rather cumbersome and mostly unable identify the precise species (12). Serological approaches are frequently false positive or negative and therefore inapplicable for clinical practice (2). The current gold standard is the PCR of 16S rRNA, which provides the results much faster than all the other methods used before (41,42). Nevertheless, it still has some deficits in the exact determination of some strains and tends to be rather unreliable after genetic variations, because only one gene is sequenced. Therefore different methods are tested for their usability in recent studies.
The MLSA for example analyzes 5 to 7 different gene loci and has shown to be more suitable for complex prokaryotes like Nocardia (12).

Drug susceptibility testing should also be part of the diagnostic process, to determine the most effective antibiotic agent. The microdilution method and the test substances are based on CLSI (Clinical and Laboratory Standards Institute) recommendations are the gold standard for *Nocardia spp.* (2). Another valid and widely used procedure is the E-Test (epsilometer test) (43).

1.7 Therapy

Since nocardiosis is a rare disease, there are no prospective studies available and recommendations for antibiotic treatments are mainly based on retrospective studies, animal models and clinical experience.

Since 1940 sulfonamides are used as first line therapy against Nocardia (2). The most common substance of this group is trimethoprim-sulfamethoxazole (TMP-SMX). In vitro studies indicated a synergy between TMP and SMX leading to an improved bacteriostatic effect (17). But the in vivo efficiency still remains unclear (1). Although high-dose TMP-SMX (10-15mg/kg/d) is the treatment most frequently recommended and used against Nocardia, it does not always present the optimal therapeutic choice. For example, sulfonamides exhibit a high rate of dug hypersensitivity and intolerance, respectively, resulting in necessary changes in therapeutic regimes. Common side effects of sulfonamides like rush, massive nausea, hyperkalemia, crystalluria, myelosupression, renal dysfunction or liver toxicity frequently result in an early discontinuation of therapy (1,17,44).

Although strains like *N. otitidiscaviarum* display a low resistance against sulfonamides in both in vitro and in vivo tests, is it is shown in table 5 below. Recent studies indicate that the number of species non susceptible to TMP-SMX is increasing. *N. farcinica* and *N. nova*, for example, are showing high resistances in several studies, which cause the wide variation in effectiveness in table 5 (3). A case study made in Canada of 2010 reviewed 575 Nocardia-cases retrospectively and found 43% isolates non susceptible to TMP-SMX (6). But in comparison, another study made in 2012 with a relatively similar size of 552 isolates reached a different result with only 2.5% sulfonamide resistant strains in their population. They assumed that these different results are due to inexpediences in MIC testing procedures, but still agreed that resistances against sulfonamides are increasing (45). Therefore the initial therapy usually consists of two to three substances, depending on the severity of the underlying infection and the patient's concomitant diseases. The therapy should be adjusted by reference to the results of susceptibility tests and also adapted to clinical improvements of the patient (1).

Table 5: This table shows the antimicrobial susceptibility of frequent Nocardiae strains. The susceptibility ranges were acquired from different studies and case reports and are displayed in percent. This table has been adopted from Clark et al (44). x = insufficient data

Antimicrobial Substance	Susceptibility						
	N. asteroides complex	*N. farcinica*	*N. nova*	*N. brasiliensis*	*N. transvalensis*	*N. otitidiscaviarum*	*N. cyriacigeorgica*
TMP-SMX	79-100	20-100	47-100	80-100	44-88	68-100	78-100
Imipenem	70-100	65-100	95-100	0-52	48-90	0-32	77-100
Amikacin	85-100	100	83-100	99-100	20-82	94-100	99-100
Minocycline	43-100	9-66	16-100	31-90	16-54	38-100	14-40
Ceftriaxone	64-100	0-73	47-100	19-100	50-68	0-26	82-96
Ciprofloxacin	0-50	19-90	0-17	0-30	24-60	0-32	0-7
Amoxicillin/ Clavulanic acid	0-70	40-100	5-50	65-100	30-56	0-24	0-38
Linezolid	100	100	100	98-100	98-100	100	96-100
Moxifloxacin	50	25-88	2	x	x	x	4

The susceptibility testing procedure for Nocardiae, recommended by the CLSI, uses various antimicrobial substances, which had been grouped as primary and secondary agents due to their efficacy against Nocardiae in previous studies. The primary agents are TMP-SMX, amoxicillin-clavulanic acid, ceftriaxone, imipinem ciprofloxacin, clarithromycin, linezolid, minocycline, tobramycin and amikacin. Secondary agents are cefepime, cefotaxime, doxycycline, gentamicin, gatifloxacin and moxifloxacin (2).

Linezolid have been found efficient against all significant Nocardia strains recently (2), but should be handled carefully (especially in long time therapy), because of its high spectrum of adverse effects, like thrombocytopenia, aplastic anemia, peripheral neuropathy, lactic acidosis and myelosupression (44). Therefore and because of its relatively high price, linezolid is reserved for severe cases (46).

A monotherapy with TMP-SMX (5-10mg/kg/d divided in three doses a day) is usually a sufficient empiric therapy of primary cutaneous nocardiosis. Fluoroquinolones should only

be added on more widespread infections (8). The duration of therapy usually ranges between 3 months for immunocompetent patients and 6 months for immunocompromised patients.
Therapy of mycetomas usually consists in a combination of TMP-SMX and another agent, for example fluoroquinolones or amikacin. Minocycline and amoxicillin/clavolanate have also been successfully used on mycetomas before. In some mycetomas surgical treatment may be required (8,47).

The treatment of pulmonary nocardiosis depends on the manifestation of the disease and also the immune status of the patient. In some cases a monotherapy with TMP-SMX (15mg/kg/d i.v. divided in 3 to 4 doses) may be sufficient as treatment, assuming it is a mild form of pulmonary nocardiosis in an immune competent host. But in the usual therapy consists of two agents (8). TMP+SMX (15mg/kg/d i.v.) plus amikacin (10-15 mg/kg/d i.v.) is the conventionally used combination in most cases.
Alternatively some authors recommend imipenem (500 mg i.v. q6 h) plus amikacin (10-15 mg/kg/d i.v.) (44). Linezolid (600 mg p.o. or i.v. q12 h) can be added to the therapy regime, in severe cases.

Therapy suggestions for CNS nocardiosis and disseminated nocardiosis also consist of combination of TMP-SMX and imipenem or amikacin and imipenem. That being said, it is suspected, that a combination of imipenem and amikacin may prove itself insufficient as treatment for CNS infections, due to a β-lactame resistances of some strains and the fact, that aminoglycosides are known for a limited penetration of the nervous system (8). Third generation cephalosporins (ceftriaxone 2 g i.v. q12 h and cefotaxime 2 g i.v. q8 h) are also an excellent addition of therapy, because of their good penetrability of the nervous system.

Parenteral antibiosis should be administered at least 3 to 6 weeks and can be subsequently changed to oral forms, depending on clinical improvement, underlying and possible immune deficiency of the patient (44).
Surgical treatment or drainages may be necessary in large abscess formations, empyemas or complications like mediastinitis in course of a pulmonary nocardiosis (8,48).

