Chapter

Microbial Cystic Fibrosis

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Abstract

Cystic fibrosis (CF) is the most common genetic disease in Caucasians that increases the mortality rate. This disease retards the passage of water and salt through the cells and therefore affects the vital functions of different organs. Pulmonary cystic fibrosis is the most common and responsible for the majority of symptoms, burden of care, and mortality. The gene that causes the disease has now been identified and sequenced. The lung diseases with CF are usually have three pathological elements; mucus obstruction, inflammation, and infection. In the last century, the relationship between CF, respiratory microbiology, and inflammation has been understood with increased longevity and development of new treatments and laboratory techniques. In this chapter, we will illustrate causes of CF lung diseases and modern therapeutic strategies.

Keywords: cystic fibrosis, pathogenic bacteria, pneumonia infection, pulmonary inflammation, treatment guidelines

1. Introduction

Cystic fibrosis (CF) arises due to recessive mutations in the CF transmembrane regulator (CFTR) gene. This genetic disorder is carried out when two carrier parents transport the mutant CFTR gene to their child. Although no symptoms appear in the carriers, CF can be detected by genetic testing. CF-pulmonary diseases are usually associated with three pathological aspects; airway obstruction, infection, and inflammation. According to previous studies, children are most frequently infected by this disease with high rate of mortality. *Staphylococcus aureus* is the main cause of bronchitis, bronchiectasis, and pulmonary abscesses arising in the bronchi, which are usually accompanied by tenacious greenish-gray mucopurulent material [1, 2].

The mutation that attacks CFTR gene leads to CF, and obstruction in the airways with abnormal mucus, infection, and inflammation is present. Although the current treatments cannot halt the disease progression, good nutrition, defective mucus clearance, and treatment of inflammation and infection greatly improve CF of the respiratory system and its complications [3]. There is a controversial relationship between infection and inflammation. Some scientists think that the infection should precede the inflammation of airways, while others suggest the opposite [4].

Americans and Europeans are more susceptible to CF. One in 29 people of Caucasian ancestry is a healthy carrier of the CF gene mutation [5]. Detection of CF in early phases is very useful due to symptom reduction, health improvement, and low cost. For example, since 2010s, all American newborns undergo screening for CF to provide a chance for recovery if the disease is diagnosed. Most patients of CF must take pancreatic enzymes to digest food effectively, and some require insulin

for diabetes mellitus. The treatment cost of CF is very high because the drugs which treat and prevent the pulmonary diseases are very expensive [6]. Walaa et al. [7] report that 60 Egyptian children are affected by CF (6 months to 14 years). Salty skin is the most common symptom in the children affected with CF, because they suffer from dehydration due to loss of exuberant salty sweat. The percentage of ill males is 63%, while the percentage of ill females is 37%. Positive consanguinity of patients is 57%. 23% of patients has a positive family history of CF; the most frequent clinical presentation is pulmonary disease (84%), followed by pancreatic insufficiency (56%). The scientific material of this chapter aims to clearly interpret the roles of infection and inflammation in CF lung disease pathogenesis. Also, we will shed light on the therapeutic approaches to both infection and inflammation.

2. Microbes: CF interaction

2.1 Microbiology of CF lung disease

Severe and uncontrolled microbial infection may lead to CF. Microbes usually invade the airway luminal mucus, rather than tissues. Although *Staphylococcus aureus* is the main pathogenic agent for CF, many other bacteria are recorded with the development of both treatments and laboratory methods. The detection of pathogenic bacteria of CF depends on the cultivation of respiratory samples (e.g., sputum, bronchoalveolar lavage fluid, oropharyngeal swabs, or sinus samples) on the nutritive and selective media. Moreover, there are advanced techniques by which CF microbes are identified. Current methods mainly depend on cultivation of pathogenic bacteria on synthetic microbiological growth media and follow the incubation conditions to allow good growth and culture characteristics of pathogens [8]. Conventional techniques including microscopic and biochemical investigations revealed that the pathogenic microbes infecting CF airways usually exist in biofilms, which provide complete defense mechanism to the pathogens [9, 10].

