DOI:10.14753/SE.2019.2224

Bronchiectasis bacterial etiology and the role of pleural c-reactive protein in prediction of parapneumonic effusion

PhD thesis

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Budapest
2019

DOI:10.14753/SE.2019.2224

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List abbreviation

ABPA	Allergic bronchopulmonary aspergillosis
AFB	Acid-fast bacilli
AUC	Area under the curve
ATT	Alpha 1 antitrypsin deficiency
BAL	Bronchoalveolar lavage
BAT	Bronchiectasis and long-term azithromycin treatment
BLESS	Bronchiectasis and Low-Dose Erythromycin Study
BTS	British thoracic society
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CGD	Chronic granulomatous disease
CHF	Congestive heart failure
CRP	C-reactive protein
CVID	Common variable immune deficiency
EMBRACE	Effectiveness of macrolides in patients with bronchiectasis using azithroymycin to control exacerbations
ELOS	Endotoxin lipo-oligosaccharides
FEV1	Forced expiratory volume in 1 second
HRCT	High resolution computed tomography scans
HRQOL	Health-Related Quality of Life
IV	Intravenous
LDH	Lactate dehydrogenase
LLL	Left lower lobe
LUL	Left upper lobe
NCFBr	Non-cystic fibrosis bronchiectasis
NPV	Negative predictive value
NTHi	Non-typeable H. influenzae
NTM	Non toberculous mycobacterium
PPV	Positive predictive value
RLL	Right lower lobe
RMC	Rabin medical center
RML	Right middle lobe
ROC	Receiver operator characteristics
RUL	Right upper lobe
STREM-1	Soluble triggering receptor expressed on myeloid cells-1
XLA	X linked agammaglobulinemia
YNS	Yellow nail syndrome

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1. Introduction

1.1 The medical history of bronchiectasis

Bronchiectasis was first described by Laennec (1) in 1819 as part of a wider work describing the use of his novel invention, the stethoscope. A century later, in 1919, A. Jex-Blake delivered a lecture at the Hospital for Consumption (London. UK) on the conditions which causes bronchiectasis (2). In this pre-antibiotic era a third of patients who were identified as having bronchiectasis were secondary to an episode of pneumonia or pleurisy, a third due to chronic bronchitis and a further third due to bronchial obstruction, the majority of which were malignant tumors.

1.2 Epidemiology

Recent data on bronchiectasis epidemiology has been collected from cohorts in Finland, New Zealand and the USA (3–5). The data from Finland suggested an incidence of 2.7 per 100,000 people, while in New Zealand an overall incidence in children of 3.7 per 100,000 was noted but showed wide variations with regards ethnicity. For example, children from a Pacific Island descent had an incidence of 17.8 per 100,000 compared with an incident of 1.5 per 100,000 for those of a Northern European descent. Unsurprisingly, given the often-chronic nature of its development, the prevalence of bronchiectasis and hospital admission related to bronchiectasis increased with age. Studies from the USA estimate a prevalence of 4.2 per 100,000 people in those aged 18–34 years, increasing to 271.8 per 100,000 in people aged >75 years (5).

1.3 Etiology

Non-cystic fibrosis bronchiectasis (NCFBr) is characterized by irreversibly damaged and dilated bronchi with impaired muco-ciliary clearance leading to recurrent bacterial infection. Inflammation within the bronchial wall can be the result of an infection within the airway, inhalation of injurious agents or an endogenous condition such as an autoimmune disease (Table 1).

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There are several causes of bronchiectasis:

Table 1- Etiologies of bronchiectasis (9-40).

Structural lung conditions
Williams-Campbell syndrome
Mounier Kuhn syndrome
Ehler Danlos syndrome
Toxic damage to airways
Inhalation injury
Aspiration secondary to neuromascular disease
GERD
Obstruction of single bronchus
Tumor
Foreign body
Defects of mucociliary clearance
Ciliary dyskinesia (primary, secondary)
Channelopathies (CFTR dysfunction, ENaC dysfunction)
ABPA (Allergic bronchopulmonary aspergillosis)
Immunodeficiency
Common variable immune deficiency (CVID)
X-linked agammaglobulinaemia (XLA)
Chronic granulomatous disease (CGD)
Antibody deficiency with normal Ig
Secondary immunodeficiency (Hematological malignancy, post bone marrow transplantation, immunosuppressive drug)
Infections
Childhood infections
Tuberculosis
Pneumonia
Measles
Whooping cough
Nontuberculous mycobacteria
Bronchiectasis in systemic disease
Inflammatory bowel disease
Connective tissue disease
Yellow nail syndrome
Idiopathic bronchiectasis

1.3.1 Abnormal structural lung conditions

Congenital disorders affecting the structure of the bronchial tree can lead to bronchiectasis through a direct effect on the bronchial wall.

Williams–Campbell syndrome was first described in 1960 after the case reports of five children were studied by Williams and Campbell (6). Histological examination of the bronchial wall revealed a deficiency or absence of cartilage, mostly from the third division of the bronchi down.

Mounier–Kuhn syndrome (tracheobronchomegaly) is characterized by dilatation of the trachea and large bronchi, usually presented in young adults with central lobes bronchiectatic disease (Table 2). Its underlying pathology is not clearly understood but histological examination has shown atrophy of airway cartilage and smooth muscle.

1.3.2 Obstructive airways disease

Asthma and COPD conditions would lead to bronchiectasis as both have clearly been shown to cause airway inflammation and structural blockage of airways, either through bronchospasm or fixed airways obstruction, in the case of COPD.

1.3.2.1 Asthma

A number of studies have highlighted the presence of airway remodeling in chronic asthma patients using high-resolution CT (HRCT) scanning techniques. The airway remodelling can vary from mild airway wall thickening to blatant bronchiectasis. Bronchial wall thickening has been found in up to 82% of asthmatic patients in a cohort (7) and in patients with mild asthma (8). As bronchial wall thickening is indicative of airway inflammation this suggests that a significant number of patients with asthma are at risk of developing bronchiectasis.

The prevalence of bronchiectasis in these studies is estimated at 17.5–40% (8). In the largest of these studies, which comprised of 463 patients with severe asthma, 40% of patients were shown to have evidence of bronchiectasis on HRCT scans (7-8). However, study participants were selected for HRCT on the basis of clinical indication, the most common being a suspicion of bronchiectasis.

These studies suggest that bronchiectasis is associated with a more severe obstruction and is more apparent in patients who present with a longer history of asthma symptoms, consequently a subgroup of severe asthma patients appear to be at risk of developing bronchiectasis (7-8).

1.3.2.2 COPD

COPD and bronchiectasis share common symptoms of cough with sputum production and susceptibility to recurrent exacerbations driven by new or persistent infection. COPD is diagnosed on the basis of poorly reversible airflow obstruction and is therefore a physiological diagnosis. While bronchiectasis is diagnosed in the presence of airway dilatation and airway wall thickening on imaging (usually computed tomography (CT)), and is therefore a structural diagnosis. Physiological criteria for the diagnosis of COPD and structural criteria for the diagnosis of bronchiectasis create the possibility for individual patients to fulfil both, resulting conceptually in either co-diagnosis or an overlap syndrome between the two conditions. A study of moderate-to-severe COPD patients demonstrated the prevalence of bronchiectasis to be up to 50% (9).

1.3.2.3 α1-antitrypsin deficiency (ATT)

α1-antitrypsin deficiency is classically associated with predominantly lower lobe emphysema. Bronchiectasis has also been associated with the enzyme deficiency, whether this is a direct consequence of the deficiency or secondary to the emphysema-associated airways obstruction is less clear. In a study of patients with severe AAT deficiency the vast majority of subjects had some evidence of bronchiectasis on a HRCT scan (70 out of 74 subjects), with 27% having clinically significant bronchiectasis with a correlation between forced expiratory volume in 1 second (FEV1) and bronchial wall thickness (10).

1.3.3 Defects of mucociliary clearance

Abnormalities of cilia structure and/or motility cause a decreased mucus clearance from the lung. These abnormalities can be due to:

A) Primary ciliary dyskinesia (PCD)- is a genetically heterogenous disorder with mutation in genes (DNAI1 and DNAH5) which code for proteins responsible for assembly of outer dynein arms.

As cilia are present throughout the body, patients with PCD will often present with multiple symptoms such as sinusitis, recurrent otitis media, infertility and defects of organ lateralisation with situs inversus or situs ambiguus. The triad of bronchiectasis, chronic sinusitis and situs inversus is also known as Kartagener's syndrome.

B) Secondary ciliary dyskinesia- A number of noxious agents, both organic and inorganic, have been shown to affect the function of cilia in human airway epithelia. Certain

bacteria, such as *Pseudomonas aeruginosa* and *Haemophilus influenzae*, have been shown to disable mucociliary clearance by releasing products that inhibit ciliary beat frequency, allowing them to persist and propagate infection (11-12). Inhaled inorganic substances such as diesel particles (13) and cigarette smoke (14) have also been shown to have a direct effect on ciliary function, inhibiting ciliary beat frequency. It is important to note here that no causal role for tobacco smoking and the development of bronchiectasis has been made, indeed outside of COPD bronchiectasis appears to be a disease of the nonsmoker.

Aspiration of gastric contents is a well-recognized, but perhaps under diagnosed, cause of bronchiectasis. While aspiration of both acid and nonacid stomach contents leads to direct inflammation of the bronchial wall, ciliary function may also be affected by these agents.

1.3.4 Channelopathies

Defects in the ion channels of the epithelial layer can lead to dehydration of the airway surfaces, thereby affecting the depth of the periciliary layer and bringing the cilia into contact with the viscous mucus layer, further impeding its function. The most widely recognized of these defects is that found in CF. Here, the loss of a chloride channel known as the CF transmembrane regulator (CFTR) protein leads to the inability of the epithelial cells to excrete chloride.

1.3.5 Allergic bronchopulmonary aspergillosis (ABPA)

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary condition caused by a hypersensitivity reaction to the ubiquitous environmental fungus Aspergillus fumigatus. It is most commonly seen in patients with pre-existing asthma or CF and is clinically characterized by recurrent wheeze, pulmonary infiltrates and the development of bronchiectasis. The hypersensitivity reaction has mixed features of immediate hypersensitivity (type I), antigen–antibody complexes (type III) and inflammatory cell responses (type IV) (15). The inflammatory cell response seen in ABPA shows a predominance of T-helper cell type 2 (Th2) cells leading to a release of cytokines mediating allergic inflammation (as opposed to the Th1, cytotoxic pathway) (16). The type I hypersensitivity reaction causes local degranulation of mast cells and histamine release leading to bronchoconstriction. The combination of airway inflammation, which leads to viscous, eosinophil-laden mucus, plugging and airway obstruction and

bronchospasm leads to a reduction in mucociliary clearance and the development of bronchiectasis. As such bronchiectasis in ABPA is common. In three large case studies it was found that central bronchiectasis was present in 69–76% of patients with ABPA (17-19).

1.3.6 Immunodeficiency

1.3.6.1 Primary Immunodeficiency

Defects in the immune system leave the lungs vulnerable to infection and in some cases the development of bronchiectasis can be the first indication of immunodeficiency. The most common forms of primary immune deficiencies observed in patients with bronchiectasis are common variable immune deficiency (CVID), X-linked agammaglobulinaemia (XLA) and chronic granulomatous disease (CGD).

Common variable immune deficiency (CVID)- is characterized by reduced levels of immunoglobulins (Igs) with associated recurrent bacterial infections. An increased risk of autoimmune conditions and malignancy has also been identified. The majority of patients present with recurrent pulmonary infections at a mean age 29 years (20). CVID is the most common primary immune deficiency to cause bronchiectasis. A case series undertaken in a UK population identified 68% of the patients with CVID as having evidence of bronchiectasis (21)

X-linked agammaglobulinaemia (XLA)- is caused by a mutation of a tyrosine kinase gene that is involved in the development of B- lymphocytes, leading to an absence of circulating B-lymphocytes and the absence of Igs. Given the severity of the immune deficiency it usually presents much earlier than CVID, usually being diagnosed in early childhood (22). Despite treatment with replacement Igs, chronic lung disease can still develop with the risk of developing bronchiectasis increasing with age (23)

Chronic granulomatous disease (CGD)- is a group of disorders characterised by a loss of phagocytic NADPH oxidase, without which phagocytes are unable to produce the reactive oxygen species required to kill ingested bacteria. Infections are mainly due to *Staphylococcus aureus, Serratia marcescens, Salmonella sp., Klebsiella sp.* and *Burkholderia cepacia.*

Antibody deficiency with normal Igs- In a study of patients with bronchiectasis and normal IgG levels, 11% were shown to have specific antibody production deficiencies with an inability to respond to pneumococcal and *H. influenzae* vaccines (24).

1.3.6.2 Secondary Immunodeficiency

The development of bronchiectasis in HIV-infected patients has been noted in a number of case series. While recurrent pulmonary infection is likely to be the major factor in the development of bronchiectasis in these patients, the development of lymphocytic interstitial pneumonia may also be implicated (25).

1.3.7 Infections

A number of childhood respiratory infections have been implicated in the pathogenesis of bronchiectasis. The most widely recognized infectious causes of bronchiectasis are measles and pertussis infection in the West (26), with tuberculosis being a major cause elsewhere. Globally, *Mycobacterium tuberculosis* infection remains a major cause of morbidity and mortality and a significant cause of bronchiectasis. In developed countries with screening programs and adequate access to treatment, the incidence of new infections remains low. However, the incidence of nontuberculous mycobacterial (NTM) pulmonary infections is increasing. These mycobacteria vary in pathogenecity with *Mycobacterium avium* complex (MAC) being the most pathogenic whilst other organisms, such as *Mycobacterium gordonae* and *Mycobacterium abscessus*, act as opportunistic pathogens and are only found in patients with underlying lung diseases.

The second of these clinical forms is also known as ‘‘Lady Windermere syndrome’’, and was first described in 1992 in a case series of 29 predominately elderly, female patients (27). The patients had MAC infection with bronchiectasis predominantly affecting the middle lobe and lingula. The authors postulated that persistent voluntary cough suppression could lead to chronic inflammatory processes in these poorly draining lung regions which are susceptible to MAC infection (27).

1.3.8 Bronchiectasis in systemic diseases

1.3.8.1 Inflammatory bowel disease

The development of bronchiectasis in patients with ulcerative colitis is well recognized phenomenon and the subject of a number of case series (28). Classically, bronchiectasis develops after resection of the large bowel, suggesting a common immune system response that becomes concentrated on the bronchial wall after the bowel is removed. The common embryonic origin and similar structures of bowel and bronchial wall (columnar epithelial and submucosal glands) add weight to this theory. The link between Crohn’s

disease and bronchiectasis is less clear with only a small number of case reports detailing their coexistence (29), perhaps too few to determine a definite association.

1.3.8.2 Connective tissue disease

A number of connective tissue diseases have been noted to be associated with bronchiectasis, largely based on case series reviews of small numbers of patients. The clearest association is that between rheumatoid arthritis and bronchiectasis (30). Associations between bronchiectasis and Sjogren's syndrome (31), systemic sclerosis (32), systemic lupus erythematosus (33), ankylosing spondylitis (34-35) and relapsing polychondritis (36) have all been made in small case series reviews.

1.3.8.3 Yellow nail syndrome (YNS)

YNS is a rare syndrome that was first described in 1964 by Samman and White (37) and is characterized by bronchiectasis, lymphoedema and a characteristic appearance of the nails. The underlying pathological defect is not clear, although a recent study revealing an association with chronic rhinosinusitis suggests a possible defect in an inflammatory pathway or mucociliary clearance rather than a structural defect within the lung itself (38).

