Diplomarbeit

Retrospective determination of frequency of *Pseudomonas aeruginosa* colonization in Non-CF-bronchiectasis patients in Styria

-

Immunodeficiencies as risk factor for *Pseudomonas aeruginosa* colonization in Non-CF-bronchiectasis

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Eidesstattliche Erklärung

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Graz, am 12.06.2020 *Javier-Fernando Noriega Urena eh*

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1 Zusammenfassung

1.1 Ziel

Unter Non-CF-Bronchiektasen (NCFB) versteht man Bronchiektasen, die nicht auf dem Boden einer Zystischen Fibrose (CF, Mukoviszidose) entstehen. Bronchiektasen prädestinieren für eine chronische bakterielle Besiedlung der betroffenen Atemwege, was wesentlichen Einfluss auf den klinischen Verlauf der betroffenen meist chronisch pulmonal erkrankten Patientinnen und Patienten haben kann (1). In den letzten 10 Jahren nahm das wissenschaftliche Interesse an Non-CF-Bronchiektasen international deutlich zu, sodass sich inzwischen eine breite klinische Forschungslandschaft entwickelt hat. Nach unserem Kenntnisstand liegen diesbezüglich keine veröffentlichten Daten oder Studienergebnisse aus Österreich vor. Ziel dieser Arbeit ist es eine Non-CF-Bronchiektasen Kohorte, welche von der pulmologischen Spezialambulanz für pulmonale Infektionen der klinischen Abteilung für Lungenerkrankungen des LKH Graz (Universitätsklinik für Innere Medizin der Medizinischen Universität Graz) betreut wird, ätiologisch detailliert zu analysieren und zusätzlich die Häufigkeit einer *Pseudomonas aeruginosa* Kolonisation innerhalb dieser Kohorte zu bestimmen. Des Weiteren wird der Frage nachgegangen, ob Immundefekte bzw. immunsuppressive Therapien Risikofaktoren für eine *Pseudomonas aeruginosa* Kolonisation darstellen.

1.2 Methoden

Retrospektiv wurden die Daten von 119 Patientinnen und Patienten mit Non-CF-Bronchiektasen in die Analyse einbezogen. Bei den Analysen über Immundefizienz bzw. immunsuppressiven Therapien wurden zwei Personen ausgeschlossen (n = 117), da der Zusammenhang zwischen der Immundefizienz bzw. der immunsuppressiven Therapie nicht eindeutig eruierbar war.

Die Patientinnen und Patienten wurden im Zeitraum zwischen dem 11. Dezember 2003 und 09. Januar 2019 in der Spezialambulanz für pulmonale Infektionen vorstellig. Erhoben wurden das Alter, Geschlecht, die zugrunde liegende Ätiologie, Ergebnisse der Sputumkultur, *Pseudomonas* Antikörper und der Status der Immundefizienz bzw. einer immunsuppressiven Therapie. Die Datenerhebung erfolgte mittels der steirischen elektronischen Krankenhausdatenbank – MEDOCS.

1.3 Resultate

Das mittlere Alter der Kohorte betrug 64 Jahre mit einer klaren Dominanz der Altersgruppen zwischen 46 bis 85 Jahren. In allen Altersklassen, die 18-25-jährigen ausgenommen, waren mehr Frauen als Männer zu finden.

Bezüglich der Ätiologie waren postinfektiöse Bronchiektasen (17,6%) und Bronchiektasen aufgrund von rheumatischen Erkrankungen (16,8%) am häufigsten nachzuweisen. Idiopathische Bronchiektasen und COPD-assoziierte Bronchiektasen (beide jeweils in 14,3% der Fälle) waren ebenfalls noch in mehr als 10% der Fälle auffindbar. Die übrigen Ätiologien lagen unter einer Häufigkeit von 10% (Asthma 8,4%, weitere Ursachen 5,9%, ABPA 5,0%, Primäre Ig-Defizienzen 5,0%, Hämatologische Erkrankungen ohne sek. Ig.-Mangel 3,4%, ILD 3,4%, postradiotherapeutische Bronchiektasen 3,4%, Hämatologische Erkrankungen mit sekundärem Ig-Mangel 2,5%).

Sputumkultur-Ergebnisse lagen von 71 der 119 Patientinnen und Patienten (59,7%) vor. In 39,4% der Fälle konnte kein Pathogen nachgewiesen werden. *Pseudomonas aeruginosa* war bei 31,0% der Kulturen nachweisbar. In 21,1% der Fälle konnte *Haemophilus influenzae* nachgewiesen werden. Außerdem untersucht wurde die Häufigkeit von *Staphylococcus aureus* (19,7%), *Klebsiella spezies* (14,1%), *Aspergillus fumigatus* (8,5%), *Stenotrophomonas-/ Achromobacter spezies* (2,8%), *Burkholderia spezies* (1,4%), *Nocardia spezies* (1,4%) und *Streptococcus penumoniae* (1,4%).

Pseudomonas Antikörper wurden in 36 Fällen von 119 (30,3%) überprüft. Davon waren 27,8% der Patientinnen und Patienten positiv und 72,2% negativ.

In 30 Fällen von 119 (25,2%) waren sowohl Sputum-Ergebnisse für *Pseudomonas aeruginosa* als auch *Pseudomonas* Antikörper Ergebnisse vorhanden. In 30,0% der Fälle waren Antikörper nachweisbar, wobei davon auch 55,6% eine positive *Pseudomonas aeruginosa* Kultur aufgewiesen haben. Von den 70,0% Antikörper negativen Patientinnen und Patienten, wurde in 33,3% dennoch eine *Pseudomonas aeruginosa* positive Kultur nachgewiesen.

Von unserer Kohorte mit 117 Patientinnen und Patienten konnte in 33 Fällen (28,2%) eine Immundefizienz bzw. immunsuppressive Therapie nachgewiesen werden. Von diesen 33 Patientinnen und Patienten zeigte sich ein Frauenanteil von (75,8%).

Daten zur Immundefizienz bzw. immunsuppressiven Therapie und Sputumkultur lagen für 69 von 117 Patientinnen und Patienten (59,0%) vor. 21 dieser 69 (30,4%) zeigten sich immungeschwächt. Davon war in 47,6% der Fälle eine positive *Pseudomonas aeruginosa* Sputumkultur nachzuweisen (p= 0,064).

Daten zum Vergleich zwischen Immundefizienz bzw. immunsuppressiver Therapie und *Pseudomonas* Antikörpern waren nur für 36 von 117 Fällen (30,8%) verfügbar. Dabei waren 10 immungeschwächte Patientinnen und Patienten unter den 36 analysierten (27,8%) inkludiert. 20,0% der immungeschwächten wiesen einen positiven *Pseudomonas* Antikörpertest auf.

1.4 Fazit

Ab einem Alter von 46 Jahren sollte nach stattgehabten schweren Infektionen (beispielsweise schweren Pneumonien oder Mykobakteriosen) bei persistierenden respiratorischen Beschwerden eine Bronchiektasen Erkrankung in Betracht gezogen werden. Dasselbe gilt insbesondere auch für COPD Patientinnen und Patienten mit auffällig starker Hustensymptomatik, chronisch produktivem Husten oder gehäuften Exazerbationen. Dabei ist zu berücksichtigen, dass Frauen eher von Bronchiektasen betroffen sind als Männer. Bei schwer therapierbaren Infektionen muss außerdem an eine mögliche Kolonisation mit *Pseudomonas aeruginosa* oder *Haemophilus influenzae* gedacht werden.

Nach unseren Daten hat sich zwar ein Trend aber letztendlich kein signifikanter Zusammenhang zwischen Immunsuppression und *Pseudomonas aeruginosa* Besiedlung gezeigt. Aufgrund der geringen Fallzahl ist aber davon auszugehen, dass sich in größeren Studien möglicherweise ein Zusammenhang ergibt.

2 Abstract

2.1 Aim

NCFB refer to bronchiectasis not based on cystic fibrosis (CF). It predisposes for bacterial colonization which may impact clinical outcome (1). To our knowledge, regarding NCFB no representative data are available from Austria.

We aim to investigate the prevalence of *Pseudomonas aeruginosa* colonization in patients treated at a specialized clinic for chronic pulmonary infections at the Division of Pulmonology at Medical University Hospital of Graz. Furthermore, we examine the underlying etiologies in our subjects. Moreover, we considered whether immunodeficiency represents a risk factor for *Pseudomonas aeruginosa* colonization in NCFB. Our immunodeficient patients suffered either from an immunocompromising disease or immunosuppressive therapy.

2.2 Methods

We reviewed 119 cases retrospectively using the electronic Styrian Hospital network, MEDOCS. Due to inclusion criteria our immunodeficiency-based statistics only included 117 patients.

All patients applied to the Division of Pulmonology at Medical University Hospital of Graz between December 11, 2003 and January 9, 2019. Collected data included age, sex, underlying etiology, sputum culture results, *Pseudomonas* antibodies and immunodeficiency due to a medical condition or based on immunosuppressive therapy.

2.3 Results

Median age of our cohort was 64 years with most patients between 46 to 85 years. Rising age was associated with more NCFB. All age groups except the 18 to 25-year-old contained more women than men.

Most frequent underling etiology was postinfectious bronchiectasis (17.6%) and bronchiectasis due to rheumatic diseases were the second most (16.8%). Idiopathic bronchiectasis and bronchiectasis based on COPD were represented with 14.3%, respectively. The remaining etiologies all had an amount of under 10 percent (Asthma 8.4%, other rare etiologies 5.9%, ABPA 5.0%, primary Ig-deficiency 5.0%, hematological disease without secondary immunoglobulin deficiency 3.4%, ILD 3.4%, post radiotherapeutic 3.4%, hematological disease with secondary immunoglobulin deficiency 2.5%).

Sputum culture results were available in 71 of 119 cases (59.7%). In 39.4% no pathogen was detectable. In 31.0% sputum cultures were positive for *Pseudomonas aeruginosa* and in 21.1% for *Hemophilus influenzae*. Furthermore, we investigated the appearance of *Staphylococcus aureus* (19.7%), *Klebsiella species* (14.1%), *Aspergillus fumigatus* (8.5%), *Stenotrophomonas-/ Achromobacter species* (2.8%), *Burkholderia species* (1.4%), *Nocardia species* (1.4%) and *Streptococcus pneumoniae* (1.4%).

Besides the sputum cultures we also collected data for *Pseudomonas* antibodies. In 36 of 119 cases (30.3%) those results were available. Of our 36 patients 27.8% showed positive *Pseudomonas* antibodies and 72.2% negative antibodies.

In 30 out of 119 cases (25.2%) both sputum culture results and *Pseudomonas* antibody results for *Pseudomonas aeruginosa* were available. 30.0% showed positive *Pseudomonas* antibodies and of these we found 55.6% with also positive *Pseudomonas aeruginosa* cultures. Nevertheless, among the 70.0% antibody negative patients 33.3% had a *Pseudomonas aeruginosa* positive culture.

Of our cohort with 117 patients we considered for immunodeficiency analyses 33 cases (28.2%) revealed as immunodeficient with a portion of 75.8% women.
Data for both immunodeficiency and sputum culture were available in 69 of 117 (59.0%) cases. 21 out of 69 (30.4%) showed immunodeficiency. Of those 21 we found 47.6% with positive *Pseudomonas aeruginosa* sputum cultures (p=0.064).
The comparison between immunodeficiency and *Pseudomonas* antibodies was possible with 36 of 117 patients (30.8%). 10 of 36 were immunodeficient (27.8%). 20.0% of these immunodeficient patients revealed positive *Pseudomonas* antibody tests.

2.4 Conclusion

In patients of age 46 or elder bronchiectasis should be considered if they suffer from recurrent pulmonary infections or COPD and especially if they are female. In case of therapy resistant pulmonary infections, a *Pseudomonas aeruginosa* or *Hemophilus influenzae* colonization may be the cause.
However, our results showed a trend but did not prove a correlation between immunodeficiency and *Pseudomonas aeruginosa* colonization. Nevertheless, major data size may show different results.

