Treatment of pulmonary exacerbations in cystic fibrosis

People with the genetic condition cystic fibrosis suffer repeated respiratory tract infections from an early age, which are often superseded by chronic airway infection, with resultant progressive loss of lung function and premature death from respiratory failure. Acute pulmonary exacerbations contribute to diminished quality of life, reduced lung function, morbidity and mortality in cystic fibrosis, and remain a major obstacle to improving long term outcomes. This article discusses current diagnostic and therapeutic strategies employed in the treatment of pulmonary exacerbations, with a focus on critical appraisal of the evidence based on which strategies are founded. Areas of uncertainty are highlighted and current and potential future research directions are discussed.

KEYWORDS: antibiotics ■ cystic fibrosis ■ definition ■ etiology ■ infection ■ treatment

Daniel J Smith[†1,2,3], David W Reid[1,2,3] & Scott C Bell[1,2,4]

[1]Department of Thoracic Medicine, The Prince Charles Hospital, Rode Road, Chermside, Brisbane, Queensland, 4032, Australia
[2]School of Medicine, The University of Queensland, The Prince Charles Hospital, Queensland, Chermside, Brisbane, Queensland, 4032, Australia
[3]Queensland Institute of Medical Research, Brisbane, Queensland, 4006, Australia
[4]Queensland Children's Medical Research Institute, Level 4 Foundation Building, Royal Children's Hospital, 300 Herston Road, Herston, 4029, Queensland, Australia
[†]Author for correspondence:
Tel.: +61 731 394 770
Fax: +61 731 395 630
daniel_j_smith@health.qld.gov.au

Advances in cystic fibrosis (CF) care, including better nutrition, aggressive treatment of infection, and the development of specialist center care, have led to substantial improvements in life expectancy [1]. However, the majority of patients still die from complications associated with chronic respiratory sepsis before their fifth decade of life. Equally, improvements in survival have come at the cost of treatment-related morbidity including the emergence of multidrug resistant infections, complications of indwelling intravenous lines, and allergic and toxic effects of antibiotics [2–4]. Awareness of the potential pitfalls of treatment places the onus on the treating CF team to ensure that therapies are used in a way that maximizes their effect, while minimizing exposure to harm.

Pulmonary exacerbations are characterized by increasing respiratory symptoms and acute deterioration of lung function. They are associated with significant morbidity and mortality and may accelerate lung function decline [5,6], as well as being associated with diminished quality of life [7] and increased healthcare costs [8].

The availability of high-quality clinical trials to guide therapy in CF is limited and this is particularly true of exacerbations. Similarly, there have been multiple studies into airway inflammation and bacterial infection during exacerbations, but no therapies have been developed based on new knowledge generated. Thus, treatment decisions are often based on clinical experience and anecdote. Barriers to study design include inadequate cohort sizes to adequately powered single center trials, limited pharmaceutical industry investment in multicenter studies, and differences in study designs required to satisfy drug licensing authorities internationally.

The aim of this article is to critically appraise the current evidence base on which strategies for treatment of pulmonary exacerbations are founded, and discuss how treatment is commonly implemented where evidence is lacking. We highlight current areas of uncertainty, and propose areas in which further research is required.

Defining an exacerbation

In 1994 the Cystic Fibrosis Foundation (USA) convened a consensus conference to define outcome measures for use in CF clinical trials [9]. One of the eight 'top priority' issues identified was the development of a standard definition of a pulmonary exacerbation that could be applied consistently within clinical trials. Despite this welcome initiative, 17 years later no universally accepted definition has been realized. The development of an exacerbation definition is confounded by the natural day-to-day variability of symptoms, poor correlation between biomarkers and clinical outcomes [10], and differences in hospital admission and antibiotic administration policies between CF centers and across healthcare settings [11].

The absence of a universally accepted definition of what constitutes an exacerbation makes direct comparison between rates and severity of pulmonary exacerbations reported in clinical trials difficult. Other important uses of a definition include the ability to track changes in disease

future medicine part of fsg

epidemiology, and their utility as decision aids with respect to determining when treatment of an exacerbation can be safely discontinued.

In attempting to formulate a standard definition of pulmonary exacerbations, several authors have adopted the approach of performing component analysis of previous studies to examine for the factors most likely to prompt the treating physician to make the diagnosis of an exacerbation [12–14]. In these studies, symptoms generally outperformed physical examination findings and investigations. Increased cough, and increased sputum volume or purulence, and decreased appetite and weight were found to be most predictive (Table 1). The findings of these studies have not been incorporated into a unifying definition and instead, multiple different and invalidated scoring systems remain the norm [15,16].

A fall in lung function is usually seen during a pulmonary exacerbation, therefore incorporating spirometric measures into the definition of a pulmonary exacerbation may be reasonable. However, in up to a quarter of patients lung function fails to return to baseline by the end of treatment suggesting its use in guiding treatment duration may be limited [17].

Given the hypothesis that exacerbations are precipitated by an imbalance between host immunity and infection, research has, and continues to focus on identifying biomarkers capable of detecting exacerbation onset and recovery. The ideal biomarker of exacerbations should maintain its sensitivity with varying disease severity, be minimally impacted on by independent factors (e.g., age and sex), and respond rapidly with clinical improvement and resolution of inflammation [10].

Table 1. Symptom profiles used in previous attempts to define a pulmonary exacerbation.

Signs and symptoms (new or increased)	Fuchs	Ramsey	ARIC	RSSQ
Pulmonary signs and symptoms				
Increased dyspnoea with exertion	X			X
Decreased exercise tolerance				X
Increased work of breath			X	
Cough	X	X		X
Day cough			X	
Night cough			X	
Wet or congested cough			X	
Chest congestion	X			X
Frequency of cough	X			
Cough up mucus	X			
Wheezing			X	
Hemoptysis/coughing up blood	X		X	X
Sputum volume	X	X	X	X
Change in sputum appearance		X		X
Change in sputum colour			X	X
Change in sputum consistency			X	X
Increased respiratory rate		X		
Decreased lung function	X	X		
Upper respiratory tract symptoms				
Sore throat/runny nose		X	X	
Sinus pain/tenderness	X			X
Change in sinus discharge	X			X
Constitutional and GI signs and symptoms				
Malaise/fatigue/lethargy	X			X
Abdominal pain				
Fever	X	X		X
Decreased appetite/anorexia	X	X		X
Weight loss		X		X
Work/school absenteeism		X		X

ARIC: Acute Respiratory Illness Checklist; RSSQ: Respiratory and Systemic Questionnaire (© BoehringerIngelheim). Reproduced with permission from [42].

Levels of the acute phase reactant, C-reactive protein (CRP), increase in the presence of systemic inflammation, and in particular in the setting of bacterial infections. In CF, CRP levels often increase at the onset of an exacerbation, and decrease with the administration of antibiotics [18,19]. However CRP levels have been shown to return to normal before an improvement in either clinical status or lung function is seen, suggesting it has limited utility as a guide to discontinuation of therapy [20]. Furthermore, changes in CRP with treatment vary widely between individuals, and have even been shown to increase in some patients despite clinical response [20].

Airway inflammation in CF is neutrophil predominant [21]. The chemokine IL-8 is a major neutrophil chemoattractant, and can be detected in high levels in CF sputum [18]. Studies have demonstrated a fall in sputum IL-8 levels in response to antibiotic therapy, and a correlation with improvement in lung function [18]. However, results have been inconsistent, both between studies and between individuals within studies [10,19,22]. This variability may reflect differing sampling techniques (induced vs expectorated sputum) and the response of IL-8 may depend on the degree of disease severity (i.e., less reduction in IL-8 in the setting of more advanced lung disease). Another confounding factor is the anatomical heterogeneity in lung disease and difference in sputum purulence depending on sample origin within the lung. Similar difficulties have been found with a range of other pro- and anti-inflammatory cytokines (including IL-6, IL-10, TNF-α) and neutrophil products (including neutrophils elastase complexes and myeloperoxidase) that have been assessed as biomarkers [10,22].

Neutrophil elastase (NE) is a proteolytic enzyme released by activated neutrophils that is believed to contribute to host tissue damage at sites of inflammation. Neutrophils derived from patients with CF spontaneously release increased amounts of NE, and NE can be detected in high concentrations in airway secretions [10,23]. A number of studies have reported a fall in NE in response to treatment of an exacerbation, but again results between studies have not been consistent [23,24]. Calprotectin is also produced by activated neutrophils in response to pro-inflammatory cytokines and has been validated as a fecal marker of inflammatory bowel disease [25], but it is also detectable in CF serum and airway secretions. A recent study has demonstrated decreases in sputum and serum calprotectin levels with treatment of pulmonary exacerbations and changes in sputum calprotectin demonstrated a stronger correlation with clinical improvement when compared with IL-8, VEGF or myeloperoxidase [19]. Future studies will delineate whether this marker will prove to be more specific than its predecessors.

Exhaled breath condensates (EBC) measure volatile substances that are produced in the lungs and appear in expired air. They have potential advantages over sputum sampling by providing a summated assessment of the lung, and can be measured in both young children, and patients unable to produce expectorated sputum samples. The reported differences in EBC from CF patients when compared with healthy controls include lower pH, nitric oxide and ammonia concentrations, and increased concentrations of IL-6, IL-8, leukotriene B4 and nitrites [26–30]. However, as with sputum biomarkers, reported changes during exacerbations and their treatment have been inconsistent [27–29].

An alternate approach to measuring the inflammatory response is to measure changes in airway microbial flora. At the most basic level this can be achieved by determining sputum bacterial concentrations before and after treatment. When this has been done most studies report a fall in bacterial load accompanying clinical improvement [31]. However, airway infection in CF is polymicrobial [32], and it is still not known which organisms are the most important during exacerbations, and consequently which should be monitored to indicate response [33].

In the setting of chronic bacterial infection, the growth of subpopulations of bacteria with increased virulence may also be important. Detection of either these subpopulations or the virulence factors that they produce may facilitate diagnosis of exacerbations. *Pseudomonas aeruginosa* (the major pathogen of chronic CF airways infection) is capable of rapid changes in both genotype and phenotype during exacerbations [34]. Potential indicators of *P. aeruginosa* virulence detectable in CF sputum and EBC include pyocyanin, quorum sensing (QS) signals, exotoxins and pyoverdine, as well as the appearance of *P. aeruginosa* small colony variants [35–38]. There are currently only limited clinical data to support the use of virulence factors to monitor response. Grimwood *et al.* demonstrated an increased concentration of *P. aeruginosa* exo-enzymes in CF patients' sputum during acute exacerbations compared with stable controls, and that concentrations fell in response to antibiotics [39]. In a more recent study, Fothergill

et al. examined a panel of *P. aeruginosa* virulence factors and described differing patterns of response to antibiotics in each of the three patients studied [34].

Future perspective

Future research will need to focus on longitudinal studies correlating biomarkers with changes in lung function, symptoms and antibiotic response. It is unlikely that a single biomarker will be pathognomonic of a pulmonary exacerbation. However, it may be possible to develop a panel of markers that will increase diagnostic certainty and the ability to monitor the response of host and pathogen to treatment more reliably.

Epidemiology & impact of exacerbations

Data from patient registries demonstrate that the proportion of patients requiring intravenous antibiotics and hospital admissions for respiratory exacerbations increases with age, and is mirrored by a decline in lung function [40–42]. These findings are further supported by data from the Epidemiological Study of CF (ESCF), which reported that the rate of intravenous antibiotic use (at least one course per year) increased from only 23% in those aged under 6 years to 63% of patients aged over 18 years [14].

Pulmonary exacerbations have a significant impact on long-term lung health, with 25% of patients who suffer a pulmonary exacerbation failing to recover their baseline lung function following intravenous antibiotic treatment [17]. Furthermore children having a single, and adults having greater than three exacerbations in a year experience an accelerated decline in lung function in the subsequent three years [6]. These data are supported by a model predicting 5-year survival in CF, which demonstrated that each pulmonary exacerbation experienced annually was equivalent to a reduction in FEV_1 predicted of 12% [5]. Other impacts of exacerbations include an increased need for domiciliary oxygen, non-invasive ventilation and diminished health-related quality of life [7,43]. Severe pulmonary exacerbations necessitating intensive care unit (ICU) admission are associated with increased mortality, with in-hospital survival rates as low as 27% in patients requiring invasive ventilator support [43,44].