2.2 CF respiratory pathogens

2.2.1 Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium and is the first CF respiratory pathogen. Children are more susceptible to CF lung diseases than adults, and they are usually affected by *S. aureus*, which has been associated with higher airway inflammation [11, 12] and lung dysfunctions [13, 14]. This infection can be lethal when associated with *Pseudomonas aeruginosa* [15]. This association may lead to worse outcomes, if *P. aeruginosa* is associated with specific subtypes of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) and small-colony variants of *S. aureus* (SCVs). *S. aureus* infection in adults is harmless than in children, because lung functions are better [16] and there are lower complications [17]. Accordingly, the pathogenicity of *S. aureus* has two levels: the first is high when the infection occurs in children or in the absence of *P. aeruginosa*, and the second is very high (extreme) when the host is infected by specific subtypes of *S. aureus* (such as MRSA or SCVs). On the other hand, *S. aureus* may be nonpathogenic, but just serves as a marker of early or mild disease as with children and adults, respectively [18].

The two subtypes of *S. aureus* which are mentioned above (MRSA and SCVs) as well as oxacillin-resistant *S. aureus* (ORSA) are usually identified either by their resistance to these β-lactams or by carriage of the *mec*A gene, which encodes this resistance. The subtypes of *S. aureus* SCVs are slow-growing, antibiotic-resistant variants

that are difficult to detect with conventional cultures and require special laboratory methods. SCVs are associated with decreasing lung functions, and they may be treated with antibiotics including aminoglycosides and sulfonamides [19]. MRSA are tightly accompanied with CF lung disease especially in the last 20 years. A lot of people are infected with MRSA due to hospitalization and worse use of antibiotics [20–22]. Many studies reported that MRSA are associated with CF lung disease in particular decrease of lung functions [23]. Moreover, MRSA is an independent risk factor for decreasing lung functions and respiratory exacerbations [24] and for increased mortality [25]. There are some similarities between the two subtypes (MRSA and SCVs): antibiotic treatment, antibiotic resistance, and higher lung disease severity.

In some countries where CF lung disease is spreading, anti-staphylococcal agent is provided as a prophylaxis approach during childhood particularly when *P. aeruginosa* is detected early [26]. Many of antibiotics are used to eradicate *S. aureus* and *P. aeruginosa* as co-infectious agents of CF lung disease, but the most commonly used antibiotics are aminoglycosides and sulfonamides [27].

2.2.2 Pseudomonas aeruginosa

After overcoming *S. aureus* by effective anti-staphylococcal agents, *P. aeruginosa* became the most common and important pathogen related to CF-pulmonary diseases [28]. *P. aeruginosa* infection is associated with decreasing lung functions, severe inflammation of the respiratory tract, a greater risk of respiratory exacerbations, and high rate of mortality [29]. Early detection of *P. aeruginosa* is a helpful factor for full eradication, while chronic infection cannot be eradicated. Also, eradication of *P. aeruginosa* can be carried out by using antipseudomonal bioagents [30]. In contrast with *S. aureus* infection, *P. aeruginosa* infection is higher in adults than in children. At the end-stage of CF-pulmonary disease, *P. aeruginosa* is only present as a main pathogen for respiratory tract 31].

Despite *P. aeruginosa* usually producing numerous toxins as virulence factors, it may loss these virulence factors or their regulatory genes during chronic CF infections [32]. After invasion of lungs with *P. aeruginosa*, the mucoid colonies are formed due to exuberant production of alginate as one of the phenotypic changes due to chronic infection of CF-pulmonary disease [33]. The mucoid texture provides high rates of persistence and resistance for many antimicrobial agents as well as full adaptation to the respiratory airways. *P. aeruginosa* may be epidemic or non-epidemic, but the former is associated with worse outcomes such as high rate of mortality and requirement for lung transplantation [34].

Although *P. aeruginosa* is a multidrug resistant pathogen and usually leads to severe pulmonary CF, it leads to worse outcomes when associated with MRSA and SCVs. Prophylaxis by using of antibiotics is not recommended in the recent approach of *P. aeruginosa* treatment due to severe adverse events of antibiotics, but if *P. aeruginosa* is early detected, the treatment course with antibiotics must begin for complete eradication and to decrease the risk of exacerbations. Recently, inhaled antibiotics such as tobramycin and aztreonam are sufficient for eradication without any additional oral antibiotics such as ciprofloxacin [35]. Although inhaled antibiotics are sufficient for *P. aeruginosa* treatment without oral antibiotics, the clinical reports are revealing that, the combination between two classes of antibiotics leads to longer periods of clinical stability than does a single class [36].