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Glossary and Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
ACT	Airway clearance technique
AIDS	Acquired immunodeficiency syndrome
BALF	Bronchoalveolar lavage fluid
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
COPD	Chronic obstructive pulmonary disease
CPAM	Congenital pulmonary airway malformation
CR	Complement receptor
CT	Computed tomography
CVID	Common variable immunodeficiency
DLCOcSB	Diffusing capacity of the lung for carbon monoxide
DNase	Deoxyribonuclease
ELISA	Enzyme-linked immunosorbent assay
ERS	European Respiratory Society
ExoS	Exotoxin S
ExoT	Exotoxin T
ExU	Exotoxin U
ExoY	Exotoxin Y
FEV_1	Forced expiratory volume in one second
Fcγ	Fragment crystallizable gamma
FVC	Forced vital capacity
HAP	Hospital acquired pneumonia
HCAP	Health care associated pneumonia
HIV	Human immunodeficiency virus
HNPs	Human neutrophil peptides
HRCT	High-resolution computed tomography
ICAM-1	Intercellular adhesion molecule-1
ICU	Intensive care unit
IgA	Immunoglobulin A

IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ILD	Interstitial lung disease
IL-17	Interleukin 17
NCFB	Non-cystic fibrosis bronchiectasis
Non-CF-Bronchiectasis	Non-cystic fibrosis bronchiectasis
NTM	Nontuberculosis mycobacterium
PA	Pseudomonas aeruginosa
PAMP	Pathogen-associated molecular pattern
PCD	Primary ciliary dyskinesia
POH	$PcrV_{28-294}$-$OprI_{25-83}Hcp1_{1-162}$
Primary Ig-deficiency	Primary immunoglobulin deficiency
PRRs	Pattern recognition receptors
QS	Quorum sensing
RANTES	Regulated And Normal T cell Expressed and Secreted
RIG-1	Retinoic acid inducible gene I
T3SS	Type 3 secretion system
Th17 cells	T helper 17 cells
TNFα	Tumor necrosis factor alpha
VAP	Ventilator associated pneumonia

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3 Introduction

Bronchiectasis is no longer an orphan disease as assumed when it was first described in 1819 by René Laennec (2). By now it is considered as a potentially severe lung disease associated with poor outcome especially if it occurs alongside chronic obstructive pulmonary disease (COPD) (3). Notwithstanding the seriousness of this condition current epidemiological data are scarce (4).

However, a number of researchers have reported an increase in incidence and prevalence depending on gender and age (5-13). Additionally, epidemiological variations between ethnicities have been revealed (11, 14).

It is recognized that Bronchiectasis contributes a severe economic burden, hence this disease and it´s treatment is going to be more important prospectively (15).

Current treatment is based on the comprehension according to the vicious cycle concept of this disease. It contains preventing or suppressing acute and chronic bronchial inflammation (including infection), improving mucociliary clearance and reducing the impact of structural lung disease (16).

This study is focused on non-cystic fibrosis bronchiectasis (Non-CF-Bronchiectasis, NCFB).

Chronic airway infection is common in bronchiectasis and deteriorates pulmonic function. One of the most frequent organisms responsible for chronic airway infection in bronchiectasis, beside *Haemophilus influenzae,* is *Pseudomonas aeruginosa* (16). It has been revealed that chronic *Pseudomonas aeruginosa* colonization of bronchiectasis is associated with an increased risk of mortality, hospital admissions, frequent exacerbations and a worse quality of life (1). Furthermore, changes in lung function and radiological severity were reported(1). Therefore, those patients should obtain a specific therapy to suppress or eradicate colonization, if possible. Depending on individual situation, mostly either macrolides or inhaled antibiotics are appropriate (16).

3.1 Bronchiectasis

Bronchiectasis means irreversible pathological dilatation of the airways. Productive cough with production of thick sputum is a cardinal symptom. You can differentiate between a focal and a diffuse distributional pattern. In addition, they are categorized as cylindrical (tubular), varicose or cystic. Tubular bronchiectasis are the most frequent ones (8).

3.1.1 Etiology

Etiology of bronchiectasis is classifiable in several ways. In general terms, they appear in two different distributional patterns – focal and diffuse. Focal bronchiectasis means bronchiectatic changes in a circumscribed region of the lung. Diffuse bronchiectasis is described as bronchiectatic changes all over the lung (8).

In Table 1 lung involvement patterns and their principles are depicted. As shown, focal Bronchiectasis only arise from broncho-obstruction among others caused by aspiration or tumor mass. Whereas diffuse bronchiectasis has various underlying factors and frequently arise from infectious or systemic conditions (8).

Major Etiologies of Bronchiectasis and Proposed Workup	
PATTERN OF LUNG INVOLVEMENT	**ETIOLOGY BY CATEGORY (EXAMPLES)**
Focal	Obstruction (aspirated foreign body, tumor mass)
Diffuse	Infection (bacterial, nontuberculous mycobacterial)
	Immunodeficiency (hypogammaglobulinemia, HIV infection, bronchiolitis obliterans after solid organ or stem cell transplantation)
	Genetic causes (cystic fibrosis, Kartagener´s syndrome, α_1 antitrypsin deficiency)
	Autoimmune or rheumatic causes (rheumatoid arthritis, Sjögren´s syndrome, inflammatory bowel disease); immune-mediated disease (allergic bronchopulmonary aspergillosis)
	Recurrent aspiration
	Miscellaneous (yellow nail syndrome, traction bronchiectasis from postradiation fibrosis or idiopathic pulmonary fibrosis)
	Idiopathic

Table 1 Major Etiologies of Bronchiectasis and Propose Workup. Adapted from: Jameson et al., Harrison´s principles of internal medicine 2018, Vol. 2, Chapter 284, p. 1983

As illustrated in figure 1 distributional pattern could also give suggestion to the underlying etiology itself.

Table 1 Classification of bronchiectases based on their distribution.

Diffuse bronchiectases	Apical predominance	• Cystic fibrosis • Sarcoidosis
	Anterior predominance	• Infection due to nontuberculous mycobacteria • Adult respiratory distress syndrome
	Basal predominance	• Chronic aspiration sequelae • Ciliary dyskinesia • Pulmonary fibrosis • Immunosuppression-associated bronchiectasis • Bronchiolitis obliterans
	Without regional predominance	• Mounier–Kuhn syndrome • Williams–Campbell syndrome
Central Bronchiectases	• Allergic bronchopulmonary aspergillosis	
Focal Bronchiectases	• Post-radiotherapy fibrosis • Tuberculosis sequelae • Bronchial atresia • Extrinsic compression • Foreign body • Endobronchial neoplasm • Bronchial stenosis	

Figure 1 Classification of bronchiectasis based on their distribution, Bueno J, Flors L. The role of imaging in the diagnosis of bronchiectasis: the key is in the distribution. Radiologia. 2018;60(1):39-48.

Although, there are many different disorders inducing bronchiectasis, their frequency is not alike. Because different trials reveal diverse results for several reasons, a systematic literature review seems to provide valuable data. Gao and Guan *et al.* have conducted a systematic literature review which encompass 56 articles. Idiopathic etiology of bronchiectasis was the most common and found in 44.8%. Following most frequent known etiologies were post-infective (29.9%), immunodeficiency (5.0%), COPD (3.9%), connective tissue disease (3.8%), ciliary disfunction (2.5%) and Allergic bronchopulmonary aspergillosis (ABPA) (2.6%), Figure 2 (17). The study population included 8608 patients, but total amount of etiologies was 8656, which means dual and triple etiologies for bronchiectasis were verified in some patients (17).

Furthermore, significant demographic differences in etiology have been determined among the assessed studies. Prevalence of idiopathic bronchiectasis was significantly higher in Asia and Oceania (59.2% and 67.0%) in relation to Europe (41.1%), South America (37.3%),

Africa (25.0%) and North America (6.6%) (P<0.001). Additionally, the prevalence of post-tuberculosis Bronchiectasis was significantly higher in Asia (68.5%) when compared to Europe (53,7%) (P<0.001) (17).

Moreover, the review depicted the importance of the etiology referred to disease management or required genetic screening. In 18.3%, which is equal to 1577 patients, as many as one etiology affected the patient's treatment or led to genetic screening (17).

Risk factors	Total number	% of total
Idiopathic bronchiectasis	3857	44.8
Post-infective bronchiectasis	2574	29.9
Immunodeficiency	429	5.0
Chronic obstructive pulmonary disease	333	3.9
Connective tissue disease	328	3.8
Allergic bronchopulmonary aspergillosis	223	2.6
Ciliary dysfunction	218	2.5
Asthma	120	1.4
Inflammatory bowel disease	66	0.8
Obstructive	67	0.8
Aspiration/esophageal reflux	64	0.7
Congenital malformation	33	0.4
α_1-Antitrypsin deficiency	36	0.4
Diffuse panbronchiolitis	27	0.3
Pink's disease	20	0.2
Yellow nail syndrome	11	0.1
Others[†]	250	2.9

[†]Other aetiologies including sinobronchial syndrome (n = 27), amyloid (n = 1), smoke inhalation (n = 1), eosinophilic bronchiolitis (n = 1), Young's syndrome (n = 26), bronchiolitis obliterans (n = 3), vasculitis (n = 5), interstitial lung disease (n = 63), cystic fibrosis or cystic fibrosis transmembrane conductance regulator related bronchiectasis (n = 20), systematic disease (n = 47) and other unreported (n = 42).

Figure 2 Risk factors of bronchiectasis in adults (n=8608 patients), Gao YH, Guan WJ, et al. Aetiology of bronchiectasis in adults: A systematic literature review. Respirology (Carlton, Vic). 2016;21(8):1376-83

3.1.2 Epidemiology

Due to a lack of data epidemiology of bronchiectasis is not known exactly. To our knowledge there are only a few studies who assessed epidemiology especially of Non-CF bronchiectasis.

The majority of publications found, that bronchiectasis in general occur more often in women than in men (5, 12, 18). This trend may reverse in higher age, as one study showed that NCFB are more frequent in males beyond 65 years than in females (9). Diel *et al.* found men as the predominant gender for NCFB from age 45 on (19). In terms of age 64 or younger bronchiectasis are more common in women than in men, too (9).
Undisputed is the fact that incidence of bronchiectasis is increasing with age (5, 7, 9, 11, 12)

The overall prevalence rate in Germany was 67 per 100 000 (9). In United Kingdom Quint *et al.* disclosed an increasing point prevalence in women between 2004 and 2013 from 350.5 per 100 000 in 2004 to 566.1 per 100 000 in 2013 (5). The mean point prevalence increased from 301.2 per 100 000 in 2004 to 485.5 per 100 000 in 2013 (5). In the United States studies also exposed an increasing prevalence with numbers from 271.8 to 812 per 100 000 (7, 12)

Although, those trials reveal epidemiological data of bronchiectasis, different inclusion criteria of these works should be considered. Moreover, it is believed that due to more frequent use of high-resolution computed tomography more patients are diagnosed with bronchiectasis (12).

3.1.3 Pathogenesis

The vicious cycle hypothesis is a widespread explanation for pathogenesis of bronchiectasis. Chronic bronchial infection leads to inflammation and impaired mucociliary clearance, which then causes structural lung damage (16).

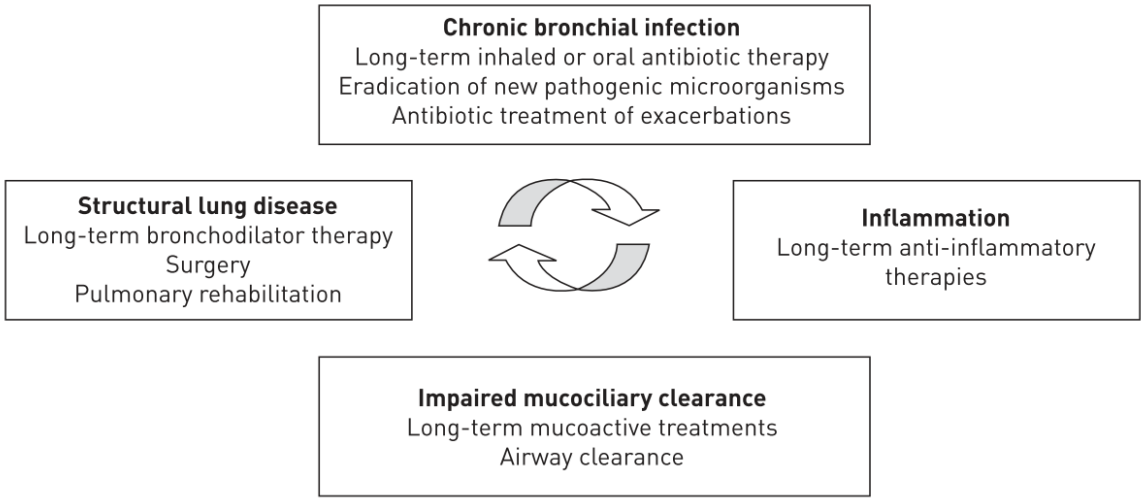

Figure 3 Treatments for bronchiectasis according to the vicious cycle concept. Polverino E, et al. European Respiratory Society guidelines for the management of adult bronchiectasis. Eur Respir J 2017; 50: 1700629

Although several trials investigated immunological processes of bronchiectasis some questions remain to be unannounced.

