Strategies to prevent kidney injury from antibiotics in individuals with cystic fibrosis

Naomi Ditchfield,

Keele University

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ABSTRACT

Kidney damage in cystic fibrosis (CF) patients is most commonly caused by antibiotics, such as aminoglycosides, which are used to treat *Pseudomonas aeruginosa* (PA). I conducted a survey of UK CF centres which showed a high rate of use of aminoglycosides in patients with no evidence of PA. I also conducted a Cochrane systematic review to assess the benefits and harms of strategies that may reduce or prevent kidney damage that is caused by intravenous antibiotic treatment. First, I attended necessary training courses for conducting a systematic review. I then wrote the protocol for the review and sent it off for peer review. I responded to the peer review comments making necessary adjustments to the protocol and it was then published. I then began the review process which involved running the searches and screening the results. We identified 54 studies that may be eligible for inclusion in the review. I was able to perform quantitative analysis and quality assessment on 4 of these. 2 studies looked at different combinations of intravenous antibiotics with no combination being more effective at preventing kidney injury. A study addressing time of dosing of tobramycin, showed a statistically significant increase in urinary excretion of KIM1 in the evening group with a mean difference of 0.73 (95% CIs 0.14 to 1.32), p=0.018 when compared to the morning group. Another study reviewed the use of nebulised tobramycin compared to intravenous tobramycin in acute exacerbations. There was a statistically significant increase in urinary excretion of protein, NAG, AAP and β2-Microglobulin in the intravenous group compared to the nebulised group suggesting intravenous tobramycin is more nephrotoxic. Morning dosing of tobramycin and using nebulised tobramycin instead of intravenous tobramycin for acute exacerbations may reduce kidney injury. Larger studies are needed to assess these strategies further.

FOREWORD

Motivation

Months before starting my masters, I met up with my supervisors to discuss the type of project I was interested in. As a medical student I have always sought the most up to date research when writing up my notes but would always wonder how reliable the information was. I chose to undertake a Cochrane review as I thought I would enjoy seeking out the most recent and relevant information and it would give me an opportunity to learn how to assess the quality of research. I thought that by carrying out a systematic review it would improve my statistical knowledge and skills. As a doctor it is important to be able to read a paper and interpret data in order to give your patients the most informed advice. I have had an interest in Paediatrics since the beginning of medical school and would like to specialise in this area when I have completed my foundation training.

Acknowledgements

I would like to thank my lead supervisor, Dr Will Carroll, for his continued support and encouragement during the year. Dr Carroll acted as the second reviewer, he independently screened the studies for the review, completed data extraction and assessed the risk of bias in the included studies. I would also like to thank my co-supervisor Dr Francis Gilchrist for his help preparing the questionnaire and for his guidance on my thesis. Dr Gilchrist acted as the external arbiter in our Cochrane review, resolving any conflicts between myself and WC in the screening, data extraction and assessment of bias.

I am very grateful to Keele University Medical School for allowing me to intercalate and to the Research Institute of Primary Care and Health Sciences for providing me with this opportunity. I am particularly grateful to Nadia Corp who helped me to design a search strategy and without her I would have struggled. Zara Richards helped me to fill in the required paperwork and was always on hand when I needed assistance. Thank you to Stephen Parton at the library who helped me access the required databases.

I would like to thank Nikki Jahnke and Tracey Remmington, the managing editors of the Cochrane review group, for answering my many emails, guiding me through the review process. I would also like to thank the Cystic Fibrosis and Genetic Disorders group for their peer review comments that helped to improve my protocol. I am thankful to Ashma Krishan, the group's statistician, who provided support on statistical queries. I would also like to thank Jessica Green, a previous intercalator, who helped to design the proposal for the Cochrane Review and was on hand to offer support and advice.

Finally, I would like to acknowledge my family and friends for their support over the year. A special thanks to my mum who shows interest in all my work and inspires me to follow a career you love.

I am responsible for the presentation and writing of the findings in this thesis. I was responsible for writing the Cochrane protocol and responding to the peer review comments we received. I received guidance and feedback on my writing from Dr Carroll and Dr Gilchrist. For the Cochrane review, I received advice from my supervisors (Dr Carroll & Dr Gilchrist), the clinical review groups managing editors (Nikki Jahnke and Tracey Remmington) and the groups statistician (Ashma Krishan).

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LIST OF ABBREVIATED PERSONS

ND: Naomi Ditchfield, author of the thesis and intercalating medical student

WC: Dr Will Carroll, lead supervisor and consultant respiratory paediatrician at University Hospital of North Midlands

FG: Dr Francis Gilchrist, co-supervisor and consultant respiratory paediatrician at University Hospital of North Midlands

NJ: Nikki Jahnke, managing editor of Cochrane Cystic Fibrosis and Genetic Disorders Group

TR: Tracey Remmington, managing editor of Cochrane Cystic Fibrosis and Genetic Disorders Group

AK: Ashma Krishan, Cochrane Cystic Fibrosis and Genetic Disorders Group statistician

LIST OF ABBREVIATIONS

AAP: Alanine amino-peptidase

AKI: Acute kidney injury

CF: Cystic fibrosis

CFALD; Cystic fibrosis-associated liver disease

CFRD: Cystic fibrosis-related diabetes

CFTR: Cystic fibrosis transmembrane regulator

CI: Confidence interval

CKD: Chronic kidney disease

CRG: Cochrane review group

DIOS: Distal intestinal obstruction syndrome

ENaC: Epithelial sodium channel

ESKD: End stage kidney disease

GIV: Generic inverse variance

GRADE: Grading of Recommendations, Assessment, Development and Evaluation

IL-18: Interleukin-1

ISRCTN: International Standard Randomised Controlled Trials Number

KIM-1: Kidney injury molecule 1

MD: Mean difference

MECIR: Methodological Expectations for Cochrane Intervention Reviews

MeSH: Medical subject headings

NAG: N-acetyl-β-D-glucosaminidase

NGAL: Neutrophil gelatinase-associated lipocalin

NSAIDs: Non-steroidal anti-inflammatory drugs

OGTT: Oral glucose tolerance test

OR: Odds ratio

PA: *Pseudomonas aeruginosa*

RCT: Randomised controlled trial

RevMan®: Review Manager 5.3

RR: Risk ratio

RRT: Renal replacement therapy

SD: Standard deviation

SMD: Standardised mean difference

UK: United Kingdom

WHO ICTRP: World Health Organisation International Clinical Trials Registry Platform

CHAPTER 1: AIMS AND OBJECTIVES

1.1 Aims

This thesis aims to determine the evidence-base for strategies to prevent kidney injury in those receiving intravenous antibiotics that have cystic fibrosis (CF).

1.2 Objectives

1. Conduct a Cochrane systematic review to determine the evidence-base assessing the benefits and harms of strategies (such as altering the type and dose of intravenous antibiotics, the avoidance of other nephrotoxic drugs alongside the intravenous antibiotics and the use of adjuvant medication including statins and fluids) to reduce or prevent kidney injury in people with CF which occurs as a result of intravenous antibiotic treatment.

2. Conduct a survey to evaluate the current use of intravenous antibiotics in children and adults with CF across centres in the United Kingdom.

CHAPTER 2: BACKGROUND OF CYSTIC FIBROSIS

2.1 Overview

This chapter will cover the background of cystic fibrosis (CF) including its epidemiology, aetiology, diagnosis, clinical features, management, prognosis, and reviews research addressing future therapies that may be used in the treatment of CF.

2.2 Introduction

Cystic fibrosis is an inherited life-limiting condition caused by a mutation in the cystic fibrosis transmembrane regulator (CFTR) gene. The disease is inherited in an autosomal recessive pattern which means the individual requires two copies of the faulty gene, one from each parent, to inherit the disease. The faulty gene causes an absence or reduction in the number of CFTR channels which results in stickier mucus in the affected individuals compared to the normal population. This affects multiple organ systems including the lungs, the pancreas, the gastrointestinal tract, the reproductive tract, and the hepatobiliary system.

2.3 Epidemiology

In the UK around 1 in 25 people are carriers of a faulty CFTR gene. The Cystic Fibrosis Registry in 2016 identified 10,461 people suffering from CF in the UK. This equates to about 1 in 2,500 live births each year. The population of those affected by CF is made up of 53.2% males and 46.8% females. The median age of those affected in 2016 was 20 years old.[1]

2.4 Aetiology

2.4.1 CFTR discovery

CF was first discovered over 400 years ago, but it was not until 1989 that the CFTR gene was discovered by Dr Lap-Chee Tsui and his team.[2] It is a large gene that is located on the long arm of chromosome seven (7q31.2).[3] As mentioned earlier, CF is caused by mutations in the CFTR gene. The CFTR gene codes for the CFTR protein which is a member of the ABC transporter family (ATP (adenosine triphosphate) -binding cassette). It is made up of two repeated units that are joined together by an R domain. Each unit contains a membrane-spanning domain (MSD), which are made up of six transmembrane regions, and a nucleotide-binding domain (NMD).[4]

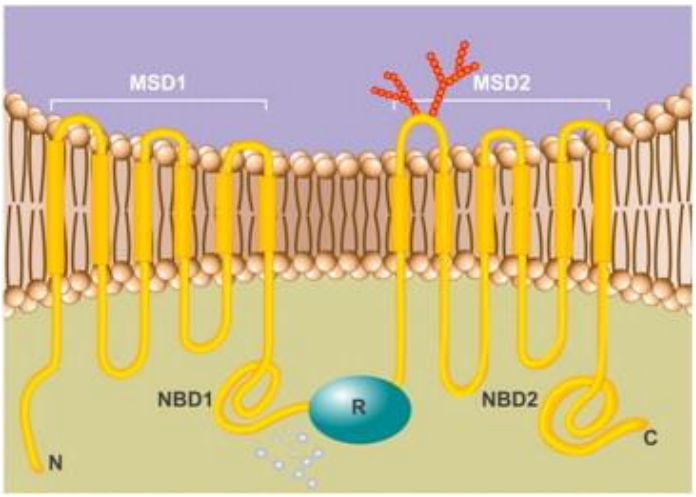

Figure 2.1 - CFTR protein[5]

The CFTR channel is mainly found at apical epithelial surfaces including the respiratory system, reproductive system, gastrointestinal system, sweat duct and the pancreas. However, recent research has suggested it may be found in non-epithelial tissue such as smooth muscle cells in the airways, vascular smooth muscle, cardiac myocytes, skeletal muscle, neuronal cells, immune cells and possibly erythrocytes.[6–13] The levels of CFTR

channels in these locations is lower, however, it still may cause some of the symptoms experienced in CF which will be discussed later.

2.4.2 CFTR mutations

The Cystic Fibrosis Mutation Database (CFTR1) currently lists 2027 mutations in their database.[14] Phe508del, previously known as ΔF508, is the most common mutation with 82.4% of CF patients in Europe in 2016 having at least one copy.[15] The highest allele frequency of Phe508del is found in Denmark.[15] Certain mutations have a higher prevalence in different countries due to a founder effect. For example, Gly551Asp mutation is most frequent in Ireland with an allele frequency of 8.78%.[15] The W1282X mutation is most frequently found in Israel, particularly in Ashkenazi Jews, with an allele frequency of 23.28%.[15]

Historically, little attention was paid to the precise genotype. Whilst some genotypes were associated with milder disease and pancreatic sufficiency, the type of defect was less important. However, over the last decade it has become apparent that certain genetic defects are more, or less amenable to treatment with small molecules including CF potentiators and CF correctors. These hold considerable promise and may offer significant improvements in life expectancy.

The different mutations can be split into five different classes, however, some mutations may fit into more than one category.

Class I: Defective protein synthesis

Most types of mutation can result in premature stop codons. These lead to the production of an abnormally short protein product. The usual 1480 amino acid protein is therefore shorter than expected. These CFTR proteins are not simply defective but the majority of

protein product is degraded before it reaches the cell surface. Typically, this results in little or no functional CFTR reaching the cell membrane and CFTR activity is very low. The genotype from premature stop codons always ends with an 'X'. Examples of this class of mutations include G542X, W128X, R553X and R1162X.

Class II: Abnormal protein folding, processing, and trafficking

Missense mutations and in-frame deletions lead to a CFTR protein being created, but it is misfolded, so it is then degraded in the endoplasmic reticulum. Therefore, very little or no CFTR protein reaches the epithelial cell surfaces. Examples of this class of mutation include Phe508del, N1303K and I507del. Phe508del is the most common mutation in this class but also out of all the mutations. The Phe508del mutation is where three nucleotides coding for phenylalanine at amino acid position 508 are deleted.

Class III: Defective regulation

Some missense mutations can cause a CFTR that is created and moves to the cell surface but that does not open. It is known as a 'gating mutation' as there are normal amounts of CFTR at the cell surface, but they are non-functioning. Examples of this class of mutations include Gly551Asp, S549N and G1349D.

Class IV: Decreased conductance

Some missense mutations lead to a CFTR protein that is created and moves to the epithelial cell surface but has a 'misshaped pore' preventing movement of Cl^- through the channel. Examples of this type of mutation include R117H, D1152H and R347P

Class V: Reduced abundance

Some missense mutations affect splice sites or the CFTR promoter which means a functional CFTR is made and moves to the epithelial cell surfaces but there are reduced

amounts of it. Examples of this class of mutation include 3849+10kbC→T, 2789+5G→A and A455E.

Class VI: Reduced stability

Some mutations can cause an increase in CFTR turnover at the cell surface meaning although the CFTR at the cell surface is functional, it is often unstable. Examples of this class of mutation include 120del23, N287Y and 4279insA. Many people, including the CF Trust, do not recognise this class of mutation perhaps due to its more recent discovery.

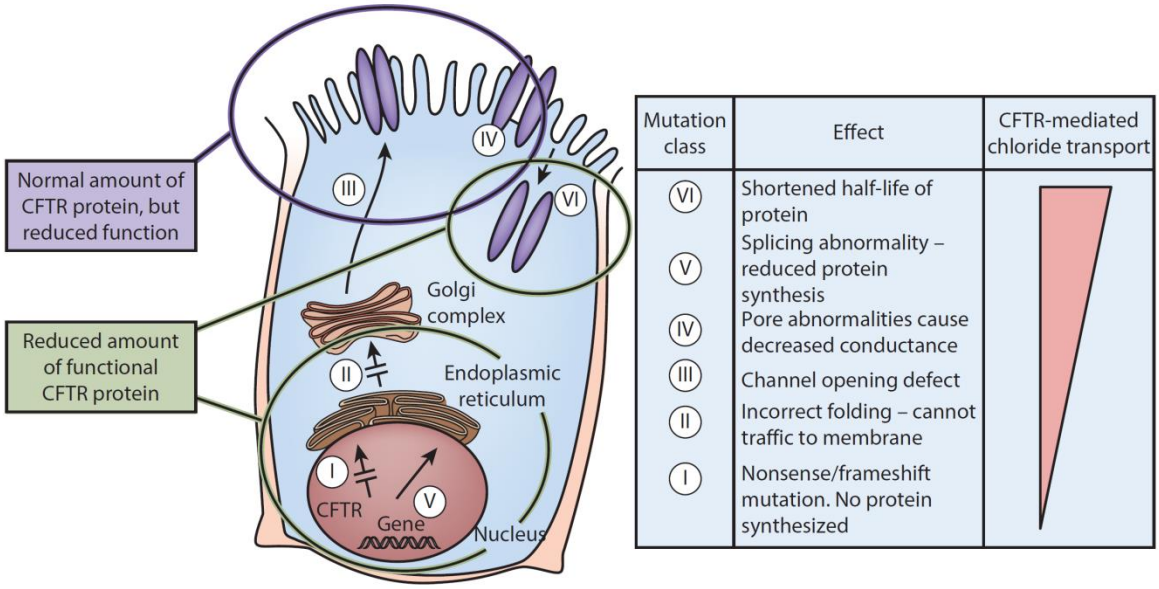

Figure 2.2 - CFTR mutations[16]

2.4.2 Role of CFTR protein

The CFTR protein functions slightly different in sweat glands than other epithelial surfaces such as respiratory, pancreatic and airway epithelia.

CFTR permits two physiologically relevant anions to pass through the cell membrane, chloride, and bicarbonate. It is also permeable to other anions of less certain significance including iodide, bromide, fluoride and glutathione.[5] Flow through the channel is thought to be controlled by cAMP (cyclic adenosine monophosphate)-dependent PKA (protein

kinase A) phosphorylation of the R domain and also by binding of ATP to the NMD regions which both induce conformational changes in the channel.[17]

Sweat gland

The most important anion which can pass through the CFTR is chloride (Cl^-). Sweat (NaCl and water) is secreted onto the skin surface and when the water evaporates this cools the skin down. In the sweat gland epithelium, CFTR absorbs Cl^- that is on the surface of the skin to control levels of NaCl in the body.[18] The CFTR protein also regulates the epithelial sodium channel (ENaC). A normal CFTR protein is required for ENaC to be able to absorb excess Na^+ from the skins surface.[19,20]

Individuals with CF have a reduced number or faulty CFTR proteins in the sweat ducts hence less Cl^- can be absorbed from the skins surface.[18,21,22] ENaC activity is reduced in CF patients due to a non-functioning CFTR, and so less Na^+ is reabsorbed back into the body.[20] This means that people with CF have more NaCl in their sweat and this is something mothers may notice when kissing their baby.

Intestinal, pancreatic and airway epithelia

A normal CFTR protein secretes Cl^- across the epithelium onto the luminal surface of the pancreas, intestines and airways.[23] In these locations CFTR normally downregulates ENaC function which is to absorb Na^+ from the lumen.[24] Therefore, with a normally functioning CFTR, Cl^- is being secreted into the lumen, small amounts of Na^+ are being absorbed from the lumen and therefore, very little H_2O is absorbed from the lumen.

In individuals with CF there is reduced secretion of Cl^- into the lumen of the intestines, pancreatic ducts and airways.[25,26] With a non-functioning CFTR, it cannot regulate ENaC activity as effectively and so this leads to more absorption of Na^+ from the lumen.[26–28]

With very little Cl^- or Na^+ left in the lumen, more H_2O is drawn away from the lumen back into the cells which leaves a dehydrated mucus coating the epithelial cells.

2.5 Diagnosis

2.5.1 Newborn screening

At five days of age, new-born babies are offered the Guthrie test which screens for nine genetic conditions including CF. This test involves taking four drops of blood from the babies' heel and placing them on a card. Parents will receive the results within 6-8 weeks but usually sooner if they are found to be positive. In 1979, it was discovered that all people with CF had raised levels of serum immunoreactive trypsinogen (IRT) in the first few months of life.[29] This suggested that dried-blood spot assay may be useful for screening for CF. In 1980 screening for CF based on IRT began in East Anglia. Raised IRT is not specific for CF and so early screening required a second-tier test which was usually to repeat IRT levels a few weeks later. Screening for CF has been carried out universally in the UK since October 2007. Since discovery of the CFTR gene, the second-tier test now involves genetic analysis testing of the blood to look for any CFTR mutations if the initial IRT is at or above the 99.5th centile. A cut of at the 99.5th centile has been shown to have a sensitivity of 97%.[30]

If babies have mutations in both CFTR genes, then it is presumed they have CF and then are referred to a specialist for assessment and sweat testing. Babies with only one mutation are usually carriers, however, it may be that they carry a rare gene that has not been detected and so they should have a second IRT which if normal means they are a carrier. If the second IRT is abnormal, then again they should be referred to a specialist and for further mutation analysis.[31] Babies that are screened are on average diagnosed at

1 month but only diagnosed at 6 months if clinically diagnosed.[32] Reviews have conflicting evidence regarding the benefit of early detection but recent evidence suggests there is a decrease in mortality in those that are detected via screening.[33] It also improves nutritional status as pancreatic enzyme replacement can be started sooner.[34] If results are inconclusive or false-positive results are received then this can create extra anxiety in the parents. However, if babies aren't screened and start to develop symptoms, this can be even more worrying for the parents especially if the diagnosis if missed originally.

2.5.2 Sweat test

The gold standard for diagnosis of CF is a sweat test which was first discovered in 1959.[35] After 2 weeks since birth a sweat test can be performed on the infant given they weigh more than 2kg and are systemically well at the time of testing. If they weigh less than this, it can be difficult to obtain enough sweat. If the infant is experiencing symptoms them the sweat testing can be attempted from 7 days of age, however may need to be repeated if not enough sweat was collected.[36] Sweat production is stimulated on the flexor surface of the forearm by iontophoresis of pilocarpine into the skin. Skin that is eczematous should be avoided as this can cause a false positive result. The sweat is collected on a piece of filter paper over a standardized period of time, usually between 20-30 minutes. The chloride levels are measured, and these results are interpreted based on the age of the child. In all ages groups a sweat chloride of over 60 mmol/L is defined as a positive test and this child is likely to have CF. In a child under 6 months of age a sweat chloride under 30 mmol/L is defined as a negative test and CF is considered unlikely. If they score between 30-60 mmol/L, then this level is classed as intermediate and requires further assessment. In someone over age 6 months a level below 40 mmol/L is normal, and these patients are unlikely to have CF. However, a level between 40-60mmol/L in people over 6 months is again classed as intermediate and requires further

investigation.[36] In the majority of people with CF this test gives a positive result confirming the diagnosis, however 1-2% of cases that have CF will have a negative sweat test result which may be due to specific mutations.[37] Other examples of false negatives include corticosteroid use or oedema commonly due to hypoproteinaemia.[38] There are other causes of a positive sweat test other than CF, these include adrenal insufficiency, hypothyroidism, malnutrition, G6PD deficiency and nephrogenic diabetes insipidus, however these diseases present differently to CF.

2.5.3 Faecal elastase

Once a diagnosis of CF has been confirmed it is necessary to determine whether the individual is pancreatic sufficient. This involves collecting a stool sample and detecting faecal pancreatic elastase-1 via enzyme-linked immunosorbent assay. Faecal elastase-1 does not degrade on its journey through the gastrointestinal system and so low levels in the stool suggest pancreatic insufficiency.[39] However, this is an indirect method of testing pancreatic exocrine function and so they are less specific and sensitive when compared to a direct method. This is particularly important in those with mild pancreatic insuffiency who may be missed because of this.[40] Advantages of indirect testing includes it is less invasive, less time consuming and less costly.[41]

2.5.4 Genetic screening

Following diagnosis if the individual has any siblings these are tested for CF in case it has been missed on screening and other family members can be screened to check for carrier status. Being a carrier can cause anxiety and depression as it creates uncertainty particularly if they are considering having children.

2.5.5 Antenatal testing

For those that are at increased risk of having a child with CF they may choose to have antenatal testing. This involves the fetus being tested for CF by amniocentesis or chorionic villus sampling.