1.3.9 Idiopathic bronchiectasis

In two large studies (39-40), which identified the cause of bronchiectasis in adults, a significant proportion of patients (26% and 53%, respectively) were found to have no identifiable cause and were labeled as having idiopathic bronchiectasis, the majority of whom were found to be female and nonsmokers. As all the patients studied had undergone rigorous clinical testing and their history had been reported, leading to the exclusion of all known causes, including genetic disorders, it is unlikely under recognition of known causes of bronchiectasis could have occurred.

Finally, another etiology of bronchiectasis is traction bronchiectasis. It is distortion of the airways secondary to mechanical traction on the bronchi from fibrosis of the surrounding lung parenchyma. Although the airways may become dilated in this situation, the other manifestations of bronchiectasis are lacking such inflammation-infection cycle. Traction bronchiectasis tends to have an upper lobe distribution in cases of radiation fibrosis and sarcoidosis, while the lower lobe is predominantly involved in cases of interstitial lung disease/ idiopathic pulmonary fibrosis (ILD/IPF).

Table 2- Bronchiectasis lobar distribution and associated diseases (17,22,27).

Location	**Disease**
Focal	
	Congenital bronchial atresia
	Foreign body
	Broncholithiasis
	Endobronchial neoplasm
Diffuse	
Upper lung	
	Cystic fibrosis
	Sarcoidosis
	Progressive massive fibrosis of pneumoconiosis
	Radiation fibrosis
Central lung	
	Allergic bronchopulmonary aspergillosis
	End stage hypersensitivity pneumonitis
	Mounier Kuhn (also lower lobe if repeated infections)
Lower lung	
	Usual interstitial pneumonia (IPF)
	Nonspecific interstitial pneumonitis
	Hypogammaglobulinemia
	Lung and bone transplantation
	Chronic aspiration
	Idiopathic
Right middle lobe and lingula	
	Atypical mycobacterial infection
	Immotile cilia syndrome

1.4 Diagnostic evaluation

1.4.1 Clinical features

The classic clinical manifestations of bronchiectasis are cough and the daily production of mucopurulent and tenacious sputum lasting months to years. The older literature also described "dry bronchiectasis" with episodic hemoptysis and no sputum production, but this presentation is less common (41).

Other, less specific complaints include dyspnea, wheezing, and pleuritic chest pain. Patients often report frequent bouts of "bronchitis" requiring therapy with repeated courses of antibiotics. There is generally a past history of repeated respiratory tract

infections over several years, although a single episode of severe bacterial pneumonia, pertussis, tuberculosis, and Mycoplasma infection can also result in bronchiectasis.
In a retrospective chart review of 103 patients with bronchiectasis who presented to a referral center, the following clinical findings were documented (41):

1) Symptoms – cough (98% of patients), daily sputum production (78%), dyspnea (62 %), rhinosinusitis (73 %), hemoptysis (27 %), and recurrent pleurisy (20 %).

2) Physical findings – crackles (75 %) and wheezing (22%) were common, with digital clubbing occurring in only 2 % of patients.

1.4.2 Laboratory tests

The following studies are typically part of the initial evaluation of a patient with bronchiectasis (42):

1.4.2.1 Routine tests performed

1) Serum immunoglobulins (IgG, IgA, IgM) and serum electrophoresis.
2) Specific antibody responses to tetanus and *Streptococcus pneumoniae* and *Haemophilus influenzae* type b capsular polysaccharides.
3) IgE and IgG to Aspergillus fumigatus +/- RAST testing.
4) Sputum for routine culture and for Acid fast bacilli
5) Autoantibodies (according to local policies but may include rheumatoid factor or anti-cyclic citrullinated protein antibody, Anti-nuclear antibody and testing for Sjogrens syndrome).

1.4.2.2 Tests performed in specific circumstances

1) Genotyping and sweat testing for cystic fibrosis:
    a) Age <40 years or
    b) Any age with persistent colonization by *Staphylococcus aureus* or *Pseudomonas aeruginosa* or
    c) Evidence of mal-absorption, male infertility or
    d) Upper lobe disease.
2) Ciliary investigations (otitis media, chronic upper respiratory tract symptoms since childhood, dextrocardiaand infertility).
3) Bronchoscopy

a) Localized disease, bronchoscopy may be indicated to exclude proximal obstruction.

b) For patients in whom serial testing of sputum does not yield microbiological information and who are not responding well to treatment, bronchoscopic sampling of lower respiratory tract secretion may be indicated.

c) Bronchoscopy is indicated if high resolution CT (HRCT) suggests atypical mycobacterial infection and sputum culture is negative.

d) Cytological examination of bronchoscopic specimens can provide evidence supporting gastric aspiration.

4) Referral to clinical immunology specialist- clinical suspicion of immunodeficiency

5) α-1 antitrypsin level- basal emphysema.

1.4.3 Radiology

1.4.3.1 Plain radiograph

Chest x-rays are usually abnormal, but are inadequate in the diagnosis or quantification of bronchiectasis. Tram-track opacities are seen in cylindrical bronchiectasis, and air-fluid levels may be seen in cystic bronchiectasis (Figure 1). Overall there appears to be an increase in bronchovascular markings, and bronchi seen end on may appear as ring shadows (43). Pulmonary vasculature appears ill-defined, thought to represent peribronchovascular fibrosis (43-44).

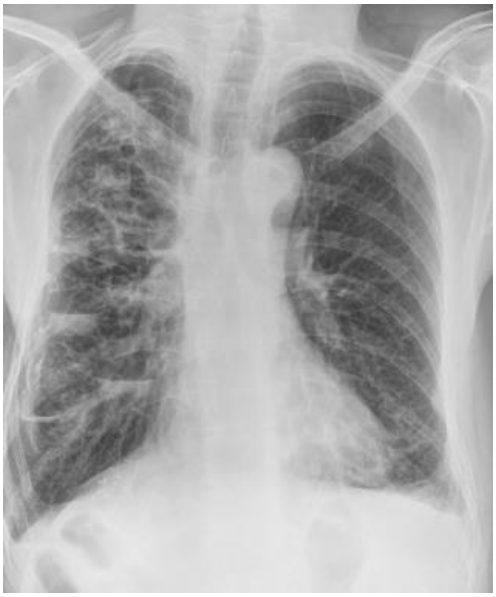

Figure 1- Chest radiography showing cystic bronchiectasis with multiple cystic airspaces (45).

1.4.3.2 CT scan

The CT signs of bronchiectasis were first described by Naidichet et al. in 1982 (46). Although initial studies using 8–10-mm slice thickness showed low sensitivity (47-49), the advent of HRCT led to markedly improved sensitivity, resulting in HRCT replacing bronchography as the diagnostic reference standard.

Optimal HRCT technique is important for maximizing diagnostic accuracy. Importantly, thin slices of 1–2 mm and a high-resolution lung reconstruction algorithm are used to optimize spatial resolution.

A number of features are helpful in diagnosing bronchiectasis (Figure 2) (43-44):

1) Bronchus visualized within 1cm of pleural surface
    A) Especially true of lung adjacent to costal pleura
    B) Most helpful sign for early cylindrical change
2) lack of tapering
3) Increased broncho-arterial ratio (BAR):
    A) Diameter of a bronchus should measure approximately 0.65-1.0 times that of the adjacent pulmonary artery branch
    B) Between 1 and 1.5 may be seen in normal individuals, especially those living at high altitude
    C) Greater than 1.5 indicates bronchiectasis
4) A number of ancillary findings are also recognized:
    A) Bronchial wall thickening: normally wall of bronchus should be less than half the width of the accompanying pulmonary artery branch
    B) Mucoid impaction
    C) Air-trapping and mosaic perfusion

DOI:10.14753/SE.2019.2224

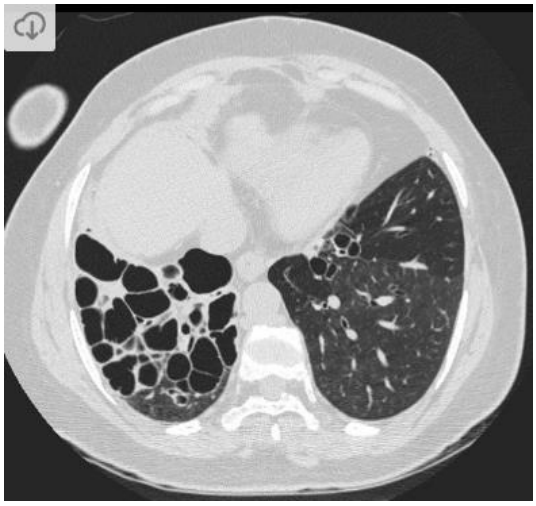

Figure 2 - High-resolution computed tomography image showing cystic bronchiectasis in the right lung (45).

1.5 Microbiology of non-CF bronchiectasis

Patients with bronchiectasis are commonly colonized with potentially pathogenic microorganisms in the airways (50). These microorganisms can cause lung infections and may produce a number of inflammatory mediators that can lead to progressive tissue damage and bronchial obstruction. The phenomenon of chronic infection, bronchial inflammation and progressive lung injury is called cole's ‘‘vicious cycle’’ hypothesis (Figure 3) and is also the reason why prompt evaluation of infection is important (51).

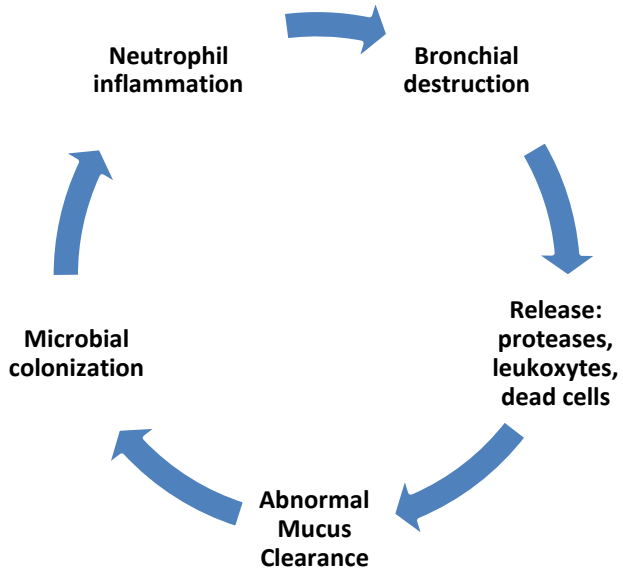

Figure 3 - Bronchiectasis pathophysiology (52).

The most commonly cultured pathogens associated with the sputum of NCFBr are *Haemophilus influenzae* and *Pseudomonas aeruginosa*, with many isolated strains showing significant antibiotic resistance (53-55). Characterizations of the most common bacteria in bronchiectasis are:

1.5.1 *Haemophilus influenzae*

H. influenzae has been reported in 14–52% of patients with non-CF bronchiectasis. It is a Gram-negative coccobacillus with specific growth requirements, which can be difficult to isolate in the laboratory if mixed with other flora. Some *H. influenzae* possess a polysaccharide capsule and can be typed using type-specific anti-capsule antisera. Those with the type B capsule (Hib) can cause invasive infection with bacteraemia, and are most familiar as a cause of meningitis or epiglottitis. The use of Hib vaccine has greatly reduced the incidence of these life-threatening conditions. *H. influenzae* with capsule types other than type B are relatively rare and are far less pathogenic. The non-encapsulated strains, referred to as non-typeable *H. influenzae* (NTHi), are also less pathogenic than Hib and only rarely cause bacteraemia. They live as commensals in the human upper respiratory tract but can cause otitis media, sinusitis and conjunctivitis, often following a primary viral infection. NTHi are a common cause of lower respiratory infection in patients with underlying respiratory abnormalities including non-CF bronchiectasis (53). The Hib vaccine does not prevent infection with NTHi as it only contains the *H. influenzae* type B capsule antigen.

NTHi could be an oral contaminant in expectorated sputum; however, studies using a protected specimen brush (PSB) at bronchoscopy found NTHi in significant numbers in non-CF bronchiectasis, confirming its presence in the lower respiratory tract (54). In contrast *Haemophilus parainfluenzae*, a common commensal organism found in the upper respiratory tract, may be cultured from sputum but was not found in PSB samples. In patients with COPD the presence of NTHi in sputum was associated with raised inflammatory cytokines, whereas patients with *H. parainfluenzae* in sputum had similar levels of cytokines to those who had no microorganisms cultured from their sputum, suggesting that even if present in the lower tract it does not have a direct pathogenic role (56-57).

There is little published data on the epidemiology of *H. influenzae* in non-CF bronchiectasis. It may be cultured repeatedly from the same patient over several years, but without typing data it is not known if this is the persistence of a single strain or

repeated episodes of infection. In COPD exacerbation NTHi were found in higher numbers and with the appearance of a new strain (58-59). However, other prospective study in COPD using molecular typing of *H. influenzae* and direct analysis of amplified DNA from sputum showed persistence of the same strain over prolong periods (60).

NTHi have various properties that can help explain their pathogenicity and ability to persist in the lung. They can adhere to mucus and to various cell types in the human respiratory tract using pili and other adhesion molecules. Virulence factors include the endotoxin lipo-oligosaccharides (LOS) and immunoglobulin (Ig) – A protease (61).

NTHi may be able to evade the immune response by varying its surface antigens. Mechanisms: include phase variation of LOS (62), and changes to outer membrane proteins (63).

The prevalence of antibiotic resistant NTHi increases over time in patients with non-CF bronchiectasis (53). Many are resistant to amidopenicillins (e.g. amoxicillin, ampicillin) either due to production of b-lactamase or alteration of penicillin binding proteins. Quinolone resistance is now recognized and resistance rates to trimethoprim and tetracycline are rising.

1.5.2 *Pseudomonas aeruginosa*

P. aeruginosa is a versatile non-fermentative Gram-negative bacillus that is found in a range of environments. *P. aeruginosa* is one of the most common isolates found in 12–43% of non-CF bronchiectasis patients (64). Stable patients with *P. aeruginosa* have poorer lung function and more sputum production when compared with patients with other potentially pathogenic microorganisms (64). It has been associated with a poorer quality of life and more frequent hospital admissions (65). There is debate over whether infection with *P. aeruginosa* leads to a faster decline in lung function as is seen in CF, or whether it is a marker of more damaged lungs (66-67).

P. aeruginosa possesses a range of virulence factors, although their expression may differ between isolates that cause acute infection and those responsible for chronic infection. Flagella, type IV pili lipopolysaccharide and exopolysaccharides contribute to the adherence to cells and surfaces. Type I and type II secretion systems export protein toxins, such as alkaline protease, elastase, exotoxin A and phospholipase C, while type III secretion systems inject exoenzymes directly into eukaryotic cells. Other extra-cellular

virulence factors include rhamnolipids, pyocyanin and hydrogen cyanide (68). Another pathogenicity factor is the ability to form alginate-enhanced biofilms (69) which contributes to the persistence of the organism rather than acute tissue damage and, together with other adaptations, promotes chronic infection.

P. aeruginosa can develop resistance by either:

1) Producing enzymes that destroy the antibiotic such as AmpC b-lactamase, carbapenemases or aminoglycoside modifying enzymes.

2) Modifying the antibiotic target, such as gyrA for quinolone resistance.

3) Reducing exposure either by a decrease in permeability or increased removal of the antibiotic from the bacterial cell (efflux). Efflux mechanisms often affect more than one class of antibiotics and therefore contribute to multi-drug resistance (70).

1.5.3 *Streptococcus pneumoniae*

S. pneumoniae is a Gram-positive coccus appearing in pairs and in short chains. It may be a harmless commensal in the oro-pharynx but can cause severe and invasive disease. It can cause otitis media, sinusitis, lower airway infections in patients with damaged lungs such as non-CF bronchiectasis or COPD, but it is rare in CF. Although

S. pneumoniae can be found in up to 37% of patients with non-CF bronchiectasis, very little has been published on its role in this condition.