As far as we know today bronchiectatic formation arises from interplay between neutrophil granulocytes, macrophages, T-cells, eosinophil granulocytes, epithelial cells and immunoglobulin, complement and antimicrobial peptides (20).

Neutrophil granulocytes

Neutrophil-dominant inflammation is denoting for bronchiectasis (21, 22). Sputum neutrophils are increased in patients with bacterial infection compared to patients without airway pathogens (23). Furthermore, levels of sputum neutrophils are higher in bronchiectasis infected with *Pseudomonas aeruginosa* than with other bacteria (23). Neutrophil granulocytes are increased in bronchiectatic biopsies and there was demonstrated a coherence between tumor necrosis factor alpha (TNFα) positive cells and neutrophils (22).

Recruitment of neutrophils is driven by chemoattractants such as interleukin 8 and leukotriene B4, interleukin-1 beta and TNFα (20, 24). The recruitment is followed by transendothelial migration of neutrophil granulocytes as depicted in figure 4.

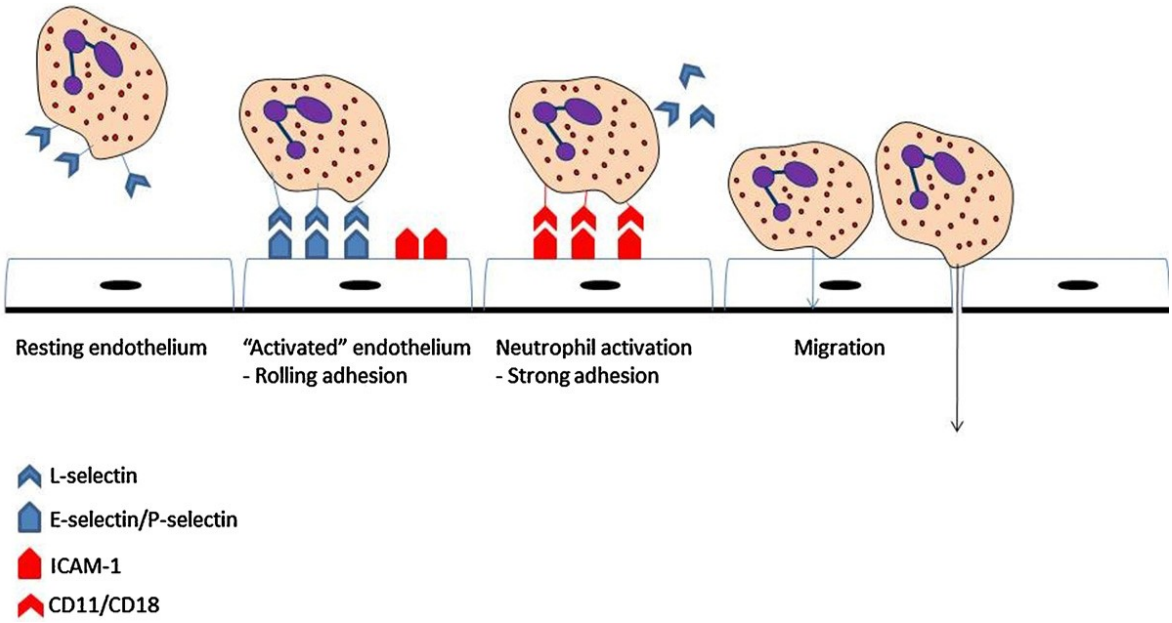

Figure 4 Neutrophil migration. Abbreviations ICAM-1 = intercellular adhesion molecule-1. Chalmers JD, Hill AT. Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis. Molecular immunology. 2013;55(1):27-34.

Neutrophil phagocytosis relies on Fcγ- and complement receptors (CR) such as FcγRIIB (CD16), FcγRIIA, (CD32), CR1 (CD35) and CR3 (CD11b/CD18).
Phagocytotic targets are opsonized with immunoglobulin G which will be recognized by Fcγ-receptors. Further, CR1 identifies complement components C3b/C4b placed on microorganisms. Complement component iC3b is recognized by CR3 (20).
Pathogens were incorporated in neutrophil phagosome and eliminated by aid of toxic contents of neutrophil granules (20).

Bacterial killing of neutrophils in patients with bronchiectasis is impaired. It has been discovered that the inflamed environment in bronchiectasis must have an impact on neutrophil phagocytotic function because airway neutrophils showed lower bacterial eradication when compared to blood neutrophils (25, 26). Moreover, viability of blood neutrophils is extended and apoptosis is decelerated (26). Furthermore, deficient oxidative burst worsens neutrophils phagocytotic ability in comparison to normal functioning neutrophil granulocytes (27).

High levels of human neutrophil peptides (HNPs) which are substantial for bacterial killing are found in bronchiectasis. They also contribute to malfunctioning neutrophil bacterial phagocytosis (28).

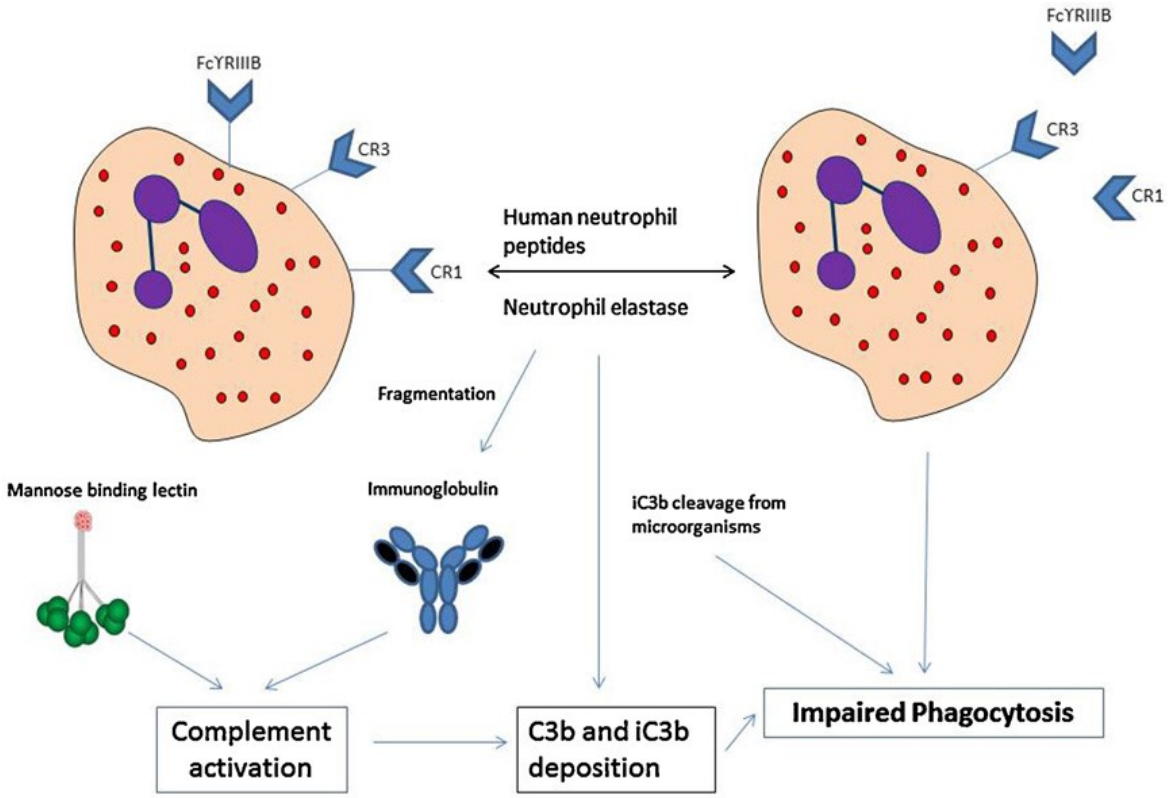

Figure 5 Neutrophil phagocytic impairment in bronchiectasis. Abbreviations CR1 = complement receptor 1, CR3 = complement receptor 3. Chalmers JD, Hill AT. Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis. Molecular immunology. 2013;55(1):27-34.

Neutrophil elastase as a mediator of neutrophils is released by degranulating neutrophil granulocytes. An abundance of neutrophil elastase in bronchiectasis exceeds natural antiprotease defenses. Therefore, its damaging property can take full effect. In vitro epithelial cell damages, slowing ciliary beat frequency and impaction on goblet cells has been verified. Moreover, neutrophil elastase has pro-inflammatory function and injures elastin, basement membrane collagen and proteoglycans. It is associated with lung function, radiological severity and 24h sputum volume as markers of disease magnitude (20, 21).
Neutrophil elastase also affects neutrophil phagocytosis as it segregates FcγRIIIb, CR1 and iC3b (20).

Macrophages

Biopsies of bronchiectasis show a hyper cytosis of macrophages (22). Via inflammatory mediators such as tumor necrosis factor alpha, interleukin-8 and leukotriene B4 they serve as coordinating factor in inflammatory reactions (20).

Efferocytosis, which means removing apoptotic inflammatory cells, is an important function of macrophages and necessary to cure airway inflammation (29). This function is affected by neutrophil elastase. It cleaves the phosphatidylserine receptor on phagocytes, decreases apoptotic cell clearance and sustains airway inflammation (30).

T cells

Presence of T cells in bronchiectasis inflammation has been proved but there was no certain ratio found between CD4+ and CD8+ T cells (31, 32). Tan *et al.* demonstrated a higher number of Th17 cells in non-CF bronchiectasis compared to healthy controls in children. Th17 cell levels in children were similar in CF and non-CF bronchiectasis (32). Equally, interleukin 17 (IL-17) was elevated in bronchoalveolar lavage fluid (BALF) (32). In addition, IL-17 levels were raised in BALF of adults, too but no correlation between *Pseudomonas aeruginosa* infection and IL-17 levels could be detected (33).

Eosinophils

In some patient's sputum eosinophils are elevated and it correlates with worse spirometry. Altogether not much is known about the role of eosinophils in bronchiectasis (20).

Epithelial cells

Epithelial cells express intercellular adhesion molecule-1 (ICAM-1) because of inflammatory processes. ICAM-1 is crucial for recruitment and better adherence of inflammatory cells. Moreover, it is necessary for neutrophil-dependent pathogen elimination (34). Serum levels of ICAM-1 are elevated in patients with infected bronchiectasis respectively (35). Furthermore, it has been determined that amount of ICAM-1and TNF-α in sputum correlate (36).

Cystic fibrosis transmembrane conductance regulator (CFTR) gene

CFTR gene was assumed to contribute to development of diffuse bronchiectasis in patients with non-CF bronchiectasis. A trial investigated this circumstance with the aid of nasal

potential difference measurement. The results showed that one mutation in CFTR gene may be co-responsible for emergence of bronchiectasis (37).

Vitamin-D deficiency

Vitamin-D deficiency was examined in relation to its impact on patients with bronchiectasis. It was presumed that there is an association between Vitamin-D deficiency and airway infections. Patients with Vitamin-D deficiency were more often chronically colonized with bacteria, had more exacerbations and inflammatory markers in sputum were increased. In addition, lung function decreased more quickly for 3 years follow-up. *Pseudomonas aeruginosa* was the second most isolated bacteria.
Nevertheless, the mechanism between Vitamin-D deficiency and exacerbations remains unclear (38)

3.1.4 Clinical manifestations

Cardinal symptoms of bronchiectasis are chronic productive cough, thick mucopurulent greenish, yellowish sputum and dyspnea. Further symptoms include chronic rhinosinusitis, fatigue, hemoptysis or chest pain (39, 40).
On physical examination bi-basal crackles and wheezing of the lung are found mostly (8, 40). Moreover, investigation of the hands might show clubbing of the digits as a sign of impaired lung function (8).
Spirometry findings encompass mild to moderate airway obstruction but overlap of pulmonary function test results with additional conditions like COPD should be considered (8).

Acute exacerbation of bronchiectasis is often characterized by worsening of symptoms and changes of sputum presentation such as specks of pus and increased volume. Notwithstanding, it should always be considered that typical signs of infection could be lacking even during exacerbation (8).

3.1.5 Diagnosis

A chest radiograph, lung function tests and sputum bacteriological culture should be performed at baseline investigation in patients with bronchiectasis typical symptoms,

initially (41). Lung function tests comprise forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), lung volumes and diffusion capacity (41).
Nevertheless, because of higher sensitivity high-resolution computed tomography (HRCT) is imaging modality of choice as it is essential to acknowledge the diagnose (41, 42).