2.6 Respiratory system

2.6.1 Pathophysiology

The entire respiratory system is significantly affected by the presence of reduced CFTR function. In the upper airway, this results in significant sinus disease. In the lower airway, there is a repeated cycle of infection and inflammation that leads to progressive lung disease with bronchiectasis and eventual destruction of normal lung parenchyma. This is because people with CF are more 'vulnerable' to infection. The 'vulnerability' arises from a weakened host immune response, chronic inflammation, decreased mucociliary clearance and a dehydrated mucus layer causing mucus stasis and adhesion eventually leading to mucus plugs.

In normal individuals the airways are rich in antimicrobial proteins including lactoferrin, antimicrobial peptides and reactive oxygen species which kill and/or supress antimicrobial growth. It is the submucosal glands that secrete these antimicrobial protein, however in CF these glands can be obstructed due to mucus plugging and so this secretion does not happen as easily.[42] Secondly, due to the CFTR dysfunction there is less bicarbonate secreted onto the lung epithelial surface and so it is more acidic.[43] Studies in CF pigs have shown that this acidification can reduce function of one of the antimicrobial peptides.[44] It is likely that these antimicrobial peptides can only supress bacterial growth for a limited period of time and rely on the mucociliary clearance to happen in order to

maintain normal lung sterility.[45] Unfortunately in CF there are areas of absent and reduced mucociliary clearance.[46]

As described above studies have found the presence of CFTR in macrophages. Macrophages have an important role in the inflammatory response and killing inhaled microbes. CFTR deficient macrophages have been shown to be related to infection in mouse models.[47]

There is a hypothesis that the CF airways are 'hyper-inflammatory' due to bronchoalveolar lavage samples from infant CF patients having neutrophilic inflammation in the absence of bacterial infection. [48] However, mucus plugging itself can cause a pro-inflammatory environment which may be due to how the mucus can trap inflammatory cells.[49]

Later in CF disease pathogenesis, chronic inflammation promotes airway remodelling which involves goblet cell hyperplasia causing mucin hypersecretion, which exacerbates the 'relative' dehydration of mucus, and airway epithelial thickening.[50]

After airway mucus plugging, the airways are then susceptible to infection. It is likely that infection arises after mucus plugging as virtually all the bacteria that infect the CF airway lumen are contained within the mucus plaques rather than the epithelial surface.[51] Biofilms can form in CF mucus which can trap bacteria and make them more resistant to antimicrobial agents and so cannot be eradicated as easily.[52]

Airway epithelial cells use the oxygen from the airway lumen for cellular metabolism rather that surrounding vasculature. With mucus plugging the epithelium struggle to receive this oxygen as the diffusion distance is further. With bacteria infecting the mucus and consuming oxygen this further lowers the amount of oxygen available to epithelial

cells. This changes the nature of the inflammatory process with levels of certain inflammatory mediators rising. The hypoxic environment provides the perfect environment for colonisation with anaerobic bacteria known as the CF microbiome. This includes organisms such as *Prevotella sp, Veillonella sp* and *Streptococcal sp*.[53] It is not yet known whether the presence of these organisms is pathogenic or not.

In CF the mesh size of the mucus is a lot smaller than mucus found in normal patients. Less neutrophils can migrate through the mesh to reach bacteria in the mucus meaning less bacteria are killed. As the mucus is then persistently infected with bacteria this causes release of neutrophil chemotaxis agents which bring more neutrophils to the site. This persistently high level of neutrophils causes a large amount of antimicrobial substance (e.g. elastase) to be released into the lumen. In particular, elastase can cause airway wall damage which produces the bronchiectasis seen in CF patients.[54]

2.6.2 Respiratory tract infection

Respiratory infection is the hallmark of CF, and it presents early in life in most individuals. Infection with bacteria, viruses and fungi can all result in respiratory morbidity, with increased symptoms and reduced lung function.

Bacterial infection and the CF lung

In the beginning, CF patients are likely to suffer from intermittent bacterial infection of the airways and then as the disease progresses can become chronically infected. Earlier in life the most common bacteria seen is *Staphylococcus aureus* but by late teens the most prominent organism is PA.[15] In the UK in 2016, 44.2% of adults (≥16 years) had chronic PA growth with 32-35 being the most common age group growing it.[1] The 2016 annual data report demonstrates a statistically significant drop in chronic PA growth in most age

groups compared to 2008.[1] Other organisms that CF patients may grow include *Burkholderia cepacia* complex, *Haemophilus influenzae, Achromobacter xylosoxidans, Stenotrophomonas maltophilia* and non-tuberculous mycobacterial such as *M. avium* complex and *M. abscessus* complex.[1,55,56]

Viral infection in CF

As with unaffected individuals, children and adults with CF are more likely to suffer from symptomatic viral respiratory tract infections in the autumn and winter. There is no evidence that CF patients are more prone to viruses than normal individuals, however, the outcome is often worse for them, with more prolonged symptoms, a greater decline in lung function and a higher likelihood of hospitalization.[57–59] Respiratory viruses can be complicated by secondary bacterial infection and it has been suggested that they may be related to the first isolation of PA.[60,61]

Fungal infection in CF

Fungal infection and sensitisation with subsequent reaction to fungal spores can lead to significant respiratory complications in children and adults with CF. It is thought that there is an increased prevalence of growth in *Aspergillus* due to the antibiotic regimens in particular the use of long term prophylactic antibiotics.[62,63] *Aspergillus* causes a range of conditions including aspergillus bronchitis, allergic bronchopulmonary aspergillosis (ABPA), aspergilloma and aspergillosis. ABPA is a hypersensitivity disease where there is an excessive immune response to *Aspergillus* antigens. This may present acutely with a wheeze, cough, dyspnoea, increased sputum production, exercise intolerance and reduced pulmonary function on testing. It can be diagnosed with raised serum IgE levels and a positive skin prick test to *Aspergillus*.

2.6.3 Respiratory morbidity in CF

Some patients with CF can be asymptomatic, however, other patients can experience cough, dyspnoea, and sputum production most days which are managed with medications. There are periods of time when their symptoms are much worse than their normal state and this is described as a pulmonary exacerbation. A pulmonary exacerbation can present as increased cough, increased dyspnoea, fever, increased sputum production, change in colour of the sputum to green/yellow and decreased exercise tolerance. During these exacerbations patients may have new X-ray changes and decreased pulmonary function on testing and so these episodes need to be managed promptly.

Eventually, patients may develop type 1 (hypoxic) respiratory failure which then causes pulmonary hypertension and cor pulmonale. Type 2 (hypoxic and hypercapnic) respiratory failure may develop and patients may require non-invasive ventilation. Respiratory failure is the leading cause of death in CF patients with up to 65% of mortality caused by it.[64]

2.6.4 Other respiratory complications

CF patients may experience other respiratory complications such as sinusitis, nasal polyps, pneumothorax, bronchiectasis, atelectasis, haemoptysis, and pulmonary hypertension.

2.6.5 Management of the respiratory system

Organisation of care and monitoring

CF clinics are held on different days for patients that are colonised with PA to those non-colonised to prevent cross infection. CF patients attend clinics regularly for monitoring of their clinical status. During clinics they are seen by several professionals including doctors, physiotherapists, dieticians, psychologists, and nurses. The doctor examines the patient, enquires about any recent changes in symptoms and monitors their compliance with treatments. Figure 2.3 highlights some of the signs that may be seen in a patient with CF. Pulmonary function tests are performed each clinic to ensure the patient is not acutely unwell and all patients are asked to provide a sputum sample or cough swab.

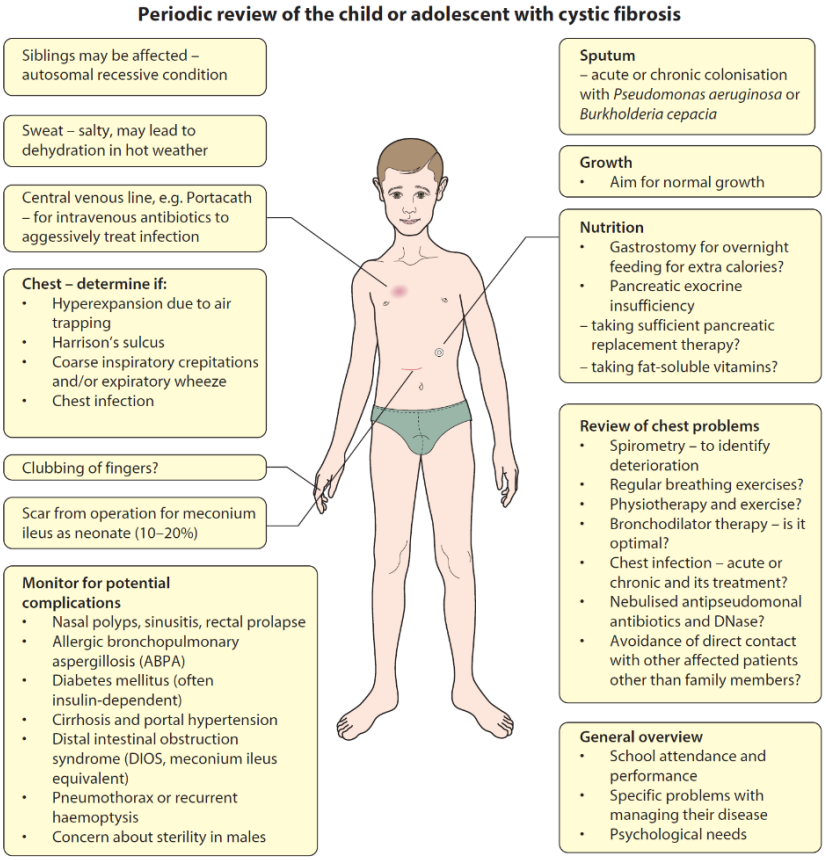

Figure 2.3 - Clinical signs seen in CF patients[16]

Antibiotics

Antibiotics can be used in the prophylaxis of infection for example oral flucloxacillin is given to children up until the age of 6 and leads to fewer infections with *Staphylococcus aureus* but does not significantly improve clinical outcomes such as lung function.[65] Oral antibiotic use at the start of a viral respiratory exacerbation is often encouraged to reduce the risk of a secondary bacterial infection, however, there is no evidence to support this.[66]

When a new organism is grown doctors should start the patient on an eradication regimen to prevent deterioration. Eradication regimens vary for different organisms and may vary from centre to centre but doctors can use the CF Trust guidelines for direction.[66] They recommend nebulised colistin and oral ciprofloxacin for the first isolate of PA given the patient is well.[66]

If eradication fails, patients may develop a chronic infection and inhaled and intravenous antibiotics may be used to control the infection. Those with chronic PA should be started on twice daily inhaled tobramycin or colistin to prevent a decline in lung function and to reduce the need for intravenous antibiotics.[66,67] In some centres they may use regular courses of intravenous antibiotics (e.g. every 3 months) to prevent deterioration, however, there is a lack of evidence to support this.[68]

Macrolides may have another role in CF other than being anti-bacterial. It is thought that macrolides such as azithromycin and erythromycin may have anti-inflammatory properties. A Cochrane review looking at long term use of azithromycin showed that when used for over 6 months it can improve respiratory function, decrease risk of exacerbations and reduce the need for oral antibiotics.[69]

Intravenous Antibiotics

For acute infections, a course of oral antibiotics is usually prescribed but intravenous antibiotics may be required if the infection is more severe.

For most organisms a single agent can be used, however for those with chronic PA infections with an acute exacerbation it is recommended to use two agents (a β-lactam or an anti-pseudomonal penicillin along with an aminoglycoside).[66,70] Courses of antibiotics are usually prescribed for two weeks but can be given for longer if required.

Intravenous antibiotics can be administered via an intravenous cannula; however, venous access can become troublesome after many courses. In this case, a vascular access device may be required such as a peripheral inserted central catheter (PICC) or an implantable device such as a port. Ports can be used for longer term delivery of medications and can last for years. They are placed under the skin and so a needle is required to access them.

In those with chronic infection or in those experiencing regular exacerbations they will require more frequent courses of intravenous antibiotics. As well as this if the infection is difficult to clear, patients may require more prolonged courses of intravenous antibiotics too. As with all medications, antibiotics can have many side effects. In particular, aminoglycosides are known to cause long term damage to the kidneys and can affect hearing. Kidney damage is a major concern, which will be discussed in further detail in the next chapter.

Physiotherapy

The physiotherapist's role is to educate the patients in chest care which includes airway clearance techniques and to encourage regular exercise. There are many airway clearance techniques, but physio can advise on choice of treatments to individualise care. This could

be active cycle of breathing, autogenic drainage, postural drainage and percussion, Aerobika Flutter or Acapella. They also monitor patients' lung function to recognise deterioration in patients and collect sputum to encourage early detection and treatment of acute infections.

Mucolytics

Mucolytics are medicines that can loosen the mucus, making it less sticky and easier to clear by coughing or mucociliary clearance. Dornase alfa is a highly purified solution of recombinant human deoxyribonuclease (rhDNase) which is an enzyme that can break down DNA. This reduces the stickiness of the sputum making it easier to clear. A Cochrane systematic review demonstrated that Dornase alfa was able to improve the FEV_1 by 9.51% (95% CI 0.67 to 18.35) when compared to placebo in trials when used for up to one month.[71] It also showed that it can reduce the number of pulmonary exacerbations in people with CF however evidence is conflicting regarding whether it improves quality of life.[71]

Nebulised hypertonic saline is also commonly used as a mucolytic however it does not have as strong an evidence base. By depositing hypertonic saline into the airways, water may travel down an osmotic gradient across the epithelial cells into the lumen of the airways rehydrating the mucus.[72] 7% hypertonic saline has been shown to improve lung function in the short term, however, has not shown the same effects in the long term.[73] It can improve quality of life and can reduce frequency of pulmonary exacerbations.[73]

Anti-inflammatory agents

Corticosteroids have a potential role in CF due to their anti-inflammatory effects. Oral steroids may slow the progression of lung disease, however, they should be used with caution due to the risk of bone disease which is already more prevalent in CF patients.[74]

There is insufficient evidence to recommend inhaled corticosteroids for reducing inflammation in CF.[75]

Non-steroidal anti-inflammatory agents (NSAIDs) have been shown to slow the rate of decline in lung function in patients with CF.[76] However, NSAIDs can have important side effects including gastritis and ulcers and can potentially be nephrotoxic.

Bronchodilators

CF causes an obstructive respiratory disease pattern and so patients may benefit from bronchodilators.[77] Evidence is limited regarding their use but they are often prescribed to CF patients.[78] However, it does add to the already heavy treatment burden and so adherence may be poor.

Lung transplant

Eventually patients with respiratory failure that are not responding to medical therapy will require lung transplantation, although many patients will die while on the waiting list. There are some contraindications to transplantation including HIV, hepatitis B and active tuberculosis (TB). In some centres *Burkholderia cepacia* and multiple drug resistant PA infections may be regarded as contraindications too.[79] Survival is variable and depends on multiple factors but one study showed a 1 year survival of 82% and 10 year survival of 51%.[80]

2.7 Pancreas

2.7.1 Pathophysiology

The pancreas secretes a bicarbonate rich isotonic fluid and digestive enzymes into the pancreatic lumen. The fluid secreted into the pancreatic lumen dilutes the pancreatic

enzymes and helps them move faster into the intestinal lumen. The bicarbonate rich fluid help to neutralize the acidic gastric contents when they reach the duodenum.

2.7.2 Pancreatic insufficiency

With less Cl^- secretion due to dysfunctional CFTR, the mucus is thicker and less HCO3- is secreted in the pancreatic luminal fluid, making it more acidic.[81] The pancreatic ducts get obstructed meaning transport of the bicarbonate and enzymes from the pancreas to the intestines is reduced. With reduced flow of bicarbonate to the duodenum the intestinal pH is more acidic which can denature the few lipase enzymes that do manage to reach the intestinal tract.[82] As the enzymes get stuck in the pancreas, they then begin to destroy the pancreatic ducts themselves and eventually the acini, replacing it with fat. This is known as pancreatic insufficiency.

Pancreatic insufficiency is prevalent in up to 87% of the CF population and the risk increases with age.[83] By identifying the genotype of a CF patient, the risk and severity of pancreatic insufficiency can be calculated. If the patient carries two 'severe' mutations it is likely that they will be pancreatic insufficient.[84] If the patient carries at least one 'mild' mutation such as Class IV and V mutations, they are unlikely to be pancreatic insufficient, however it cannot be ruled out completely.[84,85] Pancreatic insufficient individuals have reduced levels of amylase, protease and lipase causing inability to break down complex carbohydrates, proteins and fats respectively. This presents with abdominal pain and distension, diarrhoea, steatorrhea, floating stools, flatus, insatiable appetite, malabsorption, and weight loss.

As described above, CF patients can have poor fat absorption which is due to pancreatic insufficiency. The vitamins that are absorbed with fat (A, D, E and K) are therefore also

poorly absorbed and pancreatic insufficient CF patients may become deficient in these vitamins.[86]

2.7.3 Cystic fibrosis-related diabetes

Eventually, surrounding tissue including endocrine tissue such as β cells are destroyed and this can lead to cystic fibrosis-related diabetes (CFRD). When β cells are destroyed there is impaired insulin secretion leading to insulinopaenia. This leads to an impaired glucose tolerance which is known as the pre-diabetic state. However even in CF patients with a normal glucose tolerance test they can be relatively insulinopaenic.[87] This may be due to the fact that alpha (α) cells may also be destroyed in CFTR. Alpha cells produce glucagon which help with hepatic gluconeogenesis and with reduced glucagon in the blood less glucose is released in to the blood. With further destruction of β cells, CFRD can occur, commonly between the ages of 18-21 years.[88,89] In 2016 the UK CF annual registry report identified 7212 patients aged 10 or over on treatment for CFRD which equates to 29.5% of the CF population (over or equal to 10).[1] The prevalence of CFRD is greater in female than it is in men and it tends to present earlier in females which may be due to an earlier onset of puberty and the associated increase of insulin resistance at this time.[89,90] As the onset is usually insidious in nature, the classic features of diabetes (polyuria, polydipsia, weight loss) are uncommon with only a third presenting in this way.[89] Others that are diagnosed may be identified through screening.

2.7.4 Pancreatitis

Pancreatitis is a rare complication of CF with an incidence of 1.24%.[91] It is much more common in patients that are pancreatic sufficient and occurs in up to 20% of these patients.[92] The pathogenesis for this is mainly unknown, however, it is thought that the reduced flow of bicarbonate leading to a more acidic duct can activate trypsinogen to

trypsin. This active trypsin may cause autodigestion and local inflammation.[93] Pancreatitis in CF patients presents the same way as it does in a non-CF patient with abdominal pain usually in the epigastric region that may radiate to the back, nausea, vomiting and sometimes fever.

2.7.5 Management

Pancreatic insufficiency

During each clinic appointment the patients weight, height and BMI are taken and in paediatrics they plot these on a growth chart. Dieticians are needed in the management of CF patients to ensure they are gaining weight sufficiently by advising on food intake. CF patients have a greater energy demand than normal individuals and it may be hard to meet this with food alone.[94,95] Therefore, the dietician may recommend high-calorie supplement energy drinks and if that does not work the patient may need tube feeding.[96]

CF patients that are pancreatic insufficient are required to take Pancreatic Enzyme Replacement Therapy (PERT). This comes in the form of Creon which are capsules that contain enzymes including protease, amylase and lipase. The dietician plays an important role in educating regards to Creon dose and will monitor patients' symptoms to decide if they require more or less PERT.

Pancreatic insufficient patients should have their vitamin A, D and E levels monitored once a year but more frequently if they have been previously deficient. In some centres they give vitamins supplementation prophylactically but in other centres they will only give vitamin supplementation if the levels are low. The Cystic Trust recommends that Vitamin D, E and K supplementation should be given routinely if the patient is pancreatic insufficient whereas Vitamin A should only be supplemented if their levels are low.[96]

Cystic fibrosis-related diabetes

The CF Trust recommends that CFRD is screened for annually in those with CF over 10 years old by performing an oral glucose tolerance test (OGTT).[70] As the child reaches late teens or if they experience symptoms of hyperglycaemia such as weight loss, polyuria and polydipsia they are screened more regularly. An impaired glucose tolerance test is common and can be normal for a CF patient. A 'diabetic OGTT' can also return to normal in some cases. Therefore, clinicians may choose to undergo further testing such as continuous glucose monitoring before commencing treatment. Treatment of CFRD is with insulin and they are reviewed regularly at a specialist CF diabetic clinic to ensure they are on the correct regimen.

2.8 Gastrointestinal tract

2.8.1 Meconium ileus

Meconium ileus is the earliest manifestation of CF which is a type of bowel obstruction that occurs in the neonatal period. It is caused by dysfunctional CFTR channels resulting in reduced bicarbonate secretion and hence a more acidic intestinal luminal pH.[97] The mucus in the gut is therefore thick and dehydrated. Meconium ileus is seen in up to 20% of neonatal CF patients and is more common in those with more 'severe' genotypes (class I-III mutations) and those that are pancreatic insufficient [98,99] However, there are cases of it occurring in CF patients that are pancreatic sufficient and also in non-CF patients. It can present in the antenatal period seen on ultrasound scans as a hyperechoic bowel. It can also present when feeding is initiated with bilious emesis and abdominal distension or with the delayed passage of meconium. The first presentation may also be of

perforation with peritonitis and signs of shock.[100] Meconium ileus may cause volvulus which is where the intestines twist on its mesentery preventing blood supply to it.

2.8.2 Constipation

Constipation is often confused with Distal Intestinal Obstruction Syndrome (DIOS), however, their pathophysiology is different. Constipation may occur due to factors unrelated to CF as it can do in normal individuals such as low fibre diet and poor fluid intake. In CF constipation may be associated with high doses of pancreatic supplements.[101,102]

2.8.3 Distal intestinal obstruction syndrome

Distal intestinal obstruction syndrome (DIOS) occurs in 10-22% of CF patients over their lifetime and has a recurrence rate of up to 50%.[103,104] The mucus in the intestines is thicker and stickier in CF patients and it combines with viscid faecal material. This creates a mass that is often found in the distal ileum and caecum and when it sticks to the intestinal walls can be difficult to pass.[105] The mass can either block the lumen fully (complete DIOS) or partially (incomplete DIOS). DIOS presents with abdominal pain, right iliac fossa mass, constipation and later on bilious vomiting if there is complete obstruction.