Given the clinical impact of pulmonary exacerbations in CF identification of factors that may predispose to their occurrence are important in planning preventative strategies. However, to date only one prospective study has examined factors that predict pulmonary exacerbations [45]. This study, performed in a cohort of patients chronically infected with multidrug resistant organisms (e.g., *P. aeruginosa*, *Burkholderia* spp) identified female sex, poor lung function, previous pulmonary exacerbations, and the use of inhaled corticosteroids as potential risk factors. Interestingly, a negative correlation between age and exacerbations was demonstrated, which the authors postulated may represent a survivor effect. A recent *post-hoc* analysis of data from the ESCF including 16,000 patients similarly identified decreased lung function, female sex, and previous exacerbations, as well as *P. aeruginosa* infection as significant risk factors for exacerbations regardless of age [46]. Other risk factors identified from smaller retrospective studies include, low socioeconomic status and acute viral infection [47–49].

Etiology of exacerbations

Pulmonary inflammation and infection begins a short time after birth [50,51]. Initial intermittent infection is often followed by the establishment of chronic infection, often with Gram-negative bacteria. Factors predisposing to the establishment of chronic infection are beyond the scope of this article, but excellent reviews of this topic are available [21,52].

Once chronic infection is established a vicious cycle of infection and inflammation develops and acute pulmonary exacerbations are presumed to occur when this balance is perturbed. Proposed mechanisms for the development of an exacerbation include acute respiratory viral infections, acquisition of a new bacterial strain, a change in the resident bacterial flora, or an acute increase in airway inflammation – potentially as a consequence of altered host immunity.

Virus induced exacerbations

An association between viral infections and pulmonary deterioration in CF was first described over 25 years ago [53]. In this sentinel study of children with CF, isolation of a virus at the time of a pulmonary exacerbation resulted in accelerated progression of lung disease. In subsequent studies, rates of viral isolation at the times of pulmonary exacerbations of between 9% and 46% have been described [48,54,55], with the highest reported rates in those studies that have used molecular detection techniques.

The viruses detected depend on the sampling technique (nasopharyngeal aspirate compared with cough swab and bronchoalveolar lavage) and the age of the study population. However,

the viruses are in general similar to those seen in people without CF, with rhinovirus, respiratory syncytial virus (RSV), influenza A and B, and para-influenza virus IV being the most commonly detected [48,54,55].

Debate exists about whether viruses trigger exacerbations in their own right, or act indirectly by increasing the virulence of existing airway bacteria. Possible mechanistic links between viral infection and bacterial virulence include virus-induced reduction in alveolar macrophage antibacterial responses and facilitation of bacterial binding to epithelial cells [56,57]. However other authors highlight the poor correlation between viral infection and increased bacterial number to argue that viruses have direct effects on airway inflammation [48]. There is also evidence that the anatomical site of viral infection within the airway may be important, for example lower respiratory tract viruses (including RSV and influenza) may influence the rate of lung function decline, whereas upper respiratory tract viruses (including rhinoviruses) appear to have less impact [55].

In 2009 the emergence of the first influenza pandemic to occur in the modern era of CF care gave an insight into how a novel viral infection may affect people with CF. Initial single center reports in the wake of the pandemic from Australia and UK reported that in general patients had mild clinical course [58,59]. Subsequently a large multinational study (24 European centers, one USA center) reported the outcomes in 110 cases from an at-risk population of greater than 4500 (incidence 2.3%). While most patients again suffered a self-limiting illness with a return to baseline lung function within 30 days of infection, there was significant short-term morbidity with two thirds requiring intravenous antibiotics and 50% being admitted to hospital. Patients with severe lung function impairment prior to infection were most severely affected, with six patients in this group requiring ICU admission and three dying [60].

Further work is required to delineate the pathogenic potential of specific viruses, including to determine the optimum sampling site and processing technique for diagnosis, and to investigate the role of antiviral agents in treatment of pulmonary exacerbations.

The role of chronic *P. aeruginosa* infection in exacerbations

By the age of 18 years, 80% of patients with CF will have chronic infection with *P. aeruginosa* [40,41]. Following its establishment, *P. aeruginosa* undergoes phenotypic and genetic adaptations that support persistent infection [61]. Arguably the most important of these is the transformation from a planktonic free-living state, to a 'mucoid' phenotype typified by the production of alginate, and the formation of bacterial communities encased within a 'biofilm' of extracellular glycoproteins. Such changes offer protection from host immune defenses and limit antibiotic penetration [62]. Biofilm dwelling bacteria are not eradicated by the host immune response, but their presence probably provides stimulus for chronic inflammation.

Biofilm dwelling bacteria employ density dependent cell-to-cell communication (QS) to ensure survival of the colony within the constraints of its environment. It has been proposed that relatively inert biofilms periodically expel colonies of immunogenic planktonic bacteria in response to environmental stimuli, which can induce an acute exacerbation [63]. This theory is supported by data showing that in 94% of patients with chronic *P. aeruginosa* infection the same strain is isolated during periods of clinical stability and acute exacerbations [64].

Culture negative exacerbations

In 20% of pulmonary exacerbations the typical bacteria of the CF airway are not detected, particularly in children [65]. Although some exacerbations may be caused by viruses, the etiology of the remainder are unexplained. Recent interest has focused on anaerobic bacterial pathogens as the polymicrobial nature of infection in the CF lung becomes apparent [32]. Thick mucus within the airways creates anaerobic and microaerobic niches and studies utilizing anaerobic culture and nonculture molecular techniques have provided compelling evidence that CF airways are chronically infected with anaerobic bacteria [32,33,66]. A number of the anaerobes that have been detected are known pulmonary pathogens responsible for nosocomial pneumonia and lung abscesses [67,68]. It is therefore possible that anaerobes, which are not detected by standard sputum culture techniques, play an important role in triggering pulmonary exacerbations. More studies are required to assess the clinical impact of anaerobes in the CF airway [33,69].

Fungi may also have a role in exacerbations. *Aspergillus* species are isolated in half of all CF sputum samples [70], and allergic bronchopulmonary aspergillosis (ABPA) is relatively common [71]. Although a 'flare' of ABPA may mimic a pulmonary exacerbation, it can usually be differentiated by the presence of airway

'casts', accompanied by elevated blood IgE, peripheral blood eosinophilia, and positive *Aspergillus* precipitins [71]. However, recent evidence demonstrating an increased rate of lung function decline and pulmonary exacerbations in patients with chronic *Aspergillus fumigatus* infection, independent of the formal diagnosis of ABPA suggests that airway fungal infection may have a pathogenic role in exacerbations [72].

Gastro-esophageal reflux disease (GORD) is common in CF, occurring in up to half of patients regardless of symptoms or acid suppression treatment [73]. Post lung transplantation, untreated GORD is associated with an increased rate of lung function decline and onset of chronic rejection [74], but its impact in CF prior to transplantation is poorly understood. There is evidence that GORD is associated with increased cough, and lung function decline [73], but the role of GORD in provoking exacerbations has not been studied. In CF patients who are 'frequent exacerbators', GORD may be an important contributor and should be actively sought and treated when present. Use of acid suppression treatment may neutralize the pH of fluid aspirated, but may also inadvertently increase the microbial burden of gastric fluid aspirated [75]. Further research is needed in this area, but early surgical fundoplication may be the preferred option when it can be performed safely [76].

Future perspective

Much is still unknown about the etiology of pulmonary exacerbations in CF. Future research will focus on identifying mechanisms by which infectious and noninfectious stimuli trigger an increase in the host immune response in order to identify common pathways that may be manipulated to reduce airway inflammation and remodeling.

The role of sputum microbiology in guiding the treatment of exacerbations

Traditional sputum microbiological techniques for routine CF respiratory samples use selective media to specifically culture known pathogenic bacteria, followed by determination of antibiotic susceptibility based on the minimum inhibitory concentration *in vitro*. When a single organism is responsible for pulmonary infection (e.g., community acquired pneumonia) susceptibility profiles are valuable in guiding antibiotic therapy [77,78]. However, CF pulmonary infection is polymicrobial and using routine culture and sensitivity results as a guide to antibiotic therapy may not be ideal. Culture-independent (metagenomic) microbiological techniques that utilize bacterial RNA present in specimens to generate bacterial profiles, suggest that culture-dependent techniques detect only a minority of the organisms that exist within the infective milieu of CF sputum [79]. Standard culture techniques also under-represent the phenotypic diversity among detected bacteria. Fothergill *et al.* have recently demonstrated immense heterogeneity in genotype and phenotype (including antibiotic resistance) between apparently identical morphotypes of *P. aeruginosa* cultured from a single sputum sample [34]. In comparison, current standard microbiological practices would only select a limited number of morphotypes for susceptibility testing, potentially resulting in a resistance profile that misrepresents the *P. aeruginosa* population as a 'whole' in an individual patient.

The efficacy of routine microbiology is also compromised by the difference in behavior of the most common CF pathogen, *P. aeruginosa* when growing in a biofilm compared with routine pathology laboratory culture systems. For instance biofilm dwelling *P. aeruginosa* are believed to be up to a 1000-times more resistant to antibiotics than planktonic growing bacteria [80].

These limitations of established antibiotic testing methods most likely explain the poor correlation between conventional susceptibility profiles generated by single agent antibiotic testing, and clinical response to treatment [81]. Interestingly, despite a lack of evidence of improved outcomes most clinical guidelines for CF care still advocate the use of antibiotic sensitivity profiles to select 'appropriate' antibiotic regimens [82,83].

Given the shortfalls of traditional single agent sensitivity testing methods a number of alternate strategies are currently being investigated. The recognition that a number of antibiotics exhibit synergistic effects when co-administered *in vitro*, has led to the development of techniques to test the sensitivity of *P. aeruginosa* to combinations of antibiotics [84]. However, application of these methods to guide clinical practice has been limited, with similar clinical and bacteriological responses observed in patients receiving treatment based on single agent sensitivity testing results compared to multiple combination bactericidal testing in a randomized control trial [85].

In vitro biofilm models for assessment of antibiotic susceptibility have been investigated [86,87]. To date, only one study has reported outcomes in patients with antibiotics selected by such techniques and this study also failed to demonstrate superior clinical or microbiological outcomes using the biofilm model [88]. Reasons for this failure may include the confounding effect of different environmental conditions in the lung that were not replicated within the biofilm model (e.g., oxygen concentration and pH) and the need to also account for the fact that a polymicrobial infection exists in the CF lung.

Future perspective

As we begin to more fully understand the complexity of airway infection in CF it is clear that novel microbiological diagnostic methods are required, which more accurately represent resident flora. The emergence and implementation of metagenomic techniques to characterize the bacterial diversity of airway secretion will hopefully improve our understanding of how polymicrobial infections respond to antibiotic treatment. The application of bioinformatics may also provide greater insight into the expression of bacterial resistance genes on a 'whole population' basis that will more accurately predict the response to treatment.

Treating pulmonary exacerbations

Antibiotics are used routinely to treat pulmonary exacerbations in patients with CF, yet this is based on limited evidence including only two small randomized clinical trials [89,90]. Despite neither study providing clear evidence of improved clinical end points in the antibiotic treatment arms, undertaking further placebo-controlled trials would be considered unethical, given the strong anecdotal evidence for benefit.

In selecting an antibiotic regime for an individual patient important considerations include, the specific bacterial pathogens present within the airways, preferred location of treatment (hospital vs home), route of administration, how many antibiotics to use, and which ones to prescribe [91]. These decisions are often influenced by exacerbation severity, available intravenous access, previous antibiotic allergies, adherence and the support available to the individual patient if home-based therapy is to be considered.