Chest X-ray

In general sensitivity of chest radiography for the diagnosis of bronchiectasis is low. Nonetheless due to its widespread availability and low radiation dose it remains an important first step in the diagnosis of any disease of the chest.
Typical signs of bronchiectasis in chest x-ray include "tram tracks" or "tram lines" (Fig. 6) (8, 42). The finger-in-glove sign is another X-ray finding possibly consistent with bronchiectasis but even other conditions (Fig. 7) (42-44).

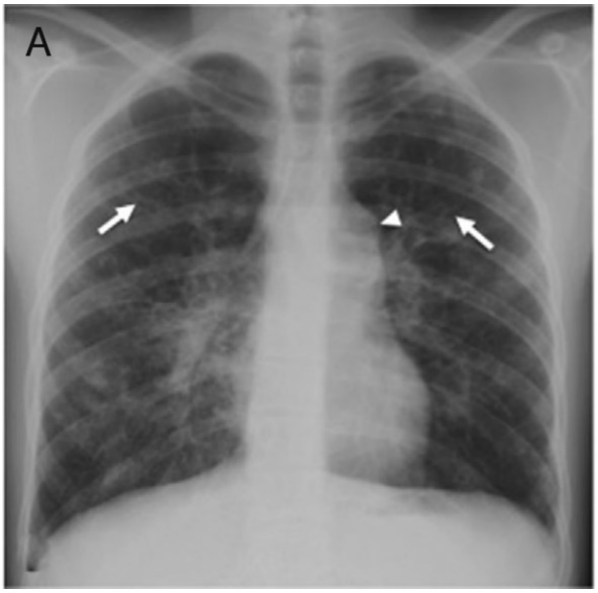

Figure 6 Tramlines in bronchiectasis (arrow). Bueno J, Flors L. The role of imaging in the diagnosis of bronchiectasis: the key is in the distribution. Radiologia. 2018;60(1):39-48

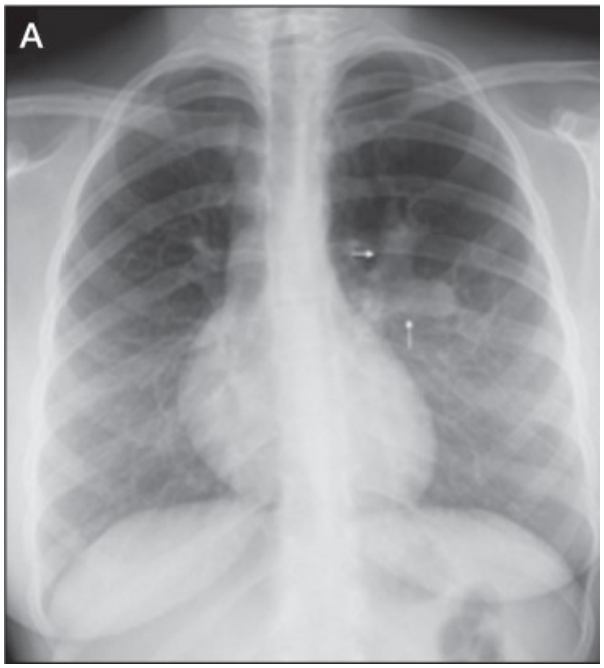

Figure 7 Finger-in-glove sign (arrows). Ariza Prota MA, Diez Jarilla JL, Prieto A, Pando Sandoval A, Casan P. Finger-in-glove sign in congenital bronchial atresia. Canadian respiratory journal. 2015;22(5):255.

HRCT

Imaging modality of choice is the high-resolution CT-Scan while volumetric CT-Scan has a higher sensitivity but also goes along with higher radiation doses (41).

A spiral CT with 1mm slices should be performed enable to spot pathologies in all parts of respiratory tract(40).

The “signet-ring sign” represents bronchiectasis in CT-scan. It is formed by the bronchus and its accompanied artery where the artery depicts the signet. That implies when bronchiectasis is suspected the bronchus will be viewed in relation to its artery (21, 42).

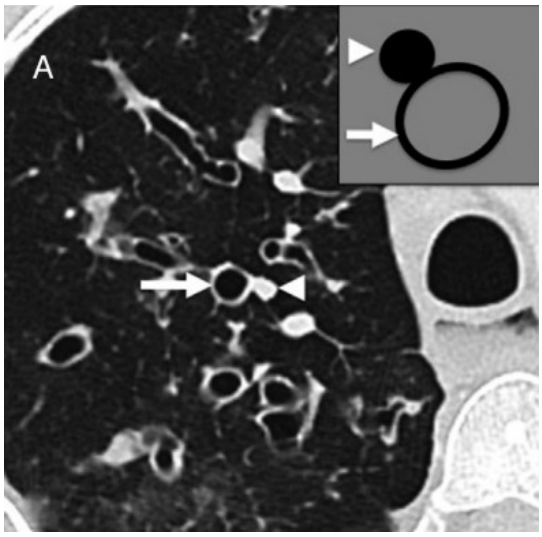

Figure 8 Signet-ring sign'. Bueno J, Flors L. The role of imaging in the diagnosis of bronchiectasis: the key is in the distribution. Radiologia. 2018;60(1):39-48.

Etiological tests

In recently diagnosed bronchiectasis The European Respiratory Society guidelines for the management of adult bronchiectasis recommends differential blood count, serum immunoglobulins (total IgG, IgA and IgM) and testing for allergic bronchopulmonary aspergillosis (ABPA) (16). Recommended ABPA screening tests involve determination of total IgE antibody level, specific IgG antibodies to *Aspergillus* and either positive immediate skin test to *Aspergillus* antigen or increased levels of IgE antibodies against *Aspergillus* (16).

Occasionally due to certain clinical features additional tests are justified. In patients with radiological or clinical signs like weight loss or hemoptysis consistent with nontuberculosis mycobacterium (NTM) infection, a single bronchoalveolar lavage or mycobacterial cultures of sputum on three successive days are appropriate (16).

If clinical signs for cystic fibrosis (CF) are present, investigation of sweat chloride, CFTR gene mutation analysis and other biomarkers of CFTR- mediated chloride ion transport are required to exclude CF (16).

Nasal nitric oxide, high-speed video analysis, transmission electron microscopy, immunofluorescence and/ or genetic analysis are necessary when there is suspicion for primary ciliary dyskinesia (16).

Alpha1- antitrypsin deficiency should be considered if there is evidence for basal emphysema or if airway obstruction appears early in life (16).

3.1.6 Treatment

Management of bronchiectasis is based on treating active infection and improving mucociliary clearance to reduce microbial pathogens and to prevent recurrent infection (8).

In acute exacerbations a sputum sample should be examined before administration of systemic antibiotics. Unless initial antibiotic therapy is not successful the microbiological test result from sputum is useful to spot sensitive antibiotics. Duration of treatment is suggested with14 days. Due to a paucity of studies comparing longer and shorter antibiotic treatment there is no evidence for this suggestion. Nonetheless shorter antibiotic administration may be promising for slight exacerbation, when patients are weak, if bacteria are highly sensitive to antibiotics or in case that the patient recovers fast.
Re-evaluation is appropriate if there is no enhancement of patient's condition after 14 days of therapy (16).

A long-term antibiotic treatment, as per guidelines 3 months or more, is suggested for bronchiectasis sickened people with a minimum of three or more exacerbations per year. Antibiotic therapy consists of an inhaled antibiotic and/or macrolide. Appropriate inhaled antibiotics are nebulised colistin or gentamicin (16). Nebulised aztreonam failed to convince in a Phase III study because of frequent side effects and no enhancement in quality of life (45). Suitable macrolides are azithromycin and erythromycin (16). As listed in table 2 other oral antibiotics are required under certain circumstances.

Table 2 and 3 summarize the recommendations respective administration of long-term inhaled antibiotics, macrolides or other antibiotics based on European Respiratory Society (ERS) guidelines for the management of adult bronchiectasis (16).

Administration of long-term inhaled antibiotics is justified under the following circumstances	**Quality of evidence (+++ high; ++ moderate; + low)**
Bronchiectasis plus chronic *P. aeruginosa* infection	+ +
Bronchiectasis without *P. aeruginosa* infection and antibiotic prophylaxis per os is 1. **Contraindicated or** 2. **Not tolerated or**	+

3. **Not effective**	

Table 2 Administration of long-term inhaled antibiotics is justified under the following circumstances

Administration of long-term macrolides or oral antibiotics is justified under the following circumstances	**Quality of evidence (+++ high; ++ moderate; + low)**
Bronchiectasis plus *P. aeruginosa* infection and inhaled antibiotic is 1. **Contraindicated or** 2. **Not tolerated or** 3. **Not practicable**	+
Bronchiectasis plus *P. aeruginosa* infection plus many exacerbations while taking an inhaled antibiotic: Macrolides supplementary or instead of inhaled antibiotics	+
Bronchiectasis not infected with *P. aeruginosa*	+ +
Bronchiectasis not infected with *P. aeruginosa* and macrolides are: **1. Contraindicated or** **2. Not tolerated or** **3. Not effective** **Administration of long-term oral antibiotics according to sensitivity and patient's acceptance**	+

Table 3 Administration of long-term macrolides or oral antibiotics in adults is justified under the following circumstances

As first line therapy in adults with bronchial infection by *Pseudomonas aeruginosa* the ongoing use of nebulized antibiotics is recommended by ERS guidelines. This recommendation is also supported by Araujo *et al.* (46) whose trial demonstrated that increasing mortality in chronic bronchial infection by *Pseudomonas aeruginosa* is linked to an annually number of exacerbations.

The use of macrolides to eradicate Pseudomonas aeruginosa belongs to second line therapy (16). Macrolide usage is associated with a grow of resistance rate and side effects especially

diarrhea (47-49). By contrast trials investigating nebulized colistin, dual release liposomal ciprofloxacin or gentamicin there was no increase in resistance reported (50-52). Given the increased risk of bronchospasm during inhaled antibiotic usage, a test dose under surveillance and combination with pre- and post- spirometry is recommended. Additionally, the prescription of a short-acting bronchodilator should be considered to obviate bronchospasm (16).

Prior to antibiotic treatment, sputum samples are required to define underlying microorganisms. Furthermore, an active NTM infection must be precluded in advance of a long-term macrolide monotherapy to prevent development of macrolide resistance in NTM. Despite the effectiveness of antibiotics general treatment modalities like airway clearance and treatment of actual etiology must be maximized (16).

Eradication therapy

Isolation of *Pseudomonas aeruginosa* in bronchiectasis is common. According to guidelines an eradication antibiotic treatment should be provided. As you can see in figure 3 there are three possible eradication treatment pathways for *Pseudomonas aeruginosa*. Nevertheless, none of these pathways can be backed up by evidence. Sputum sampling for *Pseudomonas aeruginosa* should be repeated after each step. If the bacterium is still traceable it is recommended to progress to the next step.

Continuous sputum controls are necessary in patients with new diagnosed *Pseudomonas aeruginosa* infection. In clinically stable patients a sputum sample is recommended at least once per year. Furthermore, eradication treatment must not be started if complete pathogen clearance is unlikely. (16)

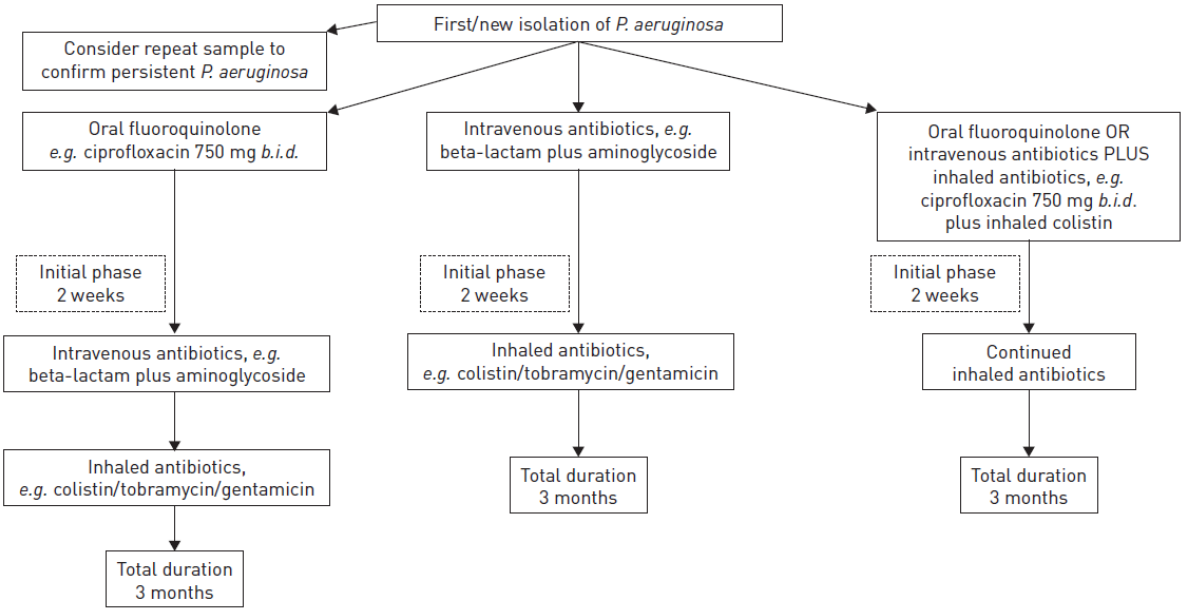

Figure 9 Three possible and alternative eradication treatment pathways based on what is commonly used in clinical practice, Polverino E et al. ERS guidelines for the management of adult bronchiectasis. The European respiratory journal. 2017;50(3).