2.8.4 Other gastrointestinal complications

A rectal prolapse is defined as a circumferential, full-thickness protrusion of the rectal wall through the anal orifice.[106] There is very little literature regarding rectal prolapses but a study in 1958 found that 22.6% of patients with CF will experience it.[107] Factors that contribute to rectal prolapse in CF patients include voluminous faeces with poorly digested food, frequent bowel movements, and increased intra-abdominal pressure due to pulmonary hyperinflation and coughing.[107,108]

With thick mucus accumulating in the intestines, bacteria can colonise the intestines irregularly which is known as small bowel bacterial overgrowth. Symptoms of this are non-specific and include bloating, abdominal pain, and malabsorption.

CF patients have an increased risk of gastrointestinal tract cancers perhaps due to the chronic inflammation.[109] There is also an increased risk of hepatobiliary and pancreatic malignancies compared to the normal population.[110]

It is thought that other GI complications are more common in CF patients including intussusception, appendicitis, gastro-oesophageal reflux disease (GORD) and peptic ulceration. [111–114]

2.8.5 Management of constipation and DIOS

Management of constipation involves increasing fibre intake, ensuring optimal hydration and the use of laxatives. DIOS is managed with laxatives and fluids initially, although it may progress to require colonoscopy with local installation of Gastrografin or even surgery.[115,116]

2.9 Hepatobiliary system

2.9.1 Cystic fibrosis-associated liver disease

It is thought that up to 37% of CF patients develop cystic fibrosis-associated liver disease (CFALD) and it is now the third leading cause of death among CF patients.[117] It is usually a complication that presents in childhood or early teens and it represents a more severe phenotype of CF. Some patients will be asymptomatic, but it can progress to multilobular cirrhosis with or without portal hypertension. Patients may experience jaundice, varices, ascites, coagulation abnormalities and encephalopathy.

2.9.2 Gallbladder disease

Gallbladder disease is prevalent in around 4% of CF patients and presents in similar ways to in non-CF patients.[118] Gallstones in CF may be associated with biliary stasis due to thickened secretions and abnormal bile acid synthesis with high levels of cholesterol.[119]

2.9.3 Management

CF patients are examined regularly for signs of liver disease such as hepatomegaly and splenomegaly. Liver disease is also monitored for once a year as part of their annual review by abdominal ultrasound and liver function tests. Ursodeoxycholic acid is often used in CFALD as it can improve bile acid flow which may be helpful in CF patients whose bile ducts are blocked with thick secretions.[120] However, a Cochrane review showed that the evidence is inconclusive regarding its use as we cannot speculate that as it reduces liver enzymes it must reduce liver damage.[121] Patients who develop cirrhosis will inevitably require a liver transplantation.

2.10 Reproductive system

The majority of men with CF are infertile (over 98%) due to congenital bilateral absence of the vas deferens and concomitant absence of the seminal vesicles.[122,123] This may be due to abnormally viscous secretions in the fetus due to the defective CFTR channel and this may impact the embryonic development of these tissues. It is important that young men are still advised to use contraception to prevent other problems including sexually transmitted diseases.

On the other hand, women with CF have an anatomically normal reproductive tract. However, they do go through puberty later than non-CF patients, with the average age of

menarche being 14.4 years compared to 12.9 years in the general population.[124] This may be due them having a lower BMI which can also cause anovulatory cycles and secondary amenorrhoea. It has been demonstrated that women with CF have stickier cervical mucus which may reduce the passage of sperm through the tract.[125] However, they should still be given appropriate advice regarding contraception as most women with CF are fertile.

2.11 Bone disorders

There is a wide variation in reported prevalence of bone disease in CF with some studies reporting up to 70% of individuals with CF having reduced bone density (osteopaenia or osteoporosis) and up to 24% may have osteoporosis.[126,127] There are several factors in CF that may contribute to this including vitamin D deficiency, low BMI, calcium deficiency, chronic inflammation, CFTR dysfunction in osteoclasts and osteoblasts, cystic fibrosis-related diabetes and steroid use.[128–133]

2.12 Psychological problems

Children with CF spend a lot of time in hospitals for clinic appointments and inpatient admissions. They are required to undergo many medical procedures and interventions such as venepuncture, cough swabs, chest percussion and examination from a young age. This can create anxiety and stress for the children and may also create resentment towards their doctor.

Cystic fibrosis can have a major impact on the family of the child with the condition. Other siblings that do not have CF may become jealous as parents may have to spend a lot more time with the child with CF for physiotherapy and appointments.[134] Parents may have to take more time off work for appointments and periods of illness. They may choose

to resign and to become a full-time carer for their child especially if they have a more severe phenotype. This can create financial stress on the family although they can seek help with this. As the individual with CF gets older they may also struggle themselves with unemployment and increased sick leave.

During school years, children with CF may feel as though they do not fit in with their peers. This may be due to the fact they may struggle to keep up with sports, are required to take Creon and other medications and produce excessive sputum. This may cause the child to feel embarrassed and ashamed and this can lead to low self-confidence.

Rates of anxiety and depression may be higher in CF patients when compared to normal individuals however the evidence in conflicting.[134,135] Psychological management is necessary from an early age in CF patients and psychologists regularly see these patients to ensure they are getting the correct support.

2.13 Prognosis

Median life expectancy rose to 43.5 years of age in 2016 compared to 32.2 years in 1998.[1,136] If the life expectancy continues to rise at this rate, then we can expect people with CF to live much longer.

One factor that affects prognosis is gender, for example the median life expectancy in 2016 for a male was 47.1 years compared to 40.1 years for a female. The predominant hormone found in females is 17β-estradiol (E2) and this has been shown to decrease chloride secretion which dehydrates the airway surface liquid further.[137] Oestrogen promotes alginate production by PA and causes mucoid conversion of PA which both increase resistance to antibiotics and hence clearance from the lungs.[138] When there are higher levels of oestrogen levels during the menstrual cycle, there are more respiratory

exacerbations. This may be due to high levels of oestrogen causing more mucoid conversion.[139] Interestingly, women on the combined oral contraceptive pill are less likely to experience respiratory exacerbations.[139] Studies have suggested that women become colonized with certain organisms such as PA, MRSA, *Haemophilus influenzae*, *Aspergillus* species, earlier in their lives than men.[138] Once chronically infected with PA they are likely to decline quicker and have poorer survival rates.[140]

Genotype can also affect prognosis with people with the Phe508del mutation having more severe disease and poorer prognosis.[141] Other mutations have been implicated with less serious disease and better prognosis. Those with absent or greatly reduced levels of the CFTR protein such as those with Class I-III mutations have poorer prognosis than those with Class IV-VI mutations.[142] However when discussing with parents of a new-born child recently diagnosed with CF, you must be careful as disease severity and prognosis can be very variable. However being homozygous for Phe508del is high attributable to pancreatic insufficiency with more than 99% having it.[141] Pancreatic insufficient patients are twice as likely to have severe lung disease with reduced FEV_1 than those who are pancreatic sufficient.[143] Pancreatic insufficiency causes nutritional deficits which again is a risk factor for poorer prognosis.

Those chronically infected with organisms such as PA, *Staphylococcus aureus* and *Burkholderia cepacia* complex show greater decline in FEV_1 which itself is a risk factor for poorer prognosis.[144] Many CF related complications can also have an impact on prognosis. Cystic fibrosis-related diabetes also increases the rate of decline in lung function and these patients often have poorer nutritional status which both reduce prognosis.[88] Cystic fibrosis-associated liver disease is the third most common cause of death in CF patients with a mortality rate of 2.5%.[144]

2.14 The future

2.14.1 Genotype specific small molecule therapy

With understanding that CF is caused by reduced, absent or poorly functioning CFTR channel on epithelial surfaces this suggests a potential solution would be drugs that modulate CFTR function. Three main approaches have been suggested with the most successful being 'potentiators' which increase the function of the CFTR channels that are already there on the epithelial cells. The next approach is 'correctors' which allow delivery of more CFTR channels to the surface of the cell. Finally, 'production correctors' allow more production of the CFTR channel.

These three approaches would be used dependant on the class of mutation. For example, as we know in Class III and IV mutations the CFTR channels reach the cell surface but are dysfunctional, therefore potentiators could be used in these two classes to increase chance of the channel opening. The most common type of class III mutation is the Gly551Asp mutation, previously known as G551D, which accounts for about 4% of all CFTR mutated alleles.[145] In vitro, potentiators increase the likelihood of the Gly551Asp CFTR channel opening, allowing more successful chloride transport.[146]

The most common CFTR mutation is Phe508del and is found in around 90% of people with CF, on at least one of their chromosomes.[145] This is a class II mutation which causes a misfolding of the CFTR protein and therefore it is degraded intracellularly before it can even reach the surface.[147] However, in vitro studies have shown that if the cells are treated pharmacologically or if the cell temperature is lowered then more CFTR is able to reach the cell surface.[148] Therefore a 'corrector' approach may be needed for this type of

mutation. When Phe508del CFTR is able to reach the surface it then behaves similarly to a class III mutation, by not opening and so a 'potentiator' could be useful too.[149]

Ivacaftor

Ivacaftor (VX-770) was identified through high-throughput screening and its success in vitro led to phase II clinical trials on patients with Gly551Asp mutations, which showed a partial improvement in nasal potential difference and significant reduction in sweat chloride levels after 28 days in those receiving 150mg of ivacaftor compared to placebo. This reduction in sweat chloride levels suggests that the ivacaftor was successful at potentiating the CFTR channels. There was no significant difference in FEV_1 in those on ivacaftor compared to placebo, however, a major limitation of this study was the small study sample (39 participants).[150]

Following on from this two larger Phase III clinical trials; ENVISION and STRIVE, were undertaken evaluating the efficacy on patients with Gly551Asp mutations.[151,152] Both trials were double blinded RCTs where participants were randomly assigned to receive either placebo or 150mg of ivacaftor every 12 hours during a 48 period. The difference between them was STRIVE look at patients aged 12 years and over, whereas ENVISION looked at children aged 6-11 years. I will focus on the results of STRIVE as it was much larger study, so it is more likely to be representative. The results of both studies were however very similar with both studies showing a significant improvement in lung function with ivacaftor. In STRIVE there was a change in the percent of predicted FEV_1 from baseline through to week 48 that was 10.5 percentage points greater with ivacaftor than placebo ($P<0.0001$). STRIVE showed that participants receiving ivacaftor were 55% less likely to experience a pulmonary exacerbation at week 48 than those receiving placebo ($P<0.001$). This finding was not seen in the ENVISION study, however this may

be due to the younger age of participants and milder disease in those in this study. Both studies demonstrated a rapid reduction in sweat chloride levels with ivacaftor meaning that it is working effectively as a potentiator to open the CFTR channel and allow reabsorption of more chloride. STRIVE showed an average reduction in sweat chloride levels of 48.7 mmol/L compared with 0.8mmol/L in the placebo group at 24 weeks. Ivacaftor can reduce the number of days spent in hospital and can reduce the number of exacerbations requiring intravenous antibiotics.[152] It can also improve respiratory symptoms and aid weight gain.[151,152] In both studies there were a similar number and nature of adverse effects in both the treatment group and placebo group suggesting there are no concerns regards to safety of ivacaftor.

There is evidence in vitro, that Ivacaftor is successful in potentiating CFTR function in other class III and IV mutations as well.[153,154] Further clinical trials have indicated that ivacaftor may be useful in patients with non-Gly551Asp CFTR gating mutations including G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P and G1349D.[155] It has been shown to increase FEV_1, decrease sweat chloride levels and improve quality of life in these patients.[155] It may be useful in the class IV mutation R117H but only in those with more severe respiratory disease.[156]

Ivacaftor has been tested in those homozygous for Phe508del CFTR mutations but unfortunately did not have any statistically significant effect on FEV_1.[157] This finding however may reflect the fact that there is very little CFTR reaching the surface of the cells and so there is little CFTR to potentiate. This suggests that these patients may also require a ‘corrector’ as well as a ‘potentiator’.

Lumacaftor

Lumacaftor (VX-809) is a CFTR 'corrector' that in vitro can improve processing of cells that are homozygous for Phe508del which reduces degradation of the CFTR channel and hence more are placed on the cell surface.[158] An RCT in patients that are homozygous for Phe508del showed that Lumacaftor was able to reduce sweat chloride levels compared to placebo. There was, however, no statistically significant difference in lung function or CFQ-R scores between intervention group and comparison group. This is disappointing, however this may be due to a small sample size or down to the fact that although there are more CFTR proteins reaching the cell surface they are still not opening effectively.[159]

Lumacaftor and ivacaftor

As mentioned earlier, using a corrector along with a potentiator in CF may be effective in patients with the Phe508del CFTR mutation as they allow more CFTR protein is able to get to the cell surface and more is able to function. RCTs have demonstrated that patients receiving a combination of lumacaftor and ivacaftor compared to placebo have a statistically significant increase in predicted FEV_1, reduced rate of pulmonary exacerbation, increase in BMI and improvement in CFQ-R score.[160,161] There have been seven episodes overall of deranged liver function which resolved when the medication was stopped. Further studies are required in order to look at the long-term effects of this treatment.

2.14.2 Gene therapy

At its heart, CF is a genetic disease. In common with many single gene disorders, it was hoped that correction of the basic cellular defect could be achieved by replacing the defective gene.[162] However, despite over 30 years of considerable effort, progress with gene therapy has disappointing.

The main issues are that vectors, such as adenovirus that permit efficient gene penetration into the respiratory epithelium have been shown to be remarkably proinflammatory and harmful to the lungs. Whereas, safer, non-viral transfection attempts with, for instance, liposomal preparations have resulted in limited gene transfection.

The search for therapeutic advances do continue and there is limited evidence for some benefit. A randomised, placebo-controlled trial tested the efficacy of CFTR gene therapy administered by nebulizer using the non-viral liposome complex pGM169/GL67A. It showed a statistically significant increase in lung function in the gene therapy group when compared to placebo. More research in this field is needed to look at long term effect.[163]

CHAPTER 3: RENAL DISEASE IN CYSTIC FIBROSIS

3.1 Introduction

In this chapter, I will define renal disease and the epidemiology of renal disease in CF patients. I will go on to discuss the main causes of this with the most important and common being medications, particularly aminoglycoside antibiotics. I will discuss detection of renal disease and introduce the main focus of this thesis which is looking at strategies to prevent renal disease caused by intravenous antibiotics. People with CF are living longer and so it is now more important than ever to prevent long term complications, such as renal disease, of medications that are used routinely in CF.

3.2 Definitions of renal disease

Damage to the kidneys can result in what is known as an 'acute kidney injury' (AKI), which is defined as any of the following:[164]

- an increase in serum creatinine of at least 0.3 mg/dL within 48 hours;
- an increase in serum creatinine to 1.5 times baseline (which is known, or presumed, to have occurred within the previous seven days); or
- urine volume less than 0.5 mL/kg/h for six hours.

This definition is the KDIGO definition and it is commonly used by nephrologists. There are other definitions of AKIs including the RIFLE and AKIN classification which can be seen in the image below.

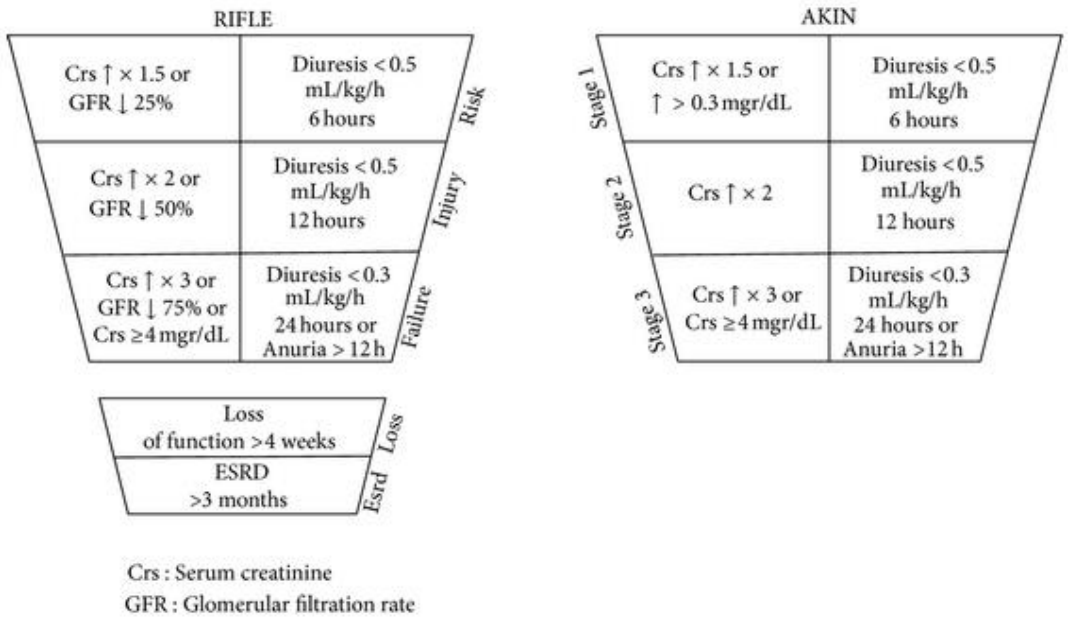

Figure 3.1 – RIFLE and AKIN classification of an AKI[165]

AKI's extend hospital stay and often shorten antibiotic treatment courses. It is possible for an AKI to resolve and for the individual to regain normal kidney function. However, recent evidence suggests an AKI, particularly if a person suffers multiple occurrences, may initiate the development of chronic kidney disease (CKD).[166]

CKD is defined as abnormalities of kidney structure or function (e.g. albuminuria or glomerular filtration rate (GFR) below 60 mL/min/1.73 m²), present for longer than three months, with implications for health.[167] It has been reported that CKD can have many negative implications on a person's life and approximately 25% of people with CKD have been found to have depression, which is a higher rate than the general population.[168] CKD can progress through different stages, ranging from 1 to 5; stage 5 is the most severe and is also known as end-stage kidney disease (ESKD) or kidney failure.[167] One study in the UK estimated that in all patient populations CKD costs the NHS £1.44 billion to £1.45 billion per year.[169]

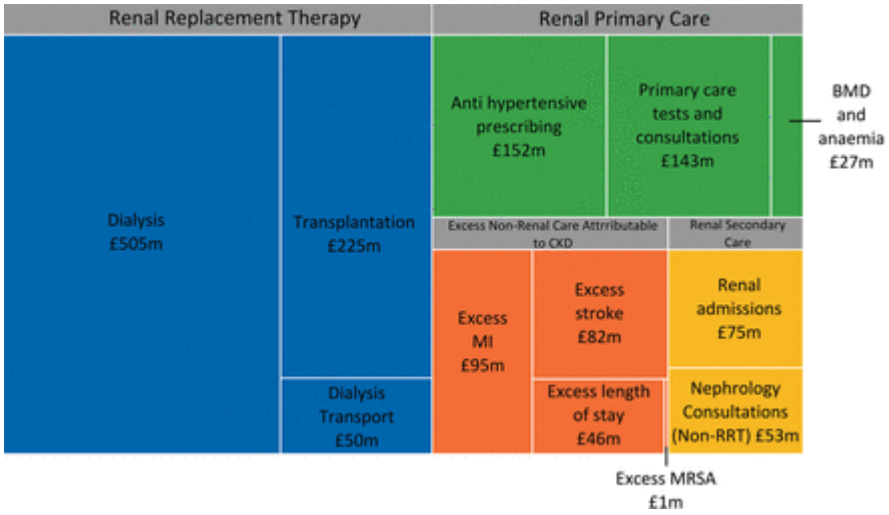

Figure 3.2 - Costs attributable to CKD[169]

CKD may progress to ESKD, defined as GFR less than 15 mL/min/1.73m². ESKD has many complications including anaemia, electrolyte disturbances, bone disorders, uraemia, and hypertension. People with ESKD require a form of renal replacement therapy (RRT) to correct any electrolyte or metabolic disturbances; RRT encompasses kidney transplantation and dialysis. Individuals receiving RRT are less likely to be employed than age-matched participants, in particular those receiving haemodialysis, which can cause a financial burden for themselves and their family.[170] Transplantation is preferable as it reduces the mortality risk and improves quality of life (QoL) when compared with dialysis.[171]

3.3 Epidemiology of renal disease in cystic fibrosis

A UK study published in 2007 estimated that the incidence of acute renal failure is between 4.6 and 10.5 cases per 10 000 people with CF per year.[172]

The annual prevalence of chronic kidney disease (CKD) (stage 3 or over) in individuals with CF is much higher than that seen in age-matched controls within the general population. A study in the US estimated the mean annual prevalence of stage 3 or greater

CKD to be 2.3%.[173] The overall incidence rate of stage 3 or greater CKD in CF patients was 4 events per 1,000 person-years of follow-up.[173] Both the prevalence and incidence increase with age with the prevalence being as high as 19.2% in patients over the ages of 55.[173] A UK study of adults with CF showed 31-42% had a creatinine clearance that was below the normal range, highlighting renal disease may be more prevalent than we think.[174] A cross sectional retrospective study in Canada identified the incidence of CKD as 6% in children aged 2-18 years. However, in this study Prestidge measured GFR by plasma disappearance of Technetium-99 m diethylenetriaminepentaacetic acid (mGFR), rather than the conventional method of measuring creatinine clearance (eGFR).[175] Soulsby identifies three methods of assessing renal function; using serum creatinine, using creatinine clearance and using isotopic or non-isotopic clearance rates.[176] Different studies have measured for renal function in different ways and so it is impossible to collate this information. Each method of assessment comes with its own limitation and so novel ways of detecting early renal function decline are needed.

3.4 Aetiology of renal disease in cystic fibrosis

The cystic fibrosis transmembrane conductance regulator gene (CFTR) is expressed in all segments of the nephron.[177] Inactivation of the CFTR protein has been shown to cause low molecular weight proteinuria.[178] However, the only clinically significant primary renal disease seen in CF is nephrocalcinosis. The majority of renal disease in CF is secondary, caused by a complication of other organ involvement or chronic infection or, most commonly, due to nephrotoxic medications. Renal disease can also be seen in CF patients as an incidental comorbidity.

3.4.1 Drug nephrotoxicity in CF

The most common cause of renal disease in CF is drug nephrotoxicity with the most commonly implicated drugs being aminoglycoside antibiotics. Aminoglycosides are bactericidal antibiotics that bind to the bacterial 30S ribosomal subunit inhibiting protein synthesis which is necessary for growth. Aminoglycosides are eliminated from the body by renal clearance through glomerular filtration, but some are re-absorbed back into the proximal tubule. This is mediated by a receptor called megalin.[179] This causes an accumulation of aminoglycosides in the proximal tubule epithelial cells. This can result in apoptosis and necrosis of these cells due to mitochondrial dysfunction and release of reactive oxygen species.[180,181] Patients may present with non-oliguric acute renal failure, with little change to the urine dipstick results, and can be reversed on cessation of the drug.[182] People with CF often have increased renal clearance hence requiring higher doses of drugs in order to achieve therapeutic levels.[183] An association has been found between the cumulative lifetime dose of intravenous aminoglycosides and long-term renal damage.[174] Gentamicin is more harmful to the kidneys and so tobramycin is the aminoglycoside of choice in most centres.[184] Other intravenous antibiotics can too cause kidney damage including beta lactams and colistin, however, aminoglycosides are by far the worst.

