Chapter

Recent Approach in Microbial Pathogen Complications in Patients with Cystic Fibrosis

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Abstract

Cystic fibrosis (CF) is a multisystem genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Microbial infection is the defined characteristics of cystic fibrosis airway disease. This infection is caused by bacteria, fungi, and viruses which increase complications leading to patient death. Additionally, bacterial pathogens including *Haemophilus influenza*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, nontuberculous mycobacterium (NTM) species, and MRSA are attributed to pulmonary infections. Subsequently, fungal pathogens such as *Candida* sp. and filamentous fungi such as *Aspergillus fumigatus* can also lead to pulmonary infections. On the other hand, *Pseudomonas aeruginosa* is the most common bacterial pathogen leading to complications in CF distal airways disease. Also, *Aspergillus fumigatus* can lead to aspergillus lung diseases including allergic bronchopulmonary aspergillosis and aspergilloma formation. Control of pathogenic microorganisms associated with cystic fibrosis may prevent pulmonary complications and has the potential to improve the prognosis of this life-limiting disease.

Keywords: cystic fibrosis, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Haemophilus influenzae*, MRSA, CFTR, NTM, microbial infection, respiratory diseases

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive disease which basis on a dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) protein [1]. It is an inherited chronic disease that remains a common cause of morbidity and mortality in affected patients [2]. Also, it is a monogenic autosomal recessive condition affecting approximately 1 in 3000 of the Caucasian population and involves multiple organs, predominantly the lungs, gastrointestinal tract, pancreas, and liver [3].

Mutations in CFTR gene are resulted in defective mucociliary clearance leading to the production of thick and sticky bronchial mucus. This mucus facilitates the entrapment of viruses, bacteria, and fungal spores and acts as a suitable environment for growth of these microorganisms [4]. High morbidity and mortality rates are secondary to recurrent respiratory infections, which, when associated with this obstructive lung disease, lead to respiratory insufficiency [5]. Complex microorganisms and microbial communities can be identified in the distal airways in a

variety of respiratory diseases, including CF [6]. Chronic infection is the defined characteristics of CF airway disease. Conditions within the airways of patients living with CF are conducive to colonization by a variety of opportunistic bacterial, viral, and fungal pathogens [7].

Also, bacterial strains that colonize the respiratory tract include *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* [8], and *Burkholderia cepacia*; these have been recorded as the main common CF pathogens. Traditionally, *Pseudomonas aeruginosa* has been regarded as the main pathogen in CF, and chronic infections have been linked to disease morbidity and mortality [9]. Little attention has been paid to the role of *Aspergillus* sp. and other filamentous fungi in CF. It has become more apparent, however, that *Aspergillus* sp. may play an important role in chronic lung disease in CF [10]. This chapter discusses the complications of microorganisms in patients with cystic fibrosis, including bacterial, fungal, and viral pathogens and their effects on this disease leading to an increased risk of mortality.

2. Defining causes and history of cystic fibrosis

Cystic fibrosis is a multisystem genetic disease that affects children and young adults [11]. It is caused by mutations in CFTR gene leading to defective or insufficient amounts of functional CFTR protein and causes abnormalities in chloride (Cl), bicarbonate (HCO_3), and sodium (Na) transport across cell membranes with serious consequences on multiple organs. The CF lung disease is characterized by infection and inflammation with eventual bronchiectasis and eventual respiratory failure causing death in over 90% of CF patients [3].

Cystic fibrosis is an autosomal recessively inherited disorder caused by the presence of one of more than 1500 possible mutations in CFTR gene with an occurrence of the clinical disease being 1 in 2500 live births. This mutation leads to the nonfunction or loss of function of CFTR (a cyclic AMP-regulated chloride ion channel) leading to defective chloride ion transport through epithelial cell surfaces [12].

Since its first description in 1938, study of the genetics, pathophysiology, and clinical manifestation of the disease has led to the creation of new therapies and significantly improved quality of life and survival [2].

In CF, the biology and treatment strategies are important to understand for several reasons. Firstly, it is the most common cause of chronic respiratory failure in children and adults. Secondly, CF is a common reason for the dysfunction of pancreatic exocrine in children and adults. Thirdly, the majority also develop pansinusitis. Other potential consequences of CF include diabetes, liver disease, bone disease, and infertility [3].

Advances in research of CF have given a roadmap for the understanding of pathophysiology studies and treatment stages for other severe airway diseases, including chronic obstructive pulmonary disease, non-CF bronchiectasis, and asthma [11].