2.2.3 Burkholderia cepacia complex

Burkholderia cepacia complex (BCC) is a group of Gram-negative bacteria and is comprised of at least 18 species. Of these, two species are the most common and

associated with CF lung infections and disease, *B. cenocepacia* and *B. multivorans*, but the latter is more distributed than the former. Nevertheless, *B. cenocepacia* is associated with more rapid lung function decline and mortality rate than *B. multivorans*. Other BCC species are less common, and their clinical associations are less well defined such as *B. gladioli* [37]. *Burkholderia* CF infections are notorious because they are associated with more severe lung disease, they are transmissible among persons with CF, they are resistant to multi-antibiotics, and epidemic strains can infect CF patients after internal contact at camps and clinics [38]. Associated outcomes often range from clinical quiescence to rapidly progressive, necrotizing pneumonia and fatal septic disease "cepacia syndrome" [39]. Therapy is usually limited to specific antibiotics as needed [40, 41].

2.2.4 Stenotrophomonas maltophilia

Stenotrophomonas maltophilia is a Gram-negative bacterium, which is widely spreading in the United States in recent years as CF pathogen especially among adolescents and young adults. This bacterium has intrinsic and acquired resistance to many antibiotics. No clear evidence for treatment of this pathogen so far [42].

2.2.5 Haemophilus influenzae

Haemophilus influenzae is a Gram-negative bacterium and is firstly detected in CF respiratory cultures. This bacterium is more prevalent in children and less common in adults. Although its association with CF complications is controversial, it is associated with non-CF bronchiectasis and chronic obstructive pulmonary disease. The cultivation of this bacterium is difficult and usually requires specific conditions for detection. The recent isolates of *H. influenzae* are non-typeable and unencapsulated since the vaccine of *H. influenzae* type B (HIB) has been discovered. This bacterium is well known as resistant to β-lactam antibiotics due to its production of β-lactamase; therefore, treatments usually include a β-lactamase inhibitor such as amoxicillin-clavulanate [43].

2.2.6 Achromobacter xylosoxidans

Achromobacter xylosoxidans is a Gram-negative bacterium that is similar to *P. aeruginosa*. Although this bacterium is widely spreading in the United States, it remains low in CF lung diseases (<10%). This bacterium is associated with worse radiographic and spirometric measures of lung disease. Similar to *P. aeruginosa* and BCC, *A. xylosoxidans* is the dominant, and occasionally, bacterium is isolated from CF patients at end-stage. Some microbes are notorious due to their resistance to many antibiotics, so their treatment is limited [44].

2.2.7 Nontuberculous mycobacteria

Nontuberculous mycobacteria represent 6–30% of CF prevalence. Two groups of mycobacteria, accounting for six species, are currently considered important CF pathogens: *Mycobacterium avium* and *Mycobacterium abscessus* complexes. The treatment of nontuberculous mycobacteria has two phases: multiple intravenous antibiotics for weeks to months or multiple inhaled and oral antibiotics for months to years. Side-effects and toxicities are common and can be troublesome [45].

2.2.8 Fungi and viruses

A lot of fungi are isolated from CF patients, including yeasts such as *Candida* spp. and filamentous fungi such as *Aspergillus* spp. There is a respiratory disease known as

allergic bronchopulmonary aspergillosis (ABPA), in which the bronchia are affected by inflammation due to *Aspergillus* infection. Patients of CF and other chronic airway diseases can develop an IgE-mediated allergic airway disease known as ABPA. The treatment for which primarily involves steroids, although the addition of an antifungal such as itraconazole may allow for lower doses of steroids. Human respiratory viruses are not thought to chronically infect the CF airway, but they have been shown both to be important and common triggers of CF respiratory exacerbations [45].

2.3 CF airway microbiome

Many studies which are concerned with identification of the microbiota of the respiratory system depend on DNA-sequencing techniques. The results of these techniques revealed that there is a wide diversity of microbiota inhabiting the respiratory system. This diversity of microbiota is high in young CF patients who have better lung functions and subsequently need fewer courses of antibiotics and vice versa in the case of adults. The most dominant microbiota in infected lungs of CF patients are *P. aeruginosa*, BCC, and *A. xylosoxidans* [46].