S. pneumoniae has a polysaccharide capsule that helps evade opsonisation, and isolates lacking the capsule are avirulent. There are over 90 capsule types and the capsule type may be one of several factors that determine the pathogenicity of an individual strain (71). A polyvalent vaccine containing the most common serotypes is available and recommended for use in patients with chronic lung disease

S. pneumoniae can use a wide variety of molecules to adhere to host cells and produces an IgA protease and a toxin, pneumolysin that can promote invasion, inflammation and tissue damage (72). Pneumolysin is proinflammatory and has many actions including cytolysis, inhibition of cilial beating, and direct activation of the classical complement cascade.

The prevalence of antibiotic resistant *S. pneumoniae* has increased and in some countries very high rates of resistance to penicillin, macrolides and tetracyclines limit the treatment options. Penicillin resistance is due to modifications to penicillin binding proteins not by the production of a b-lactamase and, therefore, amoxicillin–clavulanate is ineffective.

1.5.4 *Staphylococcus aureus.*

S. aureus is a Gram-positive coccus found in clusters that may be part of the normal flora in the anterior nares, throat and on moist skin sites such as groin and axilla. Infection is by abscess formation, particularly in skin and soft tissues. It is a rare cause of characterized respiratory tract infection, but can cause severe pneumonia after influenza. It is a common cause of early infection in CF but is less common in non-CF bronchiectasis where its presence may indicate undiagnosed CF (40). There is also an association of *S. aureus* with ABPA in non-CF bronchiectasis (73).

1.5.5 Non-tuberculous mycobacteria (NTM)

Patients with bronchiectasis, as with other chronic lung diseases, are predisposed to infection with NTM. Few studies have undertaken a detailed analysis of NTM in the context of bronchiectasis. The prevalence of NTM in bronchiectasis may be higher than anticipated because of the non-specific symptoms and because routine screening is not usually undertaken. In one study, three cases of NTM were detected over 6 years in 91 patients with bronchiectasis (74). In another, NTM were found in 6% of bronchiectatic patients (55). No mycobacteria were isolated in a study of 150 patients over 3 years (40), but in this study sputum was sent only if no response to standard treatment occurred.

Patients with *Mycobacterium avium complex* infection may develop bronchiectasis over years. Middle-aged or elderly women seem particularly prone to this disease (27). However, isolation of an opportunist mycobacterial species should not necessarily be interpreted as pathogenic. A ‘one-off’ isolate may have been inhaled shortly before the sample was provided. Persistent isolation (colonization) may occur without any change in clinical status. HRCT scan features can be helpful in confirming infection.

Once an opportunist organism has been isolated, prolonged follow-up may be required to decide whether this represents colonization or infection. Careful follow-up is mandatory because colonization can change to infection (42). This will include:

1) Clinical features - deterioration favoring infection.
2) Sputum examination- (repeated culture, smear positive, heavy growth.
3) Lung function- rapid decline.
4) HRCT- exudative ‘tree-in-bud’ bronchiolitis, mucus plugging, cavitating nodules, rapid progression of disease.
5) Treatment failure.
6) The species isolated will also influence the likelihood of infection: *M. avium complex*, *M. kansasii*, *M. malmoense* (75-76).

However, there is a lack of available data on the frequency of other important pathogens such as non- tuberculous mycobacterium (NTM), as well as the role of patient age and lobar distribution of the disease on bacterial profile.

1.6 Treatment

1.6.1 Aim of the treatment

The aims of bronchiectasis management are (42):

1) Reducing symptoms reduce exacerbation frequency.
2) Preserving lung function.
3) Improving health related quality of life.

Patient education is of great importance as many patients have a high treatment burden with physiotherapy regimes, inhaled and oral therapy regimes and frequent hospital visits. Optimizing treatment compliance, rapid recognition of exacerbations and appropriate use of complex therapies requires active patient engagement. Patients should be advised to stop smoking as smoking will accelerate lung function decline and predispose to mortality (77). As with most other chronic illnesses, patients with bronchiectasis will be offered the annual influenza vaccination in accordance with national guidelines (42).

1.6.2 Airway clearance

Despite limited evidence, airway clearance techniques are widely considered a key component of management (42). There is little high-quality data comparing different techniques for effectiveness. The choice is therefore largely determined by patient choice and ease of use.

Chest physiotherapy- chest clearance techniques include physiotherapy regimes such as Active Cycle of Breathing Technique and Postural Drainage with or without adjuncts such as positive airway pressure devices, (e.g “Flutter” devices providing oscillatory positive pressure), or high frequency chest wall oscillation (78). Small, but significant improvements can be seen in exercise capacity, sputum volume and HRQOL (Health-Related Quality of Life) in patients who use chest physiotherapy compared with control (79-80). The availability of a physiotherapist or other health professional experience in teaching chest clearance techniques is invaluable for patient education.

Inhaled hyperosmolar agents- As an adjunct to standard chest physiotherapy, nebulized hypertonic saline can alter the mucus osmolality making it easier to clear (81). Hypertonic

saline can improve FEV1 when used in combination with chest physiotherapy (82). Recent trials of inhaled mannitol, another hyperosmolar agent, suggest it can increase sputum volume compared to placebo, although the overall significance of this to patients is not entirely clear (83-84).

DNase- The experience of recombinant DNase acts as a cautionary tale in extrapolating results in cystic fibrosis to patients with non-CF bronchiectasis. Despite being effective in selected patients with CF, DNase was shown to be potentially harmful in a randomised controlled trial in NCFBE, reducing FEV1 (85). It is therefore not advised for use in this group of patients, and highlights the different pathophysiology in NCFB, compared with CF associated bronchiectasis.

1.6.3 Long Term Antibiotics

Long-term suppressive antibiotic therapy aims to reduce the bacterial load in the airways, interrupting the 'vicious cycle'. According to the hypothesis, this should slow down disease progression and lead to improved symptoms and a reduction in exacerbation frequency (51).

Until recently there has been a lack of evidence to guide long term antibiotic therapy in NCFBr, but the recent publication of a number of randomized controlled trials have established clearly that long term antibiotic therapy can reduce exacerbations as well as providing other benefits.

1.6.3.1 Oral macrolide therapy

The BAT, BLESS and EMBRACE trails compare the effects of long term macrolide therapy (either 6 or 12 months) to placebo (86-88). All these trials have shown a significant reduction in exacerbation frequency compared to placebo during the treatment period. The BAT trail showed a median exacerbation frequency of 2 in the placebo group compared with 0 in the treatment group after 12 months ($P<0.001$)(87). Both 12-month trails showed a reduction in FEV1 decline for the treatment group, although these were small and of doubtful clinical significance (87-88). The main concern of macrolide therapy is a marked increase in macrolide resistance in oropharyngeal and other bacteria. The BAT trial showed macrolide resistance of 88% in the treatment group compared to 26% on placebo (87). Azithromycin was associated with increased gastrointestinal side effects in the BAT trial, although erythromycin appeared to be better tolerated (88). There

have been other concerns regarding macrolides including an increased incidence of cardiovascular events although no cardiovascular complications were observed in these small RCT's (89-90).

Macrolides have anti-inflammatory effects including inhibition of inflammatory cell migration, cytokine secretion and attenuation of the production of reactive oxygen species (91). Whether the benefit of macrolides is attributable to their antibiotic or anti-inflammatory effect is unclear.

BTS guidelines recommend consideration of long-term oral antibiotics for patients with ≥ 3 exacerbations a year or those chronically colonized with *Pseudomonas aeruginosa* (42). These guidelines were written before the publication of the three recent trials and given that the EMBRACE trial showed benefit in patients with one or more exacerbations per year these recommendations may change. In clinical practice, macrolides are most frequently used in patients with three or more exacerbations per year, patients with *Pseudomonas aeruginosa* and also in patients with less frequent exacerbations who continue to have significant impairment of quality of life despite standard treatment.

1.6.3.2 Inhaled antibiotic therapy- Nebulized or inhaled antibiotics deliver a high concentration of the drug to the airways, reducing systemic absorption and therefore theoretically are associated with fewer side effects compared with oral therapy. A 12 months randomized control trial comparing nebulized gentamicin to nebulized 0.9% saline found a significant reduction in bacterial load, associated with decreases in exacerbation frequency and improvements in quality of life (92). This was associated with a reduction in airway and systemic inflammation (93). A 3-monthes follow-up review after treatment was stopped showed all outcome measures returned to baseline suggesting that this type of therapy needs to be continued long term for sustained benefit (92).

Pseudomonas aeruginosa colonization is associated with a worse prognosis in most studies (65). Inhaled antibiotics can suppress *P.aeruginosa* bacterial load and even achieved eradication of *P.aeruginosa* in 30% of patients treated in the nebulized gentamicin trial (92,94). Inhaled tobramycin has been successful in treating CF patients with chronic *Pseudomonas aeruginosa* infection (95). A study by Barker et al showed nebulized tobramycin also has benefits in non-CF bronchiectasis (95). During the study, tobramycin was given twice daily for 4 weeks. At 6 weeks the pathogen was eradicated in 35% of patients while all patients in the placebo group had persistent colonization. There were some adverse effects with bronchospasm and cough. Larger studies of

tobramycin are needed in non-CF bronchiectasis. Several newer agents are now in late phase clinical trials including dry power inhaled ciprofloxacin, nebulized liposomal ciprofloxacin and inhaled colistin which was the subject of a recent phase III trial demonstrating improved quality of life and a reduction in exacerbations in compliant patients.

1.6.3.3 Anti-inflammatory therapy

Bronchiectasis is thought to be predominantly a neutrophil driven disorder, and neutrophils are largely resistant to the anti-inflammatory effects of corticosteroids. There is no role for oral corticosteroids in bronchiectasis out-with the treatment of ABPA (96). Inhaled corticosteroids are currently indicated in patients with asthma, COPD or airway hyper-reactivity. They may have some benefits in bronchiectasis. Some studies have shown that regular high dose inhaled steroids reduce 24-hour sputum volume, reduce inflammatory markers in sputum and improve quality of life (97). However, they have not shown any significant improvement in lung function, or exacerbation frequency. A Cochrane review acknowledges they have short-term benefits but concludes there is insufficient evidence to recommend their routine use (97). Recent data in COPD shows an increase in pneumonia with the use of inhaled steroids (98). Whether this is true in bronchiectasis is uncertain but would be a concern in a population already at high risk of serious respiratory infections.

A large number of promising anti-inflammatory therapies specifically targeting neutrophils, such as neutrophil elastase inhibitors and CXCR2 antagonists are now entering clinical trials (99).

DOI:10.14753/SE.2019.2224

1.6.3.4 *Pseudomonas aeruginosa* eradication treatment

In keeping with recommendations in cystic fibrosis, most specialist centers will attempt eradication of *P. aeruginosa* upon first isolation. An attempt should be made to eradicate using 14 courses of oral ciprofloxacin. Failure to eradicate *P. aeruginosa* with oral treatment may lead to consideration of intravenous and/or nebulized eradication (Figure 4).

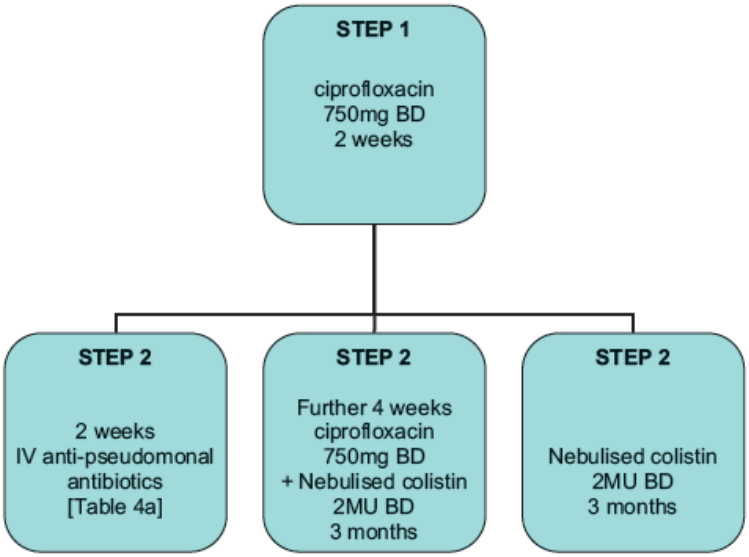

Figure 4 - The British Thoracic Society guidelines provides a useful algorithm for *Pseudomonas aeruginosa* eradication (42).

1.6.3.5 Treatment of Exacerbations

An exacerbation is defined as (Figure 5):

1) Worsening local symptoms – cough, increases sputum volume, change in viscosity, increased sputum purulence.
2) Dyspnea, increase wheeze, hemoptysis.
3) Systemic upset.

DOI:10.14753/SE.2019.2224

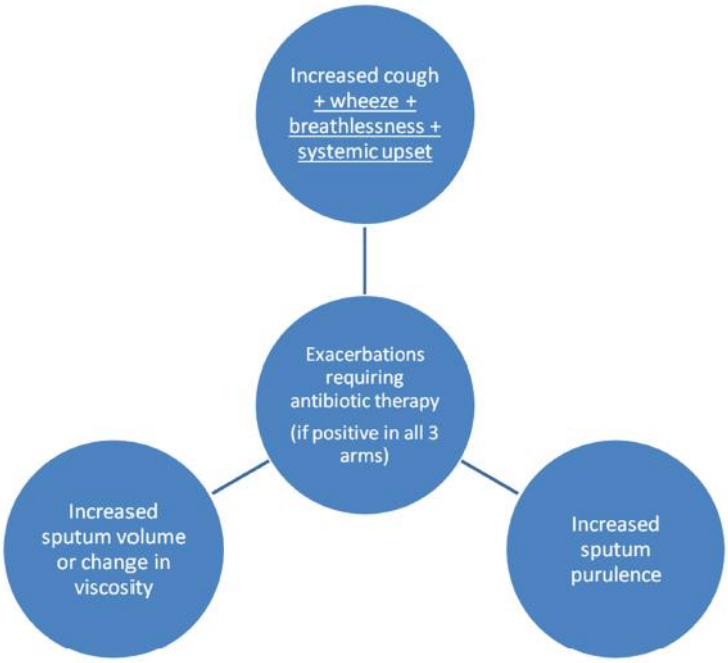

Figure 5 - Definition of an exacerbation needing antibiotic therapy (42).

A sputum sample should be obtained at the initial presentation of an exacerbation and sent for culture. While pending sputum culture results, patients should receive treatment targeted towards organisms in previous sputum samples, based on previous sensitivity results. This emphasizes the importance of sending regular sputum samples while patients are clinically stable. Due to the higher bacterial loads and difficulty in achieving antibiotic penetration into biofilms, longer courses of therapy are given in bronchiectasis and most advocate 14 days for exacerbations. (42). If there is no previous bacteriology, first line treatment is amoxicillin 500mg three time a day or clarithromycin 500mg twice a day for 14 days (Table 3). By consideration of empiric therapy in patient with no previous bacteriology, the guideline does not take in consideration certain risk factors: patient age, bronchiectasis location, disease patter (bilateral vs unilateral).

Table 3- Common organisms associated with acute exacerbation of bronchiectasis and suggested antimicrobial agents (42).