Survival of individuals with CF has improved significantly over the last half century from a median age of survival of 5 years in the 1970s to 40 years of age as of 2011. There are different reasons for improvement in clinical outcomes including the intense use of antibiotic therapy, advancement in chest physiotherapy, nutritional support, specialized CF units, and introduction of CFTR modulators; however, the majority of CF deaths still occurred in young adult especially in the ages between 21 and 30 years as a consequence of respiratory failure [13]. Recent research also suggests that even asymptomatic genetic carriers for CF may be at risk for subclinical physiological derangements, which can be exacerbated by external stresses and other environmental triggers [14, 15].

3. Microbial complications in patients with cystic fibrosis

Airways of patients with CF are usually infected with various microorganisms. In infected airways of CF patients, microhabitats can develop owing to local differences in the inflammatory reaction between the different focal areas of infection and also as a result of the competing activities of the many co-colonizing microbial populations [16]. Several bacterial strains are major causes of mortality and morbidity and have therefore been studied intensely [12].

3.1 Microbial infection in patients with cystic fibrosis

Chronic infection is a characteristic of CF airway disease. So, conditions in the airways of patients with CF are conducive to forming colonization by different microorganisms such as bacterial, fungal, and viral pathogens. Molecular identification of microorganisms has emphasized and recorded the polymicrobial nature of microbial infections in the CF airway microenvironment. Additionally, changes in airway of CF physiology through the loss of CFTR functionality lead to a variety of immune dysfunctions that permit microbial pathogen colonization and microbial persistence [7].

The prognosis of patients with the hereditary disease CF is substantially dependent on chronic respiratory infection and inflammation [17]. CF lungs are often colonized or infected with a complex microbial species, mainly composed of bacteria, provoking acute and chronic infections [18]. Airway infection accounts for 90% of the morbidity and mortality observed in CF patients. These chronic infections are typically associated with a few bacterial pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* complex [19].

High morbidity and mortality rates in cystic fibrosis patients are secondary to recurrent respiratory infections, which, when associated with this obstructive lung disease, lead to respiratory insufficiency, the main cause of death in these patients [5, 14].

CF lung disease is characterized by progressive colonization of respiratory tract infection by different bacterial strains leading to polymicrobial biofilms. Also, the emergence of nontuberculous mycobacteria (NTM) infections has caused additional challenges to patient management due to their multiresistance nature to antibiotics [20]. These are compounded by the impairment of mucociliary clearance and the inability to mobilize thick secretions within the airways. These result in mucus impaction, microorganism colonization, recurrent infections, persistent inflammation and death from respiratory failure. With improvement in CF prognosis, new challenges emerge, including the management of fungal colonization and infection [1].

3.2 Host-microbe interactions with CF lung infection

Bacterial and fungal infections are hallmarks of CF lung disease. In the era of long-term inhaled antibiotics and increasing CF patient survival, new “emerging” pathogens are detected in CF airways, yet their pathophysiological impact remains largely controversial and incompletely defined. As a consequence to chronic microbial triggers, innate immune cells, particularly neutrophils, are continuously recruited into CF airways where they combat pathogens but also lead to tissue injury through the release of oxidants and proteases. The coordinated interplay between host immune cell activation and pathogens is essential for the outcome of CF lung disease. A better understanding of this phenomena may enhance the survival in CF [21–23].

3.3 Nontuberculous mycobacteria species in patient with cystic fibrosis

NTM are wide environmental microorganisms causing chronic pulmonary infection in lung diseases such as CF. Also, pulmonary disease caused by NTM has a major threat with CF and difficult to diagnose and problematic to treat according to the US Cystic Fibrosis Foundation (CFF) and European Cystic Fibrosis Society (ECFS). Additionally, the most common NTM species identified in CF are the slow-growing *Mycobacterium avium* complex (MAC) including *M. intracellulare*, *M. avium*, and *M. chimaera* and the rapid-growing *M. abscessus* complex (MABSC) including subspecies of *M. a. abscessus*, *M. a. massiliense*, and *M. a. bolletii*. Other less common NTM species include *M. kansasii*, *M. simiae*, and *M. fortuitum* [24].

In addition, there are more than 100 types of NTM, and more are being found every year. The reported prevalence of NTM in CF varies widely from 45 to 40% with *Mycobacterium avium* complex and *Mycobacterium abscessus* complex being the most common [25, 26].

4. Airway colonization process in patients with cystic fibrosis

Colonization of microorganisms in respiratory tract infection within young CF patient includes common pathogens such as *S. aureus* or *H. influenza* then succeeded by *P. aeruginosa* infection in the latter stages of CF.