3. Immune response in CF lung disease

Destruction of the respiratory airways may carry out due to the chronic CF infections. This damage is mediated by abnormal response of the host to airway infections, which in turn leads to irreversible bronchiectasis and lung function decline [47]. Many studies report that, bacterial infection and inflammation are leading to triggering of neutrophils [48]. Moreover, the dysfunction of CF-CFTR is a main cause of altered immune defense and disorders in the airway's environment. Appearance of neutrophil elastase (NE) is a good biomarker of disease [49].

The mutant CFTR gene leads to production of an abnormal protein, resulting in abnormal transport of salt and water across lining cells of the respiratory system, digestive system, and genital tracts. Insufficient water transport to the lining cells of the airways leads to formation of more thick and viscous respiratory secretions which clog small airways. Due to water reduction, the mucus becomes stagnant and infected with bacteria such as *P. aeruginosa* that may be inhaled or brought into the lungs through the mouth. Due to stagnant mucus, infection and chronic inflammation are developed. The tenacity of stagnant mucus is increased because the inflammatory cells are trapped in it. Due to accumulation of stagnant and infected mucus inside the airways, the bronchi dilate, and subsequently their walls are weakened. This phenomenon is called bronchiectasis that results in further airflow obstruction. According to the previous case, the respiratory cycle can be called the viscous cycle in which airway obstruction, inflammation, and infection are present, which lead to decrease of lung functions, respiratory failure, and death. Decrease of lung functions especially in children can also be due to exposure to smoking and polluted air, which also leads to pulmonary exacerbations.

The defective CF gene leads to defective CFTR and thick viscous secretions, which in turn lead to bronchial obstruction then to an infection then inflammation and finally bronchiectasis. Infection, inflammation, and bronchiectasis can lead to bronchial obstruction (**Figure 1**). Infection amplifies defective CF gene, which in turn leads to defective CFTR, which activates the resident airway inflammatory cells, which stimulate neutrophils and neutrophil products such as neutrophil elastase and monocytes, and finally bronchiectasis occurs (**Figure 2**).

The surface of epithelial lining cells of respiratory airways is dehydrated and acidified due to CFTR dysfunction, and abnormal mucociliary clearance is carried

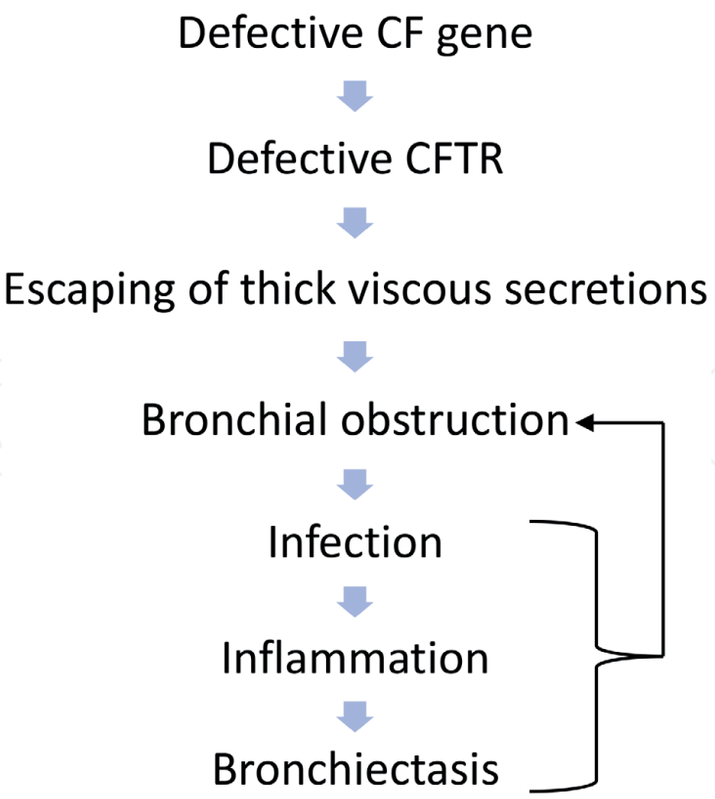

Figure 1.
Traditional pathophysiology of CF lung disease.