Anti-inflammatory Therapy

Bronchiectasis come along with inflammation of the airways. Therefore, an anti-inflammatory therapy appears to be eligible. Based on a lack of benefit in several trials the ERS guidelines for the management of adult bronchiectasis do not recommend either inhaled corticosteroid or statins as treatment for bronchiectasis (16). There was no advantage reported for lung function in any of the included studies investigating inhaled fluticasone, inhaled budesonide and atorvastatin (53-55). A further study recently published examined the effect of atorvastatin in NCFB infected with *Pseudomonas aeruginosa* in a randomized controlled trial. There was no enhancement either in cough or in lung function reported. Administration of atorvastatin reduced systemic inflammation and advanced quality of life especially the activity domain. In addition, a total number of subjects with bacterial airway colonization decreased but mean bacterial load did not alter significantly (56). However, inhaled corticosteroids prescribed for additional conditions like Asthma or COPD should not be discontinued when bronchiectasis are diagnosed (8, 16).

Mucoactive treatment

Mucus hypersecretion is common in several lung diseases like COPD and CF. Normally mucus is removed by ciliated airway epithelium, but increased mucus production

overwhelms this clearance mechanism. Therefore, mucoactive drugs aim to ease expectoration of sputum and to reduce mucus hypersecretion. Mucoactive agents comprise expectorants, mucoregulators, mucolytics and mucokinetics (57).

Long-term mucoactive treatment including a minimum of three months is suggested to be offered to adults with bronchiectasis if profit of ordinary airway clearance is absent. Although trials dealing with mucoactive agents are not consistent, a prolonged time to first exacerbation and reasonable side effects were reported (16).

However, the use of recombinant human DNase in patients with bronchiectasis is not recommended. It has been revealed that recombinant human DNase does not decrease but increase the number of exacerbations when compared to placebo (58, 59).

Mucoactive treatment should be tailored to each patient while regarding the symptoms, lung function and desires. Furthermore, tolerance testing and bronchodilators as premedication should be considered. Overall, mucoactive treatment is not easy to handle and therefore may affect the compliance (16).

Long-acting bronchodilators

Long- acting bronchodilators might be valuable ahead of physiotherapy, inhaled antibiotics and – mucoactive drugs and are suggested under those circumstances. They enhance compliance and reduce possible bronchospasms caused by inhaled agents (16).

Long-acting bronchodilators should not be prescribed consistently but diagnosis of bronchiectasis should not affect their application in additional conditions like asthma or COPD prescription, too. Moreover, ERS guidelines for the management of adult bronchiectasis suggest introducing long-acting bronchodilators tailored to patients in case of considerable dyspnea (16).

Evidence to this suggestion is indirect and restricted and based on one trial (60).

Surgical interventions

Only patient population that comes into question for surgical intervention are patients with localized bronchiectasis and regular exacerbations. Purpose of surgery is to remove the diseased lung segment to break the vicious cycle (16).

A metanalysis including 38 trials with an amount of 5541 participants revealed a reasonable operative risk profile for patients with non-CF bronchiectasis with a surgical morbidity of 16.7% and mortality of 1.5%. 66.5% of subjects were free of symptoms postoperatively and

27.5% showed a significant improvement of symptoms. Nevertheless, 9.1% of subjects had to deal with the similar burden of symptoms as prior to resection or even worse. Additionally, proportion with symptomatic non-CF bronchiectasis showed an enhancement in quality of life subsequent to surgery (61).

An immunosuppressed status, *Pseudomonas aeruginosa* infection and the quantity of residual bronchiectasis are momentous and affect the outcome (62).

Despite the benefit of surgery, the complications should not be neglected. The most frequent complications were air leak more than seven days and atelectasis in 3.6% of patients. Further ramifications were hemorrhage, bronchopleural fistula, empyema, wound infection, cardiac arrhythmias and pneumonia (61).

Surgical intervention should be considered meticulously and an experienced surgeon plus an expert respiratory physician ought to be consulted (16).

Physiotherapy

An airway clearance technique (ACT) taught by a trained respiratory physiotherapist to ease expectorate sputum is suggested by ERS guidelines especially in adult patients with chronic productive cough and issues to expectorate sputum. This should be executed once or twice daily (16, 63). A pulmonal rehabilitation program is recommended for patients with bronchiectasis and vitiated exercise capacity (16).

3.2 Pseudomonas aeruginosa

3.2.1 General description/Amplification

Pseudomonas aeruginosa refers to a gram-negative monotrichous polar mastigoted bacterium. On its surface located pili are used for movement, to create a biofilm and stick to host cells. Based on pigment production of pyocyanin and pyoverdine it appears as greenish-bluish shiny gunmetal colonies in culture medium.

Pseudomonas aeruginosa has a remarkable adaptive capacity due to a marked genome. It is able to cause infection not only in human but also other species and can even breed in disinfectants.

The bacterium is often referred to as a “water bug” because its ability to exist in moist environments such as lavatories, showers or even flower vases. Outside of the hospital it can be found in soil, plants or waters. Additionally, colonization of nose, pharynx or gut is

possible without causing symptoms. *Pseudomonas aeruginosa* can grow aerobic as well as anerobic though the presence of nitrate as an electron acceptor is necessary (64).

3.2.2 Pseudomonas aeruginosa as pathogen

3.2.2.1 Epidemiology

Pseudomonas aeruginosa is one of the most common pathogen causing nosocomial pneumonia, wound infection and urinary tract infection (64).
In patients with bronchiectasis chronic bronchial infection by *Pseudomonas aeruginosa* differs in a wide range across European countries. Prevalence data extent from 0.9% in Serbia and 12.5% in United Kingdom to 21.2% in Spain. Prevalence in Greece and Israel is rather 40% (46). A retrospective study investigating a total of six cohorts from Argentina, Brazil and Chile established chronic bronchial colonization in 39.8% of subjects (65). Whereas, in china numbers of Pseudomonas aeruginosa detection in sputum from patients with bronchiectasis have been determined in a range from 21.5% to 73.5% (66).

3.2.2.2 Pathogenesis

Pseudomonas aeruginosa is an opportunistic pathogen. It can lead to emergence of severe acute infections which are mostly nosocomial. Almost all of them are linked to host immunodeficiency like neutropenia, severe burns, cystic fibrosis or further immunocompromising conditions (67).
Neutrophil granulocytes occupy a crucial role in cellular defense against *Pseudomonas aeruginosa*, thus neutropenia is a main risk factor related to infection (64).

Consequences of infection by *Pseudomonas aeruginosa* rely upon interaction of host defense and bacterial virulence factors. Major cells of host defense include epithelial cells and phagocytotic cells such as macrophages and neutrophils (67).

Epithelial cells

Epithelial cells line the whole respiratory tract whereas ciliated epithelial cells represent more than 50% of them. Thereby they create a physical barrier against invading pathogens during breathing and entail initiation of innate and adaptive immune system. Ciliated epithelial cells do remove particles by upwards ciliary movement (67). Other epithelial cells

indeed express pattern recognition receptors (PRRs), Toll-like receptors, retinoic acid inducible gene I (RIG-I), C-type lectins and inflammasome components (68). PRRs can recognize pathogens based on their pathogen-associated molecular pattern (PAMP) and trigger an inflammatory response (68).

Phagocytotic cells

Key feature in response to bronchial *Pseudomonas aeruginosa* infection is the presence of neutrophil granulocytes (67). Among others recruitment is controlled by chemoattractants such as interleukin 8 (20, 24).

Neutrophils destroy bacteria by molecules like reactive oxygen and nitrogen species and nonoxidative molecules like defensin antimicrobial peptides, lysozyme and neutrophil elastase but also cause tissue damage because of degranulation. Longevity and apoptosis of neutrophils confines the harm (67).

Similarly, macrophages claim an important role in bacterial lung infection by *Pseudomonas aeruginosa,* however hyper-activation can tarnish bacterial clearance and affects morbidity and mortality (69).

Macrophages serve as they phagocytose, sequester antigens, and secrete small amounts of cytokines and chemokines in the steady state (67).

Virulence factors of *Pseudomonas aeruginosa* isolated from acute infections differ from those obtained from chronic infections. Flagella and pili and a downregulation of virulence mechanisms like type 3 secretion system were found to be absent in bacteria from chronic infections. Moreover, they show increasing formation of biofilms and overexpression of the exopolysaccharide alginate (67).

Flagella and type 4 pili

Flagella and pili refer to filamentous appendages that occur as cytodendrites on *Pseudomonas aeruginosa*. The bacterium possesses a single polar flagellum and multiple pili on both poles. The flagellum contributes to motility through corkscrew motion. Furthermore, it is essential for bacterium adherence to host epithelial cells and thereby causing an inflammatory response.

Pili serve for twitching motility but also trigger formation of biofilms and lead to accumulation of the bacilli. With the aid of pili *Pseudomonas aeruginosa* can form

microcolonies, the bacteria can concentrate on one location and presumably shelter itself from the host immune system and antibiotics. An impairment of flagellum or pili function lessens the virulence (67).

Type 3 secretion system

Type 3 secretion system (T3SS) allows *Pseudomonas aeruginosa* to inject toxins into host cells with a needle like appendage. It is not only found in *Pseudomonas* but in many morbific gram negative bacteria. The T3SS harms the host cell membrane whereupon effector proteins such as ExoS, ExoT, ExU and ExoY were injected. ExoS and ExoU are of capital importance. ExoS disrupts the actin cytoskeletal organization while ExoU, likely 100 times more potent than ExoS, readily leads to cell necrosis. Overall, precise function of the toxins and their impact to pathogenesis remains obscure (64, 67).

Quorum sensing and biofilm

Quorum sensing (QS) refers to a mechanism that controls and regulates the number of bacteria based on environmental alteration. Many bacteria have QS in common. It affects the biofilm formation and probably governs around 10% of genes in the genome and over 20% of the expressed bacterial proteome (67).

Executive molecules in QS are autoinducers that assist specific transcriptional regulators as cofactors when the number of bacteria reaches a limit. The level of autoinducers rises and falls proportional to bacteria count. Once a specific amount of autoinducers is achieved an activation of certain downstream genes ensues which concerns the whole bacterial population (67).

Pseudomonas aeruginosa renders three different autoinducers which regulate cell survival, biofilm formation and virulence. Biofilms, especially the extracellular polymeric substance (EPS), builds up a physical and chemical strength against outside influences. Additionally, biofilms contain to a surface attached mutually linked bacteria. Their transcriptional profile and growth differ in biofilm. Last-named presumably occurs because of relative shortage of oxygen and lack of nutrition which further might explain the antibiotic resistance and therefore reflects a serious medical problem (67)

Proteases

To date known proteases secreted by *Pseudomonas aeruginosa* are Alkaline protease, the two elastases LasA and LasB and Protease IV. It is known that they occupy functions in eye infections and sepsis(67).

Alkaline protease destroys host complement proteins and host fibronectin and contributes to protection of *Pseudomanas aeruginosa* against immune detection through interaction with flagellin signaling (67).

LasA and LasB are controlled by the Ias quorum-sensing system. LasBs skills are more extensive than LasAs and it is assumed that LasA the promotes proteolytic function of LasB (67).

Proteas IV destroys complement proteins, immunoglobulins and fibrinogen which erosions in ocular infections. Furthermore, through destruction of surfactant protein A and D it supports Pseudomonas aeruginosa persistence during infection(67).

Lipopolysaccharide

Lipopolysaccharides build by *Pseudomonas aeruginosa* with the major impact to infections are Lipid A and O-polysaccharide. They are located on the outside of the outer membrane. Their function encompasses a contribution to antigenicity, inflammatory response, exclusion of external molecules and reciprocity with antibiotics. Two types of O-Polysaccharides, named A-band and B-band, exist. Because the B-band possesses higher antigenic quality its production is discontinued in many chronic *Pseudomonas aeruginosa* isolates to hide from host immune system (67).