It is well established that *Pseudomonas aeruginosa* is a frequent and virulent pulmonary pathogen in patients with CF [27]. After a period of intermittent colonization, the organism becomes permanently established and is difficult to eradicate. Most patients with CF become chronically infected with wild-type *P. aeruginosa* strains in early childhood [28]; prevalence increases with age, so that as many as 80% of patients with CF are infected by the time they reach 20 years [27]. During the years following initial colonization, the wild-type strains uniformly mutate into mucoid variants [28]. Conversion to the mucoid phenotype is thought to be driven by the unique CF microenvironment [29–31]. For patients with CF, this conversion results in a significant increase in morbidity and mortality accompanied by a measurable decline in pulmonary function [28]. The mucoid matrix is believed to allow the formation of protected biofilm microcolonies [30, 32] and provide increased resistance to opsonization, phagocytosis, and digestion [33]. Furthermore, resistance to various antibiotics is increased [27, 34].

The potentiality of pathogenic bacterial colony morphotypes in *P. aeruginosa* evolved to a non-swimming phenotype form. So, motility is considered as one of the first steps of *P. aeruginosa* in CF lungs that lead to adaptation steps including biofilm formation and progress to chronic infection. Also, impaired swimming motility seemed to be a candidate to disease marker of *P. aeruginosa* infection development. So far, the pathological changes in the lungs are best studied due to the high mortality rates linked to poorer lung function and recurrent development of infections [18].

Also, the lungs of people with cystic fibrosis are predominantly colonized with *Pseudomonas aeruginosa* using the following mechanism: firstly, reduced mucociliary clearance combined with the malfunction of antibacterial peptides; secondly, impaired defense of the lungs due to low levels of glutathione and nitrous oxide; thirdly, reduced ingestion of bacteria by lung cells; and finally, increased numbers of bacterial receptors [35].

On the other hand, earlier age of infection with *P. aeruginosa* in our population was strongly associated with greater likelihood of severe lung disease later in life, most particularly in those subjects who acquired *P. aeruginosa* before the age 5; the

observed association was stronger in females than in males. *P. aeruginosa* infection may be a cause of more severe CF lung disease but may also be a marker of some other processes determining lung function in children with CF [36].

5. Fungal infection in patients with cystic fibrosis

Fungi are generally divided into molds or yeasts with the latter circumferentially shaped with a one-celled thallus. Molds also known as filamentous fungi grow as branching cylindrical hyphae [1]. Aspergillosis is the most well-characterized and well-recognized *Aspergillus* disease in CF, but reported prevalence varies significantly and is likely to be underdiagnosed. Aspergilli are saprophytic, spore-forming, filamentous fungi found ubiquitously in the environment. *A. fumigatus* is the most prevalent species causing human disease and is the species most frequently isolated from the respiratory secretions of CF patients [37].

Yet, the relative contribution of other emerging pathogens, such as *S. maltophilia*, *Aspergillus*, *Candida*, and *Scedosporium* species, remains less precisely defined. *Aspergillus fumigatus* is the only species that is associated with an increased risk for infection with *P. aeruginosa* [9]. *Candida* sp. is found in as many as 70% of patients with CF. The clinical significance of *Candida* in patients with CF is not clearly understood, but most clinicians discount these organisms as not significant [38]. Fungemia caused by *Trichosporon* has been reported in two patients with CF, who were double lung transplant recipients [39]. Some species such as *Exophiala dermatitidis* grow as unicellular fungi (yeast) at human body temperature but in the case of filamentous fungi at room temperature. *Trichosporon* species as of any *Candida* species may produce true mycelium in culture conditions or in host tissues. So, there are variations in isolation and purification of yeast and filamentous fungi. Additionally, some fungal strains are thermotolerant filamentous mycota such as *Aspergillus fumigatus* which are the most common, and others include *Scedosporium* species and *E. dermatitidis* [1]. *A. fumigatus* is frequently detected in respiratory secretions of both adults and children with CF. Once present in the airways, *Aspergillus* can exacerbate lung inflammation, establish infection, and trigger hypersensitivity responses [10].

In patients with CF, complications increased when exposed to *A. fumigatus* spores causing impaired mucociliary clearance and defective innate immune responses leading to accumulation and persistence of fungal spores within the smaller airways. Also, germination of spores leads to the formation of fungal hyphae and release of antigens, phospholipases, proteases, and other virulence factors which damage the airway of epithelial cells and allow a large dose of antigenic factors access to the interstitial and vascular compartments [40].