Since there are no official recommendations for the optimal duration of an antimicrobial treatment, authors recommend a long term treatment of several months to surely prevent a

relapse nocardiosis. Therefore, even though some studies reported that pulmonary nocardiosis may usually be cured in approximately 2 to 4 months, it is suggested that pulmonary and soft tissue infections ought to be treated for at least 3 months in immunocompetent patients and 6 months in immunocompromised patients, which must also be adapted to the course of disease. Cerebral infections ought to be treated for at least 12 months and must exhibit radiographic improvement, before medical treatment can be discontinued. Following the antimicrobial treatment, it is suggested to monitor patients for early detection of relapses. This monitoring usually includes periodical CT or MRI screenings of patients with recent CNS nocardiosis (44).

The outcome of nocardiosis, especially of disseminated cases, is better, the sooner a definite diagnose has been made and appropriate therapy has been initiated (15). While the mortality rate of CNS nocardiosis ranges in between 30% to 53%, skin and soft tissue infections can be treated successfully most of the time. Relapses usually occur after abbreviated therapy durations with less than 3 months. Therefore exact monitoring of therapy adherence is highly recommendable (44).

1.8 Purpose and relevance of the study

Although nocardiosis is a rare disease, epidemiologic studies indicate that the incidence has been increased during the last decades (8)**.** Therefore and because of the potentially foudroyant course of disease the awareness for nocardiosis should be raised especially for specific collectives of patients. Unfortunately the therapeutic decision may difficult in many cases, since some *Nocardia spp*. findings alone or alongside other microorganism can also represent harmless colonization and not infection. Furthermore, true nocardial infections cause unspecific clinical diseases, which can easily confounded with other life-threatening infections. These difficulties and uncertainties in the interpretation of clinical and microbiological findings lead to under- and overtreatment of patients, with relevant impact on the outcome.

To improve the accuracy of clinical decisions we created a structured algorithm for the differentiation between nocardial colonization and infection. Using clinical, radiological and microbiological data from patients with a positive nocardia culture we retrospectively applicated this decision guidance to the information available at the time of nocardia

determination. The results of the classification process (suspected colonization versus suspected infection) were finally compared with clinical outcome parameter.

2 Methods

2.1 Preparation and recruitment

To obtain an adequate patient population a retrospective study design was chosen. Included were all patients with Nocardia findings (nocardial isolates) confirmed by culture or PCR in clinical samples during a period of 12 years from the years 2001 until 2013. No other inclusion criteria were given. For that purpose two microbiological laboratories in Graz (the Institute of Hygiene, Microbiology and Environmental Medicine and the Institute of Hospital Hygiene and Microbiology) were contacted, by what data of 62 patients with microbiologically confirmed Nocardia isolates had been obtained. The ethic approval was requested and has been approved. Before further analysis of the data was performed, proposed diagnostic criteria for differentiation between infection and colonization were established:

Table 6: Diagnostic criteria for nocardial infection; Note: If the criteria mentioned above are not applicable, the nocardia finding will be presumed as colonization

Criteria for nocardial infection	
Clinical, radiological and microbiological criteria must be fulfilled to regard as real infection	
Clinical Criteria	
	1. Progressive pulmonary disease, focal extrapulmonary disease (primarily abscesses) or fever of unknown origin (FUO) **A N D** 2. Exclusion of other infections (e.g. infections due to other bacteria (including mycobacteria) or fungi, with good response to short term, antimycobaterial or antifungal therapy, respectively)
Radiological criteria (not needed in primary cutaneous lesions)	
	1. Newly occurred pulmonary infiltrates, nodules, cavitations or exudative pleural effusions **O R** 2. Newly occurred extrapulmonary exudative effusion or abscess formation
Microbiological criteria	
	1. ≥ 2 positive culture of ≥ 2 separately obtained sputa or 1 positive culture from sputum in case of immunosuppression **O R** 2. ≥ 1 positive culture from a BAL, transbronchial biopsy **or** any other sterile compartment (e.g. brain, blood culture, liver)

2.2 Data ascertainment

Patient names were matched with potentially existing medical records archived in the database of the KAGes hospitals. For that purpose, the hospital information system openMEDOCS was used. Older data was acquired via Auraweb. The collected data was transferred into a sheet made with Microsoft Excel 2010 for facilitated comparability.
The following parameters were collected:

General informations	• Date of birth and age at admission • Sex • Country of origin
Medical background	• Preexisting illness (pulmonary and other) • Known immunodeficiency (HIV, malignancies, transplant, known leucocytopenia, steroid therapy if > 10 mg/d prednisone or equivalent)
Specific medical information	• Weight loss and BMI change, respectively • Admission diagnosis • Clinical symptoms (fever, cough, sputum, dyspnea, cutaneous or subcutaneous infection signs) • Laboratory parameter [CRP (mg/dl), leucocytes (G/ml)] • Treatment (Y/N); duration of treatment • Antibiotic agents used • Outcome
Radiographic findings	• Localization and patterns • C-CT or C-MRI done (Y/N)
Microbiological information	• Cultivation period • Sample type (sputum, BAL etc.) • Nocardia species • Resistance testing (Y/N) • Resistance pattern

Patients with insufficient or non-existing medical data were excluded. On basis of the previously observed parameters, the diagnostic criteria mentioned above were applied.
An immune deficiency was assumed, if a patient had either a solid organ transplantation, a hematologic malignancy (e.g. lymphoma), chemotherapy for a solid organ malignancy, a disease severely affecting the immune system (e.g. HIV, drug induced cytopenia) or received a prolonged high dose glucocorticoid therapy or any other immune suppressive treatments (e.g. > 10 mg/d prednisone or equivalent for more than 3 month, MTX, azathioprine, cyclophosphamide).

2.3 Data evaluation

The patients were allocated into two groups, according to the results of the diagnostic criteria (see table 6). Patients, who matched all of the three criteria, were classified as infected ("infection group") and those who only partially applied – or not at all - to the criteria were classified as colonized ("colonization group").
Depending on the received antibiotic treatment, the groups were subdivided again in "insufficient treated" and "sufficient treated" patients. The therapy was assumed to be sufficient, if the used antimicrobial substances were both in line with the performed resistance testing and applied for at least three months. In cases of no resistance testing, the therapy was be still seen as sufficient, if the applied antimicrobial substances had been proven to be effective against Nocardia in previous studies [i.e. TMP-SMX, imipenem, amikacin, 3rd generation cephalosporins (e.g. ceftriaxone, cefotaxime), amoxicillin/clavulanic-acid, linezolid, and fluoroquinolones (particularly moxifloxacin)]. The microbiological characterization was not part of this study, therefore the assessed E-tests will not be shown in detail. The patients who were classified as "insufficient treated" and "sufficient treated" were compared using a fourfold table. The outcome of the patient was also evaluated (survived or deceased). In patients, who died after a relevant Nocardia finding, a real infection was assumed. The functionality of the classification model has been evaluated by comparing the therapy and outcome of the patients and significance was calculated by using the chi-square test and fishers-exact test. P-values under 0.05 were assumed to be significant.

3 Results

3.1 Data assignment process

After the first analysis of the medical history from primarily included patients (n=62), we had to exclude 24 before using the diagnostic scheme above. 4 of those 24 had not any further medical data in our database, besides the positive Nocardia findings of the microbiological institutes. In 20 of those 24 medical data were available, but they had to be excluded anyway because of flaws in documentation or problems in the follow-up.