Exotoxin A

Exotoxin A triggers apoptosis and therefore leads to cell death. Furthermore, it inhibits protein synthesis which also ends up in host cell death (67).

Lipases and phospholipases

They degrade surfactant by cleaving surfactant lipids and phospholipids and destroy erythrocytes through a specific hemolytic phospholipase (67)

Pyocyanin

This pigment responsible for *Pseudomonas aeruginosa* conspicuous bluish-greenish color leads to oxidative stress in host cells. Moreover, it interrupts host catalase and mitochondrial

electron transport. Pyocyanin pigment causes apoptosis in neutrophils and hinders removal of apoptotic material by macrophages. Furthermore, it restrains cilia function and secretion of interleukin 8 and RANTES by epithelial cells of the airway.
Phagocytotic cells express reactive oxygen and nitrogen species. Pyocyanin is assumed to be protective against them and therefore against phagocytosis (67).

Iron chelation

Siderophore and Pyoverdine tap host iron reservoir because of low free iron levels in host. An interplay between iron-bound pyoverdine and Pseudomonas aeruginosa causes an increase of exotoxin A production and of pyoverdine itself (67).

3.2.2.3 Clinical manifestation

Pseudomonas aeruginosa can afflict multiple regions of the body such as lungs, eyes and urinary tract. However, it has a potent predilection for the lungs (70).
Especially nosocomial pneumonia by *Pseudomonas aeruginosa* is associated with poor outcome. A study conducted in Rome revealed data with total intensive care unit mortality of 44.5% and overall hospital mortality of 47.3%. Aforesaid trial investigated health care associated pneumonia (HCAP), hospital acquired pneumonia (HAP) (in intensive care unit [ICU] and non-ICU patients) and ventilator associated pneumonia (VAP) (71).

Urogenital infections can also be triggered by *Pseudomonas aeruginosa* often related to catheters, obstructions or manipulation of the urogenital tract system (64).
Whirlpool dermatitis, nail infection and otitis externa known as swimmers ear belong to spectrum of ambulant infections. Contamination of contact lenses solution by *Pseudomonas aeruginosa* may cause ulcerative keratitis (64). Furthermore, *Pseudomonas aeruginosa* is a common bacterium in endocarditis due to intravenous drug abuse (72)

Those localized infections may serve as origin of sepsis. Once most patients with bacteremia were neutropenic or suffered from burn injury. Latter suffer less from bacteremia nowadays instead bacteremia is seen most frequent in patients in intensive care unit (70).
Initial symptom of bacteremia is fever. In advanced cases additional features involve shock and hypothermia. Distinguishing feature to other gram-negative sepsis are certain skin

lesions such as ecthyma gangrenosum. It primarily arises in patients with neutropenia or AIDS as basic condition and is highly indicating for *Pseudomonas aeruginosa* (70).

A recent trial found mortality rate subsequent to bacteremia caused by urinary tract infection of 14.5% with shock in 45.2% of the patients (73). Another study revealed data due to community-onset sepsis. Mortality was reported with 33.8% and 42.3% of cases presented septic shock (74).

3.2.2.4 Diagnosis

Pseudomonas aeruginosa is detectable by gram stain and on laboratory media such as blood and MacConkey agars. It generates a typical shiny gunmetal greenish-bluish color in culture medium where it is easy to cultivate. Moreover, it causes a characteristic lime blossom like smell and oxidase reaction is positive. The ability to grow at 42°C is not only denoting for pseudomonas aeruginosa but also a distinctive feature to other pseudomonas bacteria (64, 70). Additionally, verification of *Pseudomonas aeruginosa* through detection of *Pseudomonas aeruginosa* IgG antibodies by ELISA is clinically relevant (75).

3.2.2.5 Prevention

Objective in hospital must be to ensure a *Pseudomonas aeruginosa* free environment. Devices such as faucet water filters are auxiliary to keep lavatories clean from bacteria. Hand disinfection is inevitable to avoid transmission and spread infection (64).

In terms of prevention current research addresses vaccination against *Pseudomonas aeruginosa*. One trial investigated the protective effectiveness of a trivalent vaccine, $PcrV_{28-294}$-$OprI_{25-83}Hcp1_{1-162}$ (POH), in murine pneumonia and burn models. Results showed an increased protection in *Pseudomonas aeruginosa* lethal pneumonia and murine burn models when compared to single ingredients alone when formulated with Al $(OH)_3$ adjuvant. Furthermore, extensive protection against multiple clinical isolates of *Pseudomonas aeruginosa* have been reported. Administration of POH was followed by strong immune response. Accordingly, lower bacterial loads, reduced pathology, fewer inflammatory cytokine expression and decreased inflammatory cell infiltration were established (76). A further recently published trial examined agency of nasal PcrV adjuvanted with CpG oligodeoxynucleotide (CpG) compared with a nasal PcrV/aluminum hydroxide gel (alum) vaccine in seven groups of mice. Five groups were immunized with different vaccine components and two groups served as saline control. Anti-PcrV IgG, IgA and IgG isotype

titers have been determined after 50 days where PcrV-CpG vaccinated mice alone showed a significant rise in titers. Subsequently all groups were contaminated intratracheally with a fatal dose of *Pseudomonas aeruginosa.* Ultimately, highest survival rate of 73% was found by far among PcrV-CpG vaccinated group. Moreover, lung edema and further inflammation-related variables were less marked (77).

Vaccines with the objective to affect the flagella or pili and thereby prevent chronic infections were not beneficial yet (67).

4 Methods

4.1 Objectives

- To determine frequency of *Pseudomonas aeruginosa* colonization in NCFB patients in Styria.
- To ascertain whether immunodeficiencies or immunosuppressive therapies represent a risk factor respective *Pseudomonas aeruginosa* colonization in patients with NCFB
- To investigate etiology of NCFB in Styria

4.2 Study Design

We retrospectively revised files of 129 patients with NCFB who appeared between December 11, 2003 and January 9, 2019 at the Division of Pulmonology of the Department of Internal Medicine at University Hospital of Graz. Those patients had been either diagnosed with NCFB before their appearance at Medical University Hospital of Graz or they had been applied and diagnosed with NCFB during the mentioned period at Medical University of Graz. The Styrian database MEDOCS served for data analysis concerning our selected patients. All data evaluation and processing happened anonymously. Ethics committee at Medical University of Graz approved the purpose.

4.3 Data elicitation

We retrospectively reviewed medical records of 129 patients with diagnosed NCFB. Compiled data included gender, date of first appearance at pulmonary ambulance for NCFB at Medical University Hospital of Graz, immunodeficiency or immunosuppressive therapy, current colonization of the lungs and colonization prior to last 12 months including their pathogens and *Pseudomonas aeruginosa* antibodies.

4.4 Subjects

4.4.1 Inclusion criteria

All subjects are full-aged and were diagnosed with NCFB. All patients applied to the hospital of Medical university of Graz within the period between December 11, 2003 and January 9, 2019. Diagnosis of bronchiectasis had been made either before the defined period or during the mentioned period.

Location of the first diagnosis of bronchiectasis has not been set as an inclusion or exclusion criteria.

4.4.2 Exclusion criteria

All subjects with bronchiectasis due to cystic fibrosis were excluded in advance. During reevaluation of the underlying cause of bronchiectasis all subjects with suspicion of PCD (primary ciliary dyskinesia) were precluded as well. Furthermore, cases with questionable correlation between immunodeficiency and Bronchiectasis were excluded in certain statistics.

4.5 Diagnostic criteria of Bronchiectasis

We diagnosed bronchiectasis based on British Thoracic Society Guideline for bronchiectasis in adults BMJ 2018 as followed (78):

CT features of bronchiectasis

1. Bronchiectasis is defined by bronchial dilatation as suggested by one or more of the following:

- Bronchoarterial ratio >1 (internal airway lumen vs adjacent pulmonary artery)
- Lack of tapering
- Airway visibility within 1cm of costal pleural surface or touching mediastinal pleura.

2. The following indirect signs are commonly associated with bronchiectasis:

- Bronchial wall thickening
- Mucus impaction
- Mosaic perfusion/airtrapping on expiratory CT

4.6 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 27.

5 Results

Data from patients with NCFB who applied to the Division of Pulmonology at the Medical University Hospital of Graz between December 11, 2003 and January 9, 2019 yielded an amount of 129 subjects. Regarding to underlying causes of bronchiectasis, ten cases did not match with eligibility criteria and had to be excluded. Overall, 119 subjects remained. Those 119 patients underwent statistical analysis.

Because of eligibility criteria statistics about immunodeficiency or immunosuppressive therapy considered 117 of these 119 patients (Fig. 10).

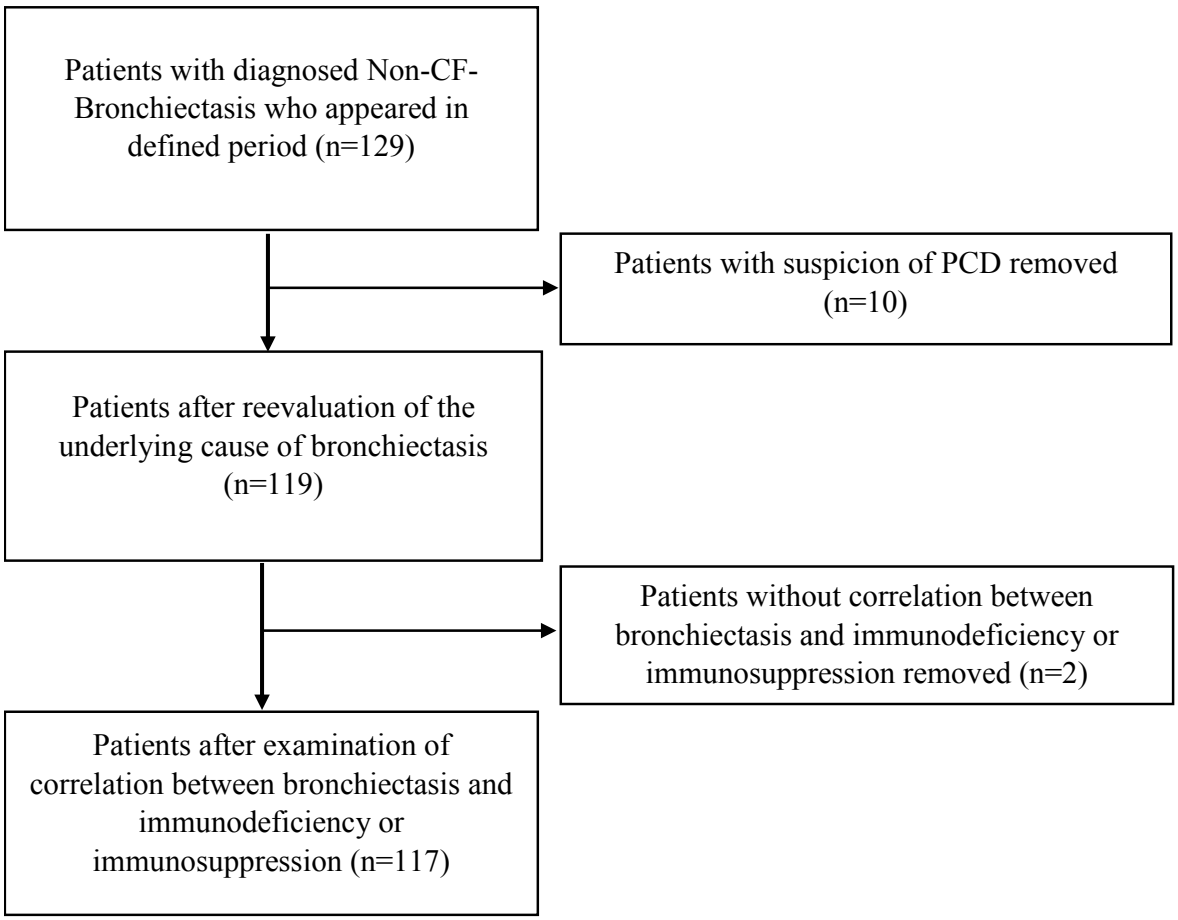

Figure 10 A flow chart showing the procedure for identifying the matching study participants

5.1 Study population

Overall, study population encompasses 119 participants with an amount of 63.0% female (n=75) and 37.0% male (n=44) persons (Table 4). Mean age is 64 years.

Unattached to sex, the most represented age group were the 76 to 85-year olds (22.7%) (Table 4, Figure 11). Based on gender, highest count of male participants was found between 66 to 75 (10.1%) years and most female participants were found with 16.0% among 56 to 65 years (Table 4).

The least existing patient group was found among age group 18-25 with 1.7% overall. All of them were male. Related to sex, this group also contained the lowest number of participants in total. 0.0% females and 1.7% males. (Table 4). This lowest count of male was additionally found between the 26-35-year old (Table 4).