Organism	Recommended first-line treatment	Length of treatment
Streptococcus pneumoniae	Amoxicillin 500 mg tds	14 days
Haemophilus influenzae (β-lactamase negative)	Amoxicillin 500 mg tds	14 days
	Amoxicillin 1 g tds	14 days
	Amoxicillin 3 g bd	14 days
Haemophilus influenzae (β-lactamase positive)	Co-amoxiclav 625 mg tds	14 days
Moraxella catarrhalis	Co-amoxiclav 625 mg tds	14 days
Staphylococcus aureus (MSSA)	Flucloxacillin 500 mg qds	14 days
Staphylococcus aureus (MRSA): oral preparations	<50 kg: Rifampicin 450 mg od + trimethoprim 200 mg bd	14 days
	>50 kg:Rifampicin 600 mg + trimethoprim 200 mg bd	
Staphylococcus aureus (MRSA): intravenous preparations	Vancomycin 1 g bd* (monitor serum levels and adjust dose accordingly) or teicoplanin 400 mg od	14 days
Coliforms (eg, *Klebsiella*, enterobacter)	Oral ciprofloxacin 500 mg bd	14 days
Pseudomonas aeruginosa	Oral ciprofloxacin 500 mg bd (750 mg bd in more severe infections)	14 days

BTS guidelines state that intravenous antibiotics may be considered in patients who fail to respond to oral therapy, or sputum culture reveals organisms to which no oral therapy is beneficial (e.g multidrug resistant *pseudomonas aeruginosa*) or where patients are systemically unwell (42). BTS guideline also suggests criteria for inpatient treatment which are:

1) Unable to cope at home.
2) Development of cyanosis or confusion.
3) Breathlessness with respiratory rate >25.
4) Circulatory failure.
5) Respiratory failure.
6) Temperature >38ºC.
7) Intravenous therapy required in patients with clinical failure after oral antibiotics

The goals of successful antibiotic treatment are defined in Figure 6.

DOI:10.14753/SE.2019.2224

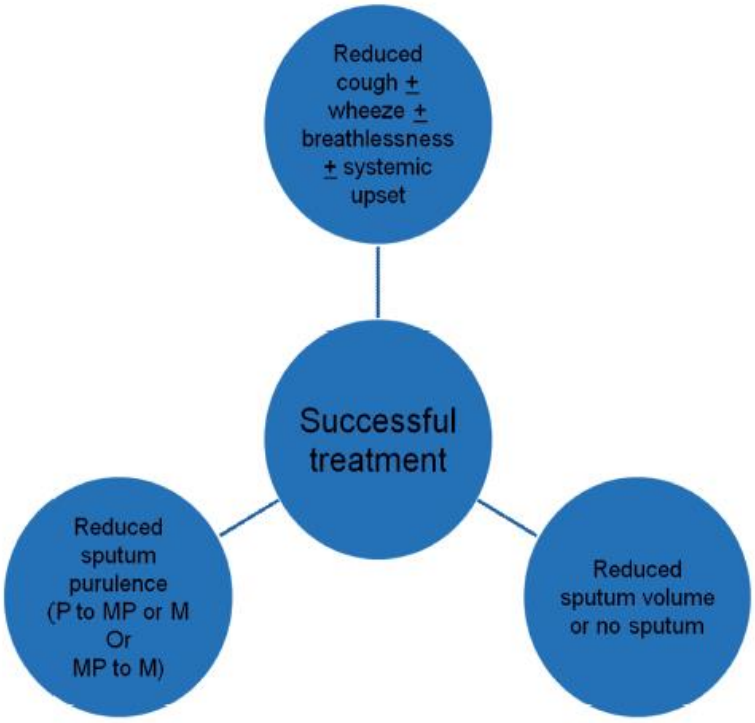

Figure 6 - Definition of successful treatment of an exacerbation.
P, purulent; MP, mucopurulent; M, mucoid.

1.6.4 Pulmonary rehabilitation

Patients with bronchiectasis with significant breathlessness benefit from pulmonary rehabilitation and should be routinely referred for this service (100).

1.7 Parapneumonic effusion

1.7.1 Parapneumonic effusion as an alarming sign in pneumonia

Despite the advent of potent antibiotics, bacterial pneumonia still results in significant morbidity and mortality in the American population. The annual incidence of bacterial pneumonia is estimated to be 4 million, with approximately 25% of patients requiring hospitalization (101). Because as many as 40% of hospitalized patients with bacterial pneumonia have an accompanying pleural effusion (102), effusions associated with pneumonia, parapneumonic effusions, account for a large percentage of pleural effusions. The morbidity and mortality rates in patients with pneumonia and pleural effusions are higher than those in patient with pneumonia alone. In one study of 1,424 patients

hospitalized with community acquired pneumonia, patients with pleural effusions were 2.7 times more likely to be treatment failure than were those without pleural effusions (103). In another study, the relative risk of mortality in patients with community acquired pneumonia was 7 times higher for patients with bilateral pleural effusions and 3.4 times higher for patients with unilateral pleural effusions of moderate or greater size as compared with other patients with community acquired pneumonia alone (104). In assessing risks of patients with community acquired pneumonia, the presence of a pleural effusion is given the same weight as a Po_2 less than 60mmHg (105). Espana et al (106) recommended that any patient with pneumonia and a loculated effusion or an effusion greater than 2 cm in thickness on the decubitus be hospitalized. Some of the increased morbidity and mortality in the patients with parapneumonic effusion are due to mismanagement of the pleural effusion (107).

1.7.2 Definition of parapneumonic effusion

A parapneumonic effusion is a pleural effusion associated with lung infection (108). Early in the course of parapneumonic effusion, the pleura become inflamed with leakage of cellular elements, protein and fluid into the pleural space, forming the effusion. Subsequent bacterial invasion results in a frank empyema, the presence of which often requires thoracentesis. A delay in the diagnosis and initiation of proper therapy for infectious effusions leads to increases in the complication rate. These delays are more common in patients with coexisting heart failure or malignancy (109-110).

Parapneumonic pleural effusions are divided into three groups or stages based upon pathogenesis.

1. Uncomplicated parapneumonic effusion — An uncomplicated parapneumonic effusion forms when lung interstitial fluid increases during pneumonia and moves across the adjacent visceral pleural membrane. The pleural fluid is characterized by "exudative" chemistries and an influx of neutrophils into the pleural space. An effusion forms when the resorptive capacity of the pleural space is exceeded; it resolves with resolution of the pneumonia.

2. Complicated parapneumonic effusion — A complicated parapneumonic effusion develops when there is bacterial invasion of the pleural space. Bacterial invasion typically leads to an increased number of neutrophils and the development of pleural fluid acidosis, which results from anaerobic utilization of glucose by the neutrophils and bacteria. In

addition, lysis of neutrophils increases the lactate dehydrogenase concentration in the pleural fluid to values often in excess of 1000 IU/L. Perhaps, because the bacterial count is low or because anaerobic cultures are not done, cultures of fluid from complicated parapneumonic effusions are sometimes falsely negative (111). Nevertheless, the typical morphotypes of anaerobes are usually seen on Gram stain and the characteristic putrid odor of the pleural fluid is considered diagnostic of anaerobic infection. Often, there is deposition of a dense layer of fibrin on both the visceral and parietal pleurae that can lead to pleural loculation (112). Whether spontaneous adhesions of the pleural space can result from pleural space inflammation without infection is unknown.

3. Thoracic empyema — Empyema develops when there is evident bacterial infection of the pleural liquid, resulting in either pus or the presence of bacterial organisms on Gram stain. A positive culture is not required for a diagnosis, since there are several reasons why bacteria may not be cultured from an empyema (see 'Bacteriology' below):

- Anaerobic organisms may be difficult to culture or anaerobic cultures are not done because they are not specifically requested
- Sampling is often performed after a patient has received antibiotics
- Sterile inflammatory fluid may be aspirated adjacent to an infected loculus of infection
- Current culture methods are insufficiently sensitive

1.7.3 Clinical presentation

The clinical presentation of the adult with parapneumonic effusion or empyema depends upon the patient's timing of presentation and immune competence, and also the specific organisms causing infection. Patients with pneumonia and uncomplicated parapneumonic effusion present earlier in the course of their pneumonia; those with empyema typically present later when bacteria from the untreated pneumonia have had time to colonize the pleural space. Infection with less virulent bacteria favors a later presentation; therefore, many empyemas complicate indolent anaerobic pneumonias, such as those following aspiration.

Common clinical features of bacterial pneumonia with parapneumonic effusion include cough, fever, pleuritic chest pain, dyspnea, and sputum production. However, patients may only have one or two of these symptoms. In general, the presenting symptoms, other than pleuritic pain and duration of fever, are not helpful in determining which patients

have pneumonia versus pneumonia with a parapneumonic effusion or empyema. Compared to those with pneumonia alone, patients with empyema may report a longer course with several days of fever and malaise rather than hours. Among patients with pneumonia, the presence of a parapneumonic effusion was associated with an increased likelihood of being admitted, a longer hospital stays, and possibly increased mortality (113).

Physical examination may identify the presence of pleural fluid when the fine or coarse crackles, egophony (also known as e-to-a changes), and increased fremitus (palpable asymmetric increase in vibration with speech) typical of consolidation are replaced by decreased breath sounds and decreased fremitus. Occasionally, egophony will still be present at the upper edge of the effusion. Dullness to percussion is a potential feature of lung consolidation from either pneumonia or pleural effusion and is thus not a useful discriminating physical finding. Although clinical findings are helpful when present, they are often absent so radiographic imaging is crucial to the complete evaluation.

Radiographic and ultrasound imaging play a key role in the evaluation and management of parapneumonic effusions and empyema (114). In all patients with pneumonia, the chest radiograph should be evaluated for evidence of pleural fluid.

The presence of a complicated parapneumonic effusion or empyema is first suggested by a chest radiograph showing a pleural-based opacity.

1.7.4 Epidemiology of parapneumonic effusion

Pleural infection is rising in incidence across all age groups worldwide, confirmed by reports from the United States, Canda, Europe and Asia (115). The mortality rate of empyema has risen alarmingly. In Utah, death rates from empyema were sixfold higher in 2000-2004 compared to 1950-1975 (116). Overall in the United States, the incidence of empyema per 100,000 persons had roughly doubled between 1996 and 2008 with roughly equal increase occurring in all age groups (117). In this study, the increase was largely due to increase in nonpneumococcal empyema and *Staph sp*. empyema (55). The explanation for the increase in empyema incidence is not clear but has been attributed at least in part to the induction of the heptavalent pneumococcal conjugate vaccine (PCV7) IN 2000. After the introduction of this vaccine, there was a reduction in invasive pneumococcal disease, but the incidences of pneumococcal empyema in children and adults have both increased (115). The decrease in incidence of empyema from serotypes

covered by the vaccine was overcompensated by an emergence of disease caused by nonvaccine serotypes (particularly serotype 1) (115).

1.7.5 Diagnostic evaluation of a pleural effusion in adults

Determining the cause of a pleural effusion is greatly facilitated by analysis of the pleural fluid. Thoracentesis is a simple bedside procedure with imaging guidance that permits fluid to be rapidly sampled, visualized, examined microscopically and quantified for chemical and cellular content.

The indication for diagnostic thoracentesis is the new finding of a pleural effusion. Observation, in lieu of diagnostic thoracentesis, may be warranted in uncomplicated heart failure and viral pleurisy. In the former setting, the clinical diagnosis is usually secure; in the latter, there is typically a small amount of fluid. However, if the clinical situation is atypical or does not progress as anticipated, thoracentesis should be performed.

Tests routinely performed on pleural fluid include cell count, pH, protein, lactate dehydrogenase (LDH), and glucose. Additional commonly performed tests in selected patients include amylase, cholesterol, triglycerides, N-terminal pro-BNP, adenosine deaminase, gram and AFB (acid-fast bacilli) stain, bacterial and AFB culture, and cytology.

Initial diagnostic clues can be obtained by gross inspection of pleural fluid as it is being aspirated from the patient's chest (118). Observations that are helpful for diagnosis are listed in the table 4.

Table 4 – Colors of pleural fluid helpful in diagnosis (109).

Color of fluid	Suggested diagnosis
Pale yellow (straw)	Transudate, some exudates
Red (bloody)	Malignancy, benign asbestos pleural effusion, postcardiac injury syndrome, or pulmonary infarction in absence of trauma
White (milky)	Chylothorax or cholesterol effusion
Brown	Long-standing bloody effusion; rupture of amebic liver abscess
Black	Aspergillus niger, Rhizomes oryzae, metastatic melanoma, pancreaticopleural fistula, crack cocaine use, bronchogenic adenocarcinoma, chronic hemothorax
Yellow-green	Rheumatoid pleurisy
Dark green	Biliothorax

The pleural fluid is next characterized as either a transudate or an exudate.

Transudates — Transudates result from imbalances in hydrostatic and oncotic pressures in the chest, as occur with CHF and nephrosis, or conditions external to the pleural space. Examples of the latter include movement of fluid from the peritoneal, cerebrospinal, or retroperitoneal spaces, or from iatrogenic causes, such as crystalloid infusion through a central venous catheter that has migrated into the mediastinum or pleural space. Nevertheless, transudates have a limited number of diagnostic possibilities that can usually be discerned from the patient's clinical presentation (table 5).

Table 5 - Causes of transudative pleural effusions (109).

Transudate etiologies	Comments
Atelectasis	Caused by increased intrapleural negative pressure
Cerebrospinal fluid leak into pleural space	Thoracic spinal surgery or trauma and ventriculopleural shunts
Heart failure	Acute diuresis can result in borderline exudative features
Hepatic hydrothorax	Rare without clinical ascites
Hypoalbuminemia	Edema liquid rarely isolated to pleural space
Iatrogenic	Misplaced intravenous catheter into the pleural space
Nephrotic syndrome	Usually subpulmonic and bilateral
Peritoneal dialysis	Acute massive effusion develops within 48 hours of initiating dialysis
Urinothorax	Caused by ipsilateral obstructive uropathy

Exudates — In contrast, exudative effusions present more of a diagnostic challenge. Disease in virtually any organ can cause exudative pleural effusions by a variety of mechanisms (Table 6), including infection, malignancy, immunologic responses, lymphatic abnormalities, noninfectious inflammation, iatrogenic causes, and movement of fluid from below the diaphragm.

Exudates result primarily from pleural and lung inflammation (resulting in increased capillary and pleural membrane permeability) or from impaired lymphatic drainage of the pleural space (resulting in decreased removal of protein and other large molecular weight constituents from the pleural space). Exudates can also result from movement of fluid from the peritoneal space, as seen with acute or chronic pancreatitis, chylous ascites, and peritoneal carcinomatosis.

Table 6 – Causes of exudative pleural effusions (109).

Exudate etiologies	Exudate etiologies descriptions
Infectious	Bacterial pneumonia, Tuberculous pleurisy, Parasites, Fungal disease, Atypical pneumonias, Subphrenic abscess, Hepatic abscess, Splenic abscess, Hepatitis, Spontaneous esophageal rupture
Malignancy-related	Carcinoma, Lymphoma, Mesothelioma, Leukemia, Chylothorax, Paraproteinemia (multiple myeloma, Waldenstrom's macroglobulinemia)
Connective tissue disease	Lupus pleuritis, Rheumatoid pleurisy, Mixed connective tissue disease, Eosinophilic granulomatosis with polyangiitis (Churg-Strauss), Granulomatosis with polyangiitis (Wegener's)
Endocrine dysfunction	Hypothyroidism, Ovarian hyperstimulation syndrome
Other inflammatory disorders	Pancreatitis (acute, chronic), Benign asbestos pleural effusion, Pulmonary embolism, Radiation therapy, Uremic pleurisy, Sarcoidosis, Postcardiac injury syndrome, Hemothorax.
Iatrogenic	Drug-induced, Esophageal perforation, Esophageal sclerotherapy, Enteral feeding tube in pleural space

1.7.6 Diagnostic criteria

The Light's Criteria Rule is a traditional method of differentiating transudates and exudates that measures serum and pleural fluid protein and LDH (119). Abbreviated versions of Light's Criteria Rule have similar diagnostic accuracy and have been recommended for clinical use (120-121).