38 from 62 patients were successfully classified upon their medical history, with 12 patients as part of the “infection group” and 26 patients as part of the “colonization group”. One patient of the colonization group was lost to follow up and had been excluded, eventually.

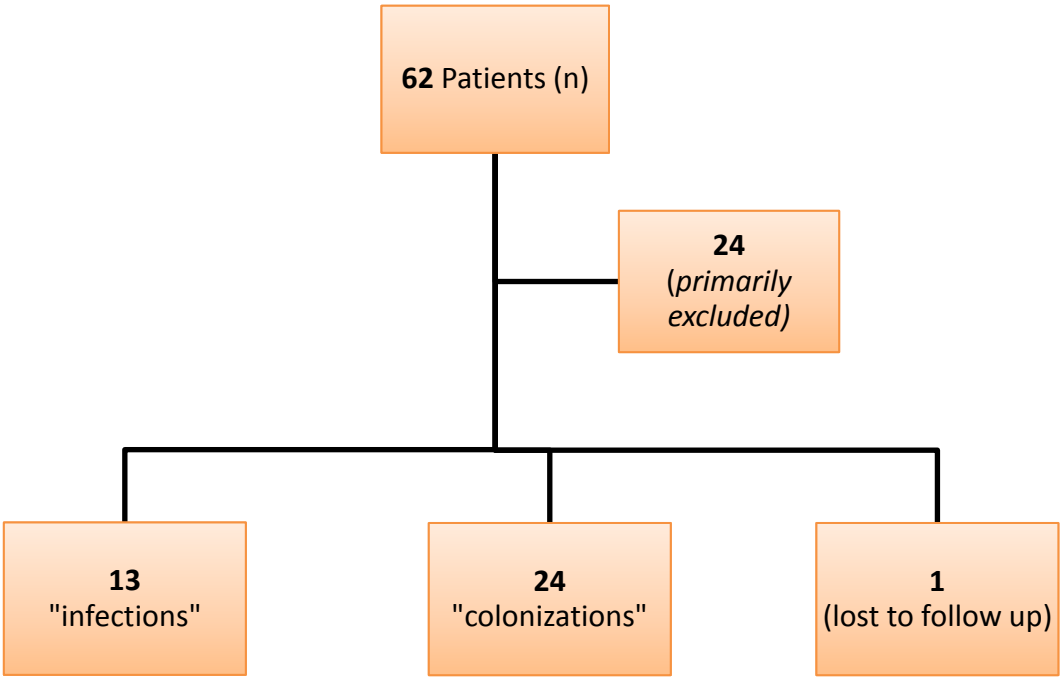

Figure 8: Overview about the number of excluded and included cases in this study.

3.2 Epidemiology

3.2.1 Gender distribution and demographic findings

11 of the 37 assessed patients were female (30%) and 27 were male (70%), which corresponds roughly to a 2.3 to 1 ratio (male to female). A more precise distribution is displayed in figure 9 below.

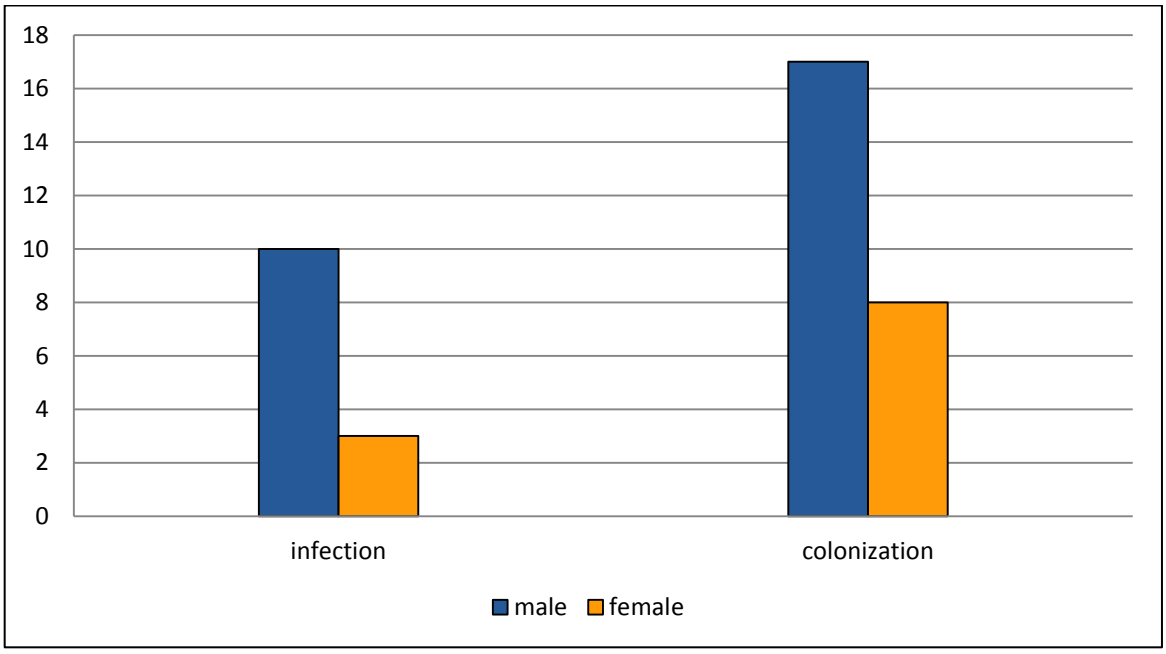

Figure 9: Gender distribution of Nocardia spp. in patients classed by the study criteria. (n=37)

Patients, classified as infection, had a male to female ratio of 3.3 to 1. The sex ratio in the colonization group was 2.1 to 1 (male to female).

The country of origin from the patients had also been assessed. In total 35 (95%) of the 37 evaluated patients were originally born in Austria. Only 2 (5%) of our patients came from another country (Afghanistan and Slovenia) and both of them were classified as colonization retrospectively.

3.2.2 Age distribution

An overview of the average age of patients at Nocardia findings had also been created. To achieve a better and more differentiated comparability between these patients, we performed an age-stratification and formed four age groups (0-20 years, 21-40 years, 41-60 years and 61-80 years). 58 patients were assigned to their specific age bands, regardless of whether they could be classified as infected or colonized in the end, because their age at the time of the Nocardia finding was still known.

The median age of patients with a positive Nocardia finding was 62 years and the average age 57 years (±18). The quantity of positive findings in the age groups 41-60 and 61-80 years was higher than in the age groups 0-20 and 21-40 years. There was also a rise of Nocardia findings from the age groups 41-60 to 61-80 years.