Some antibiotics that can be given orally for respiratory infections such as ciprofloxacin, azithromycin and beta lactams have been associated with kidney damage.[185] It is known that nebulised colistin can cause an AKI [186] and there have been cases of nebulised tobramycin damaging the kidneys.[187] By omitting the use of oral and nebulised antibiotics when using intravenous antibiotics, this may reduce the risk of kidney injury.

There is evidence that anti-inflammatory drugs can help to slow the rate of lung function decline.[76] Despite this good evidence, the use of NSAIDs as regular therapy in CF is not widespread. This may relate to concerns that ibuprofen can result in nephrotoxicity and increase the risks of pulmonary infection.[188,189] NSAIDs work by blocking cyclo-oxygenase (COX) 1 and 2 enzymes, preventing the synthesis of prostaglandins and so reducing inflammation. These drugs can cause renal damage by two main mechanisms. The first is by causing an immunological reaction which causes acute tubulointerstitial nephritis.[190] The second mechanism is by inhibiting COX 1 and 2 so reducing the number of prostaglandins formed. This means there is less vasodilatation in glomerular capillaries and arterioles. With reduced perfusion to the kidney, it is then at a higher risk of ischaemic injury due to lack of oxygen and glucose especially in those with pre-existing volume depletion.[189]

Immunosuppressive therapy following lung transplantation causes renal damage with 18% of CF patients that have had a lung transplant experiencing it within one year of the transplant.[191] Calcineurin inhibitors such as cyclosporine affect endothelial cell function, decreasing vasodilator (prostaglandins) production and hence increased vasoconstrictor (endothelin and thromboxane) production.[192] This leads to vasoconstriction of the afferent and efferent glomerular arterioles in the kidney, reducing its perfusion which predisposes the kidney to renal failure.[193,194]

Other drugs that may induce an AKI in a CF patient include ciprofloxacin, proton pump inhibitors, diuretics, azithromycin, sulphonamides, colistin, ceftazidime and rifampicin.[185]

3.4.2 Nephrocalcinosis and stone formation

Asymptomatic nephrocalcinosis was seen in up to 92% of patients with CF found at autopsy in a study by Katz *et al*, however, a limitation of this study was that it had a small sample size.[195] There is also an increased risk of nephrolithiasis with 3-6.3% of CF patients suffering from renal stones compared to 1-2% in the normal population, with the majority of these stones being calcium oxalate.[196,197] Risk factors for stone formation include low urine volume, hyper-oxaluria, hyper-calciuria and hyper-uricosuria.[195] Prolonged periods of immobilisation that can occur in CF patients, for example during a respiratory infection, can lead to hypercalcaemia and hypercalciuria.[198] Both prednisolone and loop diuretics such as furosemide increase calcium excretion and lead to hypercalciuria and so are both risk factors for stone formation.[198] Pancreatic insufficiency leads to fat malabsorption which can cause enteric hyperoxaluria. This increases the urinary calcium-oxalate saturation hence increasing the risk of stone formation.[198] Repeated antibiotic use leads to gut decolonisation of *Oxalobacter formigenes* which means gut oxalates cannot be broken down hence you get enteric hyperoxaluria. Gut oxalates are absorbed into the blood and can end up in the kidney where they get deposited and can form stones.[199] Hypercalciuria is seen in 30% with CF and may also be due to a renal Cl^- channel defect caused by CFTR mutation.[182,195]

3.4.3 Diabetic nephropathy

CFRD is prevalent in 40-50% of adults with CF and with rising life expectancy this figure is set to increase.[200] It is estimated within 5-10 years of diagnosis, 30-50% of all patients with insulin dependent diabetes will develop diabetic nephropathy.[182] Patients with CFRD are at risk of the same microvascular complications as non-CF diabetics. With

incidence of CFRD increasing, so will the incidence of diabetic nephropathy meaning more people will require RRT.

3.4.4 Rare causes of renal pathology in CF

Inflammation-associated systemic amyloidosis is a complication of CF and in most is asymptomatic but can present with nephrotic syndrome if it affects the kidneys.[201] It is thought that it arises due to a state of chronic infection. The incidence is rising due to the increasing life expectancy of patients with CF. One study showed that 33% of CF patients had systemic amyloidosis with renal involvement at autopsy, however this study was only small.[202] If the patient does experience symptoms then the prognosis is poor, although colchicine may help.[203]

Chronic infection and inflammation lead to high levels of Immunoglobulin A (IgA) in the blood. When this IgA is deposited in the kidneys it can cause further immunological reactions and lead to glomerulonephritis.[182] IgA nephropathy is the most common cause of glomerulonephritis in patients with CF.[204] The risk of this is increased if there is liver disease as this reduces the amount of immune complexes cleared from the blood and so there is more to be deposited in kidneys and other organs.[205]

Antineutrophil cytoplasmic antibodies (ANCA) can be found in CF patients that experience recurrent infections. They can cause vasculitis, a rare complication in CF, which is usually predominantly skin involvement only but there have been cases of renal involvement.[206]

Finally, tubulointerstitial nephritis can also occur in CF patients through an allergic reaction to a drug or from an infection. This can present with fever, vomiting, malaise

and nausea and there may also be signs of the allergic reaction, if this is the cause, including a rash.[182]

3.5 Monitoring kidney function in CF patients

All patients with CF are monitored for renal disease of any aetiology, usually at their annual review. Renal disease may be identified by measuring blood pressure, on routine bloods or on urine dipstick.

Renal disease is often asymptomatic in the early stages, the first indication of renal disease may be proteinuria on a urine dipstick or abnormalities in eGFR, urea or creatinine. However, GFR is insensitive in early renal damage as up to 30% of nephrons can stop working before GRF decreases.[207] This is because the other nephrons compensate by increasing their glomerular filtration.[208] Creatinine and electrolytes including magnesium are also insensitive.[209,210] Therefore research has been conducted in search of earlier markers of renal dysfunction caused by aminoglycosides.

3.6 Detecting renal disease caused by antibiotics

As mentioned above, aminoglycosides accumulate in the kidneys in the proximal tubule epithelial cells which can cause damage to these cells. This is known as acute tubular necrosis which can cause leaking of electrolytes, proteins, and certain tubular enzymes.

Raised levels of tubular enzymes including N-acetyl-β-D-glucosaminidase (NAG), alanine amino-peptidase (AAP), neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18) and collagen IV may indicate nephrotoxicity.[211–216] In animal studies, KIM-1 outperforms other markers of nephrotoxicity and it is currently the only regulated proximal tubule biomarker that is

used in drug development.[217,218] Currently, there is very little known about these enzymes but we do know they are markers of early kidney damage. An increase in these biomarkers does not necessarily indicate a clinically significant decrease in renal function. The use of these biomarkers for monitoring raises many questions including whether they should be used to make decisions regarding stopping antibiotics. Further research is needed to identify how much of an increase in these biomarkers is needed for there to be clinically significant renal disease (e.g. decrease in eGRF or rise in creatinine and urea).

Table 1 – Biomarkers of kidney injury

Alanine amino-peptidase (AAP)	Enzyme located on plasma membranes including renal tubular epithelial cells.
Collagen IV	Forms the main collagen component of the basement membrane including in the kidneys.
Cystatin C	This is a protein found in most cells and is normally filtered out of the blood by the kidneys.
Interleukin-18 (IL-18)	This is a cytokine produced by inflammatory cells in the kidneys during an acute kidney injury.

Kidney injury molecule 1 (KIM-1)	This is a transmembrane protein found in the proximal tubules with increased expression following an injury.
N-acetyl-β-D-glucosaminidase (NAG)	This is an enzyme that is found in high concentrations in the lysosomes of proximal tubule cells in the kidneys.
Neutrophil gelatinase-associated lipocalin (NGAL)	This is a protein produced by renal epithelium that is damaged.

3.7 How can renal disease caused by antibiotics be prevented in cystic fibrosis?

Whilst kidney damage as a result of antibiotic treatments is common, it is not inevitable. We understand how various treatments work, and this suggests several different strategies that might be utilised to prevent or minimise nephrotoxicity. We hypothesised that there were at least four potential strategies.

3.7.1 Risk minimisation strategies for individuals requiring aminoglycosides

Children or adults with new or established PA infection often require broad-spectrum antibiotics in order to successfully eradicate colonisation or suppress growth during pulmonary exacerbations. Aminoglycosides are antibiotics used to treat respiratory infections; they can, however, be harmful to the kidneys. There have been several important studies examining the effects of varying the duration and frequency of

aminoglycoside treatment. Studies have also examined the relative effects of choosing one particular aminoglycoside over another.

Previous Cochrane Reviews have examined the clinical consequences of different duration and frequency of intravenous antibiotic regimens.[219,220] These concluded that there is no evidence to support a recommendation about duration of treatment, but once-daily dosing is better for kidneys than multiple-daily dosing.

3.7.2 Comparison of the relative nephrotoxicity of different antibiotic regimens

We may be able to reduce kidney damage in CF patients by identifying strategies using different types and doses of intravenous antibiotics that are less nephrotoxic. It is vital, however, that the antibiotic regimens are still as effective at clearing the respiratory infection.

3.7.3 Avoidance of concomitant nephrotoxic drugs

Another strategy to minimise kidney damage may involve the omission of other nephrotoxic drugs when using intravenous antibiotics. NSAIDs are useful in reducing inflammation in the lungs, however, they can harm the kidneys.[76] This damage may be exacerbated when prescribed alongside intravenous antibiotics and to reduce this risk the duration and frequency of NSAID use needs to be considered. Some oral and nebulised antibiotics can also cause kidney damage, by omitting these drugs while using intravenous antibiotics it may reduce the risk of kidney injury.

3.7.4 Adjuvant therapies to 'protect the kidneys'

Finally, a further strategy involves looking at the use of adjuvant medications or therapies alongside intravenous antibiotics. Adjuvant therapy describes treatment used additionally to the primary treatment. Statins are primarily used to reduce cardiovascular risk in individuals with elevated blood cholesterol. However, they may also be helpful in reducing kidney damage in those receiving intravenous antibiotics in CF. Statins work by inhibiting an enzyme (HMG-CoA reductase) which preventing the production of mevalonate and therefore reducing cholesterol synthesis, hence their use in cardiovascular disease. It has been suggested that statins may prevent nephrotoxicity.[221] Inhibition of HMG-CoA reductase results in a depletion of cellular sterols which are needed for the megalin-mediated uptake of aminoglycosides into the kidneys. Less aminoglycoside is deposited in the renal proximal tubular cells hence reducing necrosis of these cells. This was seen in vitro and then in vivo, where animal studies have shown that this effect may be limited to certain subgroups of statins with rosuvastatin having a greater effect in murine models.[222,223]

Adequate hydration is needed for kidneys to function correctly and avoiding dehydration might avoid kidney damage. We know from studies examining the use of nephrotoxic chemotherapeutic agents that hydration status is important in mitigating the harmful effects of toxins on the kidney.[224] Certainly adequate hydration or hyperhydration are likely to affect the toxicity of any drug treatment. Good hydration will tend to reduce the overall concentration of drugs in the body and within the urine. Adequate hydration ensures total body water is marginally increased. Thus, the total volume in which most drugs are distributed, the volume of distribution, is slightly larger. There is a larger effect on the cells located within the kidney as any excess fluid will be excreted as urine. The concentration of all water-soluble constituents of the blood will be lower within the

glomerular filtrate and therefore there will be a lower drug concentration throughout the renal tubules. Enteral or intravenous fluids could be used as an adjuvant therapy alongside intravenous antibiotics to reduce the risk of kidney injury.

3.8 Why is it important to prevent renal disease in cystic fibrosis patients?

With improved outcomes and longer life expectancy for individuals with CF the need to protect the kidneys from long-term damage is becoming an important consideration. Median life expectancy rose to 47 years of age in 2016 compared to 32.2 years in 1998.[83,136]

2011 was a significant landmark in the UK for individuals with CF.[83] For the first time, there were more adults than children with CF and the median age of individuals with CF has continued to increase. The median age of those affected in 2011 was 18 years old. It has moved on from a disease that was fatal during childhood for most to a condition that can be actively managed and where survival for many is likely to significantly increase. Failure to die from respiratory complications heightens the importance of 'other system disease'. There is a need to rapidly secure less nephrotoxic (and ototoxic) antibiotic treatment regimens. We are at a 'critical moment' in CF care. A holistic, multisystem view of the condition needs to be taken.

CHAPTER 4: INTRODUCTION TO COCHRANE SYSTEMATIC REVIEWS

4.1 History of Cochrane systematic reviews

In 1971, Archie Cochrane published a book, 'Effectiveness and efficiency' criticising the lack of evidence behind many medical interventions.[225] He suggested that there should be systematic reviews examining and summarising all the evidence on a particular intervention which can then be used to produce guidelines. In 1993 the Cochrane Collaboration was set up by Ian Chambers and a group of 70 other colleagues. In 2018, the Cochrane Collaboration is now made up of over 37,000 contributors from over 130 countries. The evidence can be used by healthcare providers and even patients and carers, so the reviews should be accessible to all and easy to read. Each review addresses an intervention for a certain outcome in a condition. The authors analyse the data from randomised control trials, which is the highest level of research, and can use the data in a meta-analysis. Cochrane reviews are regarded as the highest standard of evidence and are used to write guidelines.

4.2 Cochrane review group

Each contributor is affiliated to a group, for example I am affiliated to the Cystic Fibrosis and Genetic Disorders Group. The Cochrane Review Group (CRG) sent us a list of reviews of important topics highlighting the priorities. They required us to complete a title proposal form which was a brief outline of what we intended to review. Kidney injury is becoming increasingly important to prevent as people with CF are surviving longer. It is an important complication caused by aminoglycoside antibiotics (and

sometimes other antibiotics) which are needed for optimal respiratory outcomes. As suggested above there are strategies that may reduce the risk of kidney injury and so I decided to focus my Cochrane review on this.

4.3 Systematic review training

It is recommended that authors attend at I attend Cochrane systematic review author training courses. I attended RA1 and RA2 on the 4th and 5th July 2017 respectively. RA1 focused on the importance of Cochrane reviews and what was to be included in the protocol for the review. It also gave an insight into how to use the review software which was necessary to start the review. RA 2 reviewed the search process for finding potentially relevant studies for the Cochrane review. This included learning about databases and how to structure a search strategy. We also looked at the exclusion and inclusion criteria for studies and a brief look at the risk of bias. I then attended RA3 and RA4 on the 20th and 21st February 2018. RA3 looked at summary statistics, performing meta-analysis on dichotomous and continuous data and how to enter this into RevMan. We then reviewed risk of bias thoroughly following on from our brief look in RA2 and looked at how to identify heterogeneity. RA4 focused on how to analyse non-standard data and again how to enter this into RevMan. Finally, we looked at how to use the GRADE Pro software and how to create a summary of findings table on this software. I found these courses challenging as a lot of the other course delegates had more statistical experience than myself as they were further along in their career. However, I ensured I asked questions whenever I did not understand something, and this helped. We were also provided with the lecture copies of theses training sessions which I made notes on while at the training and used when I was writing the protocol and review. I also used the online webinars and the Cochrane Handbook for Systematic Reviews of Interventions for further

guidance on how to produce a Cochrane Review.[226] I was able to contact my CRG whenever I was unsure on what to do and in particular found NH and TR, the managing editors, invaluable at providing advice.

I also attended the systematic review seminars at Keele University. This included a set of six different seminars looking at writing a protocol, producing a search strategy, screening abstracts, data extraction, quality assessment and meta-analysis. These seminars provided a much more basic introduction to understanding a systematic review and I felt if I had not attended these then I would have struggled further on the Cochrane courses.

4.4 The protocol

4.4.1 What is a protocol?

Before authors can start completing their review they must publish a protocol. The protocol is a set of headings that the author must fill in and this is stored in RevMan. As authors may have knowledge on the topic areas which they are to review this can introduce bias. By defining in the protocol which studies are to be included and excluded this prevents authors from including studies that they think will show certain results. Authors should not look at results of studies that may be included before defining the protocol in order to reduce the risk of review authors' biases.[227] By clearly stating the methods in the protocol, it promotes transparency and should be described in a way that would allow someone else to carry out the review and get the same results.[227]

4.4.2 Software

The most important and helpful software for producing a Cochrane review is Review Manager (RevMan).[228] This software is free to use for authors writing and editing a protocol and then later a review. My systematic review is of an intervention, however, RevMan can also be used to produce reviews of other types including overviews of reviews, methodology reviews and diagnostic test accuracy reviews. RevMan contains headings and subheadings which form a standardised structure for producing a Cochrane review. Authors can use these headings to guide what they write about. These headings are based on The Methodological Expectations of Cochrane Intervention Reviews (MECIR).[229] These are a set of standards that protocols and reviews should adhere to and can be seen in a guidance box in RevMan if required. The Cochrane Handbook for Systematic Reviews of Interventions was also helpful for providing more guidance and detail on many areas for example types of studies to include[226]. RevMan can create comparison tables which can be used to show the included studies. It can also perform meta-analysis and create graphs to display the results of these. Drafts and final copies of reviews and protocols are stored on Archie which is a central database of all ongoing and completed reviews.

The next software I used was EndNote, which I used as my main reference manager.[230] Covidence is another reference manager that I used, however, EndNote was much better at removing duplicates.[231] Covidence was accessed by myself and WC to screen the studies and decide which ones were to be carried through to the next stages. Covidence can also be used for data extraction and for assessing risk of bias.

4.4.3 Publishing a protocol

Once the authors have completed the protocol they will then send it to the Cochrane review group. It is then sent to the editors for peer review, they will feedback with comments of changes that need to be made. This may require several attempts but once the editorial board are happy with the final protocol it can then be sent to be published in the Cochrane Database of Systematic Reviews (CDSR). By publishing the protocol, other contributors can see that this area is already being investigated, so it prevents duplication.[227]

CHAPTER 5: METHODS OF THE COCHRANE SYSTEMATIC REVIEW

During this chapter, I have followed the structure of the headings on RevMan as closely as possible to give a better insight into what the protocol and review should include.

5.1 Writing the protocol

I started writing the protocol in October 2017 and I sent it off to the editorial board on the 25th January 2018 for peer review. We received it back with changes to be made and sent it off for final submission on the 12th April 2018. It was published on the 22nd May 2018. Please see appendix 3 for a copy of the published protocol.

5.1.1 Defining the question

Before beginning designing a protocol, the author needs to formulate a review question. The question should be based on the structure of the acronym PICO – P (Participants), I (Interventions), C (Comparison) and O (Outcomes). As described in the previous chapter, I had decided to focus my Cochrane review on strategies that may reduce kidney injury caused by intravenous antibiotics in CF patients. With this in mind, it was clear that my participants would be patients with CF who are receiving intravenous antibiotics. My intervention would be any strategy that reduces kidney injury and my comparison would be to standard care or placebo. The main outcome is nephrotoxicity, however, I do have other outcomes which are described later in this chapter in more detail.

5.1.2 Choosing a title

When choosing a title for the Cochrane review, the handbook suggests a structure which is as follows; '[Intervention] for [health problem] in [participant group/location].' Based on this structure, I was able to formulate the title 'Strategies to prevent kidney injury from antibiotics in people with cystic fibrosis'. When I first designed the title, I used the word 'renal' rather than kidney, but my managing editor NJ reminded me that it was important to use terms that would be easily understood by the general public. This was a helpful reminder and I worked harder to make my protocol accessible to all. We did get feedback from a consumer during the peer review stage commenting that the word 'injury' was confusing as she thought it sounded like the kidney injury was due to an accident. However, we felt that clinical readers would associate the term 'injury' with acute kidney injury. I added 'from antibiotics' to the title after the peer review stage so that it was clearer what the injury was caused by.

5.1.3 Objectives of the review

To assess the benefits and harms of strategies (such as altering the type and dose of intravenous antibiotics, the avoidance of other nephrotoxic drugs alongside the intravenous antibiotics and the use of adjuvant medication including statins and fluids) to reduce or prevent kidney injury in people with CF which occurs as a result of antibiotic treatment.

5.1.4 Background for the protocol

The background of the protocol should include a concise description of the condition and may include information such as the epidemiology. Authors must go into detail regarding what the intervention is and how it may work. As most of this information was covered

in my background of my thesis, I will not repeat it again. The protocol should cover why it is important to do this review and as discussed above this is because people with CF are living longer and so kidney damage caused by intravenous antibiotics (particularly aminoglycosides) is becoming more important to prevent. However, aminoglycosides are needed for the best respiratory outcomes, so we must look at strategies that can reduce this damage as much as possible.

5.2 Criteria for considering studies for this review

5.2.1 Types of studies

I chose to include randomized control trials (RCTs) which are the main focus for Cochrane reviews. An RCT involves randomly allocating participants into one of two groups (or more) by methods such as a random number generator. One group will receive the intervention under investigation and the other group will receive a comparison or be a control group. All participants have an equal chance of being allocated the intervention. By allocating participants into groups randomly it tries to ensure that each group overall has similar baseline characteristics and so any confounders are equally distributed. This reduces the risk of selection bias. With my review being of an intervention, RCTs are the most appropriate as they are the highest quality comparative studies.

Quasi-RCTs are similar to RCTs in the way that they test an intervention on a set of outcomes compared to a control or comparison group. However, they differ in the fact that in quasi-RCTs, participant allocation to a group is not randomized. In quasi-RCTs participants may be allocated to a group by pseudo random sequences such as alternation or by date of birth.[232] Therefore, participants do not have an equal chance of being in the intervention group. This means that the two groups are less likely to have similar baseline

characteristics and so this can introduce bias affecting internal validity. We decided to assess quasi-RCTs using the Cochrane risk of bias tool and include them if we agreed that the groups were similar at baseline.