Also, exotic genera can be found in CF airway secretions, such as *Penicillium*, *Alternaria*, or *Scedosporium*. Some studies provide the first evidence that even healthy airways are not sterile and contain distinct fungi called "pulmonary mycobiome." The kinetics, dynamics, and disease relevance of the pulmonary mycobiome, however, are poorly understood [7]. The majority of yeasts in CF belong to *Candida* species and the most common species such as *Candida albicans* and other yeast strains recorded such as *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, and *Candida dubliniensis* [1].

6. Viral infection in patients with cystic fibrosis

Progression of CF respiratory disease is influenced by viral infection. Respiratory viral infections include respiratory syncytial virus (RSV), rhinovirus,

influenza, para influenza, and adenovirus which is RSV and influenza infection leading to decreases in lung function [41]. Also, viral respiratory infections show pronounced and long-lasting effects on patients with CF, resulting in significant declines in forced vital capacity (FVC), forced expiratory volume in 1 second (FEV-1), and significant increases of both the frequency and duration of hospitalizations especially that the frequency of viral respiratory infections is closely associated with pulmonary deterioration in patients with CF [42]. Additionally, viral respiratory infections occur in equal frequency in CF patients and healthy controls which in CF patients viral upper respiratory tract infections are associated with lower respiratory tract symptoms in 31–76%. Viral infections cause long-term respiratory morbidity in CF patients [43].

7. Prevention and control of microorganisms in cystic fibrosis

Despite the development of antibiotics and vaccines, patients with CF are still faced with many debilitating and fatal infections, which demonstrate the adaptability of microbial pathogens [44]. The choice of antibiotic depends on in vitro sensitivity patterns. Physicians treating patients with CF are increasingly faced with infection with multidrug-resistant strains of *Pseudomonas aeruginosa*. Also, innately resistant organisms such as *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans* become more prevalent [45].

Additionally, many studies resulted in strong indicator for a relevant importance of mutators in bacterial populations especially in infectious diseases. High prevalence and mutators are recorded environmentally in chronic respiratory infection with *Pseudomonas aeruginosa* in CF patients [46].

7.1 Strategies and guidelines for bacterial infection control within CF patients

Guidelines on infection control developed for general patient populations are applicable to CF patients. Historically, before 1998, guidelines on infection control were applied to acute-care hospitals. Recently it includes for non-acute-care settings. Also, it has shifted from hospitals to home and outpatient clinics to reduce days of hospitalization and provide chronic suppressive treatments. Infection control in CF guidelines has been developed by Healthcare Infection Control Practices Advisory Committee (HICPAC) and Centers for Disease Control and Prevention (CDC) [47].

Common pathogens that infect the lungs of CF patients include *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Burkholderia cepacia*. Aggressively treating pulmonary infection with antibiotics has contributed to improved survival in patients with CF but has also promoted multiple drug-resistant bacteria [48].

Antibiotic therapy leads to improvement in lung function, the best predictor of survival in CF patients which treat CF lung disease morbidity based on decisions with which antibiotics are selected according to broad empirical and based on the severity of the patient's pulmonary exacerbation, the colonizing microorganisms, and the patient's age [49].

A range of anaerobic bacterial species are present in huge numbers in the lungs of CF patients, which contribute significantly to infection and inflammation in the CF lung. So, informed alterations of antibiotic treatment to target anaerobes is done, in addition to the primary infecting pathogens, may improve management [50].

A positive approach to the protection of the lung from early in life seems to be the only way to change the slope of the survival curves [51]. Ticarcillin can be used

in the treatment of pulmonary exacerbations of cystic fibrosis due to susceptible strains of *Pseudomonas aeruginosa* [52, 53].

Also, *Alcaligenes* bacterial strains are characterized as motile, Gram-negative, producing oxidase enzyme. The most common is *Alcaligenes xylosoxidans* known as *Achromobacter xylosoxidans*. The bacterial genus is composed of three main species such as *Achromobacter piechaudii*, *Achromobacter xylosoxidans*, and *Achromobacter faecalis.* These bacterial strains observed multiple antibiotic resistance especially grouped of aminoglycosides and recorded sensitivity to cephalosporins (third generation) [54].

7.2 Inhalational antibiotics for the treatment of *Pseudomonas aeruginosa*

In recent years, there are emergences of inhaled antibiotics to treat patients with Gram-negative bacterial infection such as *P. aeruginosa* infection. The benefits of which are explained below [55, 56].