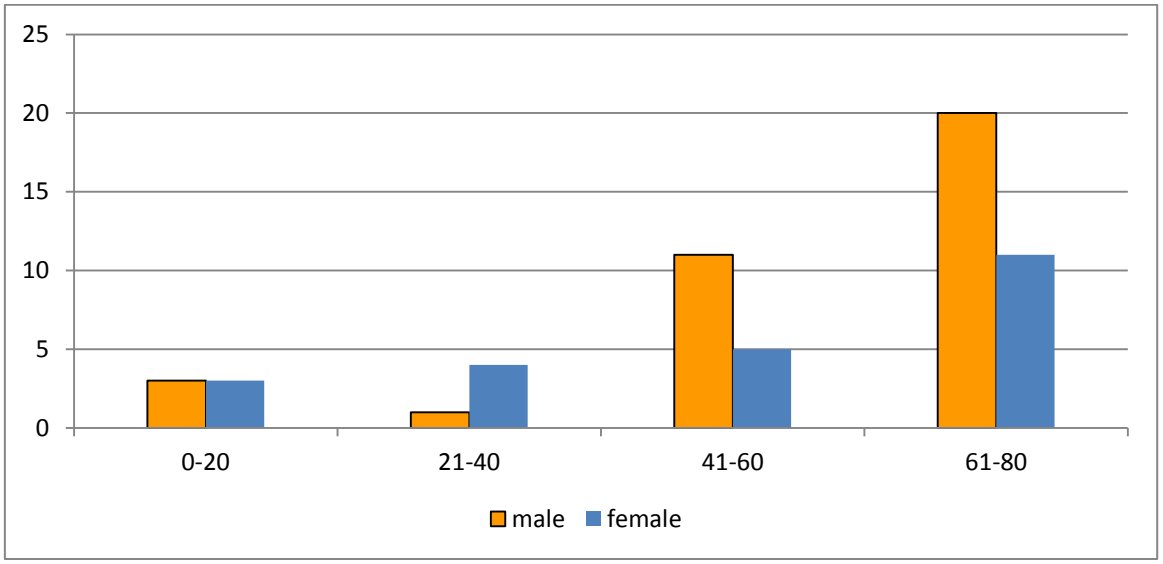

Figure 10: Age at the time of the Nocardia finding in all patients who were observed in the study. The patients have been grouped in the categories 0-20, 21-40, 41-60 and 61-80 years according to their age. (n=58)

The same age related Nocardia findings have been calculated separately for the 37 classified patients and are shown below. They have also been stratified by their age.

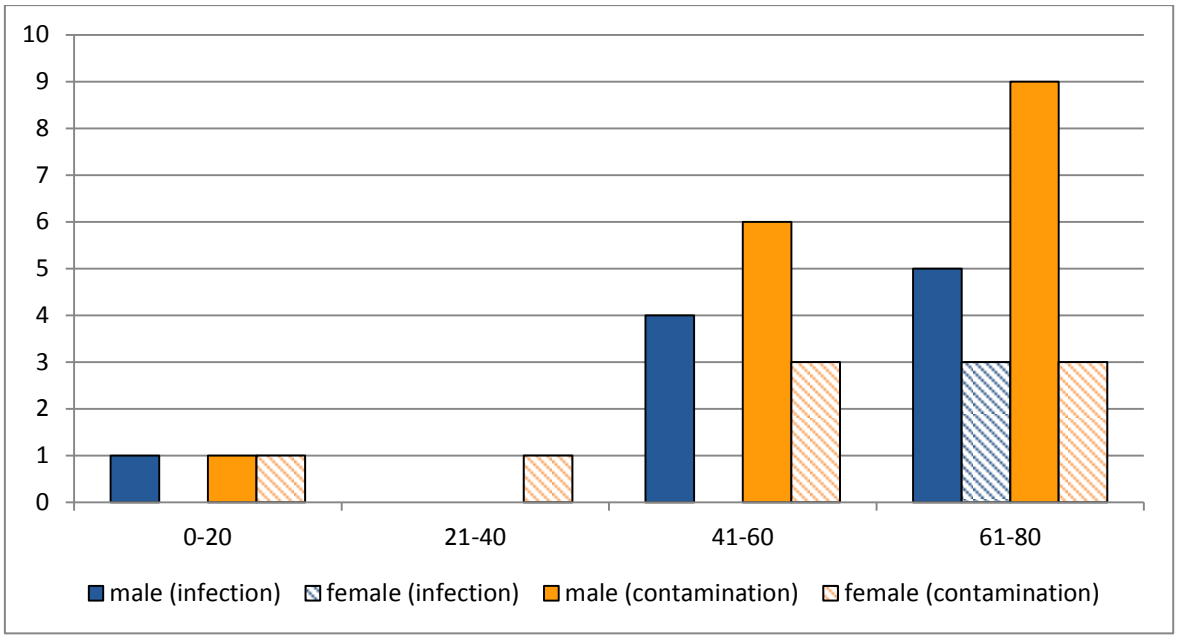

Figure 11: Patient age at the time of the Nocardia finding comparing classified patients only. Both an age- and a gender-stratification have been used. (n=37)

The highest incidence of Nocardia findings was seen in patients between the age of 61 years and 80 years. The age based median in incidence was found to be 61 years for all of the 37 classed patients. Looked at the groups separately, the median age was 61 years for the patients categorized as infected and 60.5 years for colonized patients. We found a rise in the age related incidence in both the infected and the colonized patients, displaying a 8 and 6 times higher rate of positive findings in the age-group 61-80 compared to the 0-20 years old patients. 81 percent of all findings in this study occurred after the age of 41 and 92 percent of the infections found occurred after the age of 40.

Men as well as women exhibited a higher rate of Nocardia findings in the higher age groups compared to patients in the younger age groups. The increase had been found stronger in men than in women.

3.2.3 Incidence

The incidence had been calculated with 58 patients, disregarding whether they could or could not be successfully classified, because the time of event was known for all of them. Nevertheless, the incidence had been also calculated with classified patients only and is displayed below, differentiating between infections and colonizations.

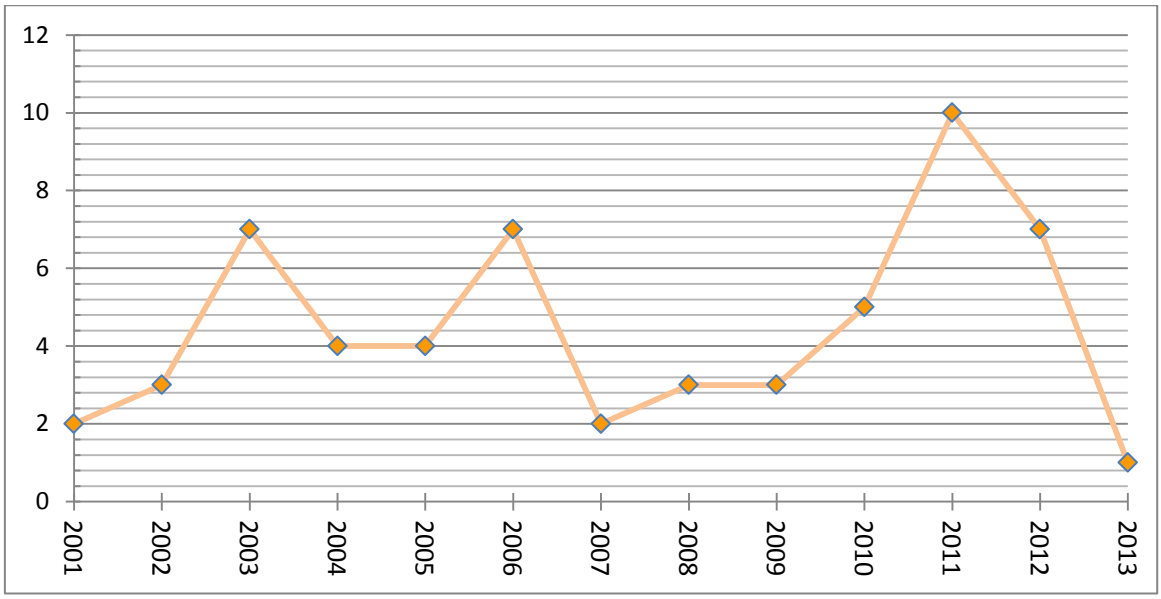

Figure 12: Quantity of Nocardia findings from 2001 to 2013[x] (n=58).
[x] The medical data of 2013 was only partially available at the time of the study (early 2013).