			Sex		
			Male	Female	Total
Age	18-25	n	2	0	2
		n %	1.7%	0.0%	1.7%
	26-35	n	2	3	5
		n %	1.7%	2.5%	4.2%
	36-45	n	3	4	7
		n %	2.5%	3.4%	5.9%
	46-55	n	7	14	21
		n %	5.9%	11.8%	17.6%
	56-65	n	4	19	23
		n %	3.4%	16.0%	19.3%
	66-75	n	12	14	26
		n %	10.1%	11.8%	21.8%
	76-85	n	11	16	27
		n %	9.2%	13.4%	22.7%
	86-95	n	3	5	8
		n %	2.5%	4.2%	6.7%
	Total	n	44	75	119
		n %	37.0%	63.0%	100.0%

Table 4 Sex-Age Crosstable, n = number, n% = number in percent

As depicted in Figure 11 bronchiectasis occurred predominately between 46 and 85-year-old people in our study population. Other age groups represented a minor part.

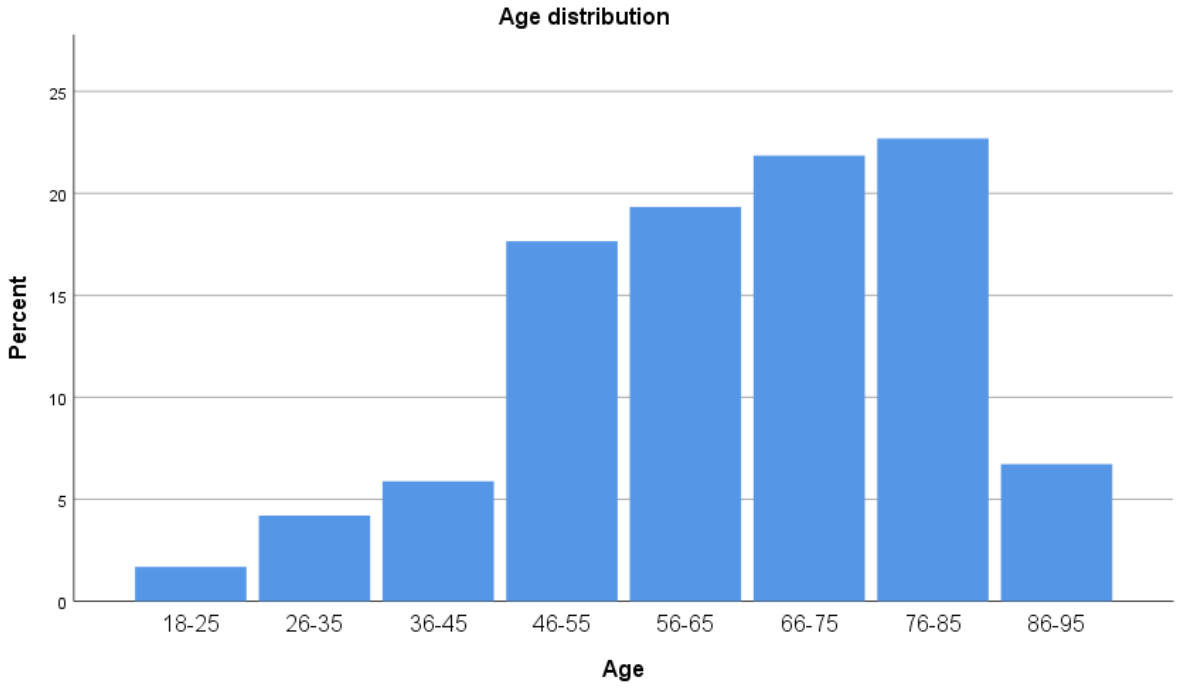

Figure 11 Age distribution among study population (n = 119). 18-25 = 1.7%; 26-35 = 4.2%; 36-45 = 5.9%; 46-55 = 17.6%; 56-65 = 19.3%; 66-75 = 21.8%; 76-85 = 22.7%; 86-95 = 6.7%

5.2 Etiology of Non-CF Bronchiectasis

		n	% of n = 119
Causes of NCFB	Postinfectious	21	17.6%
	Rheumatic diseases	20	16.8%
	COPD	17	14.3%
	Idiopathic	17	14.3%
	Asthma[#]	10	8.4%
	Other rare etiologies (single etiology < 1%)	7	5.9%
	ABPA	6	5.0%
	Primary immunoglobulin deficiency	6	5.0%
	Hematological disease without secondary immunoglobulin deficiency	4	3.4%
	ILD	4	3.4%
	Postradiotherapeutic	4	3.4%
	Hematological disease with secondary immunoglobulin deficiency	3	2.5%
	Total	119	100.0%

Table 5 Causes of NCFB; n: count; NCFB: Non-Cystic Fibrosis Bronchiectasis; COPD: Chronic obstructive pulmonary disease; [#]: Asthma without ABPA; ABPA: Allergic bronchopulmonary aspergillosis; ILD: Interstitial lung disease

Data have been reviewed in respect of etiology of NCFB (Table 5). To determine the underlying cause, we reevaluated every single case history unattached to its current diagnosis.

The ensuing sentences serve for better understanding of the above-mentioned table 5.

We summarized rheumatoid arthritis, sarcoidosis, systemic scleroderma, granulomatosis with polyangiitis, Sjögren syndrome, granulomatosis with polyangiitis, systemic lupus erythematosus and Takayasu's arteritis as rheumatic diseases.

The category “other rare etiologies” comprises congenital pulmonary airway malformation (CPAM), heterozygote CF mutation, Hunter syndrome, IgA nephropathy, Mounier-Kuhn syndrome, pneumoconiosis, and postsurgical NCFB. As primary immunoglobulin deficiencies we outlined common variable immunodeficiency (CVID), IgG-deficiency and Morbus Bruton. The hematological diseases without secondary immunoglobulin deficiency include bone marrow transplantation with consecutive graft versus host disease and B-cell chronic lymphocytic leukemia. Moreover, the hematological diseases with secondary immunoglobulin deficiency encompass immunoglobulin deficiency due to B-cell chronic lymphocytic leukemia, invasive aspergillosis based on immunoglobulin deficiency due to acute myeloid leukemia and chronic lymphocytic leukemia with subsequently occurred CVID.

Our results revealed NCFB subsequent to infection as the most frequent cause in 17.6% (21 cases) of our study population (Table 5).

16.8% of the cases showed NCFB due to rheumatic diseases. In this group rheumatoid arthritis was the most frequent cause with 25.0% (4.2% of n = 119). Second most underlying rheumatic disease was sarcoidosis with 20.0% (3.4% of n = 119), followed by systemic scleroderma (15.0%; 2.5% of n = 119), granulomatosis with polyangiitis (15.0%; 2.5% of n = 119) and Sjögren syndrome (10.0%; 1.7% of n = 119). Eosinophilic granulomatosis with polyangiitis, Systemic lupus erythematosus and Takayasu's arteritis appeared least often with each 5.0% (0.8% of n = 119).

The appearance of idiopathic NCFB and NCFB caused by COPD were equal with 14.3% (table 5). Furthermore, in 8.4% asthma was the most likely cause (table 5). Other rare etiologies occurred in 5.9% whereas congenital pulmonary airway malformation (CPAM), Heterozygote CF mutation, Hunter syndrome, IgA nephropathy, Mounier-Kuhn syndrome, Pneumoconiosis, and postoperative NCFB each had a share of 0.8% of the study population.

Moreover, ABPA and primary immunoglobulin deficiency appeared equally with 5.0%, respectively.

Among primary immunoglobulin deficiencies CVID was mostly found in 66.7% (3.4% of n = 119) while IgG-deficiency and Morbus Bruton only occurred once always (each 16.7%; 0.8% of n = 119).

In 3.4% of the cases each hematological disease without secondary immunoglobulin deficiency, ILD and post radiotherapeutic NCFB were proven (table 5).

Bone marrow transplantation with consecutive graft versus host disease (75.0%; 2.5% of n = 119) was the most common hematological disease without secondary immunoglobulin deficiency beside one case of B-cell chronic lymphocytic leukemia (25.0%; 0.8% of n = 119) in this subset.

Our analysis revealed hematological diseases with secondary immunoglobulin deficiency as the rarest cause of NCFB (table 5).

Each case of immunoglobulin deficiency due to B-cell chronic lymphocytic leukemia, invasive aspergillosis based on immunoglobulin deficiency due to acute myeloid leukemia and chronic lymphocytic leukemia with subsequently occurred CVID was represented once (each 33.3%; 0.8% of n = 119).

5.3 Pseudomonas aeruginosa colonization in NCFB

Sputum culture results were available in 71 cases. Pathogen, without clinical significance were excluded. We investigated the sputum culture results for 9 different pathogens remained. As shown in table 7, 100 pathogens were detectable overall. This implies that multiple pathogens were found in some patients, eventually.

In 39.4% no pathogens were found (Table 6). The most frequent proven pathogen was *Pseudomonas aeruginosa* with an amount of 22.0% of all pathogens detectable (9.0% male, 13.0% female) (Table 6). This is equal to 31.0% (12.7% male, 18.3% female) of the 71 patients.

		Sex								
		Male			Female			Total		
		n	% of Pathogens	% of Patients	n	% of Pathogens	% of Patients	n	% of Pathogens	% of Patients
Pathogens last 12 months	No pathogen	9	9.0%	12.7%	19	19.0%	26.8%	28	28.0%	39.4%
	Pseudomonas aeruginosa	9	9.0%	12.7%	13	13.0%	18.3%	22	22.0%	31.0%
	Hemophilus influenzae	7	7.0%	9.9%	8	8.0%	11.3%	15	15.0%	21.1%
	Staphylococcus aureus	5	5.0%	7.0%	9	9.0%	12.7%	14	14.0%	19.7%
	Klebsiella species	4	4.0%	5.6%	6	6.0%	8.5%	10	10.0%	14.1%
	Aspergillus fumigatus	1	1.0%	1.4%	5	5.0%	7.0%	6	6.0%	8.5%
	Stenotrophomonas - / Achromobacter species	1	1.0%	1.4%	1	1.0%	1.4%	2	2.0%	2.8%
	Burkholderia species	1	1.0%	1.4%	0	0.0%	0.0%	1	1.0%	1.4%
	Nocardia species	1	1.0%	1.4%	0	0.0%	0.0%	1	1.0%	1.4%
	Streptococcus pneumoniae	1	1.0%	1.4%	0	0.0%	0.0%	1	1.0%	1.4%

Table 6 Pathogens of the last 12 months – Sex Crosstabulation n: count; % of pathogens: amount of all 100 pathogens in percent, % of Patients: amount of all 71 cases in percent

Hemophilus influenza was detected in 21.1% of the patients (9.9% male, 11.3% female) with a proportion of 15.0% of all pathogens (Table 6). Furthermore, *Staphylococcus aureus* (14.0% of pathogens) was found in 19.7% of the sputum culture results (7.0% male, 12.7% female) (Table 6). 10.0% of the pathogens were *Klebsiella species* and 6.0% *Aspergillus fumigatus* (Table 6). These data correspond to 14.1% (5.6% male, 8.5% female) and 8.5% (1.4% male, 7.0% female) of the patients (Table 6). 2.0% of the pathogens were found as *Stenotrophomonas - / Achromobacter species* which is equal to 2.8% of 71 microbiological results (1.4% male, 1.4% female) (Table 6). *Burkholderia species*, *Nocardia species* and *Streptococcus pneumoniae,* each were detectable with an amount of 1.0% of the pathogens (Table 6). These are 1.4% (each 1.4% male and none female) of the patients, respectively (Table 6).

In total, more pathogens were found in women than in men (61.0% vs. 39.0%; Table 6).

Moreover, we evaluated the context between *Pseudomonas* antibodies and gender. As shown in table 7 in 36 cases, consisting of 30.6% men and 69.4% women, *Pseudomonas* antibodies data were available.

Our analysis revealed 72.2% negative and 27.8% positive antibody results (Table 7).

The negative findings contain 80.8% women and merely 19.2% men (Table 7). Among positive test results male patients were represented more frequently than female patients (60.0% vs. 40.0%) (Table 7).

Pseudomonas Antibodies - Sex Crosstabulation					
			Sex		Total
			Male	Female	
***Pseudomonas* Antibodies**	Negative	n (%)	5 (13.9%)	21 (58.3%)	26 (72.2%)
	Positive	n (%)	6 (16.7%)	4 (11.1%)	10 (27.8%)
Total		n (%)	11 (30.6%)	25 (69.4%)	36 (100%)

Table 7 Pseudomonas Antibodies - Sex Crosstabulation; n: count; %: amount of total 36 in percent

Overall, markedly more women showed negative *Pseudomonas* antibodies whereas more men were traceable among positive test results (Table 7).