Bacteriological considerations

The choice of antibiotics is based on the sensitivity profile of bacteria present on culture of airway secretions. Early infection is typically with the common respiratory pathogen *Staphylococcus aureus*, and nontypeable *Haemophilus influenzae*, which are often replaced by *P. aeruginosa* in late childhood. Other Gram-negative bacteria that may cause chronic infection in a minority of patients include *Burkholderia cepacia* complex (BCC), *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans* [92].

The realization that chronic *P. aeruginosa* infection results in increased lung function decline, exacerbation frequency, morbidity and mortality has led to the development of early detection programs that often involve invasive bronchoscopies and aggressive antibiotic regimes aimed at eradicating *P. aeruginosa* infection at the time of first acquisition [93–96]. Implementation of these regimes within pediatric centers has proven to be effective in delaying the onset of chronic *P. aeruginosa* infection by several years [96], though their impact on the development of bronchiectasis is not well established [97]. However, the long-term impact on survival, antibiotic resistance profiles, and the potential acquisition of other opportunistic bacterial pathogens is not well understood. Similar strategies have been proposed for first acquisition of other CF airway pathogens, but again the long-term efficacy of these regimens remains to be proven (Table 2).

In recent years an evolution of airway bacterial infection has been witnessed with the emergence of several new opportunistic bacteria including *S. maltophilia*, *A. xylosoxidans*, methicillin-resistant *S. aureus* (MRSA), and other Gram-negative bacteria (e.g., *Ralstonia* and *Pandoraea* species), while rates of *Burkholderia* species infection have remained constant [92]. The explanation for the changing microbial profile is unclear but may include better microbiological detection techniques, the effects of more aggressive and successful treatment of *P. aeruginosa*, increased prevalence of the opportunistic pathogens within healthcare facilities, or simply a consequence of increased survival leading to selective pressure due to greater lifetime exposure to antibiotic therapy.

Whilst BCC infections are associated with worse clinical outcomes (including reduced post-transplant survival) [98–100], the clinical importance of other *Burkholderia* species and emerging Gram-negative pathogens are unknown. However, many of these other bacteria are inherently antibiotic resistant, which may increase their potential to significantly impact on disease course and survival in CF [101–103].

Table 2. Eradications strategies for treatment of specific airway pathogens at first isolation.

Bacteria	Antibiotic	Dose	Special instructions
Methicillin-sensitive *Staphylococcus aureus*	Flucloxacillin	100 mg/kg/day oral	Two agents in combination for 2–4 weeks. If persistent infection consider 2 weeks of combination iv. agents
	Fusidic acid	750 mg daily pral (>12 years old) Dosing under 12 complicated see PI	
Methicillin-resistant *Staphylococcus aureus*	Topical mupirocin	Intranasal	Combined use of topical mupirocin (5 days) and two other oral agents for 6 weeks to 6 months
	Trimethorprim–sulfamethoxazole	160/800 mg b.i.d. oral	
	Fusidic acid	750 mg daily oral Dosing under 12 complicated see PI	
	Rifampicin	10 mg/kg/day oral Max 450 mg under 45 kg Max 600 mg over 45 kg	
Haemophilus influenzae	Amoxicillin–clavulanic acid	875/125 mg b.i.d. oral or 250/125 mg, 2 t.i.d. oral Dosing under 12 complicated see PI	Single agent for 2–4 weeks, repeated if still positive at the end of the course. Consider 2 week iv. course if remains positive
	Doxycycline	200 mg initial dose, 100 mg b.i.d. oral[†]	
	Cefaclor	500 mg t.i.d. (>7 years), 250 mg t.i.d. (1–7 years) oral	
Pseudomonas aeruginosa	Ciprofloxacin	15 mg/kg oral b.i.d. (under 5 years) 20 mg/kg oral b.i.d. (5–18 years) 750 mg b.i.d. oral (adult)	3 weeks to 3 months of ciprofloxacin combined with an inhaled antibiotic Multiple regimens have been studied [91–94]
	Colistin	1 million units b.i.d. inhaled (under 2 years) 2 million units b.i.d. inhaled (over 2 years)	
	Tobramycin	300 mg b.i.d. inhaled	

[†]*Avoid tetracyclines in patients under 12 years of age.*
b.i.d.: Twice daily; iv.: Intravenous; Max: Maximum dose; PI: Product information; t.i.d.: Three-times daily.
Data taken from the Report of the UK Cystic Fibrosis Trust Antibiotic Working Group, and the Australian Therapeutic Guidelines [82,83].

In the absence of Gram-negative organisms, choice of antibiotics is typically based on available microbiological cultures and sensitivity patterns, and single-agent treatment may be employed. In the presence of *P. aeruginosa* antibiotic therapy is directed towards this, and modified to target co-infections if clinical response is suboptimal (Table 3).

Antipseudomonal antibiotic classes

Antibiotic options for treatment of *P. aeruginosa* are limited (Table 4). *Fluoroquinolones* exert their bactericidal effect against Gram-negative bacteria through inhibition of DNA gyrase and remain the only oral agent to which *P. aeruginosa* is susceptible *in vitro*.

Aminoglycosides demonstrate dose-dependent antimicrobial effects, suggesting the peak plasma concentration achieved is the most important pharmacokinetic attribute for bacterial killing. This is supported by studies demonstrating the equivalence of once-daily administration compared with traditional multidose regimes [104,105]. The major limitation of systemically administered aminoglycosides is the potential to develop serious and permanent toxic side effects in patients with CF including nephrotoxicity, vestibular disturbance, and ototoxicity (rates of 42, 30 and 17%, respectively) resulting from long-term cumulative exposure [106–108]. Choice of individual aminoglycoside and co-administration of another antipseudomonal agent are important in limiting their toxic effects, with tobramycin being associated with less nephrotoxicity than gentamicin [109]. Similarly, the combined nephrotoxic effects of tobramycin and colistimethate sodium are greater than either agent administered alone [106]. Other factors that may limit nephrotoxicity include, once-daily dosing regimens, and morning aminoglycoside dosing [104]. Therefore despite antibiotic guidelines typically suggesting a total tobramycin dose of 10 mg/kg, careful monitoring of plasma levels is imperative to prevent both acute and long-term toxicity. The dose prescribed may often need to be substantially reduced, particularly in older patients and doses may need to be varied between admission, as well as over time. Several models for aminoglycoside monitoring have been developed, however, there is currently no consensus on the best method to employ [110].

β-lactam antibiotics (β-lactams), in contrast to aminoglycosides exert their bactericidal effects through time-dependent inhibition of bacterial cell wall synthesis, suggesting maintaining plasma levels above the minimal inhibitory

concentration (MIC) of the bacterial pathogen may be important. This led to the hypothesis that continuous infusions would have greater antibacterial effects than intermittent dosing regimens. However, to date β-lactams delivered by continuous infusion have not proven more efficacious than multiple daily doses [111]. In addition a recent study suggested that twice daily ceftazadime dosing may be equivalent to traditional three-time daily dosing [112], although the study has been criticized for being underpowered [113].

There are several classes of β-lactams including, the extended spectrum penicillins (e.g., ticarcillin and piperacillin) and third generation cephalosporins (ceftazadime), which together are the most extensively studied and widely used first-line agents [114], but are also associated

Table 3. Oral and intravenous antibiotics for the treatment of pulmonary exacerbations where *Pseudomonas aeruginosa* is not isolated†.

Bacteria	Antibiotic	Dose	Special considerations
Oral antibiotics			
Methicillin-sensitive *Staphylococcus aureus*	Di/Flucloxacillin	125 mg b.i.d. (prophylaxis) 25 mg/kg q.d. (<18 years of age) 1–2 g q.d.	Consider prophylaxis in 0–3 years of age, but currently unproven clinical benefit
	Trimethorprim–sulfamethoxazole	160/800 mg b.i.d.	
	Clindamycin	450 mg t.i.d.	
	Roxithromycin	150 mg b.i.d.	
Stenotrophomonas maltophilia	Trimethorprim–sulfamethoxazole	160/800 mg b.i.d.	Targeted treatment currently of unproven benefit
	Minocycline	200 mg initial dose, 100 mg b.i.d.‡	
	Tigecycline	100 mg initial dose, 50 mg b.i.d.‡	
Intravenous antibiotics			
Methicillin-sensitive *Staphylococcus aureus*	Di/flucloxacillin	50 mg/kg (<18 years) 500 mg to 1 g q.d.	
Methicillin-resistant *Staphylococcus aureus*	Vancomycin†	1 g b.i.d.	
	Teicoplanin	10 mg/kg b.i.d. three doses, then daily (<18 years) 400 mg for b.i.d. three doses, then daily	
	Linezolid	10 mg/kg (<12 years) 600 mg daily	
Haemophilus influenzae	Cefuroxime	50 mg/kg t.i.d. (1–18 years old) 1.5 g t.i.d.	
	Cefotaxime	50 mg/kg t.i.d. (1–18 years of age) 2 g t.i.d.	
Burkholderia cepacia complex	Ceftazidime	50 mg/kg t.i.d. (1–18 years of age) 2–3 g t.i.d.	A combination of at least two antibiotics from different classes should be used
	Meropenem	25–40 mg/kg t.i.d. (4–18 years of age) 2 g t.i.d.	
	Imipenem	22.5 mg/kg t.i.d. (<40 kg) 1 g t.i.d.	
	Piperacillin–tazobactam	90 mg/kg q.d. (children) 4.5 g q.d.	
	Trimethorprim–sulfamethoxazole	240 mg b.i.d. (6 months to 6 years of age) 480 mg b.i.d. (6–12 years of age) 960 mg b.i.d. (>12 years of age)	
	Tobramycin†	10 mg/kg daily	Plasma concentration monitoring required
Stenotrophomonas maltophilia	Ticarcillin–clavulanate	80–100 mg/kg q.d. (<18 years) 3.1 g q.d.	

This table is Intended as a guide only, antibiotic choice may vary dependent on local resistance profiles and antibiotic availability.
†Therapeutic drug monitoring is required.
‡Avoid tetracyclines in patients under 12 years of age.
b.i.d.: Twice daily; Max: Maximum dose; q.d.: Four-times daily; t.i.d.: Three-times daily.
Data taken from the Report of the UK Cystic Fibrosis Trust Antibiotic Working Group, and the Australian Therapeutic Guidelines [82,83].

with high rates of allergy [115]. Carbapenams (meropenem and imipenem) and fourth generation cephalosporins (cefepime) display activity against both Gram-positive and Gram-negative bacteria, suggesting they may have utility in treatment of co-infection with *S. aureus* and *P. aeruginosa* [116]. Meropenem has demonstrated equivalence to ceftazadime containing regimes in treating pulmonary exacerbations, with lower rates of *P. aeruginosa* resistance developing [117,118]. However, the recent emergence in CF of *P. aeruginosa* mutants producing metal- β-lactamases capable of inactivating all clinical β-lactam substrates (with the exception of aztreonam) raises concerns that increasing resistance rates will be seen in the future [119]. Meropenem also has high *in vitro* activity against anaerobic bacteria present in the CF airway secretions, suggesting it may have additional benefits compared to other antipseudomonal agents in the setting of polymicrobial infection [32]. There are no comparative trials of the cefepime to other treatment regimes in CF, and higher rates of *in vitro P. aeruginosa* resistance have been reported in comparison to ceftazidime [120]. The monobactam antibiotic, aztreonam, has a spectrum of activity limited to Gram-negative organisms. Aztreonam

Table 4. Currently licensed antibiotics for the treatment of *Pseudomonas aeruginosa* in cystic fibrosis.