Most Nocardia findings have been made in the year 2011, which contained 17.2% of all findings. In the years 2001 and 2007 the quantity of nocardial findings was the lowest during our observation period with 2 positive Nocardia findings.

The overall incidence was calculated based on the commuted area of Styria and neighboring states with estimated 1.6 million inhabitants. Incidences were calculated per 100.000 inhabitants per year and were 0.125 in 2001, 0.188 in 2002, 0.438 in 2003, 0.25 in 2004 and 2005, 0.438 in 2006, 0.125 in 2007, 0.188 in 2008 and 2009, 0.313 in 2010, 0.625 in 2011 and 0.438 in 2012. The year 2013 has been excluded from this calculation because microbiological data was only available until the midst of 2013. This means the real incidence is estimated to be between 0.125 and 0.625 per 100.000 inhabitants per year, respectively. We found no significant raise of incidence in our study.

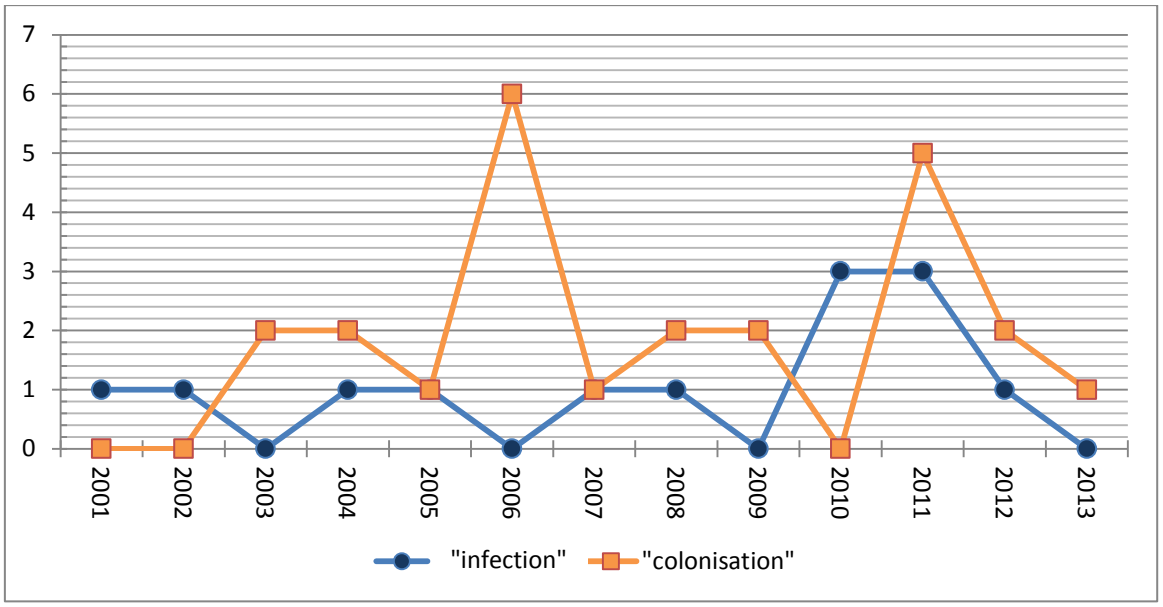

Figure 13: The quantity of "infected" or "colonized" patients after application of the classification chart (n=37).[x]

[x] The medical data of 2013 was only partially available at the time of the study (early 2013).

The incidence of Nocardia findings has also been also analyzed for infections and colonizations separately and is displayed in figure 13 above. We found a higher rate of colonizations compared to infections. We also found some peaks in the incidence, which also are congruent with the "overall" incidence in figure 12. There are two peaks in colonizations by Nocardia in the years 2006 and 2011. The infections on the other hand exhibited only one peak during the years 2010 and 2011, which contained 43% of all observed infections in this study. These peaks may be indicative for an annual fluctuation in incidence of nocardiosis.

3.3 Clinical data and Outcome

3.3.1 Overview of the assessed patients

Like mentioned above, 37 patients of the 62 patients were evaluated based upon their medical history, so that the diagnostic criteria could have been applied on them. The table 7 contains the patient ID's and the main dependent variables of the study.

Table 7: Overview of all patients with applicable diagnostic criteria. The table is not sorted by the date of diagnose but rather the Patient-ID.
[a]) Patients are only labeled as treated, if they were treated with antimicrobial substance recommended by CLSI for at least 3 months.

ID	Clinical Criteria	Radiographic Criteria	Microbiological Criteria	Classified as	Treatment [a]	Outcome
1	No	No	Yes	Colonization	+	survived
2	No	Yes	Yes	Colonization	-	survived
3	No	Yes	No	Colonization	-	survived
4	No	Yes	No	Colonization	+	survived
5	Yes	Yes	Yes	Infection	+	deceased
6	No	Yes	No	Colonization	-	survived
7	Yes	Yes	Yes	Infection	+	deceased
8	Yes	Yes	Yes	Infection	+	survived
9	Yes	Yes	Yes	Infection	+	survived
10	Yes	Yes	Yes	Infection	+	survived
11	Yes	Yes	Yes	Infection	-	deceased
12	Yes	Yes	Yes	Infection	+	survived
13	Yes	Yes	Yes	Infection	-	deceased
14	Yes	Yes	Yes	Infection	+	survived
15	Yes	Yes	Yes	Infection	+	survived
16	No	No	Yes	Colonization	-	survived
17	No	No	No	Colonization	-	survived
18	No	Yes	Yes	Colonization	-	survived
19	No	Yes	Yes	Colonization	-	survived
20	No	Yes	No	Colonization	-	survived
21	No	Yes	Yes	Colonization	-	survived
22	No	No	Yes	Colonization	-	survived

ID	Clinical Criteria	Radiographic Criteria	Microbiological Criteria	Classified as	Treatment [a]	Outcome
23	No	No	Yes	Colonization	-	survived
24	No	Yes	Yes	Colonization	-	survived
25	No	Yes	Yes	Colonization	-	survived
26	No	No	Yes	Colonization	-	survived
27	No	No	No	Colonization	-	survived
28	No	Yes	No	Colonization	-	survived
29	No	Yes	Yes	Colonization	+	survived
30	No	Yes	No	Colonization	-	survived
31	Yes	Yes	Yes	Infection	-	survived
32	No	Yes	No	Colonization	-	survived
33	No	No	No	Colonization	-	survived
34	Yes	Yes	Yes	Infection	+	survived
35	No	Yes	No	Colonization	-	survived
36	No	No	No	Colonization	-	survived
37	Yes	Yes	Yes	Infection	+	deceased

In total, 5 (38%) patients died in the course of nocardiosis. In 3 of them the findings were received post mortem.