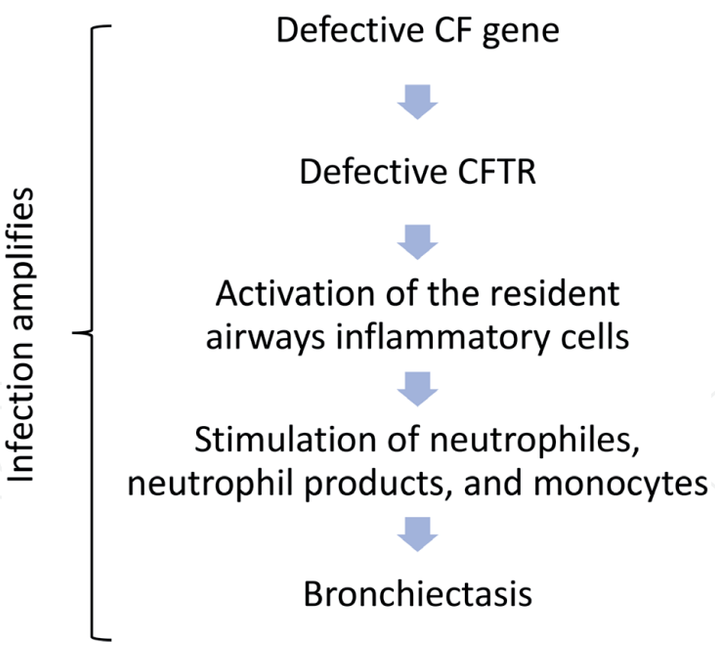

Figure 2.
Potential alternative mechanism for airway inflammation in CF lung disease.

out. Dehydration is carried out due to water loss, while acidification is carried out due to bicarbonate loss [50, 51]. The neutrophilic inflammatory response is higher in CF than in non-CF patients. However, the neutrophilic inflammatory response is reduced in neutrophil apoptosis. Neutrophils and their products are accumulated due to deficiency in mucociliary clearance and macrophage dysfunction [52]. The passage airways may destruct by the action of anti-proteases, such as alpha-1-antitrypsin, a serine protease inhibitor, and secretory leukocyte protease inhibitor. So, neutrophil

products such as proteases and elastases are released to react with anti-proteases and therefore avoid their deleterious action toward the passage airways [53, 54].

Some substances act as mediators of immune response and serve as important biomarkers of disease progression, such as neutrophil elastase, which is abundant in induced sputum in children with CF compared to control children [55]. High level of neutrophil elastase in induced sputum indicates lung dysfunctions and bronchiectasis [13]. The inflammation of the passage airways in sputum is reduced after detection and using of effective antibiotics for treatment of a CF-pulmonary diseases [56]. Inflammatory proteins are considered potential biomarkers of disease in CF. For example, the blood plasma proteins are biomarkers of CF disease [57].

The common example of immune response in CF lung disease is the immune response to *P. aeruginosa* [58]. CFTR dysfunction predisposes the host to infection with *P. aeruginosa* and then allows for chronic infection and subsequent reduced opportunity for eradication. Moreover, *P. aeruginosa* interacts with other bacterial pathogens including *S. aureus* and *B. cepacia* complex to alter the inflammatory response [59].

4. Anti-inflammatory therapy of CF

4.1 Ibuprofen

Ibuprofen inhibits neutrophil migration and aggregation [60]. It improves the lung functions especially in patients younger than 13 years. Gastrointestinal bleeding may be associated with chronic therapy. Recent studies report that high-dose ibuprofen could slow the progression of lung disease in CF, particularly in children with mild disease [61]. Despite the efficacy of ibuprofen for CF lung disease therapy, its use is uncommon compared to other CF therapies due to severe adverse effects such as kidney failure and gastric bleeding [62].