Class	Drug	Route of administration	Dose	Special considerations
β-lactams				
Extended spectrum penicillins	Ticarcillin/clavulanate†	Intravenous	80–100 mg/kg q.d. (child) 3.1 g q.d. (adult)	Need to be administered by slow intravenous infusion
	Piperacillin/tazobactam†	Intravenous	90 mg/kg q.d. (child) 4.5 g q.d. (adult)	
Third generation cephalosporin	Ceftazidime	Intravenous	50 mg/kg t.i.d. (under 18 years) 3 g t.i.d. (adult)	
Carbapenems	Meropenem†	Intravenous	25–40 mg/kg t.i.d. (4–18 years old) 2 g t.i.d. (adult)	
	Imipenem†	Intravenous	22.5 mg/kg q.d. (under 40 kg) 1 g t.i.d. (over 40 kg)	Need to be administered by slow intravenous infusion
Monobactam	Aztreonam	Intravenous	30 mg/kg (under 2 years) 50 mg/kg (2–12 years) 2 g t.i.d. (over 12 years)	
		Inhaled‡	75 mg t.i.d.	US FDA approved
Fourth generation cephalosporin	Cefepime	Intravenous	50 mg/kg b.i.d. (under 40 kg) 2 g b.i.d. (over 40 kg)	
Aminoglycosides				
	Tobramycin	Intravenous	10 mg/kg daily§	Plasma concentration monitoring required
	Tobramycin inhalation solution	Inhaled‡	300 mg b.i.d.	
Fluoroquinolones				
	Ciprofloxacin	Oral	15 mg/kg (under 5 years) 20 mg/kg (5–18 years) 750 mg b.i.d. (adult)	Mild exacerbations and eradication regimens
Polymyxin B				
	Colistin	Intravenous	25000 units/kg t.i.d. (under 60 kg) 2 million units t.i.d. (over 60 kg)	Need to be administered by slow intravenous infusion
		Inhaled‡	1 million units b.i.d. (under 2 years) 2 million units b.i.d. (over 2 years)	
Other agents				
	Fosfomycin	Intravenous	100 mg/kg (under 40 kg) 5 g t.i.d. (over 40 kg)	

†Co-administration of probenecid may be considered to increase drug levels.
‡Have not been studied in treatment of exacerbations.
§Therapeutic drug monitoring is required.
b.i.d.: Twice daily; q.d.: Four-times daily; t.i.d.: Three-times daily.

contains an unfused β-lactam ring that confers low cross-reactivity with other β-lactam drugs meaning that it can be used safely in patient with penicillin hypersensitivity [121].

Colistimethate sodium (colistin) was originally discovered in the 1940s, but seldom used for several decades due to concerns about its adverse effects. However, it has re-emerged as an antibiotic in patients with CF due to its activity against Gram-negative bacteria including multidrug resistant *P. aeruginosa*. The major adverse effects limiting its use are nephro- and neuro-toxicity [122]. Parenterally administered colistin may be most suited for patients with multidrug resistant *P. aeruginosa*, or in patients where aminoglycosides are contraindicated due to previous toxicity.

Fosfomycin is another 'forgotten' drug with efficacy against multidrug resistant *P. aeruginosa*. Despite evidence for its clinical efficacy in CF being limited to cohort and case studies only [123], *in vitro* data demonstrating its ability to penetrate biofilms, and to act synergistically with agents from each of the major classes of antipseudomonal antibiotics [124], combined with a benign side effect profile suggest it is a useful second-line agent [125]. However, potential for resistance to develop suggests its use is best used only in patients with limited antibiotic options due to allergies and toxicity of other antibiotic agents [126].

Where, how & which antibiotic?

During a severe pulmonary exacerbation, a patient's clinical status should dictate the need for hospital admission. However, many patients with milder exacerbations prefer home-based treatment, which reduces the impact on work and family commitments [127]. When comparing home with hospital treatment the available small studies present conflicting evidence. The equivalent outcomes and improved quality of life from home-based treatment demonstrated in some studies [127,128] needs to be considered in the context of other studies that have demonstrated greater improvements in lung function and weight with hospital-based treatment, as well as a reduction in the duration of therapy [129,130]. A recent, large (>1500 patients) retrospective study has supported home-based treatment demonstrating that although hospital-treated patients appear to gain greater improvement in lung function in the immediate post-treatment period, long-term outcomes, and time to next exacerbation were similar regardless of treatment location [131].

Once treatment location is decided the next consideration is how to administer therapy. As discussed previously, fluoroquinolones are the only orally administered bioavailable antipseudomonal agents. Ciprofloxacin is the most commonly prescribed quinolone, and is frequently combined with a nebulized agent in outpatient treatment regimens for mild exacerbations. Nebulization allows delivery of high drug concentration directly to the airway whilst avoiding systemic adverse effects. Unfortunately inhaled administration of antibiotics fails to treat recalcitrant bacteria sequestered in under-ventilated lung regions. Until recently aminoglycosides and colistin were the only nebulized agents in clinical use. However, multicenter Phase III randomized controlled trials (RCTs) have recently demonstrated the safety and efficacy of aerosolized aztreonam lysine in stable patients with moderate-to-severe lung function impairment [132,133], which has supported US FDA approval for use as a maintenance treatment in CF.

Despite oral and nebulized combination regimes often being used in clinical practice, there is limited evidence to support their use in the acute setting. A Cochrane review identified three small randomized controlled trials comparing ciprofloxacin monotherapy with combination intravenous therapy. No major differences in outcome were seen, but each of the trials was under-powered and the treatment arms were not blinded [134].

Intravenous antibiotic regimes remain the cornerstone of treatment of severe exacerbations in hospitalized patients. Intravenous therapy may also be delivered effectively in the home, providing patients have reliable intravenous access, are appropriately educated in self-administration and have access to 24 h medical support should difficulties arise.

Further considerations when administering intravenous antibiotics-in-the-home setting include the mode of delivery (infusion vs slow intravenous injection) and the stability of the agents in solution. A number of antibiotics demonstrate limited stability in solution (e.g., meropenem) and the patient must be educated in the sterile preparation of these prior to each administration. To overcome the obstacle of slow intravenous infusion of medication a number of small, portable pressure driven devices have been designed, which deliver a fixed rate of drug delivery and negate the inconvenience of infusion pumps and gravity driven delivery.

The superiority of combination intravenous therapy for Gram-negative nosocomial

pneumonia has been clearly demonstrated [135]. However, in the setting of chronic pulmonary infection in CF, systematic reviews comparing combination with monotherapy have failed to clearly demonstrate superiority of combination therapy [114,136,137]. However, whilst acknowledging the current lack of evidence, treatment guidelines recommend dual antipseudomomal antibiotic therapy [82,137]. No combination regimen has demonstrated clear superiority over the others, but in practice two agents from different antimicrobial classes (usually a β-lactam and aminoglycoside) are typically administered.

In selecting specific agents current practice guidelines recommend reference be made to available sensitivity profiles, and also recommend avoiding agents to which organisms are resistant [82]. However given the previously discussed limitations of routine sensitivity testing, and their poor correlation with clinical response, an alternate approach is to select antibiotics to which the individual patient has previously responded. Anecdotally, clinicians may only refer to antibiotic sensitivity profiles when second-line agents are being selected, in the situation where the patient has failed to improve with initial treatment regimen.

Finally, lifetime cumulative exposure to antibiotics results in rates of β-lactam allergy of up to three-times that of the general population, which limits treatment options in a significant proportion of patients. In work from our group, Burrows *et al.* demonstrated a prevalence of 36% for allergy to a single class of β-lactam antibiotic, and 19% of patients had multiple β-lactam allergies [115]. In some patients with multiple antibiotic allergies hospital-based desensitization protocols allow the safe re-introduction of β-lactams to which they have previously reacted [138]. However, recurrent allergy is common and patients must be closely monitored. The additional burden is that desensitization must be repeated for each individual treatment course.

Duration of therapy

There are no RCTs studying the optimum duration of antibiotic therapy for pulmonary exacerbations in CF [137,139]. Theoretical concerns that a shorter duration of antibiotic therapy may reduce the time to the next exacerbation, or increase the rate of lung function decline, need to be balanced against the potential adverse effects [2], and risk of promoting antibiotic-resistant organisms [4], which may be associated with prolonged treatment courses.

Prospective studies in the treatment of acute exacerbations of chronic bronchitis, and microbiologically proven ventilator-associated pneumonia (VAP) have demonstrated noninferiority of shorter courses of antibiotics [140,141]. Although, of note in the case of VAP, patients infected with Gram-negative organisms (commonly *P. aeruginosa*) had an increased risk of microbiological relapse with shorter treatment [141].

In a small prospective cohort study of 22 CF patients undergoing hospital-based treatment for pulmonary exacerbations, improvements in spirometry and oxygenation plateaued after 8 days of treatment [142]. These limited prospective data are supported by a recent large retrospective study of outcomes following pulmonary exacerbations in over 1500 people, which suggested that further improvements in spirometry after 8–10 days of therapy were small, and that shortening antibiotic duration did not adversely affect the time to next exacerbation. Adequately powered prospective studies are required to more fully understand the optimal duration of treatment for pulmonary exacerbations in CF [131].

New antipseudomonal antibiotic options

Development of new antibiotic agents over the past two decades has been limited. Factors that contribute to the reluctance of pharmaceutical companies to invest in antibiotic development include microbiological (e.g., bacterial adaptability leading to resistance while drugs are still in development), regulatory (e.g., need to demonstrate superiority to other agents prior to licensing) and financial (greater profitability in development of other pharmaceuticals e.g., anticancer or immunomodulatory agents) [143,144]. Therefore, recent developments have instead focused on repackaging existing agents, for example for inhalation delivery. Alongside the recently licensed inhaled aztreonam, other inhalational preparation undergoing investigation in RCTs (both Phase II and III) include levofloxacin, coformulation of fosfomycin and tobramycin, liposomal amikacin, and dry powder formulations of tobramycin, colistin and ciprofloxacin [145,146]. Although these medications are being developed primarily for use in maintenance regimens they have the potential to be studied for use in the treatment of pulmonary exacerbations.

Novel targets for drug development

The formation of a biofilm that offers physical protection to *P. aeruginosa* from host immune clearance and antimicrobial agents represents a

significant challenge to developers of new antipseudomonal agents. In an attempt to meet this challenge the focus of research has moved away from bactericidal agents to the development of agents capable of disrupting biofilms, or agents that limit biofilm dwelling bacteria access to essential nutrients.

Quorum sensing is essential to the development of robust biofilms, therefore anti-QS agents are of major interest. A recent study has reported the ability of garlic to block the production of QS molecules by *P. aeruginosa in vitro*, and disrupt biofilm development in mouse urinary and respiratory tract infection models [147,148]. A pilot placebo-controlled study has demonstrated safety and tolerability of garlic in patients with CF, but did not show any differences in outcomes compared to controls [149].

Biofilm 'dispersal' is a naturally occurring process that allows propagation of infection to other sites when available nutrients in the local environment are sufficient to support an expansion in the bacterial community. During dispersal biofilms liberate planktonic bacteria in response to environmental triggers, leaving behind an empty extracellular matrix shell. Given that *in vitro* studies suggest planktonic bacteria are significantly more susceptible to antibiotics than their biofilm dwelling counterparts, it is theoretically possible that inducing dispersal in the presence of antibiotics will enhance their bactericidal effects. Overall control of dispersal is again under the control of QS, but both reactive oxygen and nitrogen species are capable of triggering dispersal [150]. *In vitro* experiments have demonstrated the ability of nontoxic levels of sodium nitroprusside (a nitric oxide donor) to trigger dispersal and increase antibacterial killing when combined with antipseudomonal antibiotics. To date there have been no human trials of dispersal agents.

Iron is an essential nutrient to *P. aeruginosa* and the organism has become adept at scavenging iron from the environment [151]. The primary mechanism by which it obtains iron is through the production of secreted molecules with high iron binding coefficients that are capable of sequestering iron from the environment (siderophores), and delivering it to the bacterium. Interestingly, culturing *P. aeruginosa* in either iron-depleted medium, or medium containing excess iron leads to failure of robust biofilms and disruption of established biofilms [152–154]. Similar results are seen when naturally occurring and synthetic iron chelators are utilized as iron competitors [153].

A novel approach has been to conjugate antibiotics to siderophores as a 'Trojan horse' approach to delivering antibiotics to biofilm dwelling bacteria. These studies are in their infancy, but have shown encouraging results [155].

The studies discussed above offer exciting insights into potentially clinical useful antibiofilms agents. However, for the most part this research has been performed using laboratory strains of *P. aeruginosa*, which may differ considerably in their behavior compared with clinically relevant strains, and in *in vitro* biofilms models that poorly reflect the environmental conditions present in the CF lung.