The average cultivation period amounted to 23.9 days, with a minimum and maximum duration of 4 and 64 days, respectively. The average period amounted to 19 [4-61] days in the “infection”-group and 28.8 [8-64] days in the “colonization” group, respectively. The cultivation period was estimated as the timespan between dispatching of the specimen and the first written microbiological announcement of a nocardial finding. A resistance testing was performed in 9 of 27 cases (33%). 6 out of these 9 have later been classified as infection and the other 3 as colonization. All resistance testings were performed using the e-test method.

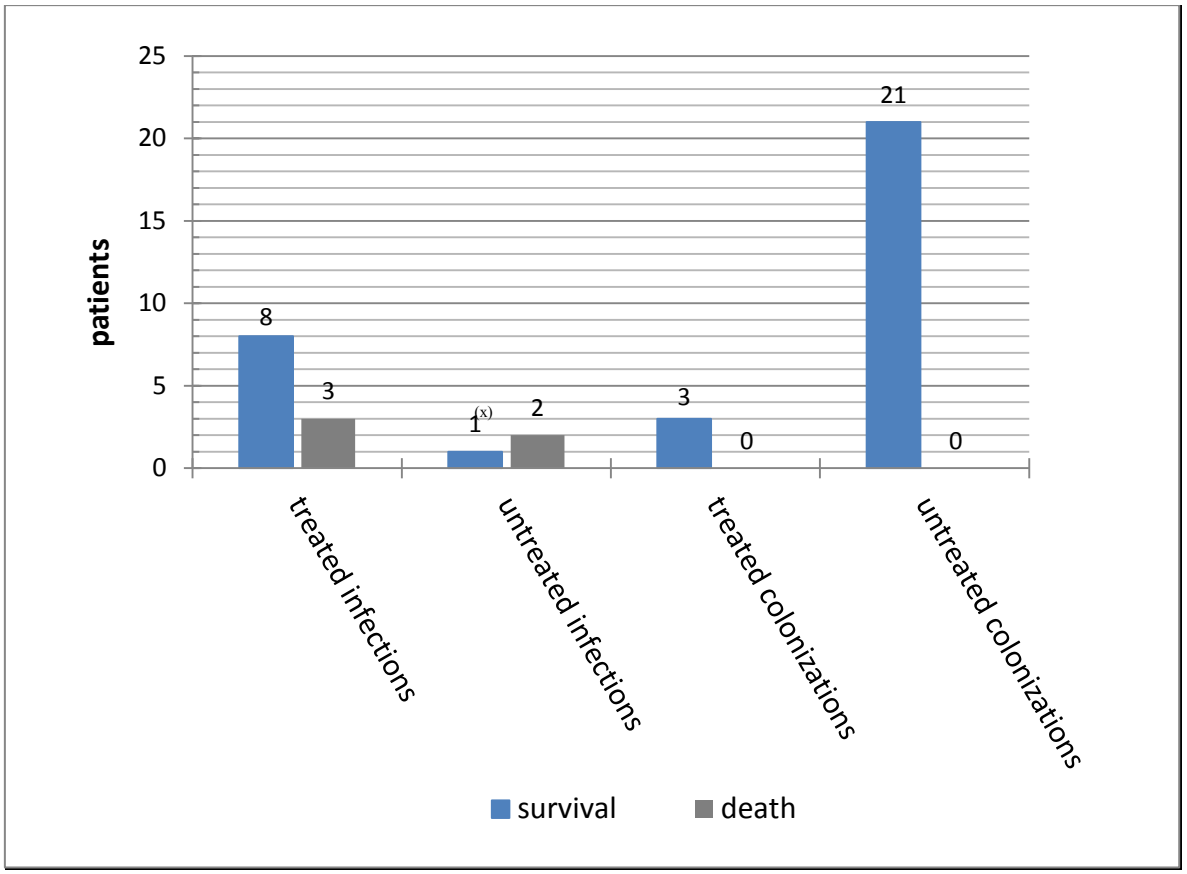

Figure 14: This diagram shows the infections and colonizations and their particular outcome (survival; death).
(x) This patient is labelled "untreated", because therapy lasted only for 1 month

10/13 (77%) infected patients received an appropriate antibiotic treatment. Comparing both groups with each other, patients in the colonization group were significantly less often treated than patients in the infectious group (p=.003). Supposing that the 3 treated presumably colonized patients could have been infections and therefore only considering untreated colonized patients, the negative predictive value for the classification was 87.5 percent.

Patients classified as colonized had a significant lower mortality compared to the patients in the infectious group (p=.002). In total 5 (38.5%) of the 13 patients considered as real nocardiosis, deceased during the course of their disease. We found a mortality rate of 25% for cutaneous nocardiosis and 44% in cases with pulmonary nocardiosis. The mortality rate for secondary nocardiosis and dissemination, respectively, was 75%.

3.3.2 Risk factors and clinical presentation

Table 8: Patients classified as „infections“ by the diagnostic criteria used in this study. This table shows the risk factors, site of infection and the specific Nocardia strain found in the patient.
[(a)] Age means the age at the time of event and not the current patient's age
[(b)] Species labeled as Nocardia spp. are not otherwise specified strains

ID	Age[(a)]	Gender	Immune deficiency	Concomitant diseases (other)	Site of Infection	Species[(b)]
5	66	Male	-	Alcoholism/ Sleep Apnea Syndrom	pulmonary	*Nocardia spp.*
7	54	Male	Heart-Tx	-	pulmonary	*N. abscessus*
8	68	Male	Leukemia/ BMT	-	pulmonary	*N. cyriacigeorgica/ asteroides/ aobensis*
9	66	Male	-	-	cutaneous (bursitis)	*N. asiatica/ abscessus*
10	9	Male	-	-	cutaneous (abscess)	*Nocardia spp.*
11	61	Female	SLE	-	pulmonary/ CNS	*N. farcinica/ otitidis/ caviarum*
12	50	Male	-	-	cutaneous (phlegmon)	*N. brasiliensis/ asiatica*
13	64	Male	Lymphoma	-	cutaneous (abscess)/ CNS	*N. brasiliensis/ harenosa/ asiatica/ transvalensis*
14	72	Female	Leukemia	-	pulmonary/ CNS	*N. nova*
15	48	Male	Cytopenia	HCV (interferon therapy)	pulmonary	*N. cerradoensis/ aobensis/ africana/ veteran*
31	48	Male	Leukemia/ BMT	-	pulmonary	*N. nova/ pseudosporangifera/ jiangxiensis*
34	61	Male	Lung-Tx	CKD, IDDM	pulmonary	*N. farcinica*
37	77	Female	-	Hypertonia, IDDM	pulmonary/ disseminated	*Nocardia spp.*

Of the 13 cases classified as infection, 9 (69%) had a verified pulmonary nocardioses and 4 (31%) a cutaneous manifestation. 31% (1 primary cutaneous nocardiosis and 3 pulmonary nocardioses) developed a secondary CNS infection. Immune deficiencies were present in 8 (62%) of all 13 infected patients, which were 7 (78%) of the 9 cases with pulmonary nocardioses and 1 of 4 cases (25%) with primary cutaneous manifestation.

Further specification of the *Nocardia spp.* has been done in 10 out of 13 patients (77%) classified as infection.