4.2 Azithromycin

Azithromycin is a broad-spectrum antibiotic belonging to macrolide group, and at the same time, it has immunomodulatory effects, so it has high effectiveness in the treatment of CF lung disease and other chronic inflammatory conditions [63]. Azithromycin may be used for a very long period (chronic azithromycin) either with or without chronic *P. aeruginosa* infection [64]. With chronic *P. aeruginosa* infection, azithromycin is taken thrice weekly for 6 months to improve forced expiratory volume (FEV) and subsequently decrease the risk of pulmonary exacerbations. On the other hand, without chronic *P. aeruginosa* infection, azithromycin could reduce 50% of pulmonary exacerbations and improve weight, but without improvement of lung functions [65]. Azithromycin is recommended for CF treatment in patients suffering from chronic *P. aeruginosa* infection and those without chronic infection aged 6 years and older [66]. Despite the high durability of azithromycin, resistant bacteria are emerging, so the treatment should be reassessed every 6–12 months. Azithromycin is prohibited for patients with nontuberculous mycobacteria (NTM) unless it is prescribed in combination with other anti-mycobacterial medications as part of NTM therapy.

4.3 Corticosteroids and leukotriene receptor antagonists

Corticosteroids, especially its systemic forms, or cortisones are powerful anti-inflammatory agents which are widely used in the treatment of CF. Although systemic corticosteroids can intensively improve lung functions, they have adverse

effects that outweigh any benefit [67]. Inhaled corticosteroids do not have any efficacy in the treatment of CF [68]. Therefore, the treatment of CF by systemic or inhaled corticosteroids is not recommended by the Cystic Fibrosis (CF) Foundation [66]. On the other hand, leukotriene receptor antagonists (LTRAs) are nonsteroidal oral medications, which are used as anti-inflammatory bronchoconstriction preventors. LTRAs block a chemical reaction that leads to inflammation in the airways. LTRAs are effective as antihistamines, and they are better than placebo, but less effective than nasal corticosteroids in improving symptoms and quality of life in patients with seasonal allergic rhinitis [69].

5. Treatment management of CF

CF carrier testing is recommended for everybody especially for Caucasian women whether they are considering pregnancy or already pregnant. CF-carrier test must be made before marriage, because the marriage of the positive CF-carriers leads offspring affected with CF, and vice versa. So, the early diagnosis of CF either before birth or for newborns allows for earlier and faster treatment in CF centers and avoidance of serious complications including poor growth. CF centers must be accredited by the CF Foundation. CF centers have multidisciplinary teams of physicians, nurses, respiratory therapists, dietitians, and social workers who can care for both adult and pediatric patients [70]. Good nutrition for affected persons with CF increases lung functions and life expectancy. Once CF disease is diagnosed, the patient must follow a nutrition program that is including a high-calorie diet, pancreatic enzymes and a liberal-fat. Essential vitamins must be supplemented to reduce the risk of deficiency of certain fat-soluble vitamins.

Although ill infants and young children with CF have intermittent cough and wheezing, structural and functional abnormalities in the lung as early as the first few months of life are detected. CF treatments include physical methods to eliminate thick secretions from the chest. CF treatments with chemical methods include prescription of different medications, such as dornase alfa and hypertonic saline as thinners of sticky airway secretions, albuterol as bronchodilator, tobramycin as inhaled antibiotic, and ibuprofen and azithromycin as anti-inflammatory drugs [71]. Preventive measures against CF or its complications necessarily require frequent follow-up for nutrition, lung functions, and screening for complications in an accredited CF center.

6. Conclusion

CF lung disease is one of the many causes of morbidity and mortality worldwide. CF lung disease has indefinite symptoms including airway obstruction, infection, and inflammation. This disease is associated with different microorganisms such as *P. aeruginosa*, *S. aureus*, and *B. cepacia* complex. Several medications are used as antimicrobial treatment for these pathogens. The airway microbiota is influenced by several factors including the environment, host, disease progression, and antibiotic treatment. Immune response to microbes in the CF airways is high due to dysfunction of CFTR protein. Although the recent therapies for airway infections and immune-inflammatory response are effective, they cannot fully stop disease progression. Today, CF lung disease has less risk because anti-inflammatory and antimicrobial therapies are in continuous development. Eventually, the authors recommend that, CF-carrier test must be made in particular before the marriage, early treatment of respiratory diseases especially if CF disease is diagnosed, avoidance of

relatives marriage because it enhances an emergence of genetic diseases including CF, and finally, the treatment with corticosteroids (cortisone) must be under full control by a physician due to its severe adverse effects.

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Conflict of interest

The authors declare no conflict of interest.

Notes

This chapter is concerned with CF-pulmonary diseases rather than other diseases of CF, because it is more widespread around the world and a common cause of morbidity and mortality especially in Caucasian areas as reported by the WHO.

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