Future perspective

Further research examining short course antibiotic regimens for treatment of pulmonary exacerbations is merited. However, the lack of defined end points to signal the end of an exacerbation, and reluctance of physicians (and patients) to deviate from current 'successful' treatment regimens will make studies difficult to design and successfully complete.

Future development of novel antibacterial agents will require assessment of their efficacy against clinical *P. aeruginosa* strains, in models developed to more accurately represent conditions in the CF airways. Given that planktonic bacteria released from biofilms are theoretically more immunogenic than their biofilm counterparts, it will be important to carefully assess any potential adverse effects of these strategies on the host inflammatory response.

Adjunctive therapies to prevent & treat pulmonary exacerbations

The proven benefits of oral corticosteroids in exacerbations of asthma and COPD have led to their use in CF exacerbations being proposed. Only one RCT has been performed to examine the effects of oral corticosteroids in CF [156]. This pilot placebo-controlled study examining the effect of a 5-day course of oral prednisolone in 24 children with CF undergoing hospital treatment for a pulmonary exacerbation demonstrated a modest (3.6%), nonsignificant improvement in FEV_1 at 14 days, but this was at the expense of increased rates of treatment related adverse events. The authors concluded that more than 250 patients are needed to adequately power a trial to detect a 4% improvement in FEV_1. Despite this lack of published evidence, a survey of UK CF physicians has suggested that many will use corticosteroids on a case-by-case basis [157].

Given the significant deleterious effects and increased healthcare costs associated with pulmonary exacerbations effective utilization of maintenance treatments to prevent their occurrence is paramount [158]. Much of the improvement seen in the health of people with CF has coincided with the development of multidisciplinary CF care teams, and wherever feasible patient care should be coordinated by a CF specialist center [159,160].

Maintenance exercise and physiotherapy regimens are vital in assisting clearance of viscous airway secretions. A number of chest physiotherapy techniques, including active cycle of breathing, percussion and autogenic drainage, as well as a range of hand-held expiratory oscillatory devices are available to enhance airway clearance, but superiority of one method over the others has not been established, and the individual method employed is usually negotiated between the patient and the expert physiotherapist [161]. Despite widespread acknowledgment of the importance of physiotherapy in maintaining lung health in CF, there are few studies that report on the impact on prevention of, and recovery from pulmonary exacerbations [158].

Inhaled mucolytic and rehydrating agents including recombinant DNAse (Dornase alfa), hypertonic saline and mannitol further facilitate sputum clearance, and have an established role in maintenance regimens [162–164]. Although the role of these agents in the treatment of exacerbations has not been established, pulmonary exacerbations are typically accompanied by an increase in volume and purulence of respiratory secretions, and clearance of these secretions is important in resolution of exacerbations. Therefore maintenance mucolytic regimens should be continued and where tolerated, intensified during exacerbations [137]. However, in clinical practice, tolerance of hypertonic saline and mannitol may be reduced during periods of heightened airway inflammation, especially in patients with severe lung disease.

The use continuous or cyclical courses of antibiotics have been extensively investigated as a method of preventing lung function decline. CF specialist centers in Denmark attribute increased survival in their patients to administration of regular parenteral antibiotics, irrespective of clinical status [165]. However, clinical trials have yet to show superiority of this approach over prescribing antibiotics based on symptoms [114,166]. An alternate approach is to use regular inhaled antibiotics either continuously or on alternate months. This approach was subject to a recent Cochrane review that identified 17 RCTs comparing maintenance inhaled antibiotic regimens to placebo. The review concluded that inhaled antibiotic use was associated with improved lung function, and a reduction in exacerbation rates, but increased rates of resistant *P. aeruginosa* were observed in the antibiotic treated patients [167]. Oral maintenance anti-staphylococcal treatment has been widely prescribed in young children in an attempt to prevent *S. aureus* infection and lung damage. Such regimens reduce rates of *S. aureus* isolation but beneficial effects on lung function or exacerbation rates have not yet been seen, and concern exists that these regimens may result in increased rates of *P. aeruginosa* acquisition [168]. Use of oral azithromycin has been shown to improve lung function and reduce exacerbations rates, particularly in patients with chronic *P. aeruginosa* infection and should be continued during pulmonary exacerbations [15,169,170].

Noninvasive ventilation (NIV) is an emerging therapy in CF [171], which may be used as a physiotherapy adjunct, or as a means of short-term ventilatory support. The need for invasive mechanical ventilation (IMV) during a pulmonary exacerbation is associated with poor survival, and IMV is a contraindication to acute lung transplantation in many centers [43]. NIV has been proposed as an adjunct to prevent the need for IMV. Although there are no RCTs comparing NIV to IMV in CF, observational studies suggest NIV treatment is associated with better outcomes during pulmonary exacerbations and can be used as a bridge to lung transplantation in patients with chronic respiratory failure [44,172–174]. These studies are likely subject to selection bias, but do support a trial of NIV in exacerbations complicated by respiratory failure. As an adjunct to physiotherapy, NIV has been shown to reduce hypoxia and improve tolerance of treatment [175].

Malnutrition is common in CF, and results in reduced lung function and survival [5,176]. Improving nutritional status using high-energy/high-protein supplements may prevent deterioration in lung function [177,178]. However, addressing dietary issues is often difficult and requires expert dietetic, nursing and psychological input in order to not only institute nutritional supplements, but also engender the behavioral changes required to maintain modifications over a prolonged period. The challenge of maintaining weight is added to by a further worsening of the already increased resting energy expenditure present in CF [142], which is often accompanied by a decrease in appetite and anorexia, as well as nausea related to antibiotics. In this setting, although oral supplements

are typically used in the first instance, enteral feeding may also need to be considered [179].

Conclusion

Despite the recognized importance of pulmonary exacerbations in lung function decline and reduced survival in CF, much is still unknown about their etiology, and treatment is still largely based on expert opinion and experience.

Future perspective

It is hoped that the emergence of molecular techniques capable of more accurately representing the bacterial diversity within respiratory secretions, a greater understanding of the biofilm model of bacterial communities, and the development of techniques to test antibacterial agents in *in vitro* models that mimic more closely conditions in the CF airways will herald advances in the treatment of exacerbations.

The polymicrobial nature of infection adds to the complexity in decision making in the treatment of pulmonary exacerbations and will make studies of antibiotic effectiveness more complex, and harder to interpret. In the absence of new classes of antibiotics, novel approaches to airway infection in CF will be required to ensure ongoing improvements in the health of people with CF.

Executive summary

- Acute pulmonary exacerbations contribute to diminished quality of life, reduced lung function, morbidity and mortality in cystic fibrosis (CF), and remain a major obstacle to further improving survival.

Definition

- The lack of a universally accepted definition of a pulmonary exacerbation prevents direct comparisons being made between clinical trials.
- Future directions:
  - Identification of biomarkers reflective of clinical status may facilitate the creation of a standard definition for application in research and clinical practice.

Epidemiology

- Risk factors for pulmonary exacerbations include previous exacerbations, reduced lung function, female sex, and chronic *Pseudomonas aeruginosa* infection.
- Future directions:
  - Monitoring the changes in the epidemiology of airway infection, including emerging bacterial pathogens patterns will be crucial in monitoring response to new therapeutic strategies.

Etiology

- Acute exacerbations occur when the balance between chronic bacterial infection and host immunity is perturbed. Some exacerbations are triggered by acute bacterial or viral infections, but in many cases the cause is unknown.
- Future directions:
  - Identification of common pathways by which infectious and noninfectious stimuli trigger acute exacerbations may allow development of novel therapeutic agents.

Microbiology

- Current culture-dependent microbiological techniques are inadequate to identify the diversity of bacteria present in the CF airway, and poorly predict response to treatment in polymicrobial and biofilm infections that frequently occur.
- Future directions:
  - Culture independent microbiological (metagenomic) techniques will more accurately represent bacteria present in CF airway infection.
  - Application of bioinformatic approaches to identification of bacterial resistance genes may allow more accurate prediction of treatment response.

Treatment

- Many aspects of treatment, including the optimum number and combination of antibiotics to use, and duration and location of treatment, are based on very limited evidence. Antibiotic options for treatment of chronic Gram-negative bacterial infections are limited, and further impacted on by development of bacterial resistance and antibiotic allergies.
- Future directions:
  - Prospective studies comparing outcomes from 'short-course' antibiotic regimens may allow reduction in cumulative antibiotic exposure.
  - Novel antibacterial agents will target biofilm growth and bacterial access to essential nutrients.

Adjunctive treatment

- Treatment in specialist centers by a multidisciplinary team including specialist nurses, physiotherapists and dieticians has significantly contributed to improved survival in CF. Adherence to maintenance therapies including physiotherapy, mucolytics and nutritional supplements are important in preventing exacerbations. Maintenance and eradication antibiotic regimens delay acquisition of chronic bacterial pathogens, but their long-term impact on outcome is as yet unknown.
- Future directions:
  - Surveillance of patients treated with *Pseudomonas aeruginosa* eradications regimens, *Staphylococcus aureus* prophylaxis, and chronic azithromycin therapy will determine the impact of these interventions on bacterial infections and long-term outcomes.

Financial & competing interests disclosure

SC Bell has received grant support from the NHMRC and from Queensland Health. Bell has also received support to undertake clinical trials by the following: Vertex, Boehringer Ingelheim, Aradigm and Mpex, in the past 5 years. He has served on advisory boards to provide advice for clinical trial design and conduct with the following: Boehringer Ingelheim and Mpex, and is also a member of the Publications Committee for an ongoing clinical trial for Vertex. DW Reid has received grant support from the NHMRC and Queensland Health. He has received a one-off grant from Novartis Pharmaceuticals within the past 5 years to undertake an investigator initiated study. DJ Smith has received a research scholarship from the School of Medicine (Northside Clinical School – The Prince Charles Hospital), University of Queensland. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet* 373(9678), 1891–1904 (2009).

2 Parmar JS, Nasser S. Antibiotic allergy in cystic fibrosis. *Thorax* 60(6), 517–520 (2005).

3 Prayle A, Watson A, Fortnum H, Smyth A. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax* 65(7), 654–658 (2010).

4 Merlo CA, Boyle MP, Diener-West M, Marshall BC, Goss CH, Lechtzin N. Incidence and risk factors for multiple antibiotic-resistant *Pseudomonas aeruginosa* in cystic fibrosis. *Chest* 132(2), 562–568 (2007).

5 Liou TG, Adler FR, Fitzsimmons SC, Cahill BC, Hibbs JR, Marshall BC. Predictive 5-year survivorship model of cystic fibrosis. *Am. J. Epidemiol.* 153(4), 345–352 (2001).

6 Sanders DB, Bittner RC, Rosenfeld M, Redding GJ, Goss CH. Pulmonary exacerbations are associated with subsequent FEV_1 decline in both adults and children with cystic fibrosis. *Pediatr. Pulmonol.* 46(4), 393–400 (2011).

▪ **Retrospective study providing strong evidence in support of the association between acute pulmonary exacerbations and accelerated lung function decline.**

7 Britto MT, Kotagal UR, Hornung RW, Atherton HD, Tsevat J, Wilmott RW. Impact of recent pulmonary exacerbations on quality of life in patients with cystic fibrosis. *Chest* 121(1), 64–72 (2002).

8 Lieu TA, Ray GT, Farmer G, Shay GF. The cost of medical care for patients with cystic fibrosis in a health maintenance organization. *Pediatrics* 103(6), e72 (1999).

9 Ramsey BW, Boat TF. Outcome measures for clinical trials in cystic fibrosis: summary of a cystic fibrosis foundation consensus conference. *J. Pediatr.* 124(2), 177–192 (1994).

▪ **Conference report addressing the need to adopt universally recognized outcome measures for use within clinical trials in cystic fibrosis, including a singularly accepted definition of what constitutes a pulmonary exacerbation.**

10 Sagel SD, Chmiel JF, Konstan MW. Sputum biomarkers of inflammation in cystic fibrosis lung disease. *Proc. Am. Thorac. Soc.* 4(4), 406–417 (2007).