Cross over trials can be used in stable, chronic conditions to evaluate the effect of interventions.[233] A participant is given an intervention and then some time later a different intervention is given. This is compared to a parallel trial where each participant receives a single intervention and is compared with participants receiving other interventions or a control group. With a cross-over trial design each participant acts as a control for themselves, removing any baseline participant variation. This means that fewer participants are required overall to achieve the same effect. As the participant gets to test each intervention, they are then able to decide for themselves which intervention they prefer. Cross-over trials should not be used if the intervention is likely to have a long-lasting effect as it means they would not be back at baseline when starting the next arm of the trial. This is known as 'carry over', when the effects of the last intervention are persisting into the next intervention. However, this can be minimised by using a 'washout period' where there is a certain amount of time before the next intervention is started. We planned to assess cross-over trials on an individual basis. If we agreed that the treatment in the first phase alters their condition meaning they will differ from their initial state when entering the next phase, then we planned to exclude the trial unless we were able to use data from the first phase only.

5.2.2 Types of participants

Intravenous antibiotics that can cause kidney damage, such as aminoglycosides, are most commonly used in CF patients due to the recurrent respiratory infections. Therefore, I required participants in the studies to have CF, diagnosed by genetic or sweat testing.

Although kidney damage is more common in adults, due to the higher cumulative total dose of aminoglycosides, I decided to look at adults and children. Children need to be included as we need to know if these strategies are safe to use on them. All participants within a group must be receiving intravenous antibiotics of any type (planned or unplanned), for a respiratory infection, for the study to be included.

5.2.3 Types of interventions

I have identified possible strategies to reduce kidney injury such as altering the type/dose of intravenous antibiotic, omission of other nephrotoxic drugs (e.g. NSAIDs, oral antibiotics and nebulised antibiotics) while on intravenous antibiotics or use of adjuvant medications when on them (e.g. fluids or statins). However, I planned to look at any strategy that may reduce kidney damage as there may be other strategies that we have not thought of. I aimed to compare these strategies with standard clinical care or placebo. One peer reviewer identified that I had not made clear what exactly the standard clinical care is and therefore I had to add more detail to this section of the protocol. Where the intervention is to omit other nephrotoxic drugs (NSAIDs, oral antibiotics and nebulised antibiotics) when using intravenous antibiotics, the standard care would be to carry on these other nephrotoxic drugs alongside them. Where the intervention is to prescribe a different type of antibiotic, in the case of a PA infection, the standard clinical care would be use of an aminoglycoside and a beta lactam which is recommended by the UK CF Trust.[66]

As mentioned earlier previous Cochrane Reviews have examined the clinical consequences of different duration and frequency of intravenous antibiotic regimens.[219,220] As this has been established, we elected not to repeat this analysis and

our protocol excluded studies that only examine the relative nephrotoxicity and clinical effectiveness of aminoglycoside dosing frequency or duration.

5.2.4 Types of outcome measures

The Cochrane Handbook describes the importance of highlighting outcomes that are meaningful to both patients and to clinicians. All outcomes that are meaningful should be included in the review even if they have not been reported in individual studies. This is because it highlights a gap in knowledge and may prompt researchers to address this in future studies. It is important that outcomes must include beneficial and undesirable effects of the intervention, therefore the clinician or patient can make an informed decision.

Primary outcomes

Primary outcomes should be the outcomes that the conclusions on the effect of the intervention can be drawn from. I included any study where nephrotoxicity was measured as an outcome. My primary outcomes are listed below.

1. Nephrotoxicity (determined by use of invasive and non-invasive biomarkers):

a) Serum (blood) creatinine levels (change from baseline, with thresholds as defined in each trial)

b) Creatinine clearance (change from baseline, using e.g. the Schwartz Estimate for children and the Cockcroft-Gault formula for adults (with thresholds as defined in each trial))

c) Urinary excretion of protein (change from baseline)

d) Urinary excretion of biomarkers of proximal tubule toxicity (e.g. kidney injury molecule-1, retinal binding protein, beta-2 microglobulin, Clara cell protein,

microalbumin, N-Acetyl-Beta-D-glucosaminidase, alkaline phosphatase, alanine aminopeptidase, gamma-glutamyl transferase or cystatin C) (change from baseline)

e) Urine output (ml/Kg/h)

The consumer mentioned in the peer review comments that many of these terms were not understandable to a lay person. The editor however commented that in order for a meta-analysis to be performed these outcome measures need to be clearly specified and so needed to remain this way. In order to make it slightly more understandable I added in a comment to the protocol describing that any changes in these markers from baseline may indicate kidney damage.

Secondary outcomes

These are the outcomes that although the main conclusions would not be drawn from, they may help to make clinical decisions. My secondary outcomes are listed below.

1. Eradication of respiratory infection (defined as negative bronchoalveolar lavage, sputum, or cough swab cultures at the end of treatment course)
2. Participant-reported symptom scores (change from baseline)
3. Lung function parameters (e.g. forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio) (change from baseline)
4. Participant-reported quality of life scores (e.g. CFQ-R)[234] (change from baseline)
5. Adverse effects of treatment

Eradication of respiratory infection should be addressed as even though a strategy may be reducing kidney injury, it is important it is as effective at clearing the respiratory infection. The lung function test is to make sure that the intervention does not cause a

deterioration in lung function or in contrast, cause an increase in lung function for some reason. Participant reported symptom scores and quality of life scores are important outcomes in any systematic review of an intervention. Adverse effects of an intervention should always be evaluated.

5.3 Search method for identification of studies

In this section I will describe how I went about designing a search strategy and the medical databases I searched. I decided that there was to be no restrictions regarding language or publication status when deciding whether to include a study.

5.3.1 Electronic searches

Bibliographic Databases

It is mandatory to search CENTRAL and MEDLINE databases and if the review author has access, EMBASE too, for relevant studies to be included in the review. Here I will describe these databases in more detail.

- Cochrane Central Register of Controlled Trials (CENTRAL) – This is a database developed by the Cochrane collaboration and is the most comprehensive list of RCTs with nearly 530,000 citations in January 2008.[235,236] It is published as part of The Cochrane Library and many of the results have come from systematic searches of other bibliographic databases (e.g. EMBASE and MEDLINE) and from handsearching. CENTRAL also includes citations to reports that are not found in other bibliographic databases, citations that are available only in conference proceedings and citations that are published in

many different languages.[237] It can be accessed for free via The Cochrane Library to all members of a CRG.

- MEDLINE – This is a database that contains more than 24 million references from 5,200 journals in around 40 different languages.[238] It contains citations from 1950 onwards and it is available to access for free through PubMed. However, I accessed it through Ovid which requires institutional access which Keele University provides. MEDLINE indexes citations by adding Medical Subject Terms (MeSH) descriptors to them.
- EMBASE – This is also a biomedical database containing 32 million plus records from over 8,500 journals in 30 languages from 1947 onwards.[239] Embase is indexed using Elsevier's Life Science thesaurus known as Emtree®. I thought I would be able to use Ovid to access EMBASE, however I had some problems with this, so I was advised to use Healthcare Databases Advanced Search (HDAS) instead.

We also searched grey literature which is literature that is not formally published such as conference abstracts, dissertations and research reports using the Open Grey database.[240] It is important to include grey literature as it can make up 10% of referenced studies in a Cochrane review.[241]

Trial Registries

- Cystic Fibrosis Trials Register – A search was conducted of this register by the CRG's Information Specialist using the following term: antibiotics. This register is made up from electronic searches of CENTRAL, MEDLINE, EMBASE and from handsearching two journals – Pediatric Pulmonology and the Journal of Cystic Fibrosis.[242] Unpublished work is identified by searching the abstract book

of three conferences: the International Cystic Fibrosis Conference, the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference.

- The World Health Organization International Clinical Trials Registry Platform - This registry platform was introduced in May 2007 in order to provide a list of ongoing trials and completed trials that could be all seen in one place.[243] It uses registers that contain trials of a certain standard.
- US National Institutes of Health Ongoing Trial Register Clinicaltrials.gov - This register contains a list of studies that are ongoing or completed that involved human participants.[244] According to the website, it currently has 267,527 (05/03/2018) trials listed.
- International Standard Randomised Controlled Trial Number (ISRCTN) Registry – This registry was launched in 2000 and contains trials involving human participants that assess a health intervention.[245]

5.3.2 Searching other resources

I also checked the bibliographies of included studies and any relevant systematic reviews identified for further references to relevant trials. I planned to contact experts and organisations in the field to obtain additional information on relevant trials however this was not necessary for my review.

5.3.3 Designing a search strategy

Usually NH, the CRG's Information specialist would design the search strategy for reviews in the group. However, she was on maternity leave at the time that I was about to design mine. Therefore, I had to design my own. Although this was challenging I had attended the Keele University seminars on systematic reviews and one of the facilitators, NC, helped with writing the search strategy. NC and NJ advised me not to make the

search strategy too narrow at first as they thought this would be a very niche area. As we did not know all the possible interventions to minimise kidney injury and there may be many of them we did not include this in the search strategy. We also did not include kidney injury, the main outcome, in the search strategy as we thought it would make the search too narrow. The search strategy was based on the eligibility criteria for the review. Please see appendix 3 for the protocol which includes copies of my search strategies for the different databases. For each of the criteria it is important to consider all terms and abbreviations that may be used for a word, the thesaurus in the databases can be helpful to find other terms.

Condition – In all studies the participants must have cystic fibrosis. I searched the terms cystic fibrosis, CF, mucoviscidosis and fibrocystic disease.

Intravenous Antibiotics – One of my criteria under types of participants was that they must be receiving a course of intravenous antibiotics in the study. For this criterion, I searched for anti-bacterial agents, antibiotics, antimicrobials, aminoglycosides, and different types of aminoglycosides (kanamycin, tobramycin, gentamycin, neomycin, amikacin, streptomycin).

Type of study – For each of the databases you must include a search for randomised controlled trials as this is what I described I would look at in my protocol. This filters out all studies that are not RCTS. However, this is not necessary to do when searching CENTRAL as there are only randomised control trials in the database.

MeSH Descriptors

Studies are tagged with subject heading-controlled vocabulary in each of the databases. These are known as ‘MeSH descriptors’. This means that when you search for this MeSH

term then it picks up all the studies tagged under that overall subject heading. For example, one of my MeSH terms was Cystic Fibrosis. In MEDLINE and EMBASE you must add a forward slash after the term e.g. “Cystic Fibrosis/”. Whereas in CENTRAL you must write “Cystic Fibrosis [Mesh descriptor]” in order to pick up the articles tagged under this subject heading. To search for the MeSH terms relevant to my search strategy, I used the thesaurus on the databases. For some of the subject headings, e.g. Aminoglycosides, I chose to explode them. This means that when the search is looking for that MeSH term it will also look for all narrower subject headings under that main heading. To do this I simply wrote “exp Aminoglycosides”.

Suffix variation

Some words have different suffixes at the end of them for example medic- may end in medicine, medicines, medication, or medications. Therefore, to ensure all possible suffix variations of the words are picked up on a search I used a truncation such as ‘medic$’. In the Cochrane database (CENTRAL) and when using HDAS for Embase, the truncation symbol is * rather than $.

Wildcards

Some words have different spellings, depending on whether the study was done in the United States or the United Kingdom. For example, kanamycin can also be spelt kanamycin. In order to ensure all studies with this word spelt either way are picked up, a # can be used in the place of the letter that can be changed. For example, in my search strategy I used kanam#cin to search for this antibiotic. This is called a wildcard, the symbols * and ? can also be used too.

Searching for nearby words

It is necessary to specify that when you ask the interfaces to search for two words that you use ‘NEAR’ or ‘ADJ’ in between them. This is because the search may default to just finding both words in the document that may be apart and unrelated. For example, in my search strategy I used ‘ADJ’, egg cystic$ adj5 fibros$. This would produce results that identified studies that had the word cystic up to 5 words away from fibrosis. If the words should be next to each other then you can put the phrase in quotation marks e.g. “anti biotic”

Boolean operators

For each concept, for example one of mine was cystic fibrosis, the search strategy should be built up with the controlled vocabulary terms (e.g. MeSH terms) and related words/synonyms for each concept. At the end of each concept, the Boolean ‘OR’ operator should be used. This means that when the search is run it will pick up any citations with any of these different ways of naming cystic fibrosis in. My other concepts are intravenous antibiotics and randomised controlled trials and ‘OR’ should be used at the end of these too. When I created my search strategy with these three sets of terms, I then joined them together with the ‘AND’ operator. This means that my search picked up results that included cystic fibrosis and intravenous antibiotics and that are randomised controlled trials. However, this can cause problems as if the article does not contain at least one term from each of the three sets, it will not be picked up. Therefore, when designing the search strategy, it is important that you have considered as many synonyms as possible.

5.4 Data Collection and Analysis

5.4.1 Selection of studies

I entered each strategy into the individual databases and then exported the results onto an online software program called Covidence. In total we had 6389 references from the search results. Cochrane recommends that authors use a reference management software as it provides a place to keep track of all references. Covidence is used to organise the references and helps by removing duplicates, in our case it removed 2108 duplicates.

When writing the protocol, I decided that we would consider trials in any language and would translate them as necessary. I also decided we would include studies published as full texts, but if there was only an abstract available for a particular study we would include it if it presents results. Where there are no results presented within the abstract or on any trials registry sites, then we have classified the study as 'Awaiting classification' until more information is available. Similarly, with ongoing studies, if the study met our inclusion criteria then we included it.

5.4.2 Screening

The first stage of screening involved looking at the titles and abstracts of studies on the reference list and deciding if they were relevant to the review. WC and myself screened the titles and abstracts independently on Covidence as Cochrane recommends that at least two people independently screen them.[246] On Covidence you have three options; yes, no or maybe and each reviewer must vote one of these options for all of the studies on the list. When both reviewers voted ‘No’ for a study, this study did not go forward to the next stage of screening. Covidence marked ‘Conflict’ for studies where myself and WC did not give the same answer. WC and I attempted to resolve these disagreements by discussion, but we relied on FG to act as an external arbiter to make the decision if we could not agree. In total we had 830 conflicts at this stage of screening.

When myself and WC both voted ‘Yes’ or ‘Maybe’ for a reference, it was taken forward to the next stage of screening. We were able to dismiss many studies from the review based on the title, for example there were many titles about ‘non-CF bronchiectasis’ and so these could be excluded straight away. Where it was not so clear from the title, I would look at the abstracts of studies and if these had not been imported then I hand searched for the abstract online. There were some abstracts that I was not able to access myself, so I had to ask the librarian at Keele University and NJ for help in sourcing these.

We took 164 studies forward to the next stage of screening. This involved obtaining and reading the full texts of these references. At this stage of screening we excluded 110 irrelevant references.

We took 54 references through to data extraction which referred to 50 trials. From these studies 8 were classified as ‘Awaiting classifications’ as it was not clear whether they had addressed nephrotoxicity, so contacted the study authors for more information. There were 30 studies that fit all inclusion criteria, however they reported nephrotoxicity as a dichotomous outcome, i.e. yes nephrotoxicity occurred, or no nephrotoxicity didn’t occur. We have contacted these study authors to ask them to provide continuous data for measures of nephrotoxicity such as creatinine etc. If we cannot get hold of the continuous data, we will analyse the dichotomous data in due course. We also have 8 classified as ‘Ongoing trials’ and we have contacted the authors for any data, however, at this stage we do not have any results to report from them. Therefore, at this stage we have only 4 studies that present nephrotoxicity as continuous data that we can analyse.

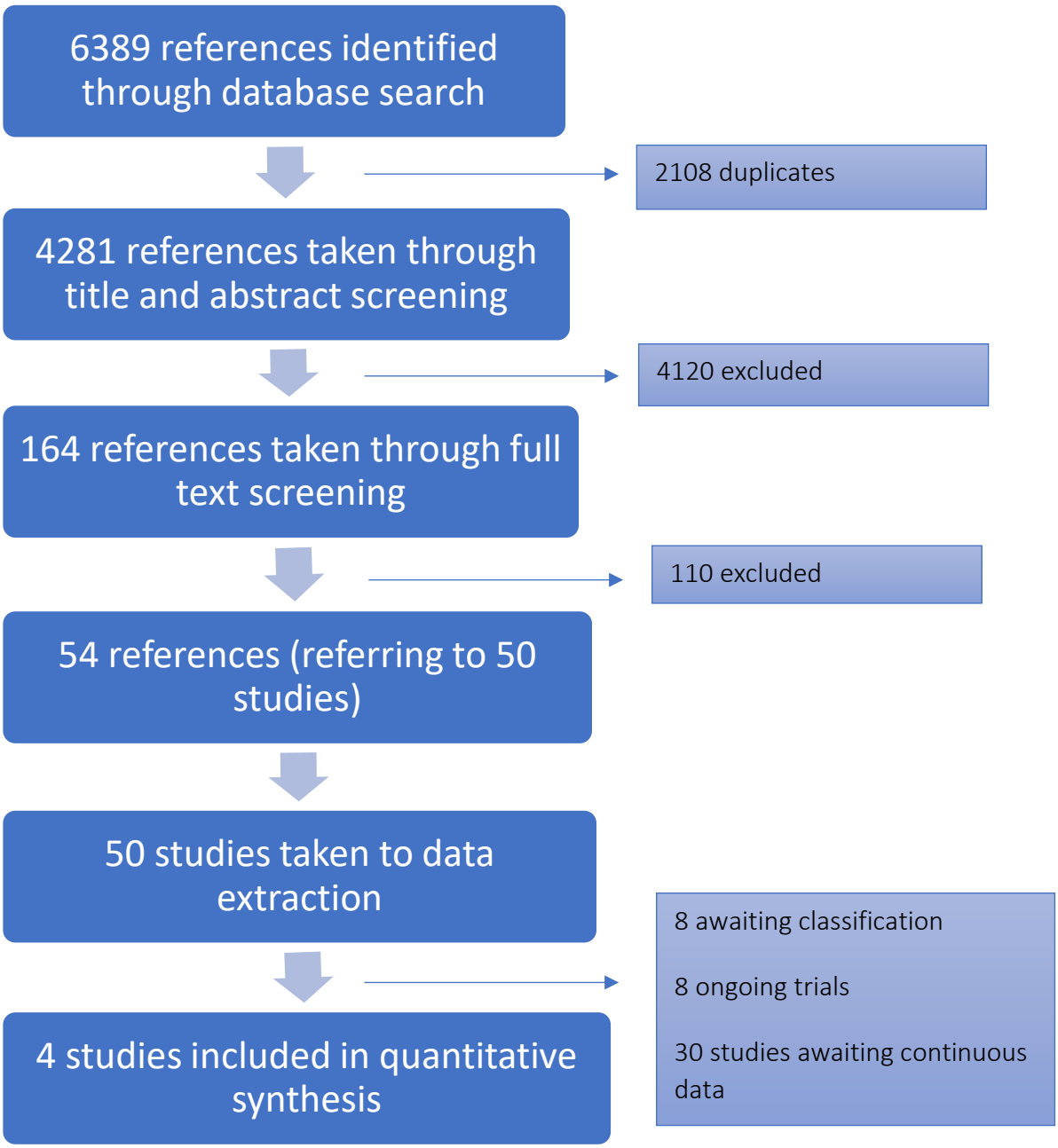

Figure 5 – Study flow diagram

5.4.3 Data extraction and management

Our CRG provided us with a paper copy of their data extraction form which both myself and WC used to extract basic information from the full text version of the included studies. Two authors should independently complete the data extraction process for the included studies.[246] I then checked for any discrepancies between our work and transferred this information to the online Covidence data extraction form where I added

more detailed information. We collected data on trial designs, participant characteristics, intervention and comparator and outcome data. We had to contact all authors for further information regarding their trial.

Data extraction forms are based on the inclusion criteria for the review and extracting the data may highlight other studies that should have been excluded. They have headings which prompt the authors on what data to extract. The forms also provide a summary of the decisions that have been made during the data extraction process. They also provide a summary of the data that can then be entered into RevMan ready for analysis.

5.4.4 Assessment of risk of bias in included studies

Myself and WC independently used the risk of bias tool as described in the Cochrane Handbook for Systematic Reviews of Interventions to assess the risk of bias across seven domains.[247] We ranked each domain as 'high risk of bias', 'low risk of bias' or 'unclear'. For all domains, where the methods have not been described clearly, we ranked this as 'unclear risk of bias'. We attempted to resolve any disagreements by discussion, but where we did not reach a decision, the third author (FG) acted as an external arbiter to mediate until we reached a final conclusion. I will now go on to describe the different types of bias and which of the Cochrane domains fit into each type. We used Covidence to input this data which was then exported to RevMan where it created a table for us.

Selection bias

This type of bias arises from any difference in the baseline characteristics of the two groups (intervention and control/comparison) due to non-random allocation to a group.

Sequence generation – This domain looks at how the participants were randomised into their groups (intervention or control/comparison). Methods such as a random number

table or computer-generated lists are appropriate and have low risk of bias. On the other hand, methods that are non-random such as sequence generated by odd or even date of birth will have a high risk of bias for this domain.

Allocation concealment – This domain looks at how the allocation sequence was concealed from the researchers and participants. If it is unlikely that the participants or investigators enrolling participants could not foresee assignment due to central allocation or sequentially numbered drug containers of identical appearances, then this study will have a low risk of bias for this domain. Where the allocation may be foreseen, for example, they used assignment envelopes that were non-opaque or used alternation then this study will have a high risk of bias for this domain.

Performance bias

This type of bias arises from the participants or personnel acting differently due to knowledge of which group the participant is in. For example, the personnel may give more attention to those in the intervention group.

Blinding of participants and personnel – This domain looks at whether the participants and personnel were blinded from knowledge of what group the participants were in during the study. The trial should state that participants and personnel were blinded or if there was no blinding the outcomes would not have been affected by lack of blinding, in order to have a low risk of bias for this domain. If there was no blinding and the outcome is likely to be affected by the lack of blinding, then this domain is deemed as high risk of bias.

Detection bias

This type of bias arises from the differences in the way outcomes are assessed (particularly subjective outcomes) due to the outcome assessors having knowledge as to which group the participant was in.

Blinding of outcome assessment – This domain looks at whether the outcome assessors were blinded from knowledge regarding which group a participant was in. Blinding of outcome assessment must be ensured for this domain to rank as low risk of bias. If there is no blinding but the outcomes would not have been affected by lack of blinding, then this can also be ranked as low risk of bias. If there was no blinding and the outcome is likely to be affected by the lack of blinding, then this will result in a high risk of bias for this domain.

Attrition bias

This type of bias arises from there being differences between the number of withdrawals from the study between the two groups.

Incomplete outcome data – This domain looks at the amount, nature, and handling of any incomplete outcome data. If there is no missing data, then this gives a low risk of bias for this domain. If the missing data is equally distributed across the intervention and control group and the reasons in both group are similar, then this can also be classed as low risk of bias. If the reason for missing outcome data is likely to be related to true outcome, then this leads to a high risk of bias for this domain.

Reporting bias

This type of bias arises when the authors do not report all outcomes that they pre-specified.