According to the traditional Light's Criteria Rule, if at least one of the following three criteria (ie, component tests of the rule) is fulfilled, the fluid is defined as an exudate (119):

1. Pleural fluid protein/serum protein ratio greater than 0.5, or
2. Pleural fluid LDH/serum LDH ratio greater than 0.6, or

3. Pleural fluid LDH greater than two-thirds the upper limits of the laboratory's normal serum LDH

Combining results of two or more dichotomous tests into a diagnostic rule, as done by the Light's Criteria Rule, wherein only one component test result needs to be positive to make the rule result positive always increases sensitivity at the expense of decreasing specificity of the rule. As would be expected, therefore, the sensitivity of the Light's Criteria Rule is higher than the sensitivity of each of the three component tests of the rule but the specificity of the rule is lower than its individual components. This tradeoff of higher sensitivity for lower specificity in the design of Light's Criteria Rule is appropriate for evaluating pleural fluid because it is important that exudates not be missed, since they can have important prognostic implications. Some transudates, however, may be misclassified as an exudate because of the decreased specificity of the rule (122).

Light's criteria have been criticized for including both the pleural fluid LDH/serum LDH ratio and the pleural fluid LDH (120) because they are highly correlated (120-123).

Alternative diagnostic criteria also exist. A meta-analysis of eight studies (1448 patients) examined pleural fluid tests and found that several tests identified exudates with accuracy similar to those used in Light's criteria, but did not require concurrent measurement of serum protein or LDH (120). Proposed two-criteria and three-criteria diagnostic rules, which require one criterion to be met to define an exudate, include:

Two-test rule

1. Pleural fluid cholesterol greater than 45 mg/dL.
2. Pleural fluid LDH greater than 0.45 times the upper limit of the laboratory's normal serum LDH.

Three-test rule

1. Pleural fluid protein greater than 2.9 g/dL (29 g/L).
2. Pleural fluid cholesterol greater than cholesterol 45 mg/dL (1.165 mmol/L).
3. Pleural fluid LDH greater than 0.45 times the upper limit of the laboratory's normal serum LDH.

The previous pleural fluid LDH cutoff point for differentiating between exudates and transudates in the traditional Light's criteria rule was 67 percent of (or 0.67 times) the upper limit of normal serum LDH. This has been changed to 45 percent, based on reanalysis of each criterion individually (120-123). All available tests may misclassify

pleural fluid as exudates or transudates when values are near the cutoff points (119-120,123). Thus, clinical judgment is required when evaluating patients with borderline test results (124).

Chemical analysis — The measurement of pleural fluid protein, LDH, glucose, pH, cholesterol, triglycerides, and amylase can provide useful information.

Protein — Most transudates have absolute total protein concentrations below 3.0 g/dL (30 g/L), although acute diuresis in heart failure can elevate protein levels into the exudative range (125-127). However, such patients have a serum to pleural fluid albumin gradient (the difference between the serum and pleural values) greater than 1.2 g/dL (12 g/L), which correctly categorizes their effusions as transudates (127-128). Elevated blood N-terminal pro-brain natriuretic peptide (NT-proBNP) also supports the diagnosis of heart failure when Light's criteria yield results in the exudative range (129).

● Tuberculous pleural effusions virtually always have total protein concentrations above 4.0 g/dL (40 g/L) (119).

● When pleural fluid protein concentrations are in the 7.0 to 8.0 g/dL (70 to 80 g/L) range, Waldenström's macroglobulinemia and multiple myeloma should be considered (130-131).

LDH — The level of pleural fluid lactic dehydrogenase (LDH) is one of the key criteria for differentiating transudates and exudates. Several specific disease associations have been noted with pleural fluid protein and LDH levels:

● Pleural fluid LDH levels above 1000 IU/L (with upper limit of normal for serum of 200 IU/L) are characteristically found in empyema (102), rheumatoid pleurisy (132), and pleural paragonimiasis (133), and are sometimes observed with malignancy.

● Pleural fluid secondary to *Pneumocystis jirovecii* pneumonia has the characteristic finding of a pleural fluid/serum LDH ratio greater than 1.0 and a pleural fluid/serum protein ratio of less than 0.5 (134). Urinothorax is another cause of elevated pleural fluid LDH associated with low pleural fluid protein levels (135).

Glucose — A low pleural fluid glucose concentration (less than 60 mg/dL [3.33 mmol/liter], or a pleural fluid/serum glucose ratio less than 0.5) narrows the differential diagnosis of the exudate to the following possibilities:

1. Rheumatoid pleurisy
2. Complicated parapneumonic effusion or empyema
3. Malignant effusion
4. Tuberculous pleurisy
5. Lupus pleuritis
6. Esophageal rupture

All transudates and all other exudates have pleural fluid glucose concentration similar to that of blood glucose.

The mechanism responsible for a low pleural fluid glucose depends upon the underlying disease. Specific examples include:

1. Decreased transport of glucose from blood to pleural fluid with rheumatoid pleurisy (136-137) or malignancy (138).

2. Increased utilization of glucose by constituents of pleural fluid, such as neutrophils, bacteria (empyema), and malignant cells (139).

The lowest glucose concentrations are found in rheumatoid pleurisy and empyema, with glucose being undetectable in some cases. In comparison, when the glucose concentration is low in tuberculous pleurisy, lupus pleuritis, and malignancy, it usually falls into the range of 30 to 50 mg/dL (1.66 to 2.78 mmol/liter).

PH — Pleural fluid pH should always be measured in a blood gas machine rather than with a pH meter or pH indicator paper, as the latter will result in inaccurate measurements (140-141). A pleural fluid pH below 7.30 with a normal arterial blood pH is found with the same diagnoses associated with low pleural fluid glucose concentrations (142). The pH of normal pleural fluid is approximately 7.60, due to a bicarbonate gradient between pleural fluid and blood (143). Thus, a pH below 7.30 represents a substantial accumulation of hydrogen ions. Transudates generally have a pleural fluid pH in the 7.40 to 7.55 range, while the majority of exudates range from 7.30 to 7.45 (142).

The mechanisms responsible for pleural fluid acidosis (pH <7.30) include:

1. Increased acid production by pleural fluid cells and bacteria (empyema) (139,144).

2. Decreased hydrogen ion efflux from the pleural space, due to pleuritis, tumor, or pleural fibrosis. Specific examples include malignancy (138), rheumatoid pleurisy and tuberculous pleurisy (136-137).

A low pleural fluid pH has diagnostic, prognostic, and therapeutic implications for patients with parapneumonic (Table 7) and malignant effusions (145). Patients with a low

pleural fluid pH malignant effusion have a high initial positive yield on pleural fluid cytology. They also tend to have a shorter survival and poorer response to chemical pleurodesis than those with a pH >7.30, although the strength of these associations do not provide prognostic value for individual patients (146-148). Clinicians should not use the pleural fluid pH as the sole criterion for the decision to recommend pleurodesis.

A parapneumonic effusion with a low pleural fluid pH (≤7.15) indicates a high likelihood of necessity for pleural space drainage (table 5) (149-150).

Nucleated cells — The total pleural fluid nucleated cell count is virtually never diagnostic. There are, however, some settings in which the count may be helpful:

●Counts above 50,000/microL are usually found only in complicated parapneumonic effusions, including empyema.

●Exudative effusions from bacterial pneumonia, acute pancreatitis, and lupus pleuritis usually have total nucleated cell counts above 10,000/microL (109).

●Chronic exudates, typified by tuberculous pleurisy and malignancy, typically have nucleated cell counts below 5000/microL (109).

The timing of thoracentesis in relation to the acute pleural injury determines the predominant cell type. The early cellular response to pleural injury is neutrophilic. As the time from the acute insult lengthens, the effusion develops a mononuclear predominance if the pleural injury is not ongoing.

Lymphocytosis — Pleural fluid lymphocytosis, particularly with lymphocyte counts representing 85 to 95 percent of the total nucleated cells, suggests tuberculous pleurisy, lymphoma, sarcoidosis, chronic rheumatoid pleurisy, yellow nail syndrome, or chylothorax (118,151). Carcinomatous pleural effusions will be lymphocyte-predominant in over one-half of cases; however, the percentage of lymphocytes is usually between 50 and 70 percent (151).

DOI:10.14753/SE.2019.2224

Table 7- Categorizing risk for poor outcome following parapneumonic effusions (151).

Pleural space anatomy	Pleural fluid bacteriology	Pleural fluid chemistry*	Risk of poor outcome	Drainage
Minimal, free flowing (<10 mm on lat decub film)¥	Culture and Gram AND stain results unknown	PH unknown	Very low	No
Small to moderate free flowing effusion (>10 mm and <1/2 hemithorax)	Neg culture and Gram stain¥¥	pH ≥7.20	Low	No††
Large, free flowing fluid (≥1/2 hemithorax)***, loculated effusion†††, or effusion with thickened parietal pleura¥¥¥	Positive culture or Gram stain	PH <7.20	Moderate	Yes
	pus		High	Yes

* pH is the preferred pleural fluid chemistry test, and pH must be determined by a blood gas analyzer. If a blood gas analyzer is not available, pleural fluid glucose should be used (P0 = glucose ≥60 mg/dL; P1 = glucose <60 mg/dL). The expert panel cautions that the clinical utility and decision thresholds for pH and glucose have not been well-established.

** Repeat thoracentesis should be considered if effusion enlarges and/or clinical condition deteriorates.

*** Larger effusions are more resistant to effective drainage, possibly because of the increased likelihood that large effusions will also be loculated.

¥ Clinical experience indicates that effusions of this size do not require thoracentesis for evaluation, but will resolve.

¥¥ Regardless of prior antibiotic use.

†† If clinical condition deteriorates, repeat thoracentesis and drainage should be considered.

††† Pleural loculations suggest a worse prognosis.

1.7.7 Challenges in diagnosis of parapneumonic effusion

The existence of inadequate diagnostic criteria is a major reason for a delay in diagnosis. Pleural leukocyte counts, effusion cells, differential counts and Light's criteria do not reliably identify an infectious etiology (110). Although a pleural pH <7.20 and pleural glucose <60 mg/dL are indications for pleural drainage, these thresholds are not sufficiently sensitive (151). Moreover, other conditions, such as malignancy, tuberculosis, rheumatoid pleurisy, and lupus pleuritis, can cause pleural fluid acidosis or low pleural glucose, demonstrating that these indicators lack specificity for infection (152). Although a pleural white blood cell count >50,000 cells/μL may help accurately diagnose parapneumonic effusions, pleural white cell counts more often range between 10,000 to 50,000. As a result, they are not sensitive for diagnostic purposes (153). Although microbiologic studies provide definitive evidence of infection, positive cultures are seen in only 60% of parapneumonic effusions, and there is often a prolonged time to culture positivity (154-155).

Because the classic pleural biochemistry testing lacks both sensitivity and specificity, the development of a novel pleural biomarker for infection has been an area of active investigation. Procalcitonin, interferon-ɣ, carcinoembryonic antigen, interleukin-6, tumor necrosis factor-α and soluble triggering receptor expressed on myeloid cells 1 (STREM-1) have been evaluated for their utility in distinguishing empyema from other types of effusions, but they are not sensitive for detection (155-157).

1.7.8 Pleural CRP for parapneumonic effusion identifying

CRP was discovered in Oswald Avery's laboratory during the course of studies of patients with *Streptococcus pneumoniae* infection (158). CRP is an acute phase protein that is synthesized by the liver in response to various stimuli (159). The induction of CRP synthesis in the liver is triggered by the production of IL-6 and TNF-α by local pleural cells (160-161). The pleural fluid CRP levels are likely to reflect the serum levels because the presence of CRP in the pleural fluid may be due to increased diffusion from the blood as a result of inflamed capillary leakage (160-161).

Pleural CRP has recently been proposed as a specific biomarker for the differential diagnosis of pleural effusions and reportedly exhibits higher sensitivity and specificity than serum CRP (162). CRP can be considered a good candidate due to its 1000-fold elevation in response to infection and the positive correlation between the serum and

pleural CRP levels (163-164). However, few studies with limited samples sets have been published on this topic (156-157, 165-167).

DOI:10.14753/SE.2019.2224

2. Objectives

2.1- Bacterial distribution profile in bronchiectatic patients

There are several points which the guideline does not address

1) By consideration of empiric therapy in patient with no previous bacteriology, the guideline does not take in consideration certain risk factors: patient age, bronchiectasis location, disease patter (bilateral vs unilateral).
2) The guideline generally recommends on taking routinely sputum bacteriology but do not make special subgroup that prone to certain infections.

Thus, the aims of this study were to assess in bronchiectatic patients with infective exacerbations the following clinical parameters:

1) The direct influence of patient age on bacterial profile
2) Characterize the lobar bacterial distribution of the bronchiectasis
3) Report the resistance rates of *P. aeruginosa* and *H. influenzae*
4) Explore the isolation rate of NTM.

2.2 Role of CRP in detecting parapneumonic effusion

We have very good criteria for defining empyema (pus appearance, pleural glucose<60, pleural PH<7.2) but with less usefulness for early detection of parapneumonic effusion before it becoming complicated.

Thus, the second aims of this study were:

1) Evaluating the sensitivity and specificity of pleural CRP levels in diagnosing parapneumonic effusions
2) Looking for the role of pleural CRP levels in distinguishing exudative from transudative effusions.
3) Evaluation the utility of pleural CRP as a novel biomarker of infection in the pleural space.

3. Methods

3.1 Study subjects of bronchiectasis bacterial distribution research

A retrospective cohort study of 339 individuals with a diagnosed infectious exacerbation of bronchiectasis occurring between January 2006 and December 2014 was conducted at the Rabin Medical Center (RMC), PetachTikva, Israel.

The criteria for inclusion in the study were as follows:

1) Diagnosis of bronchiectasis established by high-resolution computed tomography (HRCT) within <1 year before entry into the study (168).

2) Acute infectious exacerbation of bronchiectasis.

3) Performance of a bronchoalveolar lavage (BAL) and bacterial cultures.

Medical charts were analyzed for demographic data, clinical and radiological reports, and microbiologic information. We reviewed the results of the BAL cultures and HRCT to characterize the bronchiectasis lobar distribution. The BAL culture results were organized into 9 groups: no bacteria (or "normal flora"), *H. influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, other *Streptococcus* species (Groups A-F), *P. aeruginosa*, NTM, *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella*,*Proteus*, *Enterobacter*, *Serratia*) and others (*Moraxella catarrhalis*, *Stenotrophomona smaltophilia* and *Acinetobacter* species). The patient population was divided into 2 groups to compare the distribution of the BAL culture results according to age, using the sample median age as the groups' separation line. The lobar distribution of bronchiectasis in the population sample and the bacterial distribution prevalence in each pulmonary lobe were also mapped. Each bacterial presentation in bilateral disease (both lung-diffuse distribution) was analyzed and compared to unilateral (one lung-local distribution) bronchiectatic disease. Finally, antibiotic susceptibility testing was performed for all isolated bacteria.

3.2 BAL procedure

The patient was placed in the supine position for fiberoptic bronchoscopy and nasal oxygen was administered at 2–4 liters per minute. Patients received topical anesthesia with lidocaine, intravenous (IV) alfentanil 0.5 mg, and intermittent boluses of IV midazolam. No other sedating or hypnotic agents were used in this study. The fiberoptic bronchoscope (BF-1T240 Video Bronchoscope, Olympus Corporation, Tokyo, Japan; or Pentax FB-18X Fiber Bronchoscope, Pentax, Tokyo, Japan) was introduced transnasally

and advanced to target subsegmental bronchi. The BAL trap used to collect the specimen was connected to the suction channel of the bronchoscope only once distal of the vocal cords. Suction was avoided until the bronchoscope had traversed the vocal cords to avoid contamination by upper airway flora. The fiberoptic bronchoscope was wedged into a subsegmentary bronchus that was closest to the suspected infected lobe by radiology (e.g the lateral subsegment of right middle lobe (RML) bronchus or the superior subsegment of the lingular bronchus), and three aliquots of sterile saline (50 mL each) were instilled and aspirated. The first aliquot was discarded. The mean BAL fluid obtained for processing was 30 ± 10 ml.