▪ **Comprehensive review of the literature to date on the use of biomarkers detected in respiratory secretions to monitor disease severity and response to treatment, with a particular focus on current limitations and future research directions.**

11 Johnson C, Butler SM, Konstan MW, Morgan W, Wohl ME. Factors influencing outcomes in cystic fibrosis: a center-based analysis. *Chest* 123(1), 20–27 (2003).

12 Dakin C, Henry RL, Field P, Morton J. Defining an exacerbation of pulmonary disease in cystic fibrosis. *Pediatr. Pulmonol.* 31(6), 436–442 (2001).

13 Rosenfeld M, Emerson J, Williams-Warren J *et al.* Defining a pulmonary exacerbation in cystic fibrosis. *J. Pediatr.* 139(3), 359–365 (2001).

▪▪ **Prospective study in which the authors use responses to a physician completed questionnaire to develop a tool for predicting the presence of pulmonary exacerbations based on patient symptoms and lung function.**

14 Rabin HR, Butler SM, Wohl ME *et al.* Pulmonary exacerbations in cystic fibrosis. *Pediatr. Pulmonol.* 37(5), 400–406 (2004).

15 Wolter J, Seeney S, Bell S, Bowler S, Masel P, Mccormack J. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax* 57(3), 212–216 (2002).

16 Fuchs HJ, Borowitz DS, Christiansen DH *et al.* Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The pulmozyme study group. *N. Engl. J. Med.* 331(10), 637–642 (1994).

17 Sanders DB, Hoffman LR, Emerson J *et al.* Return of FEV_1 after pulmonary exacerbation in children with cystic fibrosis. *Pediatr. Pulmonol.* 45(2), 127–134 (2010).

▪▪ **Study reports upon the failure of lung function to return to baseline in 25% of patients following intravenous antibiotics, with degree of reduction in lung prior to exacerbation being most predictive of failure to recover, suggesting early intervention may improve long term outcomes.**

18 Colombo C, Costantini D, Rocchi A *et al.* Cytokine levels in sputum of cystic fibrosis patients before and after antibiotic therapy. *Pediatr. Pulmonol.* 40(1), 15–21 (2005).

19 Gray RD, Imrie M, Boyd AC, Porteous D, Innes JA, Greening AP. Sputum and serum calprotectin are useful biomarkers during CF exacerbation. *J. Cyst. Fib.* 9(3), 193–198 (2010).

20 Laguna TA, Wagner BD, Luckey HK *et al.* Sputum desmosine during hospital admission for pulmonary exacerbation in cystic fibrosis. *Chest* 136(6), 1561–1568 (2009).

21 Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest* 133(2), 489–495 (2008).

22 Chiron R, Grumbach YY, Quynh NV, Verriere V, Urbach V. Lipoxin A(4) and interleukin-8 levels in cystic fibrosis sputum after antibiotherapy. *J. Cyst. Fib.* 7(6), 463–468 (2008).

23 Brockbank S, Downey D, Elborn JS, Ennis M. Effect of cystic fibrosis exacerbations on neutrophil function. *Int. Immunopharmacol.* 5(3), 601–608 (2005).

24 Downey DG, Brockbank S, Martin SL, Ennis M, Elborn JS. The effect of treatment of cystic fibrosis pulmonary exacerbations on airways and systemic inflammation. *Pediatr. Pulmonol.* 42(8), 729–735 (2007).

25 Van Rheenen PF, Van De Vijver E, Fidler V. Faecal calprotectin for screening of patients

with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 341, c3369 (2010).

26 Carpagnano GE, Barnes PJ, Geddes DM, Hodson ME, Kharitonov SA. Increased leukotriene B4 and interleukin-6 in exhaled breath condensate in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 167(8), 1109–1112 (2003).

27 Horak F Jr, Moeller A, Singer F *et al.* Longitudinal monitoring of pediatric cystic fibrosis lung disease using nitrite in exhaled breath condensate. *Pediatr. Pulmonol.* 42(12), 1198–1206 (2007).

28 Newport S, Amin N, Dozor AJ. Exhaled breath condensate pH and ammonia in cystic fibrosis and response to treatment of acute pulmonary exacerbations. *Pediatr. Pulmonol.* 44(9), 866–872 (2009).

29 Ojoo JC, Mulrennan SA, Kastelik JA, Morice AH, Redington AE. Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. *Thorax* 60(1), 22–26 (2005).

30 Bodini A, D'orazio C, Peroni DG *et al.* IL-8 and pH values in exhaled condensate after antibiotics in cystic fibrosis children. *Int. J. Immunopathol. Pharmacol.* 20(3), 467–472 (2007).

31 Ordonez CL, Henig NR, Mayer-Hamblett N *et al.* Inflammatory and microbiologic markers in induced sputum after intravenous antibiotics in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 168(12), 1471–1475 (2003).

32 Tunney MM, Field TR, Moriarty TF *et al.* Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 177(9), 995–1001 (2008).

▪ **Demonstrates the presence of anaerobic bacteria in airway secretions from a large proportion of patients studied that are not detected by usual culture techniques.**

33 Tunney MM, Klem ER, Fodor AA *et al.* Use of culture and molecular analysis to determine the effect of antibiotic treatment on microbial community diversity and abundance during exacerbation in patients with cystic fibrosis. *Thorax* 66(7), 579–584 (2011).

34 Fothergill JL, Mowat E, Ledson MJ, Walshaw MJ, Winstanley C. Fluctuations in phenotypes and genotypes within populations of *Pseudomonas aeruginosa* in the cystic fibrosis lung during pulmonary exacerbations. *J. Med. Microbiol.* 59(Pt 4), 472–481 (2010).

35 Sanderson K, Wescombe L, Kirov SM, Champion A, Reid DW. Bacterial cyanogenesis occurs in the cystic fibrosis lung. *Eur. Respir. J.* 32(2), 329–333 (2008).

36 Schneider M, Muhlemann K, Droz S, Couzinet S, Casaulta C, Zimmerli S. Clinical characteristics associated with isolation of small-colony variants of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from respiratory secretions of patients with cystic fibrosis. *J. Clin. Microbiol.* 46(5), 1832–1834 (2008).

37 Lamont IL, Konings AF, Reid DW. Iron acquisition by *Pseudomonas aeruginosa* in the lungs of patients with cystic fibrosis. *Biometals* 22(1), 53–60 (2009).

38 Raivio TL, Ujack EE, Rabin HR, Storey DG. Association between transcript levels of the *Pseudomonas aeruginosa* regA, regB, and toxA genes in sputa of cystic fibrosis patients. *Infect. Immu.* 62(8), 3506–3514 (1994).

39 Grimwood K, Semple RA, Rabin HR, Sokol PA, Woods DE. Elevated exoenzyme expression by *Pseudomonas aeruginosa* is correlated with exacerbations of lung disease in cystic fibrosis. *Pediatr. Pulmonol.* 15(3), 135–139 (1993).

40 Cystic Fibrosis Foundation Patient Registry (2009). Annual data report. Bethesda, MD, USA (2011).

41 Cystic fibrosis in Australia: 12th annual report from the Australian cystic fibrosis data registry. North Ryde, Sydney, Australia (2011).

42 Goss CH, Burns JL. Exacerbations in cystic fibrosis. 1: Epidemiology and pathogenesis. *Thorax* 62(4), 360–367 (2007).

▪ **Comprehensive review of the available literature on the epidemiology of acute pulmonary exacerbations in cystic fibrosis.**

43 Slieker MG, Van Gestel JP, Heijerman HG *et al.* Outcome of assisted ventilation for acute respiratory failure in cystic fibrosis. *Intensive Care Med.* 32(5), 754–758 (2006).

44 Ellaffi M, Vinsonneau C, Coste J *et al.* One-year outcome after severe pulmonary exacerbation in adults with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 171(2), 158–164 (2005).

45 Block JK, Vandemheen KL, Tullis E *et al.* Predictors of pulmonary exacerbations in patients with cystic fibrosis infected with multi-resistant bacteria. *Thorax* 61(11), 969–974 (2006).

46 Vandevanter DR, Yegin A, Morgan WJ *et al.* Design and powering of cystic fibrosis clinical trials using pulmonary exacerations as an efficacy endpoint. *J. Cyst. Fib.* doi: 10.1016/j.jcf.2011.07.003 (2011) (Epub ahead of print).

▪ **The authors use data on acute pulmonary exacerbations derived from a large cohort of cystic fibrosis patients to predict the number of patients required to adequately power an interventional study, dependent on which outcome measure is selected.**

47 Schechter MS, Shelton BJ, Margolis PA, Fitzsimmons SC. The association of socioeconomic status with outcomes in cystic fibrosis patients in the United States. *Am. J. Respir. Crit. Care Med.* 163(6), 1331–1337 (2001).

48 Wat D, Gelder C, Hibbitts S *et al.* The role of respiratory viruses in cystic fibrosis. *J. Cyst. Fib.* 7(4), 320–328 (2008).

49 Emerson J, Rosenfeld M, Mcnamara S, Ramsey B, Gibson RL. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr. Pulmonol.* 34(2), 91–100 (2002).

50 Stick SM, Brennan S, Murray C *et al.* Bronchiectasis in infants and preschool children diagnosed with cystic fibrosis after newborn screening. *J. Pediatr.* 155(5), 623–628 e621 (2009).

51 Ranganathan SC, Parsons F, Gangell C *et al.* Evolution of pulmonary inflammation and nutritional status in infants and young children with cystic fibrosis. *Thorax* 66(5), 408–413 (2011).

52 Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur. Respir. J.* 23(1), 146–158 (2004).

53 Wang EE, Prober CG, Manson B, Corey M, Levison H. Association of respiratory viral infections with pulmonary deterioration in patients with cystic fibrosis. *N. Engl. J. Med.* 311(26), 1653–1658 (1984).

54 Ramsey BW, Gore EJ, Smith AL, Cooney MK, Redding GJ, Foy H. The effect of respiratory viral infections on patients with cystic fibrosis. *Am. J. Dis. Child.* 143(6), 662–668 (1989).

55 Smyth AR, Smyth RL, Tong CY, Hart CA, Heaf DP. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. *Arch. Dis. Child.* 73(2), 117–120 (1995).

56 Oliver BG, Lim S, Wark P *et al.* Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. *Thorax* 63(6), 519–525 (2008).

57 Van Ewijk BE, Wolfs TF, Aerts PC *et al.* RSV mediates *Pseudomonas aeruginosa* binding to cystic fibrosis and normal epithelial cells. *Pediatr. Res.* 61(4), 398–403 (2007).

58 France MW, Tai S, Masel PJ *et al.* The month of July: an early experience with pandemic influenza A (H1N1) in adults with cystic fibrosis. *BMC Pulm. Med.* 10, 8 (2010).

59 Nash EF, Whitmill R, Barker B, Rashid R, Whitehouse JL, Honeybourne D. Clinical outcomes of pandemic (H1N1) 2009

influenza (swine flu) in adults with cystic fibrosis. *Thorax* 66(3), 259 (2011).

60 Viviani L, Assael BM, Kerem E. Impact of the A (H1N1) pandemic influenza (season 2009–2010) on patients with cystic fibrosis. *J. Cyst. Fib.* 10(5), 370–376 (2011).

61 Rau MH, Hansen SK, Johansen HK *et al.* Early adaptive developments of *Pseudomonas aeruginosa* after the transition from life in the environment to persistent colonization in the airways of human cystic fibrosis hosts. *Environ. Microbiol.* 12(6), 1643–1658 (2010).

62 Kobayashi H, Kobayashi O, Kawai S. Pathogenesis and clinical manifestations of chronic colonization by *Pseudomonas aeruginosa* and its biofilms in the airway tract. *J. Infect. Chemother.* 15(3), 125–142 (2009).

63 Kobayashi H. Airway biofilms: implications for pathogenesis and therapy of respiratory tract infections. *Treat. Respir. Med.* 4(4), 241–253 (2005).