Selective outcome reporting – This domain looks at whether the outcomes that were mentioned in the protocol have been presented in the results of the full paper. If all pre-specified outcomes have been reported in the full paper, then this means the study is low risk of bias for this domain. If not all pre-specified outcomes have been reported then the study will have high risk of bias for this domain.

Other bias

Other risk of bias - This final domain involves looking for any other potential sources of bias in the included studies. If there are no other sources of bias, then it can be classed as having a low risk for this domain and high risk if the opposite is true.

5.4.5 Measures of treatment effect

There are 5 main types of data described in the Cochrane handbook including; dichotomous data, continuous data, ordinal data, counts and rates and time-to-event data. My outcome data is all either dichotomous or continuous data, so I will now go on to define these types of data.

Dichotomous data (binary data): Each individual gives an outcome of one of only two categories (e.g. yes/no or male/female).

Continuous data: Quantitative (numerical) data that can take any value in a specified range (e.g. height)

Dichotomous data

In my review my dichotomous data included the outcomes; adverse effects and eradication of respiratory infection. Dichotomous data can be summarised in many ways with the most common effect measures being; risk ratio (RR), odds ratio (OR), risk

difference and number needed to treat. Risk describes the probability of an event occurring. We decided to use the RR (also known as relative risk) to compare the risks of an event between the two groups (intervention group vs control group). A RR of 1 indicates that the risk of the event is the same for the intervention and control group. A RR of above 1 indicates the intervention is more likely to give the outcome. A RR below 1 indicates the intervention is less likely than the control to result in the outcome. We used 95% confidence intervals where appropriate.

The RR is calculated by:

$$Risk\ of\ event\ in\ intevention\ group =$$

$$\frac{Number\ of\ participants\ in\ intervention\ group\ that\ event\ occurred\ in}{Total\ number\ of\ participants\ in\ intervention\ group}$$

$$Risk\ of\ event\ in\ control\ group =$$

$$\frac{Number\ of\ participants\ in\ control\ group\ that\ event\ occurred\ in}{Total\ number\ of\ participants\ in\ control\ group}$$

$$RR = \frac{Risk\ of\ event\ in\ intervention\ group}{Risk\ of\ event\ in\ control\ group}$$

Peer reviewers highlighted that kidney injury may be presented as dichotomous data i.e. kidney injury or no kidney injury. As mentioned this was the case in many studies and we are in the process of contacting the study authors for their continuous data if available. If this is not possible, then we will calculate the RR.

Continuous data

In my review my continuous data included serum creatinine levels, creatinine clearance, urinary excretion of protein, urinary excretion of phospholipid markers of nephrotoxicity, urine output, lung function parameters, participant-reported QoL scores, participant-reported symptom scores. Participant reported QoL and symptom scores are not actually continuous data but in Cochrane reviews they are usually analysed as though they are.

We recorded the mean change and standard deviation (SD) from baseline for each group. However, some studies only reported a pre-intervention mean (SD) and post intervention mean (SD). We can use these two figures to calculate the mean change for each group. However, we cannot calculate the SD of the change and so these results will be reported narratively.

$$\text{Mean change} = \text{Mean post treatment} - \text{Mean at baseline}$$

There are two types of summary statistics that can be used to show the effect size i.e. the difference between two groups (intervention vs control).

The first of these is the mean difference (MD) which is used when an outcome is measured on the same scale in all studies. The MD measures the absolute difference between the mean value in two groups in a trial. We will calculate 95% confidence intervals for each MD.

$$\text{MD} = \text{Mean change of intervention} - \text{mean change of control}$$

If the MD is 0 then this indicates that the intervention has the exact same effect on the outcome as the control. When interpreting the MD, it is important to remember whether the outcome is a good one or bad outcome. For example, if the outcome is quality of life,

then any value above 0 would indicate that the intervention is better at improving quality of life than the control. However, for a bad outcome like biomarkers of proximal tubular toxicity, if the MD was above 0 then this would indicate that the intervention was causing an increase in urinary excretion of biomarkers of proximal tubular toxicity and this would be negative as it would suggest the intervention is giving more kidney damage than the control.

For some outcomes they may be measured on different scales from one study to the next. In some cases, this may be easy to resolve as you can convert them all to the same scale e.g. if one study uses pounds, but all others use kilograms, then you would convert them all to kilograms. However, with some scales we cannot convert them, for example, quality of life scales. We do not know what a score of 1 on one scale may equate to one another scale. When this occurs, we cannot use the MD and we must standardise the results of studies before combining them. In this instance we use the standardised mean difference (SMD). The calculation for this is complex, but luckily RevMan calculates most of this for you.

$$\text{SMD} = \frac{Mean\ of\ intervention\ group - Mean\ of\ control\ group}{Pooled\ standard\ deviation\ of\ both\ groups}$$

Unfortunately, the SMD does not correct for differences in the direction of the scale. For example, a score of 10 on one quality of life scale may indicate the best quality of life, whereas a score of 10 on another scale may indicate the worst quality of life. Before combining the results of the studies in a meta-analysis you must make sure all the results are running in the same direction by multiplying the results of one scale by -1. SMD can be difficult to interpret because it is units of standard deviation rather than units of an outcome scale. However, a positive SMD indicates when looking at quality of life indicates that the intervention has improved the quality of life.

5.4.6 Unit of analysis issues

We decided we would assess cross-over design trials on an individual basis to establish how much data we could include in the analysis. If the authors did not take into account the cross-over design in the analysis, any carry-over effect or within-person differences then we decided not to include them in our analysis. If this was the case, then we planned to only include data from the first phase of the trial as if it were a parallel design study. However, this would lose the advantage of the cross-over design where participants are used as their own controls.[248]

In our protocol we decided that if we found multi-arm trials with different active treatment arms e.g. statins or fluids, then we would analyse each treatment arm separately against placebo.

5.4.7 Dealing with missing data

Missing data can cause biased results and so to prevent this, we will contact study authors for any more information that we may require. If data is incomplete but partially available and we have not been able to contact the authors, then we planned to use the last available measurement. In all four studies that we have data extracted from, we have found they have given number of withdrawals and reasons for withdrawal. However, we did have to contact two study authors to find out which groups the withdrawals were from. If we find participants withdrawals are not recorded along with the reasons for withdrawal in any further studies, then we will attempt to contact the study authors as this can cause attrition bias.

Intention-to-treat analyses looks at the results of all participants and analyses them based on the group they were originally assigned to. It ignores any withdrawals, non-

compliance or swapping of groups. Ideally study authors will have performed an intention-to-treat analysis. If there was no mention of ITT analysis, then we assumed they had not performed one. We planned to undertake ITT analyses ourselves where possible, however, most trials did not report the results of participants that withdrew from the study.

5.4.8 Assessment of heterogeneity

Heterogeneity refers to the differences between studies that are being analysed in the systematic review. There are many different types of heterogeneity. Clinical heterogeneity involves differences between the participants in the study, the interventions, or the outcomes. Whereas methodological heterogeneity refers to differences in the study design which may affect the risk of bias. Both clinical and methodological heterogeneity can lead to what is known as statistical heterogeneity. This is where there are differences in the intervention effects between the studies that is more than due to just random error.

By looking for trials that report the same outcomes it may be possible to include these in a meta-analysis. The first crude method to test for heterogeneity is known as 'the eyeball test'. This is where we look at the forest plots of those studies reporting similar outcomes and see if their confidence intervals overlap. If their confidence intervals do not overlap, then there is heterogeneity. A forest plot shows the summary statistic (e.g. RR or mean difference), with a diamond symbol, for each study comparing the same outcome. The size of the diamond corresponds to the number of participants. The lines either side of the diamond represent the confidence interval which is what we look at to see if there is any overlap. A more formal test can be used to assess for heterogeneity and this is known as the chi-squared test. A large chi-squared value or a low p-value indicate heterogeneity

but should be interpreted with caution if there is a small sample size or if there are only a few studies. The chi-squared statistic can be used to work out the I^2 which is a percentage of the differences in effect estimates that is due to heterogeneity as opposed to chance. The Cochrane Handbook for Systematic Reviews of Interventions provides a guide to interpret the I^2 value:[249]

- low (might not be important) = 0% to 40%;
- moderate = 30% to 60%;
- substantial = 50% to 90%; and
- considerable = 75% to 100%.

5.4.9 Assessment of reporting bias

We planned to generate a funnel plot to attempt to identify any publication bias in the trials if we had at least 10 trials.[250] A funnel plot is a scatter plot of the estimated effects for the trials identified with the standard error on the vertical axis and effect estimate on the horizontal axis. Trials with smaller sample sizes are usually less precise and so their estimated effects are usually scattered more widely at the bottom of the plot. Trials with larger sample sizes usually have a precise effect estimate and so these trials should be scattered around the top close to the vertical line. This creates the shape of a funnel and if the plot is symmetrical it suggests there is unlikely to be any reporting bias.

We planned to compare the trial protocols with the final publication papers to see if there was any selective reporting of outcomes in the included trials. If the trial protocols were not available, then we decided to compare the outcomes reported in the results section to the outcomes reported in the methods section. We planned to extract information on the sponsors, sources of funding and competing interests of the authors to look for any

external bias. We planned to minimise publication bias by searching trial registries and contacted pharmaceutical companies for unpublished data.

5.4.10 Data synthesis

Meta-analysis refers to a statistical method that combines data from two or more studies to produce an estimate of overall effect. If there is considerable heterogeneity (I^2 over 75%), then a meta-analysis should not be carried out and so we will report these results narratively. If there is not considerable heterogeneity, then we will perform meta-analysis using the data from the included studies to create forest plots on RevMan. We planned to carry out separate meta-analyses for each of the different interventions (e.g. fluids, statins) in comparison to the control (placebo or standard clinical care). However, at this stage our studies are not looking at the same comparison and so cannot be included in a meta-analysis.

If at a later stage we are able to carry out a meta-analysis, we will use the level of heterogeneity to determine which type of analysis model to use. There are two different types of statistical models in RevMan known as the fixed effect model and the random effects model.

The fixed effect model assumes that all studies included in the meta-analysis are measuring the same true treatment effect and any differences are down to random error. We will use the fixed effect model if there is a low level of heterogeneity (i.e. I^2 less than 40%).

On the other hand, the random effects model assumes that the treatment effect is different in all included studies. The treatment effect may vary slightly in studies, for example, perhaps if they are looking at different age of participants. We will use the random effects

model when there is a higher level of heterogeneity (i.e. I^2 over 40%). The random effects models allows for heterogeneity between trials and so there will be a larger confidence interval meaning it is less precise.

5.4.11 Subgroup analysis and investigation of heterogeneity

If we are able to perform a meta-analysis and there is heterogeneity (greater than 40%) we can explore this by undertaking subgroup analyses to see if we can determine a cause for it. We will do this for our primary outcome (nephrotoxicity) only.

The subgroups that we will analyse include:

- individuals receiving planned versus unplanned antibiotic treatment;
- children (under 18 years old) versus adults.

By performing subgroup analysis, we will be able to see if the interventions affect these certain subgroups differently. For example, we may be able to see how whether being an adult effects how effective statins are at preventing kidney disease, compared to how effective they are in children.

5.4.12 Sensitivity analysis

When screening studies for eligibility for inclusion, sometimes decisions can be tricky as to whether to include them or not. A sensitivity analysis looks at removing the studies that were borderline for inclusion and then performing the meta-analysis again to see if the results are similar. Including studies with high risk of bias in meta-analyses can produce results that are not very accurate. Cross-over trials may also produce inaccurate results. By excluding these studies and then repeating the meta-analysis we can see whether the true effect of the intervention is the same. If the overall result and conclusions

are not affected by the different decision, then the results can be regarded with a higher degree of certainty. Therefore, I planned to perform sensitivity analyses including and excluding trials with high risk of bias and then cross-over trials.

5.4.13 Summary of findings table

Cochrane reviews should include a 'summary of findings table' which focuses on the main outcomes of the review. It should be as simple as possible and include information on the magnitude of effect of interventions and a summary of the quality of the evidence. The summary of findings table can have a maximum of seven outcomes and these must be the most clinically relevant outcomes to patients and health care professionals. I originally proposed to have seven outcomes in my protocol, however, peer reviewers suggested that I had missed out important outcomes, so we were allowed to include more. Our chosen outcomes were:

- Blood creatinine level
- Creatinine clearance
- Urinary excretion of protein
- Urinary excretion of biomarkers of proximal tubular toxicity
- Urine output
- Eradication of respiratory infection (defined as negative bronchoalveolar lavage, sputum, or cough swab cultures at the end of treatment course)
- Adverse effects of treatment
- Participant-reported symptom scores
- Participant-reported QoL scores

I planned to create a separate table for each treatment comparison (e.g. statins vs placebo or fluids vs no fluids).

CHAPTER 6: RESULTS OF THE COCHRANE SYSTEMATIC REVIEW

6.1 Results of the search

We have identified 54 references relating to 50 trials to be included in the review. At the moment we can only extract data from 4 of them and I have split these up into their different comparisons for analysis.

6.2 Characteristics of included studies

6.2.1 Comparison 1 – Intravenous meropenem and tobramycin versus intravenous ceftazidime and tobramycin

Latzin 2008[251]

Table 2 - Characteristics of Latzin's study

Methods	RCT Parallel design Open label Duration: 2-3 weeks Multicentre Country: Germany & Switzerland
Participants	127 participants. Randomised to receive different IV antibiotic combinations in three different scenarios: 1. Suppression therapy in those with chronic PA but not experiencing an exacerbation; 2. Acute exacerbation in those with chronic PA infection; and

	3. Eradication therapy in those with their first isolation of PA. Intervention 1: 59 participants; mean (SD) age 17.3 (8.7); 29 males, 30 females. Intervention 2: 59 participants; mean (SD) age 16.9 (7.7); 30 males, 29 females.
Interventions	Intervention 1: IV meropenem 120 mg/kg/day in 3 doses (of >50kg, 2g TDS) and IV tobramycin 9- 2 mg/kg/day in 2 doses. Intervention 2: IV ceftazidime 200-400 mg/kg/day in 2 or 3 doses and IV tobramycin 9-12 mg/kg/day in 2 doses.
Outcomes	Nephrotoxicity (serum creatinine) Lung function (FEV_1 and FVC) Inflammatory markers (CRP and leukocyte count) Full blood count Liver function Adverse effects Microbiology
Notes	I contacted Latzin to enquire about their methods of randomisation and how they concealed allocation. I also asked him for further information regarding his data tables as I struggled to work out how he calculated the mean difference.

6.2.2 Comparison 2 – Morning versus evening IV dosing

Prayle 2016[252]

Table 3 - Characteristics of Prayle's study

Methods	RCT Parallel design Open label Duration: 14 days

	Multicentre Country: UK
Participants	18 participants, aged 5-18 years, with CF that required a course of intravenous tobramycin. Intervention 1: 9 participants; median (IQR) age 12.5 (12.2-15.5). Intervention 2: 9 participants; median (IQR) age 14.5 (12.8-14.9).
Interventions	Participants received intravenous tobramycin alongside at least a second intravenous antibiotic. Intervention 1: Intravenous tobramycin administered at 08:00 Intervention 2: Intravenous tobramycin administered at 20:00
Outcomes	Pharmacokinetic measures Nephrotoxicity: Urinary excretion of biomarkers of proximal tubular toxicity (KIM1, CysC, NAGL, IL-18, NAG) Lung function (FEV_1 and FVC) Weight (kg) Melatonin levels
Notes	I contacted Prayle to check that another reference that we identified in the search was referring to the same trial which he confirmed it was. Prayle stated that 14 urine samples were collected in total and so I also enquired as to how many urine samples were obtained for each group.

6.2.3 Comparison 3 – Nebulised versus intravenous antibiotics

Al-Aloul 2014[253]

Table 4 - Characteristics of Al-Aloul's study

Methods	RCT Cross-over design Duration: 14 days

	Single centre Country: UK
Participants	20 adult (11 male, 9 female) participants with CF that were chronically infected with PA and experiencing a pulmonary exacerbation were randomised. The mean age (SD) of the participants was 22.1 (6.9) and ranged from 17-42 years. Intervention 1: 10 participants in the first arm, 10 participants in the second arm Intervention 2: 10 participants in the first arm, 10 participants in the second arm
Interventions	During each exacerbation, all patients in both groups also received IV colistimethate sodium 2 mega units three times a day. Intervention 1: Nebulised tobramycin (TOBI), 300mg/5ml, administered using a Pari LC Plus jet nebulizer and a Porta-Neb compressor at a dose of 300mg twice a day. Intervention 2: Intravenous tobramycin given at mean daily dose of 8.2mg/kg (SD 1.5) in 2 or 3 divided doses.
Outcomes	Nephrotoxicity: Serum creatinine, serum magnesium, creatinine clearance, urinalysis, urinary excretion of protein and urinary excretion of biomarkers of proximal tubular toxicity (NAG, AAP, β2 Microglobulin, Collagen IV Inflammatory markers (C-reactive protein & white cell count) Lung function (FEV_1 and FVC) Microbiology Symptom score Adverse effects
Notes	I contacted Al-Aloul to determine how he randomised the participants and how he concealed allocation. I also enquired as to whether he blinded participants and personnel. He reported in the study that he was only able to obtain 13 sputum samples, so I enquired as to what group these samples were from. Finally, I enquired as to whether two other citations that were identified from the search were references to the same trial.

6.2.4 Comparison 4 – Single IV antibiotic vs combination of IV antibiotics

Conway 1997[254]

Table 5 - Characteristics of Conway's study

Methods	RCT Parallel design Open label Duration: 12 days Single centre Country: UK
Participants	53 adult participants with CF that were chronically colonised with PA experiencing a respiratory exacerbation. 18 participants entered the study twice due to a further exacerbation later on. Of these 18, 9 were originally treated with monotherapy and 9 were originally treated with the dual therapy. On subsequent entry to the trial they received the opposite treatment to the first time they were enrolled. Intervention 1: 36 participants; mean (SD) age 21.7 (4.2) years; 17 females, 19 males. Intervention 2: 35 participants; mean (SD) age 21.2 (4.25) years; 12 females, 23 males.
Interventions	Intervention 1: IV colistin 2MU (160mg) TDS Intervention 2: IV colistin 2MU (160mg) TDS along with a second IV anti-pseudomonal antibiotic.
Outcomes	Nephrotoxicity: Serum U&Es, creatinine clearance, urine dipstick testing Lung function (FEV_1 and FVC) Oxygen saturation Clinical score

	Chest X-ray scores Inflammatory markers: White cell count, % neutrophil count, C-reactive protein Weight (kg) Blood drug levels Microbiology Adverse neurological effects
Notes	As Conway did not report the mean change with groups or mean difference between groups we were not able to use his data in our analysis. I contacted him to ask if I could have access to individual patient data. I also contacted him to see how they randomised participants and whether there was any blinding in the trial.

6.3 Risk of bias in included studies

I created a risk of bias summary using RevMan which is shown below in figure 6.1. I will now go on to explain my reasons for the decisions made on ranking.

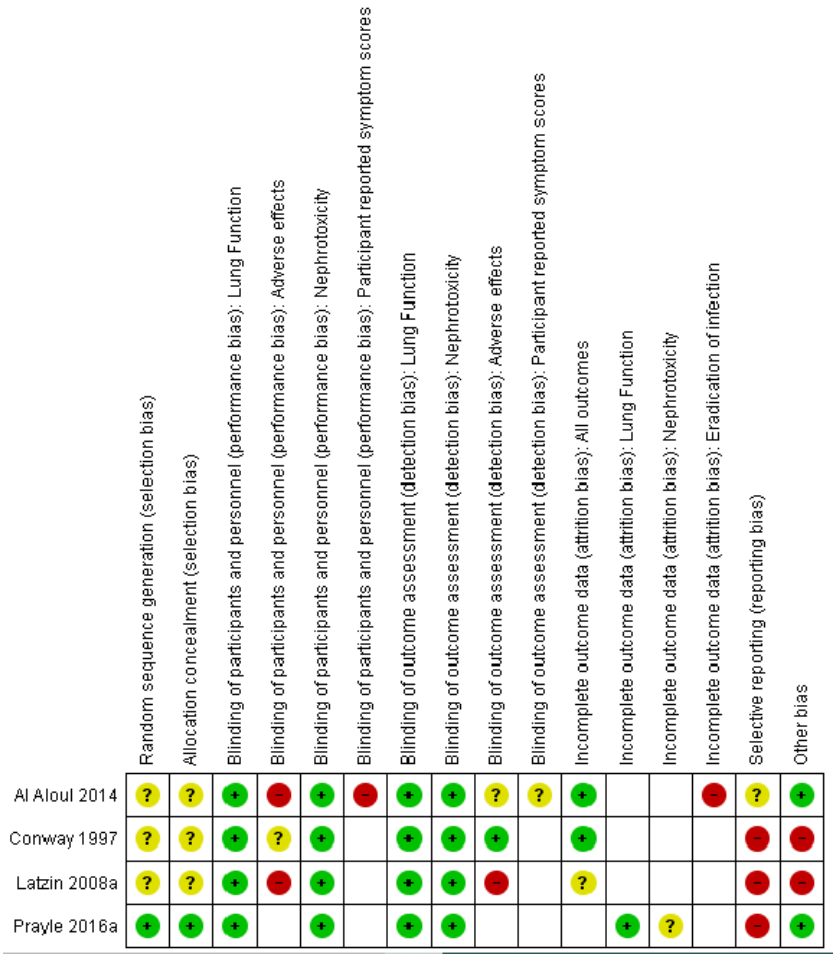

Figure 6.1 – Risk of bias summary

6.3.1 Allocation (selection bias)

Sequence Generation

All four of the studies that we have included so far were randomised. Only one of these (Prayle 2016) studies listed their method of randomisation which was a remote web-based system and so we ranked this as low risk of bias for this domain. The other three studies (Al-Aloul 2014, Latzin 2008 and Conway 1997) did not provide any information regarding how they randomised participants. We have contacted the authors to clarify this but have ranked them as unclear risk of bias for now.

Allocation Concealment

Three of our studies did not provide any information regarding concealment of allocation. (Latzin 2008, Al-Aloul 2014 and Conway 1997) We have contacted them for more information but for now we have ranked these studies to have an unclear risk of bias for this domain. As Prayle used a central web-based system it is impossible to predict what the next group assignment would be and so we ranked this domain as low risk of bias.

6.3.2 Blinding (performance bias and detection bias)

Both Prayle and Latzin's study were open label and so there was no blinding of the participants or researchers. Al-Aloul study does not state it is open label but does not mention blinding of participants or researchers. Conway's study describes blinding of outcome assessors but does not mention blinding of participants. We have contacted Conway and Al-Aloul for further information regarding blinding. As lack of blinding effects risk of bias in outcomes differently I will now describe my rankings for each outcome.