Table 9: Patients classified as „colonization" by the diagnostic criteria used in this study. This table shows the risk factors known of and the specific Nocardia strain found in the patient. [(a)] Age means the age at the time of event and not the current patient's age [(b)] Species labeled as Nocardia spp. are not otherwise specified strains

ID	Age[(a)]	Gender	Immune deficiency	Concomitant diseases (other)	Species[(b)]
1	72	Male	-	COPD, Bronchiectasis, Hypertension	*Nocardia spp.*
2	56	Male	-	Tuberculosis	*Nocardia spp.*
3	46	Male	-	Tuberculosis	*Nocardia spp.*
4	70	Male	-	Sarcoidosis, Hypertension, NIDDM	*Nocardia spp.*
6	75	Male	-	Tuberculosis	*Nocardia spp.*
16	50	Male	-	NSCLC	*Nocardia spp.*
17	79	Female	-	COPD (+LTOT), CHD	*Nocardia spp.*
18	56	Female	Cytopenia	HCV (interferon therapy)	*Nocardia spp.*
19	56	Male	-	Mounier-Kuhn-Syndrome (tracheobronchomegaly), COPD	*Nocardia spp.*
20	72	Male	-	Pulmonary Embolism	*Nocardia spp.*
21	77	Male	Leukemia	IDDM, Hyperlipidemia, AS II-III, Hypertenstion	*Nocardia spp.*
22	58	Male	-	Sarcoidosis	*Nocardia spp.*
23	64	Female	-	COPD, Hypertension	*Nocardia spp.*
24	48	Female	-	Asthma	*Nocardia spp.*
25	47	Male	-	IDDM, COPD, Hypertension	*Nocardia spp.*
26	67	Male	-	NSCLC , IDDM, COPD, Hypertension, Depression	*Nocardia spp.*

ID	Age[(a)]	Gender	Immune deficiency	Concomitant diseases (other)	Site of Infection
27	72	Male	MTX-Therapy	Psoriasis, COPD, Alcoholism, CHD, Hypertension	*Nocardia spp.*
28	80	Male	-	CKD, COPD	*Nocardia spp.*
29	34	Female	-	CF, IDDM	*N. farcinica/ otitidiscaviarum/ shimofusensis*
30	20	Female	-	CF	*N. cyriacigeorgica/ asteroides*
32	41	Female	-	-	*N. asteroides*
33	7	Male	post-TX	CF, IDDM, CKD	*N. farcinica/ otitidiscaviarum/ cyriacigeorgica*
35	71	Male	-	COPD	*N. otitidiscaviarum*
37	63	Female	-	COPD (+LTOT), CHD	*Nocardia spp.*

A specification of the strain has been done in 10 out of 24 (21%) of the colonized cases. Patients with a Nocardia infection had significant more often an immune deficiency than patients with colonizations ($p<0.01$). Preexisting pulmonary condition were present in 87.5 percent of the colonized patients, with at least 41.6 percent patients suffering from COPD. The BMI was also compared as possible risk factor, but showed no significance.

Table 10: Comparison between the infection-group and colonization-group

Infection	Patients n (%)	Colonization	Patients n (%)
Sufficient therapy	10 (77)	**Sufficient therapy**	3 (13)
Deceased	5 (38)	**Deceased**	0
Ø cultivation period (d)	19 [4-61]	**Ø cultivation period (d)**	28.8 [8-64]
Resistance testing	6 (46)	**Antibiogram**	3 (12)
Immune deficiency[(a)]	8 (54)	**Immune deficiency**	4 (17)
m:w	3.3 to 1	**m:w**	2.1 to 1

4 Discussion

4.1 Epidemiologic findings and risk factors

We analyzed the age and gender distribution, incidence and mortality in the context of suspected nocardiosis cases in Styria between 2001 and 2013.

4.1.1 Gender distribution

The general sex ratio was measured out of all Nocardia findings in this study. Furthermore, nocardial infections and colonizations have also been evaluated separately to reach a better comparability within the chosen observation period.

11 (30%) of the patients in our sample were female and 27 male (70%). This results in an overall gender ratio of 1 to 2.3 (male to female), regardless whether it had been an infection or colonization.
Considered separately, the proportion of men was even higher for the "infection-group" with a ratio of 3.3 to 1 and a bit lower in the colonization group with a ratio of 2.1 to 1.

The sex ratio as separate "risk factor" has already been mentioned by Beaman et al. in 1994. Their review analyzed 1000 patients from different studies with suspected nocardiosis and found a higher proportion of affected men with a ratio of 2.4 to 1, which is similar to the overall ratio in our study. They did also look upon whether the age reflects upon the sex ratio and they observed a rise, with a high male dominance in patients in their forties with 4:1 and only a little difference for patients in their twenties (4).

We found the same raise in male dominance for nocardiosis starting at the age groups forty upwards, as it can be seen in figure 10 above. The ratio even showed a female dominance in the age-group 21-40, which opposes the theory that estrogen may provide some kind of protective effect against nocardiosis. However our number of cases was relatively small and therefore not representative.

Interestingly, Beaman et al. also found a very different ratio for patients with AIDS and calculated a sex ratio of 4.7 to 1 (male to female). However, this ratio for AIDS patients should be considered with caution, since the collective was recruited during the 1980s (4).

They also examined whether the gender has not only an impact on the morbidity, but on the mortality of the patients as well, but found no significant differences in mortality between male and female patients in their study (4).

In this study, 4 of 5 deceased patients were male (80%) and 1 was a female (2%), resulting in a 4:1 ratio. Thus the male:female ratio of 4:1 may be higher than the overall gender ratio, but cannot be seen as significant due to the small sample size.

4.1.2 Age

Another observed attribute was the age of the patients included in our study. We found a higher incidence of nocardial isolates in patients after the age of 41, representing 81% of all patients. The average age at the time of diagnosis was 57 (±18) years. We found no significance between the incidences of patients under 40 years and over 40 years ($p=0.09$), which is most probable due to the small number of cases. The patients between the ages of 41 to 60 years and the ages of 61 to 80 did also show no significances in Nocardia findings, although the rate of positive findings was twice as high in the second age group. The higher incidence of Nocardia in elder patients is probably due to the concurrent raise of concomitant diseases and immune deficiencies, respectively.

Wang et al. analyzed 132 patients with cancer and nocardial isolates at the University of Texas from 2002 through 2012. They found a mean age for all patients with suspected nocardiosis of 59.1 years, regardless whether they had a pulmonary or cutaneous manifestation (50).
Cases of patients with suspected pulmonary Nocardiosis were analysed by Kurahara et al. in 2013. They found a mean age of 66 years in 59 observed patients (51).

4.1.3 Incidence

There are not many studies with large numbers of cases, measuring the real incidence of nocardiosis. The reason for that is mainly because most of the large studies did not particularly differentiate between nocardial infection and colonization. A study from Canada analyzed all Nocardia cases found in Quebec from 1988 to 2008 (n=575) and calculated the total incidence for Nocardia findings and infections, among others. They measured an annual incidence in between 0.33 to 0.87 per 100.000 inhabitants. However

they also did not distinguish between infections and colonizations for their incidence calculation (6).
They did also find a significant raise in incidence of Nocardia infection and colonization, respectively. They explained this circumstance with the increase of elderly people in the population, the increase in the number of immunocompromised patients, the more frequent referral of isolates to their laboratory and the improved proficiency in recovering and recognizing of presumptive *Nocardia spp.* (6).