3.3 Microbiologic samples

BAL samples were plated on blood agar, chocolate agar and MacConkey agar. All specimens were analyzed for mycobacteria using Ziehl-Nielsen stain and were cultured on Lowenstein medium and in Mycobacterium Growth Indicator Tubes (BACT MGIT 960, Becton Dickinson, USA). Negative bacterial cultures were discarded after 7 days, whereas MGIT and Lowenstein-Jensen cultures were discarded after 6-8 weeks. We used GenoType Mycobacterium DNA strip assay (HainLifescience GmbH, Nehren, Germany) for the detection and identification of mycobacteria species which were obtained from positive liquid and solid mycobacterial cultures. Susceptibility testing was performed using the National Committee for Clinical Laboratory Standards (169). Antimicrobial susceptibility testing was performed using Oxoid disks (Thermo Scientific, UK) and E tests (bio-Merieux, Marcy-L Etoile, France).

The study received ethical approval by the Helsinki Committee of the Rabin Medical Center Petach Tikvah, Israel.

3.4 Study subjects of pleural CRP research

A retrospective, single-cohort study of clinically significant pleural effusions was performed at the Rabin Medical Center in Petach Tikva, Israel. The inclusion criteria in the study were as follows:

1) Ambulatory patients who were under outpatient observation at the Rabin Medical Center Pulmonary Institute and were diagnosed with a new pleural effusion

2) Hospitalized patients who were referred for pulmonary consultation from internal medical services and received a diagnostic thoracentesis.

The study population consisted of 244 individuals who were treated at our institution between January 2011 and December 2013. The diagnoses were divided into five categories on the basis of the underlying disease.

3.5 Definitions of different types of pleural effusions

The diagnosis of malignant effusion was made when malignant cells were found on pleural fluid cytologic examination or in a biopsy specimen.

The pleural effusion was considered parapneumonic when it was associated with acute febrile illness with purulent sputum, pulmonary infiltrate responsive to antibiotic treatment or when a microorganism was identified in the pleural fluid. Empyema was defined as a thick, purulent appearing of parapneumonic effusion.

Tuberculous pleural effusion was diagnosed based on positive cultures for mycobacterium tuberculosis or when the pleural biopsy specimen revealed typical epithelioid cell granulomas.

The effusion was attributed to congestive heart failure (CHF) in individuals with findings of an enlarged heart, radiographic pulmonary venous congestion, and peripheral edema responding to diuretic treatment in the absence of malignancy or pulmonary infiltrates associated with an inflammatory process.

The diagnosis of post-lung transplantation pleural effusion was made in patients who had undergone recent lung transplantation and lacked evidence of malignancy, infection or rejection.

3.6 Laboratory Studies

Pleural fluid samples were obtained with thoracentesis before treatment soon after the diagnosis of pleural effusion. Samples were analyzed for total differential cell counts, CRP, glucose, total protein, lactate dehydrogenase (LDH), pH, amylase and cholesterol. Additionally, cytologic examination and bacterial cultures using blood agar, chocolate agar and MacConkey agar, Lowenstein medium, and Mycobacterium Growth Indicator Tubes (BACT MGIT 960, Becton Dickinson, USA) were routinely obtained for all pleural fluid samples. All specimens were analyzed for mycobacteria using Ziehl-Neelsen stain.

The supernatant was obtained by centrifugation at 300 rpm for 15 min and stored at -20°C until assayed. The clinicians who performed the laboratory studies were blinded to the clinical diagnosis of the pleural effusion.

CRP analysis was performed on a Beckman Coulter AU 2700 analyzer using a particle-enhanced immue-turbidimetric method and latex particles coated with monoclonal anti CRP antibodies. The test is linear within a concentration range of 0.008-8 mg/dl. The CRP reference range values were 0-0.5 mg/dl.

3.7 Statistical analysis

Data were collected retrospectively. Descriptive data were expressed as the mean and standard deviation (SD) or number of patients (n) and percentage (%). The differences between bacterial profiles according to age were examined using the Fisher exact test (Table 1). Statistical significance was set at $p<0.05$. A univariate logistic regression analysis was performed to determine the relationship between each bacterial infection and patient age.

To determine the variables that were most significantly associated with parapneumonic effusion, we included all pleural parameters that were traditionally used to indicate the type of effusion (PH, LDH, glucose, neutrophils, CRP) in a backward stepwise logistic regression.

To evaluate the diagnostic performance of CRP, as a marker for differentiating between parapneumonic effusions and other pleural effusions, receiver operator characteristics (ROC) analysis was performed for all significant differences between groups. ROC curves were generated by plotting the sensitivity against 1-specificity, and the area under the curve (AUC) with 95% confidence intervals (95% CI) was calculated. The optimum cut-off point based on the ROC analysis was established by selecting the value that provides the greatest sum of the sensitivity and specificity, i.e., the point closest to the upper left point of the ROC plot. For the optimum cut-off point provided by each ROC analysis, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas. To calculate the ROC curves and AUCs, we used SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

4. Results

4.1 BAL bacterial isolation

A total of 339 patients were identified as having NCFBr, with a median age of 64 years (Table 8). Of the sample population, 133 (39%) were males and 206 (61%) were females, a 1:2 relation. 319/339 (94%) underwent a single BAL procedure while 20/339 (6%) underwent a repeated second procedure during the study period.

Table 8 - BAL microbiologic profile in patients according to age.

	Age ≤ 64 years (N = 177; 67 male) % (n)	**Age >64 years (N = 162; 66 male) % (n)**	**P value**
No bacteria	37.8% (67)	40.7% (66)	NS
Haemophilus influenzae	25.4% (45)	9.2% (15)	<0.0001
Staphylococcus aureus	10.1% (18)	10.4% (17)	NS
Streptococcus pneumonia	5.6% (10)	3% (5)	NS
Other *Streptococcus* species	6.7% (12)	6.1% (10)	NS
Pseudomonas aeruginosa	9.6% (17)	17.2% (28)	0.05
Non-tuberculous mycobacterium	6.7% (12)	10.4% (17)	NS
Enterobacteriaceae	4.5% (8)	9.2% (15)	NS
Unilateral / Bilateral	59.4% (105) / 40.6% (72)	61.7% (100) / 38.3 (62)	NS

On initial assessment of 387 BAL culture results, 65.6% were positive for pathogenic microorganisms; the remainder yielded no bacteria (normal flora) (Table 9). The most common organisms were *H. influenzae* in 60 patients (15.5%), *P. aeruginosa* in 45 (11.6%) and *S. aureus* in 35 (9%). Less common organisms included NTM in 29 patients (7.5%), Enterobacteriaceae in 23 (5.9%), *Streptococcus* species in 22 (5.7%) and *S. pneumoniae* in 15 (3.9%). Of the total culture results, 25 (6.5%) were defined as "other" bacteria, including *Moraxella catarrhalis, Stenotrophomonas maltophilia* and *Acinetobacter* species.

Table 9- A total of 387 BAL cultures results in 339 patients with bronchiectasis.

Type of Bacteria	Percentage of positive cultures (n)
No bacteria	34.4% (133)
Haemophilus influenzae	15.5% (60)
Pseudomonas aeruginosa	11.6% (45)
Staphylococcus aureus	9% (35)
Non-tuberculous mycobacterium	7.4% (29)
Others	6.5% (25)
Enterobacteriaceae	5.9% (23)
Other *Streptococcus* species	5.7% (22)
Streptococcus pneumoniae	3.9% (15)
Total	100% (387)

During the study period, BAL collection was performed twice on 20 patients, of which 9 samples (yellow color) revealed the same results in both cultures (Table 10).

Table 10- bacterial cultures results in patients who underwent BAL for the second time.

Patient number	First BAL	Second BAL	Time span between BALs (months)
1	*H. influenzae*	No bacteria	12
2	No bacteria	No bacteria	10
3	*Stenotrmaltophila*	*P. aeruginosa*	24
4	*Escherichia coli*	*Escherichia coli*	9
5	*Mycobacterium simiae*	*Mycobacterium simiae*	8
6	No bacteria	No bacteria	22
7	No bacteria	*H. influenzae*	34
8	No bacteria	*Klebsiellaoxytoca*	49
9	*Streptococcus gr b*	*H. influenzae*	5
10	No bacteria	No bacteria	27
11	*H. influenzae*	*H. influenzae*	36
12	Acinetobacter sp	No bacteria	15
13	Streptococcus sp	Mycobacterium intracellulare	2
14	*P. aeruginosa*	*P. aeruginosa*	22
15	No bacteria	No bacteria	25
16	No bacteria	*Staph aureus*	11
17	*H. influenzae*	No bacteria	34
18	*Staph aureus*	*Staph aureus*	29
19	No bacteria	*P. aeruginosa*	12
20	*H. influenzae*	No bacteria	7

4.2 BAL isolation according to age

Univariate analysis revealed a significantly higher frequency of *H. influenzae* in the younger age group (25.4% in ≤64 years group *vs.* 9.2% in >64 years group, p<0.001). By contrast, *P. aeruginosa* was found at a significantly higher frequency in those aged >64 years compared with the younger patient group (17.2% *vs.* 9.6%, respectively, p=0.05). There was also a higher but not statistically significant frequency of NTM (10.4% >64 years *vs.* 6.7% ≤64 years) and Enterobacteriaceae (9.2% >64 years *vs.*4.5% ≤64 years) detected in the older age group.

Univariate logistic regression showed a similar pattern, whereby the frequency of *H. influenzae* was higher in younger patients (OR=0.969, p<0.0001, 95% CI 0.954-0.983), whereas the frequencies of *P. aeruginosa* (OR=1.027, p=0.008, 95% CI 1.007-1.048) and Enterobacteriaceae (OR=1.039, p=0.01, 95% CI 1.009-1.069) were much higher in older patients (Table 11).

Table 11 - *Univariate logistic regression of H. influenzae, P. aeruginosa and Enterobacteriaceae odds ratio (OR) according to age.*

Variable	P value	OR	95 % CI lower	95 % CI upper
Haemophilus influenzae	<0.0001	0.969	0.954	0.983
Pseudomonas aeruginosa	0.008	1.027	1.007	1.048
Enterobacteriaceae	0.01	1.039	1.009	1.069

CI confidence interval

4.3 Non-tuberculous mycobacterium (NTM)

NTM were isolated in 6.3% (29/339) of the study sample, which increased in prevalence to 10.4% (17/162) in the >64 years of age group. These NTM included *Mycobacterium avium-intracellulare* (n=14), *M. simiae* (n=8), *M. abscessus* (n=3), *M. chelonae* (n=2) and *M. fortuitum* (n=2).

The lobar distribution of NTM in all patients was as follows: 22.9% in the right upper lobe (RUL), 22.5% in the RML, 18.31% in the lingula, 14% in the right lower lobe (RLL), 12.6% in the left upper lobe (LUL), and 8.4% in the left lower lobe (LLL).

4.4 Lobar bronchiectasis distribution

There was no significant difference between the lobar distributions of bronchiectasis in the younger versus the older group (Table 1).

The general lobar distributions of bronchiectasis in all 339 patients were as follows: 25.9% in the RML, 20.7% in the RLL, 20.4% in the LLL, 13.8% in the lingula, 13% in the RUL, and 6.2% in the LUL.

4.5 Lobar bacterial distribution in bronchiectasis

In the LLL, RLL, and RML of the lung, the most frequent pathogen was *H. influenzae* (23% - 28% of the total bacterial species in each lobe), and the second most common bacterial species was *P. aeruginosa* (19% - 21% in each lobe; Figure 7). In the lingula, *H. influenzae* comprised 14% and NTM comprised 13% of all bacterial species in this lobe. In the RUL, the most common bacterial species was *P. aeruginosa*, and the second most common was NTM, at prevalence rates of 18% and 17%, respectively. Finally, in the LUL, the most prevalent bacterial species were NTM, and the second most common was *P. aeruginosa* (9% and 6% of the total lobe's bacterial species, respectively).

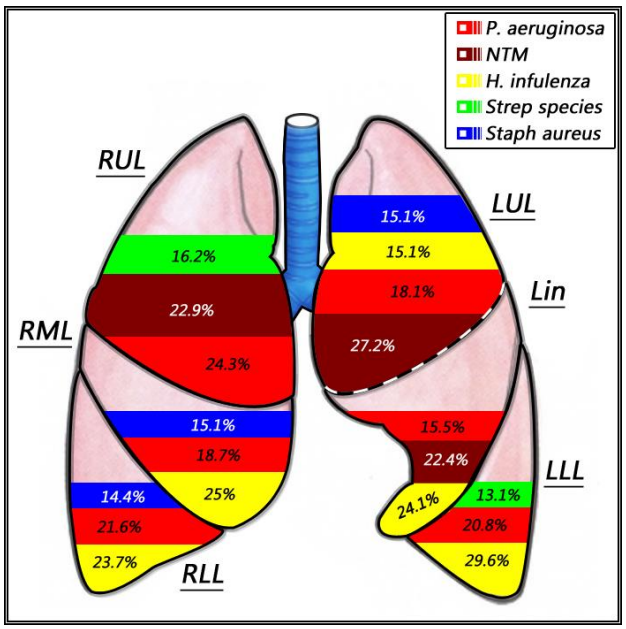

Figure 7- Lobar bacterial distribution in bronchiectatic patients. The prevalence of each bacterium is represented in each lobe. RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; Lin, lingula; LLL, left lower lobe.

NTM was found to have a significantly higher prevalence in bilateral versus unilateral bronchiectasis (65.5% [19/29] *vs.*34.4% [10/29], respectively, p=0.004). The other bacterial groups showed no statistically significant differences in their prevalence when comparing unilateral and bilateral disease (Table 12).

Table 12 - Bacterial distribution - unilateral versus bilateral bronchiectasis disease.

Type of bacteria	**Bilateral bronciectasis**	**Unilateral bronchiectasis**	P value
No bacteria	39.1% (52)	60.5% (81)	NS*
Haemophilus influenzae	38.3% (23)	61.6% (37)	NS
Staphylococcus aureus	48.4% (16)	51.5% (17)	NS
Streptococcus pneumoniae	46.6% (7)	53.3% (8)	NS
Pseudomonas aeruginosa	44.4% (20)	55.5% (25)	NS
Non Tuberculus Mycobacteria	65.5% (19)	34.4% (10)	0.004
Enterobacteriace	26% (6)	73.9% (17)	NS

*NS-non significant

4.6 Resistance to antibiotics

In this study, we reviewed the antibiotic sensitivity results of the two largest bacterial groups: *H. influenzae* and *P. aeruginosa*. A total of 60 isolations of *H. influenzae* were observed; of these, 18.3% (11/60) showed resistance to sulfamethoxazole/ trimethoprim, 16.7% (10/60) to ampicillin and 1.7% (1/60) to ciprofloxacin. No resistance to cephalosporins, tetracycline or ertapenem was observed.

Of a total of 45 isolations of *P. aeruginosa*, 17.8% (8/45) showed resistance to ciprofloxacin, 6.7% (3/45) to gentamicin and amikacin, 4.4% (2/45) to piperacillin and 2.2% (1/45) to imipenem and ceftazidime; none showed resistance to tazocin and colistin.

4.7 Pleural effusion distribution

Of the 244 patients classified as having pleural effusions, 180 (73.7%) were diagnosed with exudative effusion, 44 (18%) were diagnosed with transudative effusion and 20 (8.1%) were excluded from the study due to lack of definitive diagnosis. The exudative effusion group was further divided into the following three subgroups according to the diagnosis: 119 (53.1%) malignant effusion, 38 (16.9%) parapneumonic effusion, and 23

(10.2%) lung transplant recipients (Table 13). Tuberculous pleural effusion was not diagnosed in any patient.