Summarizing our previous results concerning *Pseudomonas aeruginosa* in 31.0%, which conforms 22 patients, sputum culture results stated *Pseudomonas aeruginosa* (Table 7). Additionally, *Pseudomonas* antibody test results were available in 30.1% of the cases or 36 patients of our original study population (n = 119).

In 25.2% (30 cases), both *Pseudomonas* antibodies and Sputum culture results regarding *Pseudomonas aeruginosa* were available (Table 8). Our analysis revealed 18 culture negative (60.0%) and 12 culture positive (40.0%) cases (Table 8). Among the 18 patients with negative *Pseudomonas* sputum culture 14 (77,7%) were negative for *Pseudomonas* antibodies while 4 (22,2%) were antibody positive (Table 8). Among the 12 patients with positive *Pseudomonas* sputum cultures 7 (58.3%) were negative for *Pseudomonas* antibodies and 5 (41.7%) patients were positive for *Pseudomonas* antibodies (Table 8).

If we assume that positive Sputum culture outcome means those patients are chronically colonized and those with negative results are not, we obtain a sensitivity of 41.7%, a specificity of 77.8%, a positive predictive value of 55.6% and a negative predictive value of 66.7% for *Pseudomonas aeruginosa* antibodies (Table 8).

Sputum culture Pseud. areug. - Pseudomonas Antibodies Crosstabulation					
			Pseudomonas Antibodies		Total
			Negative	Positive	
Sputum culture *P. aeruginosa*	Negative	n (%)	14 (77.8%)	4 (22.2%)	18 (100%)
	Positive	n (%)	7 (58.3%)	5 (41.7%)	12 (100%)
Total		n	21	9	30

Table 8 Sputum culture results of *Pseudomonas aeruginosa* – *Pseudomonas* antibodies Crosstabulation; n: count; %: percent of total 30

5.4 Immunodeficiency related to Pseudomonas aeruginosa

Due to eligibility criteria 117 patients of our primordial study population (n = 119) were included in these statistics.

Immunodeficiency - Sex Crosstabulation					
			Sex		Total
			Male	Female	
Immunodeficiency	No	n (%)	35	49	84 (71.8%)
	Yes	n (%)	8	25	33 (28.2%)
Total		n (%)	43 (36.8%)	74 (63.2%)	117 (100%)

Table 9 Immunodeficiency – Sex Crosstabulation, n: count; %: percent of total 117

As represented in table 9, from 117 patients 33 patients (28.2%) were immunodeficient. Out of 33 patients with immunodeficiency 25 (75.8%) were female. In contrast, in the non-immunodeficiency group of 84 patients, only 49 (58.3%) were female (Table 9).

Afterwards, we compared sputum culture results for *Pseudomonas aeruginosa* to immunodeficiency status of our study population (Table 10). Those data were available in 69 (59.0%) patients out of 117 (Table 10).

68.1% showed negative sputum culture results, while 31.9% were positive for *Pseudomonas aeruginosa* (Table 10). Among 47 patients with negative sputum culture 36 (76.6%) were not immunocompromised whereas 11 (23.4%) showed immunodeficiency (Table 10). Among 21 immunosuppressed patients 10 (47.6%) were *Pseudomonas aeruginosa* culture positive and in the non-immunocompromised group only 12 patients (25.0%) were found to be *Pseudomonas aeruginosa* positive (p-value 0.064) (Table 10).

Sputum culture Pseudomonas aeruginosa - Immunodeficiency Crosstabulation					
			Immunodeficiency		Total
			No	Yes	
Sputum culture *Pseudomonas aeruginosa*	Negative	n (%)	36 (75.0%)	11 (52.4%)	47 (68.1%)
	Positive	n (%)	12 (25.0%)	10 (47.6%)	22 (31.9%)
Total		n (%)	48 (100%)	21 (100%)	69 (100%)

Table 10 Sputum culture results of Pseudomonas aeruginosa – Immunodeficiency Crosstabulation; n: count; %: percent of total 69

Subsequently we compared the results of antibody testing for *Pseudomonas aeruginosa* to Immunodeficiency status.

Pseudomonas Antibodies - Immunodeficiency Crosstabulation					
			Immunodeficiency		Total
			No	Yes	
***Pseudomonas* Antibodies**	Negative	n (%)	18 (69.2%)	8 (80.0%)	26 (72.2%)
	Positive	n (%)	8 (30.8%)	2 (20.0%)	10 (27.8%)
Total		n (%)	26 (100%)	10 (100%)	36 (100%)

Table 11 Pseudomonas Antibodies – Immunodeficiency Crosstabulation, n: count; %: percent of total 36

In 36 cases (30.8%) data of both immunodeficiency status and *Pseudomonas* antibodies were available (Table 11).

Among the 26 antibody negative cases 18 (69.2%) were not immunocompromised compared to 8 (30.8%) immunodeficient cases (Table 11). Among the 10 Patients with detected *Pseudomonas* antibodies revealed 8 (80.0%) with no immunodeficiency whereas 2 (20.0%) had a weakened immune system (Table 11).

In other words, out of 10 immunosuppressed patients only 2 (20.0%) were *Pseudomonas aeruginosa antibody* positive, and out of 26 non-immunocompromised patients 8 (30.8%) were found to be *Pseudomonas aeruginosa* antibody positive (Table 11).

6 Discussion

Bronchiectasis refer to a chronic lung disease with irreversible widening of the bronchi. Main symptoms include productive cough and in some cases enormous sputum production (8).

Since epidemiological data of NCFB in Austria are missing yet, we investigated a population of 119 patients from the Medical University of Graz, to answer this question. Furthermore, we considered the correlation between immunodeficiency or immunosuppressive therapy and *Pseudomonas aeruginosa* in NCFB.

Just as described in literature our analyses also revealed an increased occurrence of NCFB with rising age with most cases in persons between 46 to 85 years (5, 7, 9, 11, 12). In our cohort 88.2% were older than 45 years (Table 4 and Figure 11).

The reasons for more frequent NCFB with rising age are not clear. It might be explainable with improved diagnostic capabilities and the constant use of CT-Scan as part of the diagnostic procedure of a lung disease. Another reason could be a rising case acquisition with expanded investigation of older people (5). Moreover, an increase of comorbidities in elderly people could lead to higher consultation rates and increasing recognition of bronchiectasis (12).

Altogether women were the predominant gender in our study population (63.0%, see Table 4). The reported results overlap with findings from the United States, United Kingdom, South Korea and many other European countries (5, 6, 12, 18, 46). In our trial only the very small subgroup of 18 to 25-year-old patients contained more men than women (2 versus 0; Table 4).

Gender distinctions are known in several airway diseases and in NCFB they are not only limited to prevalence. According to Vidaillac *et al.* males with NCFB are still more frequent in older age than women (79). Nevertheless, some studies show female predominance even among the elderly (6, 12). Altogether, data respective male/female ratio vary.

In the subgroup of immunodeficient patients' women were even more frequent as already in the whole cohort (75.8% in the immunodeficient subgroup versus 63.0% in the whole cohort

(see Table 9 and Table 4). So female sex seems to be an important risk factor to develop clinically relevant NCFB.

Based on rigorous evaluation of the patient's files we were able to ascertain an underlying etiology in nearly 85.7% of the cases (Table 5). Hence, 14.3% has been found as idiopathic bronchiectasis what is comparatively low to other studies. Gao *et al.* reported about 44.8% of NCFB were no cause could be identified in a review of 56 trials. Within the 35 included European articles in the study no reason for bronchiectasis could be spotted in 41.1% (17). Generally, the term `idiopathic bronchiectasis´ should be well considered since it is not recognizable whether those data maybe just occur because of an underlying lack of examination.

Among known etiologies postinfectious were the most frequent ones with 17.6% (Table 5). The results match with Gao *et al.* as a major etiology of NCFB. In South America and Africa postinfectious bronchiectasis are the leading cause, which can be explained by higher rates of post-tuberculosis bronchiectatic lung diseases (17).
COPD associated with NCFB entails high mortality rates (3). It is considered the main comorbidity of NCFB in Germany (COPD 41.4% and Asthma 32.8%), as lately published by Diel *et al*. (19). Interestingly, our data yielded much lower numbers for COPD (14.3%) as well as for Asthma or ABPA (8.4% and 5.0%) (Table 5). Considering the high smoking rates in Austria (80), 14.3% NCFB caused by COPD seems to be low. This discrepancy might be explained by relevant reporting bias in the study by Diel *et al.*, as they used less well evaluated data from German public statutory health insurances. Furthermore, many NCFB etiologies are frequently misclassified as COPD if only the FEV1/FVC criteria is used to classify COPD.
Notwithstanding our results from Graz only give tendencies due to minor sample size and the fact, that the analyzed cohort were recruited from a specialized outpatient clinic of a university clinic. Thus, more comprehensive studies regarding etiology of NCFB in Austria are needed.

Colonization with *Pseudomonas aeruginosa* is well known in NCFB patients (46). It is established that *Pseudomonas aeruginosa* colonization with two or more exacerbations per year, lead to higher mortality rates in NCFB (46). As expected, our data revealed *Pseudomonas aeruginosa* as the most occurring pathogen and a colonization was detected

in 31.0% of the patients (Table 6). In comparison, the US Bronchiectasis Research Registry found *Pseudomonas species* in 33% of the patients (18). Data from 10 different European bronchiectasis clinical centers including Israel were investigated by Araujo *et al.* Results showed a range of *Pseudomonas aeruginosa* colonization from the lowest in Serbia 0.9% to the highest in Israel 44.3% (46). Most of the *Pseudomonas aeruginosa* culture positive patients were female (13 out of 22; 59.1%, see Table 6), which correlates with the female dominance in the whole cohort (75 females versus 44 males, see Table 4).
With regards to immunosuppression, *Pseudomonas aeruginosa* culture positive results were more frequently found in immunocompromised (47.6%) than in non-immunocompromised patients (25.0%). Due to the small sample size, the difference was not statistically significant (p-value 0.064, see Table 10), but a trend is obvious. Therefore, an immunocompromised state should be considered as a relevant risk factor for *Pseudomonas aeruginosa* colonization and a larger sample size is needed to proof the significance.

In cystic fibrosis patient's measurement of antibodies against *Pseudomonas aeruginosa* can help to differ between intermittent and chronic *Pseudomonas aeruginosa* infection (81). Thus, a progress to chronic *Pseudomonas aeruginosa* infection and decline of lung function can be prevented by appropriate antibiotic therapy (82). However, as a previous study has shown the anti-*Pseudomonas aeruginosa* IgG test should always be used in conjunction with sputum culture results to confirm *Pseudomonas aeruginosa* colonisation. A cross-reactivity with *H. influenzae* may also lead to false positive test results (83).
In our study *Pseudomonas* antibody positivity could be explored only in a limited number of patients with results for both *Pseudomonas aeruginosa* antibodies and Sputum culture for *Pseudomonas aeruginosa* (30 out auf 119; Table 8).
We expected that patients with positive *Pseudomonas* antibodies would show more frequent positive Sputum cultures for *Pseudomonas aeruginosa* and as well negative antibodies would result in negative sputum cultures. Nevertheless, the results were different.
Pseudomonas aeruginosa sputum culture results were weakly or only moderately correlated with *Pseudomonas aeruginosa* antibody results. In culture positive patients only 41.7% were antibody positive (sensitivity) and in culture negative patients 77.8% were antibody negative (specificity) (Table 8). The data suggest a positive predictive value of 55.6% and a negative predictive value of 66.7% (Table 8). Therefore, *Pseudomonas* antibody test results are not literally valid. Nevertheless, our small data size does not permit a suitable statement on this topic.

In immunocompromised patients the sensitivity and specificity of *Pseudomonas aeruginosa* antibody results seemed to be even more limited, as the very low rate of positive antibody results (20.0%, see Table 11) do not correlate with the clearly elevated rate for positive *Pseudomonas aeruginosa* culture (47.6%, see Table 10) in immunocompromised patients. Here again, a larger representative sample size is needed for precise conclusions.

In conclusion, in this Styrian NCFB cohort older age and female gender appeared as relevant risk factors. Frequent causes of NCFB were preceding infections, rheumatic diseases, COPD, Asthma or idiopathic NCFB, and more than a quarter were immunocompromised patients. Positive *Pseudomonas aeruginosa* culture results were found in 31.0% and in immunocompromised patients in 47.6%. The diagnostic role of *Pseudomonas aeruginosa* antibody detection must be clarified in further studies with lager sample sizes.

7 References

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