64 Aaron SD, Ramotar K, Ferris W *et al.* Adult cystic fibrosis exacerbations and new strains of *Pseudomonas aeruginosa*. *Am. J. Respir. Crit. Care Med.* 169(7), 811–815 (2004).

65 Zemanick ET, Wagner BD, Harris JK, Wagener JS, Accurso FJ, Sagel SD. Pulmonary exacerbations in cystic fibrosis with negative bacterial cultures. *Pediatr. Pulmonol.* 45(6), 569–577 (2010).

66 Worlitzsch D, Tarran R, Ulrich M *et al.* Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J. Clin. Invest.* 109(3), 317–325 (2002).

67 Bartlett JG. The role of anaerobic bacteria in lung abscess. *Clin. Infect. Dis.* 40(7), 923–925 (2005).

68 Torres A, Ewig S, Lode H, Carlet J. Defining, treating and preventing hospital acquired pneumonia: European perspective. *Intensive Care Med.* 35(1), 9–29 (2009).

69 Jones AM. Anaerobic bacteria in cystic fibrosis: pathogens or harmless commensals? *Thorax* 66(7), 558–559 (2011).

70 Muller FM, Seidler M. Characteristics of pathogenic fungi and antifungal therapy in cystic fibrosis. *Exp. Rev. Anti. Infect. Ther.* 8(8), 957–964 (2010).

71 Moss RB. Allergic bronchopulmonary aspergillosis and *Aspergillus* infection in cystic fibrosis. *Curr. Opin. Pulm. Med.* 16(6), 598–603 (2010).

72 Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 137(1), 171–176 (2010).

73 Blondeau K, Dupont LJ, Mertens V *et al.* Gastro-oesophageal reflux and aspiration of gastric contents in adult patients with cystic fibrosis. *Gut* 57(8), 1049–1055 (2008).

74 Mohammed A, Neujahr DC. Gastroesophageal reflux disease and graft failure after lung transplantation. *Transplant. Rev. (Orlando)* 24(2), 99–103 (2010).

75 Sanduleanu S, Jonkers D, De Bruine A, Hameeteman W, Stockbrugger RW. Non-*Helicobacter pylori* bacterial flora during acid-suppressive therapy: differential findings in gastric juice and gastric mucosa. *Aliment Pharmacol. Ther.* 15(3), 379–388 (2001).

76 Boesch RP, Acton JD. Outcomes of fundoplication in children with cystic fibrosis. *J. Pediatr. Surg.* 42(8), 1341–1344 (2007).

77 Lim WS, Baudouin SV, George RC *et al.* BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax* 64(Suppl. 3), iii1–iii55 (2009).

78 Mandell LA, Wunderink RG, Anzueto A *et al.* Infectious Diseases Society of America/American Thoracic Society Consensus guidelines on the management of community – acquired pneumonia in adults. *Clin. Infect. Dis.* 44(Suppl. 2), S27–S72 (2007).

79 Rogers GB, Daniels TW, Tuck A *et al.* Studying bacteria in respiratory specimens by using conventional and molecular microbiological approaches. *BMC Pulm. Med.* 9, 14 (2009).

80 Rasmussen TB, Givskov M. Quorum-sensing inhibitors as anti-pathogenic drugs. *Int. J. Med. Microbiol.* 296(2–3), 149–161 (2006).

81 Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* 123(5), 1495–1502 (2003).

82 Antibiotic consensus group. Antibiotic treatment of cystic fibrosis: report of the UK cystic fibrosis trust antibiotic working group (3rd). Cystic Fibrosis Trust (2009).

▪ **Comprehensive review of antibiotic treatment of cystic fibrosis airway infections based on infecting bacteria.**

83 Therapeutic guidelines: antibiotics. Expert group for respiratory infections (14). Therapeutic Guidelines Limited, Melbourne (2010).

84 Lang BJ, Aaron SD, Ferris W, Hebert PC, Macdonald NE. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. *Am. J. Respir. Crit. Care Med.* 162(6), 2241–2245 (2000).

85 Aaron SD, Vandemheen KL, Ferris W *et al.* Combination antibiotic susceptibility testing to treat exacerbations of cystic fibrosis associated with multiresistant bacteria: a randomised, double-blind, controlled clinical trial. *Lancet* 366(9484), 463–471 (2005).

86 Hill D, Rose B, Pajkos A *et al.* Antibiotic susceptabilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *J. Clin. Microbiol.* 43(10), 5085–5090 (2005).

87 Moskowitz SM, Foster JM, Emerson J, Burns JL. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *J. Clin. Microbiol.* 42(5), 1915–1922 (2004).

▪ **Comprehensive review of the use and monitoring of aminoglycosides in patients with cystic fibrosis.**

88 Moskowitz SM, Emerson JC, Mcnamara S *et al.* Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. *Pediatr. Pulmonol.* 46(2), 184–192 (2011).

89 Wientzen R, Prestidge CB, Kramer RI, Mccracken GH, Nelson JD. Acute pulmonary exacerbations in cystic fibrosis. A double-blind trial of tobramycin and placebo therapy. *Am. J. Dis. Child.* 134(12), 1134–1138 (1980).

90 Gold R, Carpenter S, Heurter H, Corey M, Levison H. Randomized trial of ceftazidime versus placebo in the management of acute respiratory exacerbations in patients with cystic fibrosis. *J. Pediatr.* 111(6 Pt 1), 907–913 (1987).

91 Smyth A, Elborn JS. Exacerbations in cystic fibrosis: 3 – Management. *Thorax* 63(2), 180–184 (2008).

▪ **Comprehensive review of the treatment of pulmonary exacerbations in cystic fibrosis.**

92 Emerson J, Mcnamara S, Buccat AM, Worrell K, Burns JL. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. *Pediatr. Pulmonol.* 45(4), 363–370 (2010).

93 Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. *Lancet* 338(8769), 725–726 (1991).

94 Wiesemann HG, Steinkamp G, Ratjen F *et al.* Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Pediatr. Pulmonol.* 25(2), 88–92 (1998).

95 Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 358(9286), 983–984 (2001).

96 Ratjen F, Munck A, Kho P, Angyalosi G. Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 65(4), 286–291 (2010).

97 Wainwright C, Vidmer S, Armstrong D *et al.* Effect of bronchoalveolar lavage-directed therapy on *Pseudomonas aeruginosa* infection and structural lung injury in children with cystic fibrosis. A randomized trial. *JAMA* 306(2), 163–171 (2011).

98 Courtney JM, Dunbar KE, McDowell A *et al.* Clinical outcome of *Burkholderia cepacia* complex infection in cystic fibrosis adults. *J. Cyst. Fib.* 3(2), 93–98 (2004).

99 Boussaud V, Guillemain R, Grenet D *et al.* Clinical outcome following lung transplantation in patients with cystic fibrosis colonised with *Burkholderia cepacia* complex: results from two French centres. *Thorax* 63(8), 732–737 (2008).

100 Murray S, Charbeneau J, Marshall BC, Lipuma JJ. Impact of *Burkholderia* infection on lung transplantation in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 178(4), 363–371 (2008).

101 Waters V, Yau Y, Prasad S *et al. Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. *Am. J. Respir. Crit. Care Med.* 183(5), 635–640 (2011).

102 Sawicki GS, Rasouliyan L, Ren CL. The impact of MRSA on lung function in patients with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 179(8), 734–735; author reply 735 (2009).

103 Dasenbrook EC, Checkley W, Merlo CA, Konstan MW, Lechtzin N, Boyle MP. Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in cystic fibrosis. *JAMA* 303(23), 2386–2392 (2010).

104 Smyth A, Tan KH, Hyman-Taylor P *et al.* Once versus three-times daily regimens of tobramycin treatment for pulmonary exacerbations of cystic fibrosis – the TOPIC study: a randomised controlled trial. *Lancet* 365(9459), 573–578 (2005).

105 Smyth AR, Bhatt J. Once-daily versus multiple-daily dosing with intravenous aminoglycosides for cystic fibrosis. *Cochrane Database Syst. Rev.* 1, CD002009 (2010).

106 Al-Aloul M, Miller H, Alapati S, Stockton PA, Ledson MJ, Walshaw MJ. Renal impairment in cystic fibrosis patients due to repeated intravenous aminoglycoside use. *Pediatr. Pulmonol.* 39(1), 15–20 (2005).

107 Mulheran M, Degg C, Burr S, Morgan DW, Stableforth DE. Occurrence and risk of cochleotoxicity in cystic fibrosis patients receiving repeated high-dose aminoglycoside therapy. *Antimicrob. Agents Chemother.* 45(9), 2502–2509 (2001).

108 Scheenstra RJ, Rijntjes E, Tavy DL, Kingma H, Heijerman HG. Vestibulotoxicity as a consequence of systemically administered tobramycin in cystic fibrosis patients. *Acta Otolaryngol.* 129(1), 4–7 (2009).

109 Smyth A, Lewis S, Bertenshaw C, Choonara I, Mcgaw J, Watson A. Case-control study of acute renal failure in patients with cystic fibrosis in the UK. *Thorax* 63(6), 532–535 (2008).

110 Smyth AR, Prayle A. Aminoglycoside use in cystic fibrosis: therapeutic strategies and toxicity. *Curr. Opin. Pulmonary Med.* 16(6), 604–610 (2010).

111 Riethmueller J, Junge S, Schroeter TW *et al.* Continuous vs thrice-daily ceftazidime for elective intravenous antipseudomonal therapy in cystic fibrosis. *Infection* 37(5), 418–423 (2009).

112 Adeboyeku D, Jones AL, Hodson ME. Twice vs three-times daily antibiotics in the treatment of pulmonary exacerbations of cystic fibrosis. *J. Cyst. Fib.* 10(1), 25–30 (2011).

113 Smyth A, Knox A. Twice vs three times daily antibiotics in the treatment of pulmonary exacerbations of cystic fibrosis. *J. Cyst. Fib.* (2011).

114 Yang I, Kim S, Bell S. Antibiotics in COPD, bronchiectasis, and cystic fibrosis. In: *Evidence-based Respiratory Medicine.* Gibson PG (Ed.). Blackwell Publishing, Oxford, UK, 389–413 (2005).

115 Burrows JA, Nissen LM, Kirkpatrick CM, Bell SC. β-lactam allergy in adults with cystic fibrosis. *J. Cyst. Fib.* 6(4), 297–303 (2007).

116 Christenson JC, Korgenski EK, Daly JA. *In vitro* activity of meropenem, imipenem, cefepime and ceftazidime against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *J. Antimicrob. Chemother.* 45(6), 899–901 (2000).

117 Latzin P, Fehling M, Bauernfeind A, Reinhardt D, Kappler M, Griese M. Efficacy and safety of intravenous meropenem and tobramycin versus ceftazidime and tobramycin in cystic fibrosis. *J. Cyst. Fib.* 7(2), 142–146 (2008).

118 Blumer JL, Saiman L, Konstan MW, Melnick D. The efficacy and safety of meropenem and tobramycin vs ceftazidime and tobramycin in the treatment of acute pulmonary exacerbations in patients with cystic fibrosis. *Chest* 128(4), 2336–2346 (2005).

119 Walsh TR. Clinically significant carbapenemases: an update. *Curr. Opin. Infect. Dis.* 21(4), 367–371 (2008).

120 Robinson CA, Kuhn RJ, Craigmyle J, Anstead MI, Kanga JE. Susceptibility of *Pseudomonas aeruginosa* to cefepime versus ceftazidime in patients with cystic fibrosis. *Pharmacotherapy* 21(11), 1320–1324 (2001).

121 Buonomo A, Nucera E, De Pasquale T *et al.* Tolerability of aztreonam in patients with cell-mediated allergy to α-lactams. *Int. Arch. Allergy Immunol.* 155(2), 155–159 (2011).

122 Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit. Care* 10(1), R27 (2006).

123 Falagas ME, Kastoris AC, Karageorgopoulos DE, Rafailidis PI. Fosfomycin for the treatment of infections caused by multidrug-resistant non-fermenting Gram-negative bacilli: a systematic review of microbiological, animal and clinical studies. *Int. J. Antimicrob. Agents* 34(2), 111–120 (2009).