Table 13 - Clinical characteristics of the study population and pleural fluid parameters.

	Malignancy	**Heart failure**	**Parapneumonic-total effusions**	**Parapneumonic-Empyema only**	**Lung transplant**	**P value**
n	119/244 (53.1%)	44/244 (19.6%)	38/244 (16.9%)	6/244 (2.4%)	23 (10.2%)	
Male, (%)	52.9	77.3	50	60%	15	0.02
Age, years	70.9±12	76.2±10.6	64 ±17.9	59.5±19.7	58.3±6.4	<0.001
Amount, ml	1514.4±1694.5	1380.6±668.2	983.4±552.6	681.6±439.5	715.2±259.9	=0.01
CRP level, mg/dl	1.19±1.51	0.57±0.81	5.38±4.85	9.06±6.72	2.77±2.66	<0.001
WBC, K/micL	2.49±7.56	0.81±1.05	1.59±1.84	2.2±1.1	9.13±21.82	=0.003
Neutrophils, %	21.31±15.48	17.86±11.86	30.5±26.3	44.3±40	22.33±24.53	0.01
Lymphocyte, %	51.79±23.97	51.33±23.27	44.92±28.19	30.1±30	60.99±30.09	NS
Eosinophils, %	1.38±2.25	0.99±1.08	1.83±2.92	0.76±1	0.64±1.05	NS
Cholesterol, mg/dl	74.64±33.58	36.9±18.77	59.63±33.64	69±26.9	82.09±37.44	<0.001
Triglyceride, mg/dl	47.92±186.24	21.1±16.37	34.95±33.21	68.4±79	33.55±20.96	NS
Glucose, mg/dl	116.93±51.35	133.24±41.22	113.74±55.27	68±58.7	133.7±63.75	NS
Total protein, g/dl	4.31±1.06	3.07±1.05	3.22±1.16	3.98±0.96	3.51±0.84	<0.001
Amylase, U/L	100.99±309.9	48.97±23.52	37.36±21.99	37.4±25.6	35.85±16.22	NS
LDH, U/L	613.31±1327.75	405.21±1122.8	998.39±2244.33	4336.6±5235.1	2337.1±6176.01	0.01
pH	7.45±0.13	7.47±0.08	7.4 ±0.23	7.29±0.32	7.47±0.24	NS

4.8 Pleural CRP level in pleural effusions

The pleural CRP levels differed significantly among all four groups ($p<0.001$). The mean values from highest to lowest were as follows: parapneumonic (5.38±4.85 mg/dl), lung transplant (2.77±2.66 mg/dl), malignancy (1.19±1.51 mg/dl) and heart failure (0.57±0.81 mg/dl) (Figure 8). A backward logistic regression model selected CRP as the only predictor of parapneumonic effusion (OR=1.59, 95% C.I=1.37-1.89, $p<0.0001$).

DOI:10.14753/SE.2019.2224

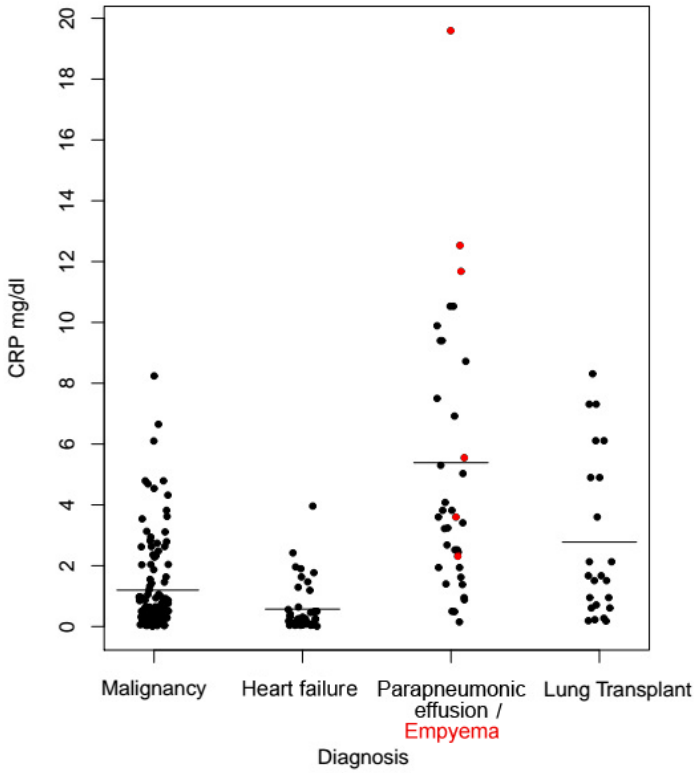

Figure 8 -Pleural fluid CRP levels in effusions secondary to pneumonia, malignancy, post-lung transplantation and heart failure. Each point represents one pleural fluid sample. The red points represent patients with empyema.

To determine the efficiency of pleural fluid CRP measurement in distinguishing parapneumonic effusion from the other 3 groups, we used ROC analysis (Figure 9A). A CRP cut-off value of 1.38 mg/dl yielded 84.2% sensitivity, 71.5% specificity, 37.6% positive predicted value and 95.6% negative predicted value. Although the area under the curve (AUC) of the pleural CRP was as high as 0.85 (Figure 9A), it was lower for the following other pleural parameters: glucose (0.54), pH (0.61), neutrophils (0.59) and LDH (0.57). As a result, we could not calculate their optimal cut-off values.

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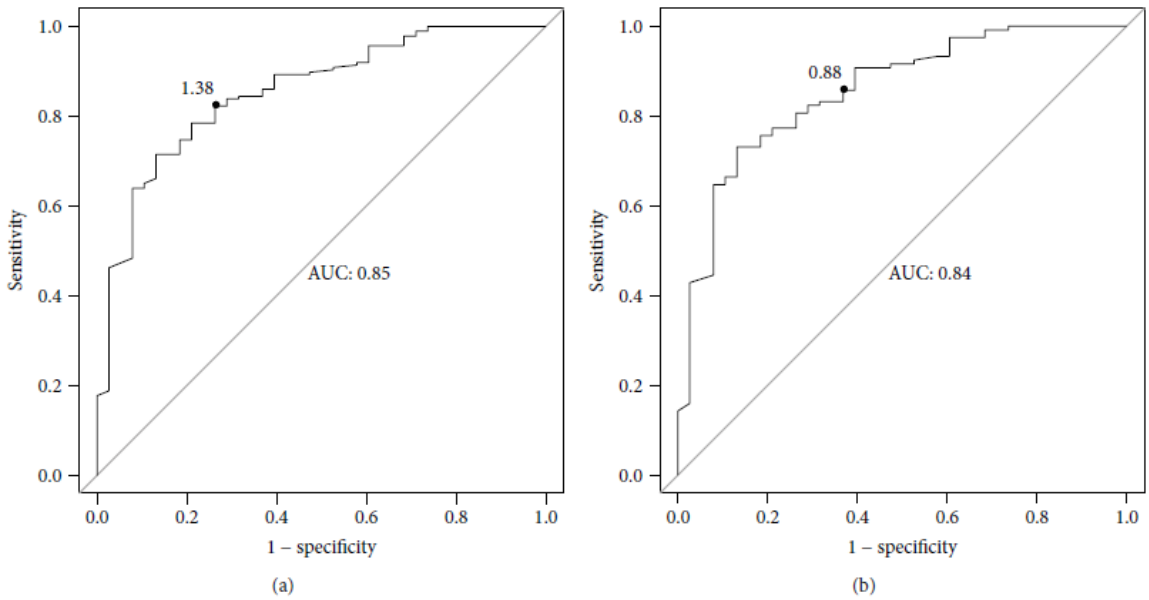

Figure 9 - Receiver operator characteristic (ROC) analysis curves of pleural fluid CRP levels for differentiating between different effusion types.

9 (A) - ROC curve of CRP levels for differentiating parapneumonic pleural effusions from other types of pleural effusions such: malignant, heart failure and post-lung transplantation.

9 (B) - ROC curve of CRP levels for differentiating between parapneumonic and malignant effusions.

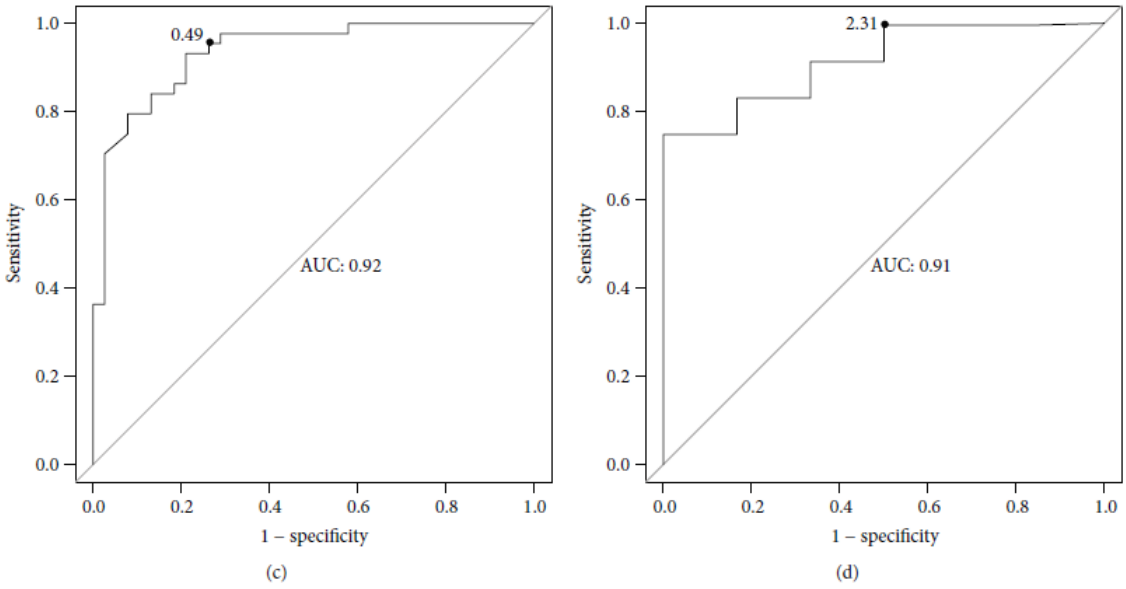

Figure 9 (C) - ROC curve of CRP for differentiating between parapneumonic and heart failure pleural effusions.

Figure6 (D) ROC curve of CRP for differentiating empyema from other types of effusion such: malignant, heart failure and uncomplicated parapneumonic pleural effusions

CRP was a good marker for distinguishing parapneumonic effusion from: post-lung transplantation effusion (1.93 mg/dl cut-off value, 75% sensitivity, 56% specificity); malignant effusion (0.88 mg/dl cut-off value, 87% sensitivity, 64% specificity- Figure 9B) and heart failure effusion (0.49 mg/dl cut-of value, 93% sensitivity, 72% specificity- Figure 9C).

CRP was a moderately good marker for differentiating between empyema and other types of effusions (2.31 mg/dl cut-off value, 83.3% sensitivity, 74.7% specificity, 8.3% positive predictive value and 99.3% negative predictive value- Figure 9D)

We also determined the efficiency of pleural fluid CRP measurements in distinguishing among heart failure effusion and the other 3 groups (Table 14). Using a cut-off value of 0.64 mg/dl, CRP exhibited 79.5% sensitivity, 59.4% specificity, 32.4% PPV and 92.4% NPV.

Table 14 - Receiver operating characteristic curve analysis of the accuracy of biomarkers for identifying parapneumonic effusions.

Biomarker	CRP optimal cut-off value (mg/dL)	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV** (%)	AUC***
PE† versus Mal‡, HF§, and LTx¶	>1.38	84.2	71.5	37.6	96.7	0.85
PE versus LTx	>1.93	75.7	56.5	71.4	61.9	0.67
PE versus Mal	>0.88	87.8	64.7	40.8	95	0.84
PE versus HF	>0.49	93.9	72.7	72	94.1	0.92
HF versus Mal, PE, and LTx	<0.64	79.5	59.4	32.4	92.2	0.76
Emp£ versus Mal, LTx, and uncomplicated PE	>2.31	83.3%	74.7%	8.3%	99.3%	0.7

†PE: parapneumonic effusions; ‡Mal: malignancy; £Emp: empyema; §HF: heart failure; ¶LTx: lung transplant; *PPV: positive predictive value; **NPV: negative predictive value; ***AUC: area under the curve.

Pleural white blood cells counts were significantly different among all four groups (p=0.003) with the following means: 9.13±21.82K/micL (lung transplant), 2.49±7.56K/micL (malignancy), 1.59±1.84K/micL (parapneumonic) and 0.81±1.05K/micL (heart failure).

DOI:10.14753/SE.2019.2224

Pleural neutrophil differentials were also significantly different among all four groups (p=0.01): 30.5±26.3% (parapneumonic), 22.33±24.53% (lung transplant), 21.31±15.48% (malignancy) and 17.86±11.86% (heart failure).

5. Discussion

5.1 Lobar bacterial distribution in NCFBr

In this retrospective cohort study of 339 individuals with infectious exacerbations of NCRBr, we found *H. influenzae* to be a more frequently associated bacterial pathogen in younger patients, whereas *P. aeruginosa*, NTM and Enterobacteriaceae were more frequently observed in the older age group. Previous studies have shown that *P. aeruginosa* pathogen carriage is associated with earlier diagnosis (54), lower FEV_1, polymicrobial colonization (170), poorer quality of life and more hospital admissions (65). Moreover, it is possible that the older patients had a longer hospital stay, longer duration of the disease and more serious lung damage; however, because this study was retrospective in nature, this information was not available.

The *H. influenzae* prevalence in our general sample was 15.2%, and it increased to 25.4% in the younger patient group. The frequency of this pathogen has been reported to range from 14% to 52% in patients with NCFBr in prior studies (53-55). Studies using a protected specimen brush have confirmed the presence of *H. influenzae* in the distal airways (54), although it is still possible that this organism represents oral contamination. We also found that (9/20) 45% of patients showed the same pathogen on repeat BAL analysis as in the initial study. This finding is supported by previous reports by Pasteur *et al*. (40) and King *et al*. (53), who found that 66% and 50% of subjects with bronchiectasis were colonized with similar bacteria when checked over 1- and 5-year periods, respectively. By contrast, McDonnell *et al*. (170) reported that 45% of patients with previous positive cultures for *P. aeruginosa* had subsequent negative cultures, most likely as a result of effective treatment of the bacterial infection. Based on these previous studies, one may conclude that previous BAL bacteriologic identification may be useful in determining which antibiotic to utilize for treatment.

Our study also showed a high prevalence of NTM as a potential bacterial pathogen in subjects with infectious exacerbations of NCFBr. We found an increased prevalence of NTM in older patients, reaching as high as 10% of the total lung bacteria in patients > 64 years of age; in comparison, other studies have reported NTM to represent up to 6% of the pathogenic profile in bronchiectatic patients (40, 55, 74). We suspect that the prevalence of NTM in bronchiectasis may be even higher, as attempts of NTM cultures are rarely reported (74). Thus, we suggest that attempts to isolate and treat NTM should

be performed more frequently. Consideration should also be given to providing surveillance screening for NTM when treating patients >64 years of age, especially individuals showing a diffuse bronchiectatic pattern on HRCT examination.

We also performed a study of antibiotic resistance among *P. aeruginosa* and *H. influenzae* isolates from patients with infectious exacerbations of bronchiectasis. For both bacteria, we did not find pan-resistance or multi-resistant strains. The highest resistance rate for *P. aeruginosa* isolates was to quinolone (17.8%), perhaps reflecting its widespread use. The resistance to alternative drugs such ceftazidime and carbapenem was very low, and no resistance was found to colistin and tazocin. Very high resistance rates for *H. influenzae* isolates to sulfamethoxazole/trimethoprim and ampicillin were found, which we speculate might be associated with frequent use of these antibiotics and longer hospitalization in our institution however this data was not available to us for analysis.