Inhaled tobramycin, inhaled colistin, and inhaled aztreonam are used to control chronic *P. aeruginosa* infections in patients with CF [57]. In CF, tobramycin inhalation solution contributed to improved survival and reduced lung function decline [58]. Subsequently, the development of antibiotic agents can achieve high tissue concentrations to control bacterial infection that is difficult to treat in hosts [59]. Inhaled tobramycin achieves a sputum concentration of drug at least 25 times the MIC, with a median serum/sputum concentration of 0.01. Such high concentrations are required for the effective killing of *P. aeruginosa* because aminoglycosides bind to mucins in sputum and reduce the availability of effective antibiotic. Also, early randomized, double-blind, placebo-controlled trials conducted in patients with CF were short-term but demonstrated positive results with the use of aztreonam solution for inhalation compared to placebo. Colistin is bactericidal and active against Gram-negative bacteria including *P. aeruginosa*. Nebulized colistin is a preferred inhaled therapy for patients with CF and chronic *P. aeruginosa* [59]. Inhaled levofloxacin was studied in patients with CF and *P. aeruginosa* lung infection. Also, nebulized levofloxacin solution (MP-376) is a novel therapy that is currently being evaluated in phase I, II, and III clinical trials among patients with stable CF and recent isolation of *Pseudomonas aeruginosa* from sputum [60].

7.3 Antifungal therapy to control yeasts and filamentous fungi with CF patients

Antifungal therapy is usually considered when the deteriorating respiratory function is not responding to antibacterial therapy and *A. fumigatus* is growing and identified in sputum cultures [61]. Long-term antifungal treatment with mold active azoles, the only class of antifungals with an oral formulation, is not without risk of toxicity and adverse events and should be carefully balanced against its benefit [10].

A. fumigatus can grow in sputum cultures, and antifungal therapy is not effective [61]. Also, *Scedosporium* sp. is the second most frequent cystic fibrosis associated filamentous fungi which is resistant to many antifungal agents [62].

In CF, *Candida albicans* causes 95% of all *Candida* infections, whereas the remainder is caused by *Candida glabrata*, *Candida parapsilosis*, and *Candida krusei*. Also, *Candida dubliniensis* has been isolated in CF; however, little is known about its potential for virulence, and treatment modality is not clearly defined [63].

7.4 Antiviral therapy to control respiratory viruses with CF patients

Respiratory viruses associated with exacerbations in CF patients and upper respiratory symptoms are strong predictors for their presence. Some studies have

suggested a role played by respiratory viruses in exacerbation of CF. The impact of respiratory viruses is likely to be underestimated due to the low detection rate by conventional laboratory methods. Molecular techniques such as multiplex PCR have improved their diagnostic accuracy identifying respiratory viruses in CF patients which are important in clinical decision-making and are potentially important as new antivirals are becoming readily available [64]. A few therapeutic options recorded to treat virus-induced CF pulmonary exacerbations include macrolide antibiotic (azithromycin). It has antiviral properties of the human bronchial epithelial cells and stimulates antiviral mechanisms in bronchial epithelial cells within the CF airway control infection with rhinovirus by reducing rhinovirus replication as an interferon pathway [65]. Also, there are exciting prospects such as antiviral host defense peptides in development and can be novel therapeutics targeting rhinovirus [66].

Additionally, CF patients infected with influenza A(H1N1) showed increased case fatality rate (CFR) and morbidity compared to patients with other chronic respiratory diseases, so, early antibiotic and antiviral treatment strategies are important to control it in CF patients [67].

8. Conclusion

This chapter concludes that complications of microorganisms associated with cystic fibrosis disease are very high and can lead to patient death. Pathogenic microorganisms isolated from CF patients included Gram-negative bacteria, Gram-positive bacteria, unicellular fungi, multicellular fungi, and viruses.

The most common Gram-negative bacterial pathogens are *Pseudomonas aeruginosa* and *Haemophilus influenzae*. Also, the most common Gram-positive bacterial pathogen is *Staphylococcus aureus* especially MRSA. Other bacterial pathogens are isolated from CF patients, such as NTM species, *Alcaligenes xylosoxidans*, and *Burkholderia cenocepacia*.

In addition, *Candida albicans* is the most common unicellular fungi, and *Aspergillus fumigatus* is the most common filamentous fungi. Influenza A and B viruses are major viruses in causing respiratory exacerbations in CF, and both viruses are more commonly detected during pulmonary exacerbations.

To prevent this microbial complication, appropriate antimicrobials can be used to treat bacterial infections, antifungal therapy for treating pathogenic fungi and antiviral to manage viral infections. With prospects, researchers must work to produce new therapeutic options to control multidrug-resistant bacteria and resistant fungi and virus. These new treatment options may further enhance the prognosis in patients with CF.

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Conflict of interest

The author declares no conflict of interest.

Abbreviations

CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator gene
MRSA	methicillin-resistant *Staphylococcus aureus*
NTM	nontuberculous mycobacteria
CFR	case fatality rate

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