We did also perform an overall incidence calculation for all Nocardia findings which were included in our study and found an incidence ranging between 0.125 and 0.625 per 100.000 inhabitants per year during our observation period. But in comparison with the study cited above, we found no significant raise in incidence of Nocardia findings over the years, which may be due to our small sample size or other yet unknown factors.

4.1.4 Morbidity and Mortality

In our study, we distinguished 9 of 13 (69%) infections with verified pulmonary nocardiosis and 4 of 13 (31%) with cutaneous nocardioses. Out of all those infections, 31% (1 primary cutaneous nocardiosis and 3 pulmonary nocardioses) developed dissemination further on. The total mortality for both pulmonary and cutaneous nocardiosis had been 38.5% in this study. Cutaneous nocardioses showed mortality rates of 25% (1/4) and the pulmonary forms a mortality of 44% (4/9). All of our cases with secondary dissemination developed a CNS nocardiosis and had a high mortality rate of 75% (3/4 patients).

Although it is known that secondary nocardiosis tends to have a poor prognosis, the total mortality in the literature is generally lower compared to our study with a morality of 25%. McNeil et al. reviewed multiple studies about nocardiosis in 1994. They assessed a mortality range for disseminated nocardiosis between 7 and 44%, which rose up to 85% in immunocompromised patients with secondary nocardiosis (52).

Even though we found a higher overall mortality in our disseminated cases, the mortality in immunocompromised patients was only 37.5% and therefore lower compared to the literature. This may be due to more intensive monitoring of immunocompromised patients and the fact that nocardial findings are frequently underrated and undertreated in

immunocompetent patients, compared to immunocompromised patients. In newer studies the mortality for disseminated nocardiosis ranged between 25% to 64% (10,20) and the crude mortality was estimated to be among 8% and 39% (8,10,11,44,53), which is similar to the mortality rate of 25% in our study. But since some of those studies did not explicatively differentiate between colonization and infection, the mortality should be probably located in the higher ranges.

4.1.5 Nocardia and other pulmonary diseases

It has been frequently reported in literature that underlying pulmonary diseases, bronchiectasis and chronic obstructive pulmonary diseases may be risk factors for pulmonary nocardiosis, probably due to defects in the cell mediated immunity. This fact should be especially considered in immune competent patients (51,54). In 23/24 (95.8%) of our cases classified as colonization, the positive Nocardia sample had been acquired from the lung. Out of these cases 16/23 (66.6%) had some form of underlying structural lung disease. Looking at them separately, we found 10/23 (43.5%) cases of COPD, 3/23 (13%) with cystic fibrosis, 2/23 (9%) with sarcoidosis and 1/23 (4.3%) with bronchial asthma. In contrast, only 2/9 (22.2%) cases with pulmonary nocardiosis had an underlying structural pulmonary defect. Additionally, both of those patients did also undergo organ transplantation (heart transplantation and lung transplantation, respectively). Therefore probably only 2/25 (8%) patients of our study with Nocardia findings in the lung were real pulmonary nocardioses, concluding that most of the Nocardia findings in the lung are in fact colonizations. The relevance of respiratory colonizations has already been reported in 1978. In this case review only 19/36 cases with confirmed *Nocardia spp.* had any clinical symptoms of disease and most of those presumably only colonized patients had an obstructive lung disease (55).

4.2 Applicability of the proposed guideline

After the retrospective analysis of patient's data and the application of our proposed classification scheme, we observed a higher rate of colonized patients in relation to patients with a presumably manifest infection. Numerical spoken, the ratio of patients with a colonization by Nocardiae and those with a nocardiosis was 1.8 to 1 (colonization to infection). It should be considered, that most of the primarily excluded patients were probably accidental findings in healthy outpatients and thus colonization as well. This

means the frequency of colonizations is probably even higher than suggested before. Not to mention all of those colonized patients who did not undergo any microbiological diagnostic procedures, who showed no symptoms to begin with and therefore did not seek any medical assistance.
This high rate of colonizations is mainly due the fact that Nocardiae can be found ubiquitous in salt- and freshwater, soil, dust and decaying matters. Therefore acquisition Nocardiae on the skin or through the respiratory system may indeed occur on frequent basis, but is usually harmless most of the time because they are opportunistic pathogens (2).

We also observed in total 5 patients with microbiological confirmed Nocardia spp, who deceased within our observation period from 2001 through 2013. Hence, all of them should be considered as real nocardial infections in retrospect. The fact, that all of those 5 have been successfully classified as infections by using our diagnostic criteria, indicates a high sensitivity of the proposed classification.
21/24 of the patients classified as colonized are considered as untreated, because either they received an insufficient antimicrobial substance, were treated too briefly for nocardial infection or did not receive any therapy at all. Even though the colonization-group received treatment significantly less often than the infection group ($p=0.003$), the mortality was still significant lower than in the infection group ($p=0.0021$). This circumstance may be indicative for a good specificity of the proposed diagnostic criteria as well. The negative predictive value for the classification was 87.5% in case all treated colonizations (3/24) were in fact treated infections. Last but not least, 6 from 37 patients (16.2%) would have been treated differently, if our proposed classification chart had been applied.

4.2.1 Impact of criteria in different manifestations

None of the patients classified as colonization, whose sample were taken from the lung, fulfilled the clinical criteria. Anyhow, some of them fulfilled either one of the radiographic criteria or the microbiological criteria or even both. Thus, the clinical criteria and evaluation of potential differential diagnoses, as for instance an acute exacerbated COPD without pulmonary infiltrates or common pulmonary infection successfully treated with a short-course of antibiotics (like for community acquired pneumonia), seemed to be of greater importance for a valid diagnosis than the other criteria. This may certainly raise the question whether all proposed criteria were of equal relevance for different manifestations

of nocardiosis, regardless the fact that all three criteria had to be fulfilled for a distinct diagnosis in this study.

In patients with a primary involvement of the skin a positive microbiological criteria with a sterile acquired sample seemed to be more determining for the final diagnosis and its outcome, because microbiological findings may be easily overlooked in primary care routine due to the fact that skin infections are all too frequently caused by *Staphylococci*, *Streptococci* or *E. coli*. For example, this study examined 5 patients on whom Nocardiae were found on or in the skin. 4/5 (80 %) were retrospectively classified as a real infection and in all of those cases the samples were obtained via sterile methods like biopsy, which makes a contamination during the extraction relatively unlikely. For example, one patient of our cases with a low malignant Non-Hodgkin-lymphoma and leucopenia (<3000 leucocytes per µl) presented himself with an abscess on the left arm. This abscess has been treated with a surgical drainage and amoxicillin/clavulanic acid for 2 weeks. Although Nocardia was verified in the drained fluid 7 days later, the antimicrobial therapy was not adapted. One month later, the patient developed a purulent leptomeningitis and deceased shortly after. This case illustrates the importance of taking all mentioned diagnostic factors into consideration.

4.3 Limitations and Future

The conclusions of this study may be limited due to several factors. First, a retrospective study design is usually error-prone itself, because the potential bias (e.g. information bias) and the problem with unseen confounding variables. Anyhow, a prospective study design is most unlikely since nocardiosis is an orphan infection.

Second, the sample size of the study was relatively low, despite the long observation period of 12 years. This circumstance again arises from the low incidence of nocardiosis. In addition, 25 patients had to be excluded during the data assessment, resulting in a further reduction of cases. To overcome the mentioned limitations, a retrospective multicenter study is already planned from our study group.

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