This study had a number of limitations, most of which derive from its observational design. Many of the individuals included in the study were receiving care in the pulmonary clinics of the hospital. The inclusion requirements of our study, which included previous HRCT and concurrent BAL isolation, precluded the inclusion of individuals not under the care of the pulmonary staff. The study conclusions, therefore, may not be generalizable to groups of patients treated by other hospital staff members. Second, we cannot entirely assume that microbiologic samples isolated from different lobes truly represent the bacteriological population in the sampled lobe, as they may instead represent bacterial colonization. Nonetheless, it may be expected that the areas of bronchiectatic lung are the most likely areas to develop acute infection. BAL isolation is a procedure that may readily provide reliable samples for culture; furthermore, this technique is associated with few complications and is readily available at our center.

The strengths of this study include the following. First, we included a large sample of bronchiectatic patients. Second, our analysis of BAL culture results provided an accurate representation of the flora of the distal airways, whereas sputum samples represent more proximal flora.

5.2 The role of pleural CRP in parapneumonic effusion detection

The present study provides evidence for the utility of the pleural fluid CRP measurement in diagnosing parapneumonic effusions. Early recognition of this diagnosis prevents possible adverse consequences from an untreated infection of the pleural cavity. We found that the pleural CRP levels were higher in parapneumonic effusion than in other effusion types, with a cut-off value of > 1.38 mg/dl. At this cut-off level, we found a low PPV but very high NPV, which implies a modest utility in confirming the diagnosis but a powerful tool for excluding it. The same is true with empyema, due to its high NPV, a cut-off value below 2.31 can theoretically decrease the necessity of pleural drainage for complicated effusions. Our results are consistent with other studies that reported higher CRP levels in parapneumonic effusions among the exudative categories, which exhibit a range of cut-off values of 3 to 9 mg/dl (109, 156-157,162,166). The differences in the absolute cut-off values can be attributed to the different analysis methods used for measuring CRP. Moreover, when the underlying cause of a pleural effusion is obscure, a high pleural CRP level combined with pleural neutrophil predominance, lower pleural glucose and lower pleural pH may shift the diagnosis towards an infectious etiology.

This study corroborates and amplifies previous investigations. Pleural CRP levels are reportedly higher in parapneumonic effusions than in other types of exudates (109,162,166). Evaluation of the pleural CRP levels is a useful test for differentiating between complicated and uncomplicated parapneumonic effusions (156,165) and between acute and chronic inflammation (166). Kapisyzi et al (171) observed that the sensitivity of pleural CRP levels was higher than serum CRP levels in distinguishing transudative from exudative effusions as well as malignant from benign effusions. A prospective evaluation of seven biological markers in patients with different causes for exudative effusions demonstrated that CRP provides the largest AUC (0.92) for distinguishing between parapneumonic effusions and tuberculosis or malignant effusions (167). Kriopoulos et al and Gabhale et al reported that pleural CRP levels provided excellent sensitivity (100%) and good specificity (79, 98.8%) at cut-off levels of 5.3 and 9.08 mg/dl, respectively, for differentiating between parapneumonic effusion and tuberculosis or malignant effusions (109, 166). A few investigative reports have suggested that the combination of pleural fluid CRP levels with neutrophil count (156, 167) or adenosine deaminase (157) to be superior to pleural CRP levels alone for predicting parapneumonic effusion.

Finally, in addition to exhibiting diagnostic value, pleural CRP levels exhibit prognostic value and can serve as a supporting tool for drainage. Porcel at al (156) found that CRP levels >10 mg/dl were associated with complicated parapneumonic effusion and were associated with the need for pleural effusion drainage. Moreover, the combination of classical biomarkers (pleural pH <7.2, LDH>100 IU/dl, glucose<60 mg/dl) improves the accuracy of detecting parapneumonic effusion (156,165).

We also found that WBC counts were higher in pleural effusion post transplantation that in parapneumonic group. Moreover, in both groups the main pleural cell differential was lymphocyte. The explanation for post transplantation pleural lymphocytosis is disruption of lymphatic flow due to severance of allograft lung lymphatics (172).

The main strengths of this study are that it demonstrates the diagnostic value of pleural CRP measurements in a large cohort of patients with varying etiologies for pleural effusion. Second, although we used several markers of inflammation individually or in combination with CRP, only CRP as a single biomarker had the highest sensitivity and specificity in differentiating between parapneumonic effusions and other effusion types. Finally, for every thoracentesis performed in this study, the same pleural biomarker panel was collected, decreasing the probability of selection bias.

A limitation of the present study is its retrospective, single-center design. A second limitation is the inclusion requirement utilizing only hospitalized or ambulatory patients under medical observation of pulmonologists necessarily excluding individuals under the care of general physicians. A third limitation is the lack of serum CRP level data which could serve for control and comparison analysis. A fourth limitation is the inability to explore the CRP trend changing level during the progression/resolving of the parapneumonic effusion, due to the availability of a single point measurement of pleural CRP to each patient. Additionally, since the decision to initiate thoracentesis was based on the judgment of the pulmonary physician, there may have been sampling bias.

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6. Conclusions

6.1 lobar bacterial distributions in patients with bronchiectasis

In conclusion, this retrospective cohort study allowed us to show the effect of age on the bacterial pathogenic profile in bronchiectatic patients with infective exacerbations. We found *H. influenzae* to be more prevalent in younger patients, whereas *P. aeruginosa*, Enterobacteriaceae and NTM were more frequently isolated from older patients. Pathogens were also associated with different lobar distributions, with the RML, RLL and LLL showing a greater tendency to develop bronchiectasis than other lobes. This study highlights the importance of prospective studies in individuals with infectious exacerbations of bronchiectasis utilizing age, lobar location and previous bacteriologic analyses to inform antibiotic treatment and determine if such treatment can decrease morbidity, mortality, recurrent hospitalizations and total cost of care while improving quality of life.

6.2 The role of pleural CRP in parapneumonic effusion detection

Pleural fluid CRP levels can be used to discriminate between parapneumonic effusions and other types of exudative effusions, which may help distinguish between exudative and transudative effusions. A CRP level >1.38 mg/dl indicates the strong possibility of a parapneumonic effusion, whereas a level <0.64 mg/dl indicates a heart failure pleural effusion. This study highlights the need for prospective studies to demonstrate the prognostic effect of pleural CRP as an effective diagnostic biomarker. If confirmed in future studies, our results support the introduction of pleural fluid CRP into clinical practice for accurate detection of patients who may benefit from the initiation of antibiotic therapy and observation for the need for chest tube drainage.

DOI:10.14753/SE.2019.2224

7. Summary

Non-cystic fibrosis bronchiectasis (NCFBr) is a major cause of morbidity and frequent hospitalization due to frequent infectious exacerbations. In addition, those infectious exacerbations can easily evolve to parapneumonic effusion, which increases the morbidity dramatically. We analyzed the influence of patient's age and bronchiectasis location on the bacterial profile of patients with NCFBr. We also studied the usefulness of pleural C-reactive protein (CRP) biomarker levels in identifying parapneumonic effusion, which is potential complication in patients with bronchiectasis.

This was a single-center, retrospective review of 244 patients diagnosed with pleural effusions and 339 subjects with bronchiectasis exacerbation. The study involved patients at the Rabin Medical Center, Petach Tikva, Israel, between January 2006 and December 2014.

We found that *H. influenzae* was more prevalent in younger patients who have bronchiectasis, whereas *P. aeruginosa*, Enterobacteriaceae and NTM predominated in older patients. Different pathogens were associated with different lobar distributions.

In the lower lobes, *H. influenzae* was the dominant species isolated, whereas in the upper lobes and lingula it was non-tuberculous mycobacterium (NTM). While *Streptococci species* were more prevalent in LLL and RUL, *P. auriginosa* was homogenously distributed in all the lobes. These findings can support decision making on empirical treatment. Overall, the RML, RLL and LLL showed a greater tendency to develop bronchiectasis than other lobes.

On the base of these findings we recommend frequent screening of NTM in older patient (age>65) with bilateral disease and upper lobes bronchiectasis.

Pleural fluid CRP levels can be used to distinguish among parapneumonic effusions and other types of exudative effusions. CRP levels < 0.64 mg/dl are likely to indicate a pleural effusion from congestive heart failure, whereas levels ≥ 1.38 mg/dl are suggestive of an infectious etiology. While we have clear criteria for empyema, we do not have such for parapneumonic effusion. In order to facilitate the initial clinical work up, we recommend using pleural CRP in addition to the conservative pleural parameters. This is especially important in patients with multiple comorbidities with nonspecific pleural effusion etiology.

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8. Összefoglalás

A nem cisztás fibrózis eredetű bronchiectasia a gyakori fertőzéses exacerbatio miatt a megnövekedett morbiditás és a frekvens hoszpitalizació egyik jelentős oka. A fertőzéses exacerbatioból könnyedén alakul ki parapneumonia - pneumónia, empyema, bronchiectasia - okozta folyadékgyülem, mely drámaian növeli a morbiditás mértékét. Nem cisztás fibrózis okozta bronchiectasiaban szenvedő betegeknél vizsgáltuk az életkor, a bronchiectasia lokalizáció és bakteriális profil befolyásoló hatását. Mindezek mellett, a C-reaktív protein (CRP) biomarker szint hasznosságát tanulmányoztuk a parapneumonia okozta pleurális effúzió felismerésében, amely a bronchiectasia potenciális szövődménye. Ez egy egy központú, retrospektív tanulmány, melyben 244 pleurális folyadékgyülemmel és 339 bronchiectasiával diagnosztizált egyed vizsgálatára került sor 2006. január és 2014. decembere közt. A tanulmányban résztvevő egyedek a petach tikvai Rabin Medical Center, betegei Izraelben.

Eredményeink szerint a fiatalabb, bronchiectasival küzdő egyének között magasabb a *H. influenzae* prevalenciája, mindemellett idősebb betegeknél a predomináns patogének köze a *P. aeruginosa,* Enterobacteriaceae és a non-tuberculous mycobacterium (NTM) sorolható. Jellemző patogén disztribúció figyelhető meg különböző lobáris lokalizációkban.Vizsgálataink alapján az alsó lebenyekből izolált domináns, fertőzést okozó baktérium a *H. influenzae*, mig a felső lebenyekben és a lingulában, e posztot az NTM veszi át. *Streptococcus spp*. prevalenciája a bal alsó valamint a jobb felső lebenyben jelentős, míg a P. aeruginosa esetében homogen disztribució figyelhető meg. Ezek az eredmények vélhetően stabilabb támaszt nyújtanak az empirikus terápia kiválasztásánál. Összegezve, a bronchiectasia kialakulásának magasabb tendenciája figyelhető meg a jobb középső, jobb alsó és bal alsó lebenyekben.

Megállapításaink alapján az NTM gyakori szűrését javasoljuk idősebb (>=65 év), bilaterális valamint felső lebeny bronchectasiában szenvedő betegeknél.

A pleurális folyadékgyülemből nyert CRP szint ideális a parapneumonia okozta pleurális effúzó és egyéb etiológiájú exudatív folyamat megkülönböztetésére. 0.64 mg/ml alatti CRP érték congestiv szivelégtelenség okozta pleurális folyadékgyülem indikátora lehet, míg >=1.38 mg/ml feletti érték fertőzéses eredetre utal. Mindamellett, hogy világos diagnosztikai kritériumokkal rendelkezünk empyema esetében, ugyan ez nem mondható el a parapneumonia okozta folyadékgyülemmel kapcsolatban. Annak érdekében, hogy

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facilitáljuk a kivizsgálást, a konzervatív pleurális paraméterek mellett javasoljuk a pleurális folyadék CRP tartalmának vizsgálatát is. Ez kifejezetten multiplex komorbiditásoktól szenvedő betegeknél fontos, ahol a mellkasi folyadékgyülem etiológiája nem specifikus.

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DOI:10.14753/SE.2019.2224

10. Bibliography of the candidate's publications

10.1 Publications related to the dissertation:

1. **Lobar distribution in non-cystic fibrosis bronchiectasis predicts bacteriologic pathogen treatment.**
   Izhakian S, Wasser WG, Fuks L, Vainshelboim B, Fox BD, Fruchter O, Kramer MR.
   Eur J ClinMicrobiol Infect Dis. 2016 May;35(5):791-6.
   Impact Factor: 2.857
2. **The Diagnostic Value of the Pleural Fluid C-Reactive Protein in Parapneumonic Effusions**
   Izhakian S, Wasser WG, Fox BD, Vainshelboim B, Kramer MR.
   Dis Markers. 2016;2016:7539780.
   Impact Factor: 2.137

The studies were presented at:

A) Annual conference of Israel society of pulmonology, June 11-12, 2015- Israel.

B) American Thoracic Society 2015 International Conference, May 15-20, 2015 – Denver, USA.

http://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference.2015.191.1_MeetingAbstracts.A1120

C) 1st World Bronchiectasis Conference, July 7-9, Hannover, Germany.

10.2 Publications not related to the dissertation

3. **Bronchoscopic Drainage of Lung Abscesses Using a Pigtail Catheter.**
   Unterman A, Fruchter O, Rosengarten D, **Izhakian S**, Abdel-Rahman N, Kramer MR.
   Respiration. 2016 Dec 13.
   Impact Factor: 2.651

4. **Short-Term Improvement in Physical Activity and Body Composition After Supervised Exercise Training Program in Idiopathic Pulmonary Fibrosis.**
   Vainshelboim B, Fox BD, Kramer MR, **Izhakian S**, Gershman E, Oliveira J.
   Arch Phys Med Rehabil.2016 Feb 8.
   Impact Factor: 3.045
5. **Characterization of Patients who were Mechanically Ventilated in General Medicine Wards.**
   Izhakian S, Buchs AE.
   Isr Med Assoc J. 2015 Aug;17(8):496-9.
   Impact Factor: 0.879
6. **Achievement of partial combined control of major diabetes targets in primary care correlates with development of chronic complications in T2DM patients--A real life data.**
   Rapoport M, Harel N, Shasha Y, Barkan R, Kitaee E, Buchs A, **Izhakian S**, Aviel-Gadot E.
   Prim Care Diabetes. 2015 Dec;9(6):412-7. Epub 2015 Jun 15.
   Impact Factor: 1.570
7. **Effect of Jewish - Arab Ancestry and Gender Matching on Clinical Outcome of Lung Transplant Patients in Israel**
   Shimon Izhakian., Walter G. Wasser., Baruch Vainshelboim., Benjamin D Fox., Mordechai R. Kramer.
   Isr Med Assoc J. 2016 Aug;18(18)470-3.
   Impact Factor: 0.879
8. **The Effectiveness of Rabbit Anti-Thymocyte Globulin in Chronic Lung Allograft Dysfunction**
   Shimon Izhakian, Walter G. Wasser, Benjamin D Fox, Baruch Vainshelboim, Jacqueline E. Reznik, Mordechai R. Kramer.
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DOI:10.14753/SE.2019.2224

11. Acknowledgement

I would like to thank and express my gratitude to the following:

Prof. Lidia Sreter, for accepting me as her student to Semmelweise university Ph.D program.

Prof. Mordechai Kramer, the head of the Pulmonary institute at Rabin Medical Center, for being my academic counselor.

Prof. Veronika Muller for arranging my Ph.D home defense.

Miss. Krisztina Tolgyesi Lovasz, the head of the Ph.D secretary office, who efficiently handled the administrative process during my studies.

Miss. Anita Marosfalvi for helping me also with the administrative process.

Finally, I wish to thank my dear family for their unconditional love and support.