Participants and personnel

Nephrotoxicity - For all four studies we judged that for the outcome nephrotoxicity the risk of bias would be low, regardless of whether participants and personnel were blinded. This is because nephrotoxicity is objective and so lack of blinding is unlikely to affect this. However, one point we did consider was that personnel may give more fluids to a certain group than the other in order to minimize nephrotoxicity.

Lung function - Similarly, for lung function, we judged that a lack of blinding would not affect the outcome therefore we rated all the four studies as low risk of bias for this domain. There is the risk that participants may put more or less effort in if they know

what group they are in. However, when lung function is conducted to ATS/ERS standard, this is effort independent once >90% maximum effort achieved. Therefore, we judged lack of blinding to be unlikely to affect this outcome.

Adverse effects - In Latzin's study the patients and personnel were not blinded and as adverse effects are very subjective lack of blinding could introduce bias. For example, if participants already have pre-conceived ideas about which treatment is best they may choose to report less adverse effects for this treatment, so we judged this domain to be high risk for bias. As Al-Aloul's study compared nebulised antibiotics to intravenous antibiotics, participants and personnel could not be blinded. Therefore, this outcome is likely to be at high risk of bias in his study. We have contacted Conway for further information regarding blinding, so we judged this domain as unclear risk of bias for now.

Participant reported symptom score - In Al-Aloul's study participants could not be blinded as it was comparing nebulised to intravenous antibiotics. Participants may display response bias when completing the symptom score questionnaire. Therefore, we judged this outcome to be at high risk of bias.

Outcome assessors

Nephrotoxicity - For all four studies we judged this outcome to be at low risk of bias whether they were blinded or not. Nephrotoxicity involves laboratory measurements which are objective and so is unlikely to be affected by lack of blinding.

Lung function - Again, for lung function we judged all studies to be at low risk of bias for this domain. This is because as mentioned above lung function is effort independent once >90% maximum effort achieved. One point we did consider was that if outcome assessors are aware of which group a participant is in they could get participants to repeat

lung function if they do not think it is satisfactory or may show more encouragement for one group than the other.

Adverse effects - Latzin's study is open label and lack of blinding of outcome assessors will introduce bias as they may prompt participants to report more adverse effects. Therefore, we judged his study to be of high risk for this domain. Al-Aloul does not report blinding of outcome assessors so we have contacted him for further information and so rate this as unclear risk of bias for now. Conway reports that the person asking about adverse effects is unaware of participants allocation, so we judged his study to be low risk of bias for this domain.

Participant reported symptom scores - As above, Al-Aloul does not mention blinding of outcome assessors so we have contacted him for further information and judged this domain to have an unclear risk of bias.

6.3.3 Incomplete outcome data (attrition bias)

Prayle reported that originally 9 participants were randomised to each group. He reported lung function data for all 18 participants, so this domain is low risk of bias for this outcome. There were 4 withdrawals, with 3 being changed to a different antibiotic and 1 withdrawing consent. It is possible that they were changed to a different antibiotic due to nephrotoxicity, so we will contact Prayle for more information. Nephrotoxicity data was only provided for 7 patients in each group due to the withdrawals. Although the number of withdrawals were equal between groups, we cannot tell exactly why patients were changed antibiotic and therefore it was hard to judge this domain and so we will rank it as unclear risk of bias for now.

Al-Aloul's study had no withdrawals and he fully reported data for each outcome for 20 participants in the nebulised group and 20 in the intravenous group. Therefore, we judged this domain as low risk for the outcomes, other than for eradication of infection. He did, however, report that only 15 sputum samples were collected and only 13 could be used. Therefore, we judged his study to be at high risk of bias for the eradication of infection outcome.

Conway's described 9 withdrawals and provided reasons for each of them. He also analysed the data as intention to treat so we judged this as low risk of bias.

Latzin originally randomised 127 participants, 64 to the ceftazidime and 63 meropenem. He describes the reasons for the withdrawals (5 did not grow PA, 2 required additional oral antibiotics and 2 had their therapy changed during the trial). Latzin reported that 4 withdrew from the ceftazidime group and 5 from the meropenem group. This would leave 60 in the ceftazidime group and 58 in the meropenem group. However, he reported data for 59 participants in each group. We have contacted him for more information on this but have judged this domain as unclear for now.

6.3.4 Selective reporting (reporting bias)

For all 4 of the studies we were unable to retrieve the study protocol, so it was difficult to assess for selective outcome reporting. Prayle reported measuring FEV_1/FVC ratio at the start and end of treatment but did not report this outcome so we judged this study to have high risk of bias for this domain. Conway reports collecting sputum samples twice weekly from each participant but does not present any results of this and so we rank this study to have a high risk of bias for this domain. Latzin reports measuring a clinical score and provides baseline clinical score data and reports there was a significant fall in both groups at day 5 and 12. However he does not provide the mean or standard deviation for

the scores at day 5 or 12 so we rank this study to have high risk of bias for this domain. Al-Aloul reports all outcomes that he states in the methods but as we were unable to access the protocol we judged this domain as unclear risk of bias.

6.3.5 Other potential sources of bias

In both Prayle and Al-Aloul's study we did not identify any other potential sources of bias so we judged this domain to be low risk of bias.

An issue that arose with Latzin's study was that we did not calculate the same mean differences as him, so we contacted him for more information. This made interpreting the tables difficult, so we judged this to have high risk of bias.

There was a unit of analysis issue in Conway's study as participants were able to enrol twice into the study. There was also an issue of a different combinations of antibiotics used in the dual therapy group and there was no individual data for each of the combinations. We therefore judged Conway's study to have a high risk of bias for this domain.

6.4 Effects of interventions

6.4.1 Intravenous meropenem & tobramycin versus intravenous ceftazidime & tobramycin

This comparison of intravenous meropenem and intravenous tobramycin compared to intravenous ceftazidime and intravenous tobramycin included one trial, with 118 participants data included in the analysis.[251] For analysis of this data, I contacted the statistician for advice as we could not calculate the same mean difference as Latzin. The study provided us with pre and post treatment means and standard deviations for each

group and an overall mean difference with confidence intervals. As we were not provided with mean changes and standard deviations for each group overall, we were not able to input this data into RevMan in a normal manner. Therefore, the statistician advised us to use Latzin's reported results and use the generic inverse variance (GIV) method on RevMan. This enabled us to input the mean difference and confidence intervals which allowed us to work out the standard error.

Primary outcomes

1. Nephrotoxicity

a. serum (blood) creatinine levels

Latzin reported the mean (SD) baseline creatinine to be 0.62 (0.20) and the post treatment mean (SD) to be 0.64 (0.25) in the meropenem group. In the ceftazidime group the mean (SD) baseline creatinine was 0.64 (0.23) and the post treatment mean (SD) was 0.65 (0.23). Latzin did not report the mean change for each group or their standard deviations but reported the mean difference as 0.03 (95% CIs of -0.0.5 to 0.10). We used the generic inverse variance method to analyse this data. I used the fixed effect model as only one study is included in this analysis at the moment so there is no heterogeneity. As there is only one study included so far in this comparison, Cochrane recommends that you turn off the button to pool the data as there is no data to pool. Below is a screenshot of the data inputted into RevMan and the corresponding forest plot. (Figure 6.2) As the

confidence intervals cross the vertical line we can conclude there is no statistically significant difference between the effect of the treatments on creatinine levels.

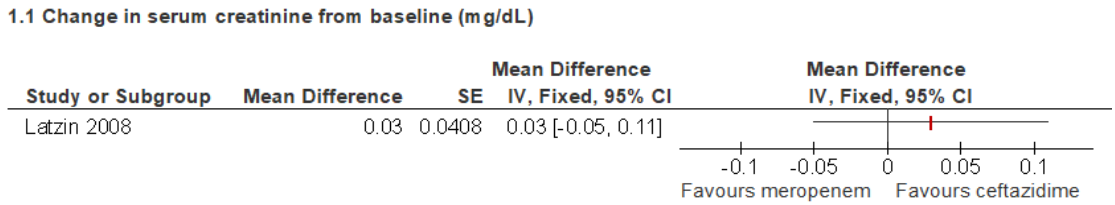

Figure 6.2 - Data analysis and forest plot of serum creatinine in Latzin's study

b. creatinine clearance

Latzin did not measure or report creatinine clearance in this study.

c. urinary excretion of protein

Latzin did not measure or report urinary excretion of protein in this study.

d. urinary excretion of biomarkers of proximal tubular toxicity

Latzin did not measure or report urinary excretion of biomarkers of proximal tubular toxicity in this study.

e. urine output (mL/kg/h)

Latzin did not measure or report urine output in this study.

Secondary outcomes

1. Eradication of respiratory infection

Latzin reported that sputum cultures were taken from participants before and after treatment. Although Latzin reported number of PA types and Pseudomonas coliform units (CFUs) this does not represent eradication of infection. In this study there was 3

different uses for the antibiotics; a group with chronic PA without an exacerbation where the antibiotics were used for suppression, a group with chronic PA experiencing an acute exacerbation and a final group who grew PA for the first time. Therefore, the outcome of eradication of infection was only relevant for group 3, however it was not reported. Latzin did, however, report the mean (SD) baseline Pseudomonas CFUs (10^8/g sputum) to be 2.37 (2.73) and post treatment mean (SD) to be 1.26 (2.06) for the meropenem group. In the ceftazidime group the mean (SD) baseline Pseudomonas CFUs (10^8/g sputum) was 2.07 (2.58) and post treatment mean (SD) to be 0.55 (0.94). Latzin did not report the mean change for each group or their standard deviations but reported the mean difference in Pseudomonas CFUs as 1.09 (95% CIs −0.12 to +2.30) and that it was not statistically significant. We were able to use this data in a generic inverse variance analysis which can be seen below. (Figure 6.3) As the confidence intervals cross the vertical line we can conclude there is no statistically significant difference between the effect of the treatments on Pseudomonas density.

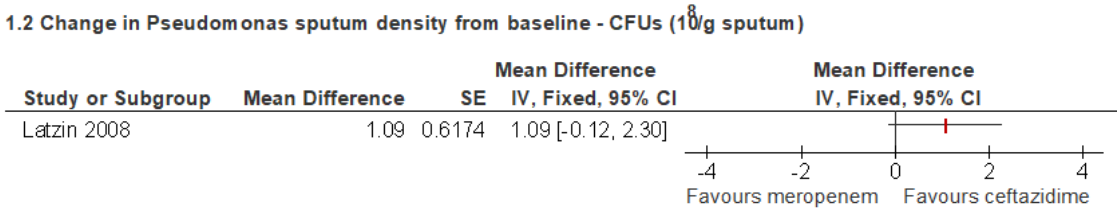

Figure 6.3 - Data analysis and forest plot of eradication of infection in Latzin's study

2. Participant-reported symptom scores

Latzin did not measure or report participant reported symptom scores.

3. Lung function parameters

a. FEV_1

Latzin reported the mean (SD) baseline FEV_1 % predicted to be 52.2 (27.1) and post treatment mean (SD) to be 57.3 (26.8) in the meropenem group. In the ceftazidime group the mean (SD) baseline FEV_1 % predicted was 55.3 (24.8) and the post treatment mean (SD) was 71.2 (21.0). Latzin did not report the mean change for each group or their standard deviations but did report the mean difference between the groups as 7.49 (95% CIs -10.44 to 25.43) and that it was not statistically significant. I inputted this data into a generic inverse variance analysis and this can be seen in the screenshot below along with the corresponding forest plot. (Figure 6.4) When looking at pre and post treatment FEV_1 % predicted it seems that ceftazidime improved it more than the meropenem. However, Latzin reports calculating the mean difference by Meropenem – Ceftazidime and gives a positive mean difference which would indicate that meropenem improves FEV_1 % predicted the most. Even though I have used their results of mean difference into the analysis, I am still not convinced they are correct and so have contacted the author for more information on how they worked it out. As the confidence intervals cross the vertical line we can conclude there is no statistically significant difference between the effect of the treatments on FEV_1.

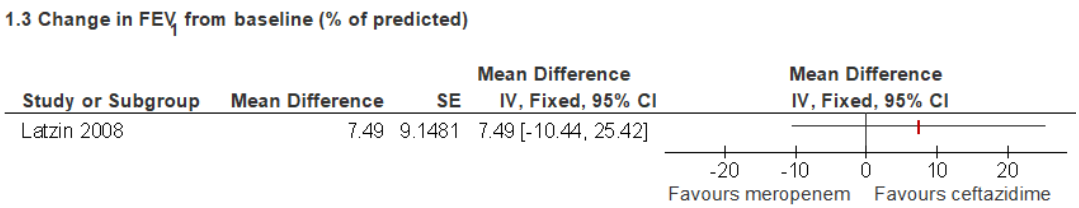

Figure 6.4 - Data analysis and forest plot of FEV_1 in Latzin's study

b. FVC

Latzin reported the mean (SD) baseline FVC % predicted to be 68.5 (24.9) and post treatment mean (SD) to be 72.4 (22.9) in the meropenem group. In the ceftazidime group the mean (SD) baseline FVC % predicted was 66.4 (20.6) and the post treatment mean

(SD) to be 71.2 (21.0). Latzin did not report the mean change for each group or their standard deviations but did report the mean difference as 1.22 (95% CIs -6.54 to 8.99) and that it was not statistically significant. Again, when looking at the results at a first glance it seems ceftazidime is better at increasing FVC % predicted and so I have contacted the author to clarify how he calculated the mean difference. Below is a screenshot of the data inputted and the corresponding forest plot. (Figure 6.5) As the confidence intervals cross the vertical line we can conclude there is no statistically significant difference between the effect of the treatments on FVC.

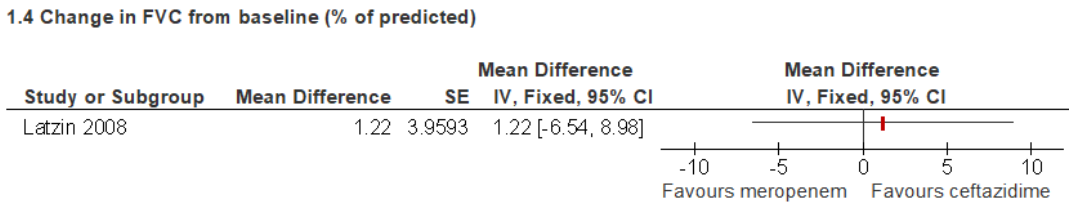

Figure 6.5 - Data analysis and forest plot of FVC in Latzin's study

c. FEV_1/FVC ratio

Latzin did not measure or report FEV_1/FVC ratio in this study.

4. Participant-reported QoL scores

Latzin did not measure or report participant-reported QoL scores in this study.

5. Adverse effects of treatment

Latzin reported 11 side effects in the meropenem group (nausea in 2 patients; headache in 2 patients; diarrhoea in 3 patients; allergic reactions in 2 patients; nose bleeding in 1 patient and fatigue in 1 patient). Latzin reported 10 patients of the CEF group (nausea in 3 patients, headache in 1 patient; diarrhoea in 3 patients; allergic reaction in 1 patient; acute pancreatitis in 1 patient and recurrent problems with the IV-line in 1 patient). Below

is the data inputted and the corresponding forest plot. (Figure 6.6) As the box is to the right of the graph that suggests that ceftazidime is favourable (i.e. gives less side effects). However, as the 'whiskers' which represent the confidence interval are cross the vertical line (line of no effect) this suggest that the result is not statistically significant.

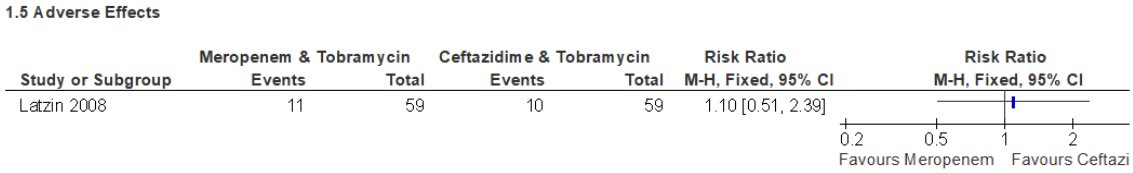

Figure 6.6 - Data analysis and forest plot of adverse effects in Latzin's study

6.4.2 Morning versus evening antibiotic dosing

This comparison of morning versus evening dose of tobramycin included one trial, with 18 participants data included in the analysis.[252]

Primary outcomes

1. Nephrotoxicity

a. serum (blood) creatinine levels

Prayle measured serum creatinine levels at the start of the study (baseline) and reported these. However, he did not measure or report serum creatinine levels at the end of the study, so we cannot calculate the mean change.

b. creatinine clearance

Prayle did not measure or report creatinine clearance in this study.

c. urinary excretion of protein

Prayle did not measure or report urinary excretion of protein in this study.

d. urinary excretion of biomarkers of proximal tubular toxicity

Prayle provided mean change and standard deviation of biomarkers for 7 participants in the morning group and 7 participants in the evening group.

Prayle reported the mean (CI) change in urinary excretion of KIM1/Cr in the morning tobramycin group to be 0.01 (-0.43 to 0.46) and 0.74 (0.26 to 1.22) for the evening group. Prayle reported a mean difference of 0.73 (95% CIs 0.14 to 1.32) with p=0.018. Below is a screenshot of the data inputted for analysis using the fixed effects model and the corresponding forest plot. (Figure 6.7) We used the fixed effects model as only one trial is included in the analysis and so there is no heterogeneity. As the p value is <0.05 we can conclude that the time of day of tobramycin dosing has a statistically significant effect on KIM1 level.

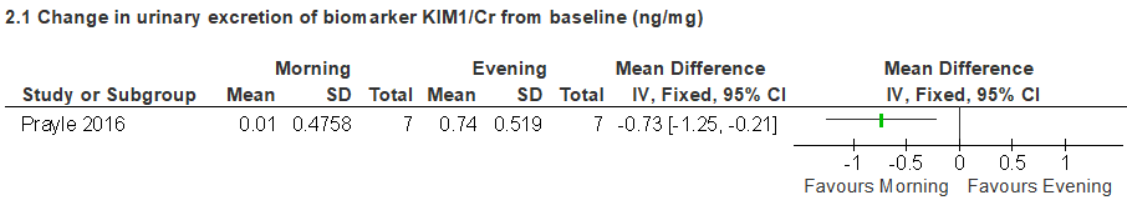

Figure 6.7 - Data analysis and forest plot of KIM1 in Prayle's study

Prayle reported the mean (CI) change in urinary excretion of CysC/Cr in the morning tobramycin group to be -16.6 (95% CIs -51.0 to 17.8) and 29.6 (- 45.2 to 104.3) for the evening group. Prayle reports a mean difference of 46.2 (95% CIs -30.7 to 123.0) with p=0.20. Below is a screenshot of this data and the forest plot. (Figure 6.8) As the p value is above 0.05 the effect of time of dosing does not have a statistically significant effect on the CysC/Cr levels.

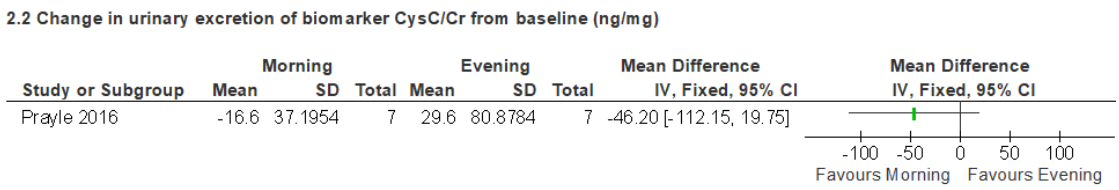

Figure 6.8 - Data analysis and forest plot of CysC in Prayle's study

Prayle reported the mean (CI) change in urinary excretion of NGAL/Cr in the morning tobramycin group to be 10.1 (-144.7 to 164.9) and 12.9 (-76.9 to 102.8) for the evening group. Prayle reports a mean difference of -0.01 (95% CIs -2.47 to 2.43) with p=0.99 which is therefore not statistically significant. From entering the data into RevMan we get a mean difference of -2.80 (95% CIs -146.19 to 140.59) with a p value= 0.9701. The difference in calculations is because Prayle performed a log transform to the data before performing the t-test. Either way the treatment effect is not statistically significant. Below is a screenshot of the data and forest plot on RevMan. (Figure 6.9)

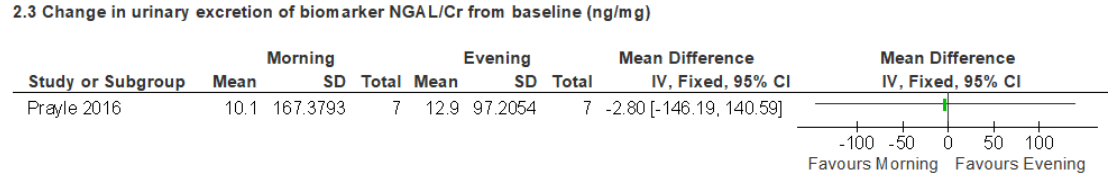

Figure 6.9 - Data analysis and forest plot of NGAL in Prayle's study

Prayle reported the mean (CI) change in urinary excretion of IL-18/Cr in the morning tobramycin group to be -5.72 (-34.3 to 22.9) and 139.3 (-221.1 to 499.7) for the evening group. Prayle reports a mean difference of 0.2 (95% CIs -0.6 to 1.0) with p=0.59. From entering the data into RevMan using a fixed effects model we get a mean difference of -145.02 (95% CIs -434.61 to 144.57), p=0.3457. Again, the difference in calculations is because Prayle performed a log transform to the data before performing the t-test. Either way the p value is greater than 0.05 meaning it is not statistically significant. Below is the screenshot of data inputted into RevMan with the corresponding forest plot. (Figure 6.10)

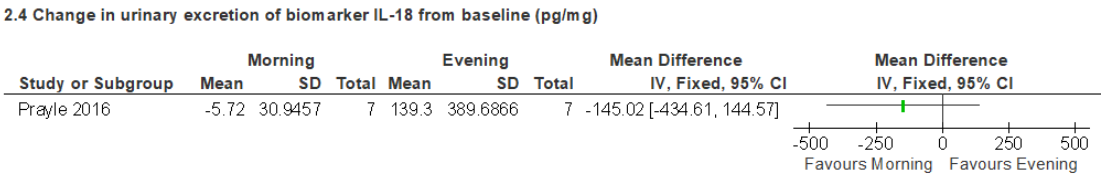

Figure 6.10 - Data analysis and forest plot of IL-18 in Prayle's study

Prayle reported the mean (CI) change in urinary excretion of NAG/Cr in the morning tobramycin group to be 0.037 (0.002 to 0.07) and 0.052 (-0.001 to 1.034) for the evening group. Prayle reports a mean difference of 0.79 (95% CIs -0.94 to 2.52) with p=0.33. From entering the data into RevMan using a fixed effects model we get a mean difference of -0.01 (95% CIs -0.07 to 0.04), p=0.5739. The difference in calculations is because Prayle performed a log transform to the data before performing the t-test. Either way the p value is greater than 0.05 meaning it is not statistically significant. Below is a screenshot of the data inputted and the forest plot. (Figure 6.11)

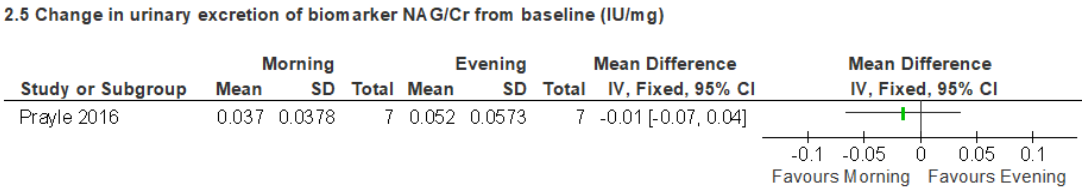

Figure 6.11 - Data analysis and forest plot of NAG in Prayle's study

e. urine output (mL/kg/h)

Prayle did not measure or report urine output in this study.