124 Kumon H, Ono N, Iida M, Nickel JC. Combination effect of fosfomycin and ofloxacin against *Pseudomonas aeruginosa* growing in a biofilm. *Antimicrob. Agents Chemother.* 39(5), 1038–1044 (1995).

125 Mirakhur A, Gallagher MJ, Ledson MJ, Hart CA, Walshaw MJ. Fosfomycin therapy for multiresistant *Pseudomonas aeruginosa* in cystic fibrosis. *J. Cyst. Fib.* 2(1), 19–24 (2003).

126 Dubois V, Arpin C, Dupart V *et al.* β-lactam and aminoglycoside resistance rates and mechanisms among *Pseudomonas aeruginosa* in French general practice (community and private healthcare centres). *J. Antimicrob. Chemother.* 62(2), 316–323 (2008).

127 Wolter JM, Bowler SD, Nolan PJ, Mccormack JG. Home intravenous therapy in cystic fibrosis: a prospective randomized trial examining clinical, quality of life and cost aspects. *Eur. Respir. J.* 10(4), 896–900 (1997).

128 Proesmans M, Heyns L, Moons P, Havermans T, De Boeck K. Real life evaluation of intravenous antibiotic treatment in a paediatric cystic fibrosis centre: outcome of home therapy is not inferior. *Respir. Med.* 103(2), 244–250 (2009).

129 Nazer D, Abdulhamid I, Thomas R, Pendleton S. Home versus hospital intravenous antibiotic therapy for acute pulmonary exacerbations in children with cystic fibrosis. *Pediatr. Pulmonol.* 41(8), 744–749 (2006).

130 Termoz A, Touzet S, Bourdy S *et al.* Effectiveness of home treatment for patients with cystic fibrosis: the intravenous

administration of antibiotics to treat respiratory infections. *Pediatr. Pulmonol.* 43(9), 908–915 (2008).

131 Collaco JM, Green DM, Cutting GR, Naughton KM, Mogayzel PJ Jr. Location and duration of treatment of cystic fibrosis respiratory exacerbations do not affect outcomes. *Am. J. Respir. Crit. Care Med.* 182(9), 1137–1143 (2010).

▪▪ **Large retrospective study demonstrating that home-based treatment may be equivalent to hospital-based treatment, and shorter courses of antibiotics may be as effective as traditional longer regimens.**

132 Retsch-Bogart GZ, Quittner AL, Gibson RL *et al.* Efficacy and safety of inhaled aztreonam lysine for airway pseudomonas in cystic fibrosis. *Chest* 135(5), 1223–1232 (2009).

133 Oermann CM, Retsch-Bogart GZ, Quittner AL *et al.* An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr. Pulmonol.* 45(11), 1121–1134 (2010).

134 Remmington T, Jahnke N, Harkensee C. Oral anti-pseudomonal antibiotics for cystic fibrosis. *Cochrane Database Syst. Rev.* 3, CD005405 (2007).

135 Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am. J. Respir. Crit. Care Med.* 171(4), 388–416 (2005).

136 Elphick HE, Tan A. Single versus combination intravenous antibiotic therapy for people with cystic fibrosis. *Cochrane Database Syst. Rev.* 2, CD002007 (2005).

137 Flume PA, Mogayzel PJ Jr, Robinson KA *et al.* Cystic fibrosis pulmonary guidelines: treatment of pulmonary exacerbations. *Am. J. Respir. Crit. Care Med.* 180(9), 802–808 (2009).

▪▪ **Provides guidance on areas of current controversy in the treatment of pulmonary exacerbations, including combination antibiotic treatment, aminoglycoside monitoring, site and duration of treatment, and antibiotic synergy testing.**

138 Burrows JA, Toon M, Bell SC. Antibiotic desensitization in adults with cystic fibrosis. *Respirology* 8(3), 359–364 (2003).

139 Plummer A, Wildman M. Duration of intravenous antibiotic therapy in people with cystic fibrosis. *Cochrane Database Syst. Rev.* 1, CD006682 (2011).

140 Falagas ME, Avgeri SG, Matthaiou DK, Dimopoulos G, Siempos II. Short-versus long-duration antimicrobial treatment for exacerbations of chronic bronchitis: a meta-analysis. *J. Antimicrob. Chemother.* 62(3), 442–450 (2008).

141 Chastre J, Wolff M, Fagon JY *et al.* Comparison of 8 vs 15 days of antibiotic therapy for ventilator-associated pneumonia in adults: a randomized trial. *JAMA* 290(19), 2588–2598 (2003).

142 Bell SC, Bowerman AM, Nixon LE, Macdonald IA, Elborn JS, Shale DJ. Metabolic and inflammatory responses to pulmonary exacerbation in adults with cystic fibrosis. *Eur. J. Clin. Invest.* 30(6), 553–559 (2000).

143 Charles PG, Grayson ML. The dearth of new antibiotic development: why we should be worried and what we can do about it. *Med. J. Aust.* 181(10), 549–553 (2004).

144 Trials and tribulations of antibiotic development. *Lancet Infect. Dis.* 8(4), 209 (2008).

145 Van Westreenen M, Tiddens HA. New antimicrobial strategies in cystic fibrosis. *Paediatr. Drugs* 12(6), 343–352 (2010).

146 Ibrahim BM, Tsifansky MD, Yang Y, Yeo Y. Challenges and advances in the development of inhalable drug formulations for cystic fibrosis lung disease. *Exp. Opin. Drug Deliv.* 8(4), 451–466 (2011).

147 Bjarnsholt T, Jensen PO, Rasmussen TB *et al.* Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. *Microbiology* 151(Pt 12), 3873–3880 (2005).

148 Harjai K, Kumar R, Singh S. Garlic blocks quorum sensing and attenuates the virulence of *Pseudomonas aeruginosa*. *FEMS Immunol. Med. Microbiol.* 58(2), 161–168 (2010).

149 Smyth AR, Cifelli PM, Ortori CA *et al.* Garlic as an inhibitor of *Pseudomonas aeruginosa* quorum sensing in cystic fibrosis – a pilot randomized controlled trial. *Pediatr. Pulmonol.* 45(4), 356–362 (2010).

150 Webb JS, Thompson LS, James S *et al.* Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* 185(15), 4585–4592 (2003).

151 Reid DW, Anderson GJ, Lamont IL. Role of lung iron in determining the bacterial and host struggle in cystic fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* 297(5), L795–L802 (2009).

152 Musk DJ, Banko DA, Hergenrother PJ. Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. *Chem. Biol.* 12(7), 789–796 (2005).

153 O'May CY, Sanderson K, Roddam LF, Kirov SM, Reid DW. Iron-binding compounds impair *Pseudomonas aeruginosa* biofilm formation, especially under anaerobic conditions. *J. Med. Microbiol.* 58(Pt 6), 765–773 (2009).

154 Musk DJ Jr, Hergenrother PJ. Chelated iron sources are inhibitors of *Pseudomonas aeruginosa* biofilms and distribute efficiently in an *in vitro* model of drug delivery to the human lung. *J. Appl. Microbiol.* 105(2), 380–388 (2008).

155 Braun V, Pramanik A, Gwinner T, Koberle M, Bohn E. Sideromycins: tools and antibiotics. *Biometals* 22(1), 3–13 (2009).

156 Dovey M, Aitken ML, Emerson J, McNamara S, Waltz DA, Gibson RL. Oral corticosteroid therapy in cystic fibrosis patients hospitalized for pulmonary exacerbation: a pilot study. *Chest* 132(4), 1212–1218 (2007).

157 Hester KL, Powell T, Downey DG, Elborn JS, Jarad NA. Glucocorticoids as an adjuvant treatment to intravenous antibiotics for cystic fibrosis pulmonary exacerbations: a UK Survey. *J. Cyst. Fib.* 6(4), 311–313 (2007).

158 Bell SC, Robinson PJ. Exacerbations in cystic fibrosis: 2. Prevention. *Thorax* 62(8), 723–732 (2007).

159 Walters S, Britton J, Hodson ME. Hospital care for adults with cystic fibrosis: an overview and comparison between special cystic fibrosis clinics and general clinics using a patient questionnaire. *Thorax* 49(4), 300–306 (1994).

160 Yankaskas JR, Marshall BC, Sufian B, Simon RH, Rodman D. Cystic fibrosis adult care: consensus conference report. *Chest* 125(Suppl. 1), S1–S39 (2004).

161 Robinson KA, Mckoy N, Saldanha I, Odelola OA. Active cycle of breathing technique for cystic fibrosis. *Cochrane Database Syst. Rev.* 11, CD007862 (2010).

162 Konstan MW, Wagener JS, Pasta DJ *et al.* Clinical use of dornase alfa is associated with a slower rate of FEV_1 decline in cystic fibrosis. *Pediatr. Pulmonol.* 46(6), 545–553 (2011).

163 Jones AP, Wallis C. Dornase alfa for cystic fibrosis. *Cochrane Database Syst. Rev.* 3, CD001127 (2010).

164 Pettit RS, Johnson CE. Airway-rehydrating agents for the treatment of cystic fibrosis: past, present, and future. *Ann. Pharmacother.* 45(1), 49–59 (2011).

165 Koch C, Hoiby N. Diagnosis and treatment of cystic fibrosis. *Respiration* 67(3), 239–247 (2000).

166 Elborn JS, Prescott RJ, Stack BH *et al.* Elective versus symptomatic antibiotic treatment in cystic fibrosis patients with chronic *Pseudomonas* infection of the lungs. *Thorax* 55(5), 355–358 (2000).

167 Ryan G, Singh M, Dwan K. Inhaled antibiotics for long-term therapy in cystic fibrosis. *Cochrane Database Syst. Rev.* 3, CD001021 (2011).

168 Smyth A, Walters S. Prophylactic antibiotics for cystic fibrosis. *Cochrane Database Syst. Rev.* 3, CD001912 (2003).

169 Saiman L, Marshall BC, Mayer-Hamblett N *et al.* Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA* 290(13), 1749–1756 (2003).

170 Kabra SK, Pawaiya R, Lodha R *et al.* Long-term daily high and low doses of azithromycin in children with cystic fibrosis: a randomized controlled trial. *J. Cyst. Fib.* 9(1), 17–23 (2010).

171 Fauroux B. Noninvasive ventilation in cystic fibrosis. *Exp. Rev. Respir. Med.* 4(1), 39–46 (2010).

172 Sood N, Paradowski LJ, Yankaskas JR. Outcomes of intensive care unit care in adults with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 163(2), 335–338 (2001).

173 Vedam H, Moriarty C, Torzillo PJ, McWilliam D, Bye PT. Improved outcomes of patients with cystic fibrosis admitted to the intensive care unit. *J. Cyst. Fib.* 3(1), 8–14 (2004).

174 Caronia CG, Silver P, Nimkoff L, Gorvoy J, Quinn C, Sagy M. Use of bilevel positive airway pressure (BIPAP) in end-stage patients with cystic fibrosis awaiting lung transplantation. *Clin. Pediatr. (Phila.)* 37(9), 555–559 (1998).

175 Holland AE, Denehy L, Ntoumenopoulos G, Naughton MT, Wilson JW. Non-invasive ventilation assists chest physiotherapy in adults with acute exacerbations of cystic fibrosis. *Thorax* 58(10), 880–884 (2003).

176 Milla CE. Nutrition and lung disease in cystic fibrosis. *Clin. Chest Med.* 28(2), 319–330 (2007).

177 Levy LD, Durie PR, Pencharz PB, Corey ML. Effects of long-term nutritional rehabilitation on body composition and clinical status in malnourished children and adolescents with cystic fibrosis. *J. Pediatr.* 107(2), 225–230 (1985).

178 Dalzell AM, Shepherd RW, Dean B, Cleghorn GJ, Holt TL, Francis PJ. Nutritional rehabilitation in cystic fibrosis: a 5 year follow-up study. *J. Pediatr. Gastroenterol. Nutr.* 15(2), 141–145 (1992).

179 Dodge JA, Turck D. Cystic fibrosis: nutritional consequences and management. *Best Pract. Res. Clin. Gastroenterol.* 20(3), 531–546 (2006).