Secondary outcomes

1. Eradication of respiratory infection

Prayle did not measure or report eradication of respiratory infection in this study.

2. Participant-reported symptom scores

Prayle did not measure or report participant reported symptom scores in this study.

3. Lung function parameters

a. FEV_1

As the FEV_1 (L) was provided as the median change and interquartile range it may suggest the data is skewed. Therefore, we chose to report this narratively as it may not be appropriate to use in an analysis. FEV_1 data was provided for 9 participants in the morning group and 9 participants in the evening group. Prayle reports the median (IQR) change (end - initial) in the FEV_1 (L) of the morning group as 0.19 (0.07 to 2.20) compared to 0.23 (0.19 to 0.28) in the evening group.

b. FVC

FVC data was provided for 9 participants in the morning group and 9 participants in the evening group. The FVC (L) is also presented as the median change from baseline and an interquartile range and was 0.32 (-0.01 to 0.38) for the morning group and 0.18 (-0.01 to 0.29) for the evening group.

c. FEV_1/FVC ratio

Prayle stated that he measured FEV_1/FVC however did not report this.

4. Participant-reported QoL scores

Prayle did not measure or report Participant-reported QoL scores in this study.

5. Adverse effects of treatment

Prayle did not measure or report adverse effects of treatment as far as we are aware in this study.

6.4.3 Nebulised versus intravenous antibiotics

This comparison of nebulised versus intravenous antibiotics included one trial, with 10 participants in each group. However, as it was a cross-over trial, all participants received both treatment arms therefore there was 20 participants' data for each arm.[253]

Primary outcomes

1. Nephrotoxicity

a. serum (blood) creatinine levels

Al-Aloul reported the mean and standard deviation of baseline serum creatinine levels and also percentage change in serum creatinine from baseline. In the nebulised group there was a mean change (SD) in serum creatinine of 4.3% (9.4) and 3.6% (10.4) in the intravenous group. He reported a mean difference between treatments of 0.7 (95% CIs -6.8 to 5.4), p=0.83. Below is a screenshot of the data inputted for analysis using the fixed effects model and the corresponding forest plot. (Figure 6.12) We used the fixed effects model as only one trial is included in the analysis and so there is no heterogeneity. As the p value is >0.05 we can conclude that there is no statistically significant difference between the use of nebulised or intravenous antibiotics on the serum creatinine levels.

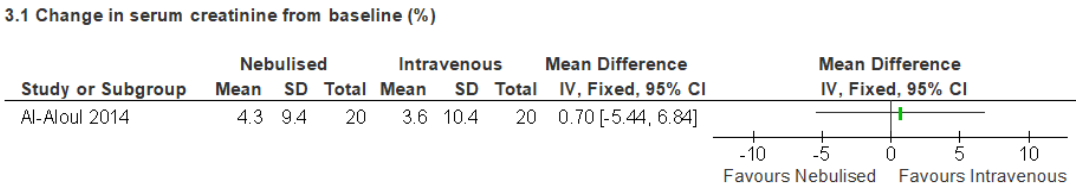

Figure 6.12 - Data analysis and forest plot of serum creatinine in Al-Aloul's study

b. creatinine clearance

Al-Aloul reported the mean and standard deviation of baseline creatinine clearance and percentage change in creatinine clearance from baseline. In the nebulised group there was

a mean change (SD) in creatinine clearance of 23.9% (48.4) and 26.1% (35.0) in the intravenous group. He reported a mean difference between treatments of 2.2 (95% CIs -24.0 to 28.4), p=0.87. Below is a screenshot of the data inputted for analysis and the corresponding forest plot. (Figure 6.13) As the p value is >0.05 we can conclude that there is no statistically significant difference between the use of nebulised or intravenous antibiotics on the creatine clearance.

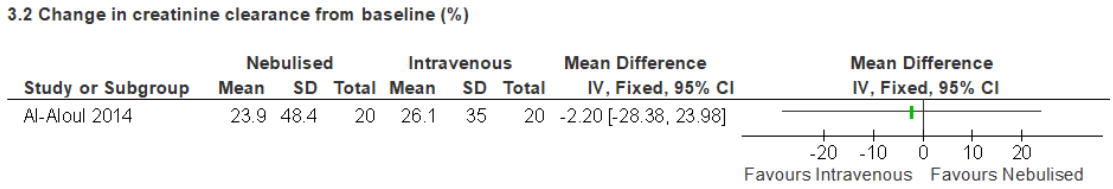

Figure 6.13 - Data analysis and forest plot of creatinine clearance in Al-Aloul's study

c. urinary excretion of protein

Al-Aloul reported the mean (SD) change in urinary excretion of protein in the nebulised group to be 0.003 (0.16) and 0.59 (0.63) in the intravenous group. Al-Aloul reported a mean difference of 0.58 (95% CIs 0.30 to 0.87), p=0.0005. Below is a screenshot showing the data inputted in RevMan and the corresponding forest plot. (Figure 6.14) As the p value is <0.05 we can conclude that the use of intravenous compared to nebulised antibiotics has a statistically significant effect on urinary excretion of protein.

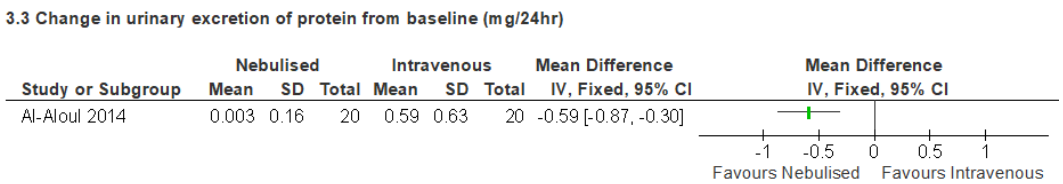

Figure 6.14 - Data analysis and forest plot of urinary protein excretion in Al-Aloul's study

d. urinary excretion of biomarkers of proximal tubular toxicity

Al-Aloul reported the mean (SD) change in urinary excretion of NAG in the nebulised group to be 0.02 (0.51) and 0.74 (0.44) in the intravenous group. Al-Aloul reported a

mean difference of 0.72 (95% CIs 0.37 to 1.07), p=0.0004 between the two groups. Below is a screenshot of this data and the forest plot. (Figure 6.15) As the p value is <0.05 we can conclude that the use of nebulised compared to intravenous antibiotics has a statistically significant effect on urinary excretion of NAG.

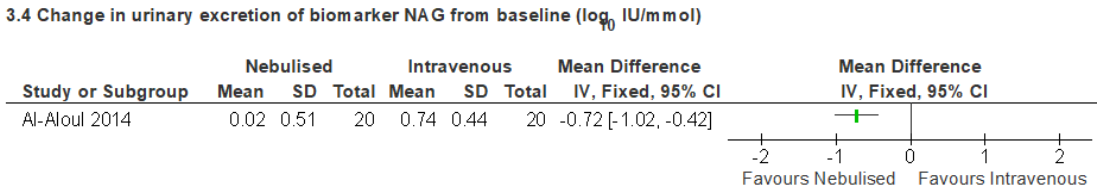

Figure 6.15 - Data analysis and forest plot of NAG in Al-Aloul's study

Al-Aloul reported the mean (SD) change in urinary excretion of AAP in the nebulised group to be -0.37 (0.69) and 0.82 (0.62) in the intravenous group. Al-Aloul reported a mean difference of 1.19 (95% CIs 0.70 to 1.68), p=0.0001 between the two groups. Below is a screenshot of this data and the forest plot. (Figure 6.16) As the p value is <0.05 we can conclude that the use of nebulised compared to intravenous antibiotics has a statistically significant effect on urinary excretion of AAP.

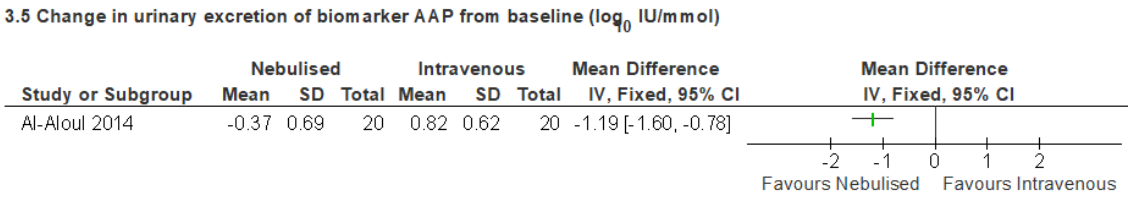

Figure 6.16 - Data analysis and forest plot of AAP in Al-Aloul's study

Al-Aloul reported the mean (SD) change in urinary excretion of β2 Microglobulin in the nebulised group to be 0.20 (0.41) and 0.64 (0.50) in the intravenous group. Al-Aloul reported a mean difference of 0.44 (95% CIs 0.16 to 0.72), p=0.0046 between the two groups. Below is a screenshot of this data and the forest plot. (Figure 6.17) As the p value is <0.05 we can conclude that the use of nebulised compared to intravenous antibiotics has a statistically significant effect on urinary excretion of β2 Microglobulin.

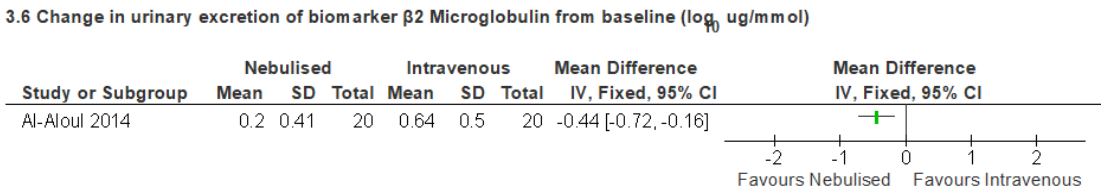

Figure 6.17 - Data analysis and forest plot of β2 Microglobulin in Al-Aloul's study

Al-Aloul reported the mean (SD) change in urinary excretion of Collagen IV in the nebulised group to be 0.16 (0.33) and 0.31 (0.41) in the intravenous group. Al-Aloul reported a mean difference of 0.15 (95% CIs 0.13 to 0.43), p=0.29 between the two groups. Below is a screenshot of this data and the forest plot. (Figure 6.18) As the p value is above 0.05 we can conclude that the use of nebulised compared to intravenous antibiotics does not have a statistically significant effect on urinary excretion of Collagen IV.

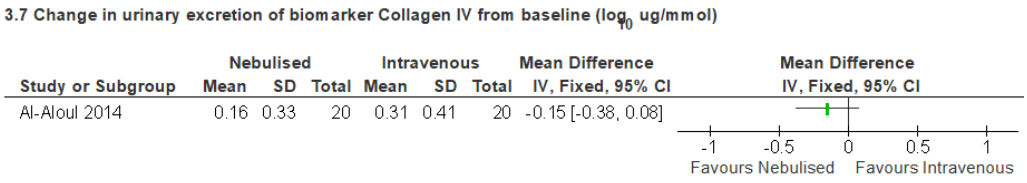

Figure 6.18 - Data analysis and forest plot of Collagen IV in Al-Aloul's study

e. urine output (mL/kg/h)

Al-Aloul did not measure or report urine output in this study.

Secondary outcomes

1. Eradication of respiratory infection

Al-Aloul did not report eradication of infection as such but reported Pseudomonas CFUs. Only 13 sputum samples could be analysed but it does not state which groups they were from and so we have contacted Al-Aloul for more information regarding this. Al-Aloul reported the mean (SD) baseline PA density to be 7.31 $\log_{10}$ cfu/mL (3.11) and post

treatment mean (SD) to be -2.41 log_{10} cfu/mL (0.97) for the nebulised group. In the intravenous group the mean (SD) baseline PA density was 6.92 log_{10} cfu/mL (1.93) and post treatment mean (SD) to be -1.56 log_{10} cfu/mL (1.31). Al-Aloul did not report the mean change for each group or their standard deviations but reported the mean difference in PA density as 0.85 log_{10} cfu/mL (95% CIs 0.03 to 1.67), p=0.05. As we did not have the mean change for each group, I had to use the GIV to input this data into my analysis. As for all other outcomes they have calculated the mean difference the opposite way around to us, I had to change the labels on this graph around so that it would be the right direction for entering their numbers straight into. Below is a screenshot of my data inputted into RevMan and corresponding forest plot. (Figure 6.19) As the confidence interval touches the line and their p value is not less than 0.05 then there is no statistically significant difference between treatments on PA density.

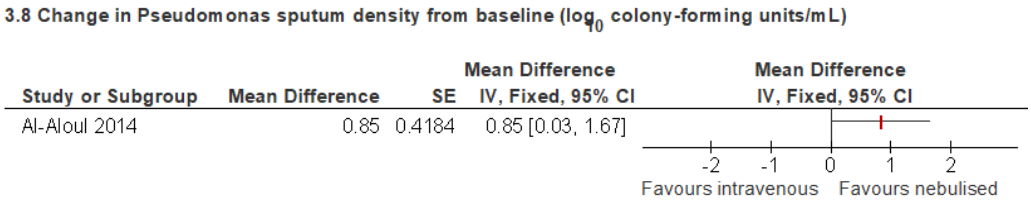

Figure 6.19 - Data analysis and forest plot of eradication of infection in Al-Aloul's study

2. Participant-reported symptom scores

Al-Aloul reported the mean and standard deviation participant reported symptom scores that were obtained at the end of the treatment. The mean (SD) participants reported symptom score for the nebulised group was 8.1 (1.3) and 8.5 (1.1) for the intravenous group. Al-Aloul reported the mean difference between treatments as 0.4 (95% CIs -0.34 to 1.14), p=0.29. Below is a screenshot of my data inputted into RevMan and corresponding forest plot. (Figure 6.20) There is no significant difference between

nebulised antibiotics when compared to intravenous antibiotics on participant reported symptom score.

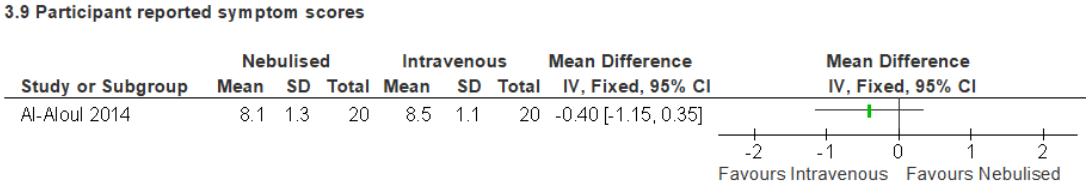

Figure 6.20 - Data analysis and forest plot of participant reported symptom scores in Al-Aloul's study

3. Lung function parameters

a. FEV_1

Al-Aloul reported the mean (SD) change in FEV_1 (% of predicted) across the course of the treatment to be 19.9 (11.3) for the nebulised group and 16.4 (8.5) for the intravenous group. The mean difference between the two groups was reported as 3.6 (95% CIs 9.7 to 2.6), p=0.26. As the p value is above 0.05 we can conclude there is no statistically significant difference between the effect of the two treatments on FEV_1 (% predicted). See screenshot below for my data analysis and forest plot. (Figure 6.21)

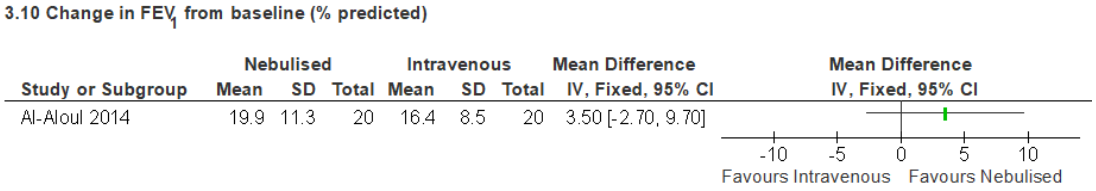

Figure 6.21 - Data analysis and forest plot of FEV_1 in Al-Aloul's study

b. FVC

Al-Aloul reported the mean (SD) change in FVC (% of predicted) across the course of the treatment to be 18.6 (14.6) for the nebulised group and 13.1 (8.6) for the intravenous group. The mean difference between the two groups was reported as 5.5 (95% CIs -2.9 to 1.9), p=0.16. As the p value is above 0.05 we can conclude there is no statistically

significant difference between the effect of the two treatments on FVC (% of predicted). See below for my data analysis and forest plot. (Figure 6.22)

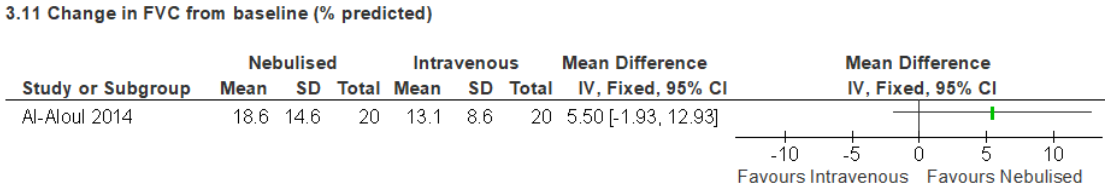

Figure 6.22 - Data analysis and forest plot of FVC in Al-Aloul's study

c. FEV_1/FVC ratio

Al-Aloul did not measure or report FEV_1/FVC ratio in this study.

4. Participant-reported QoL scores

Al-Aloul did not measure or report Participant-reported QoL scores in this study.

5. Adverse effects of treatment

Al-Aloul reported 11 adverse effects in the nebulised group and 9 adverse effects in the intravenous group. As the confidence interval crosses the vertical line (line of no effect) we can conclude that there is no statistically significant difference between treatments on adverse effects. Below is the data inputted into RevMan and the corresponding forest plot. (Figure 6.23)

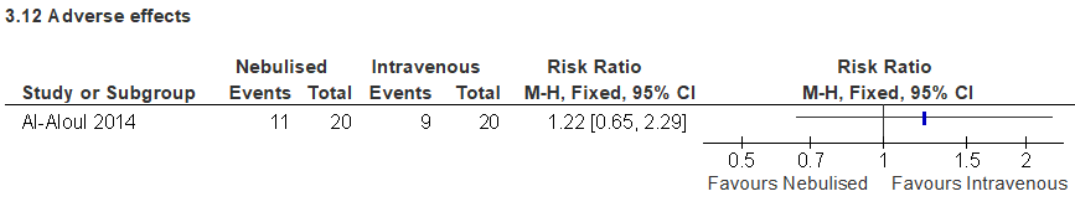

Figure 6.23 - Data analysis and forest plot of adverse effects in Al-Aloul's study

6.4.4 Single IV antibiotic vs combination IV antibiotics

This comparison of a single intravenous antibiotic compared to a combination of intravenous antibiotics included one trial, with 71 participants data included in the analysis.[254] Conway only reported the mean and standard deviation of the outcomes at different time points. He did not report mean changes for any of the outcomes or mean differences between the groups. I contacted the CRG's statistician, AK, for further advice and she suggested I would not be able to include these results in analysis and so should report them narratively. We have contacted Conway to ask if he will provide individual patient data but as this study was carried out in 1997, it is unlikely he will respond.

Primary outcomes

1. Nephrotoxicity

a. serum (blood) creatinine levels

to be completed

For the single IV antibiotic group Conway reported the mean (SD) serum creatinine on day 1 was 70 (16) and on day 12 was 72 (13). Although Conway did not report the mean change, he states that the difference is non-significant. For the combination IV antibiotic group, the mean (SD) serum creatinine on day 1 was 73 (10) and on day 12 was 71 (14). Again, Conway reports that the difference between creatinine between the time points is not statistically significant.

b. creatinine clearance

For the single IV antibiotic group Conway reported the mean (SD) creatinine clearance on day 1 was 109 (54) and on day 12 was 94 (29). Although Conway did not report the

mean change, he states that the difference is non-significant. For the combination IV antibiotic group, the mean (SD) serum creatinine on day 1 was 109 (42) and on day 12 was 91 (34). Conway reports that the difference in creatinine clearance between the time points in the combination IV group is less than 0.01 and so is statistically significant.

c. urinary excretion of protein

Conway did not measure or report urinary excretion of protein in this study.

d. urinary excretion of biomarkers of proximal tubular toxicity

Conway did not measure or report urinary excretion of biomarkers of proximal tubular toxicity in this study.

e. urine output (mL/kg/h)

Conway did not measure or report urine output in this study.

Secondary outcomes

1. Eradication of respiratory infection

Sputum cultures were collected twice weekly for microscopy, culture, and sensitivity however they were not reported.

2. Participant-reported symptom scores

Conway did not measure, or report participant reported symptom scores in this study.

3. Lung function parameters

a. FEV_1

Conway reported the mean (SD) FEV_1 (L) for the single IV group as 1.52 (0.68) for day 1, 1.58 (0.75) for day 5 and 1.66 (0.82) for day 12. Conway reported the mean (SD) FEV_1 (L) for the combination IV group as 1.62 (0.78) for day 1, 1.87 (0.93) for day 5 and 1.92 (0.89) for day 12. Conway reports that there was no statistically significant difference in the FEV_1 between the two groups on day 1, day 5 or day 12.

b. FVC

Conway reported the mean (SD) FVC (L) for the single IV group as 2.44 (1.04) for day 1, 2.45 (1.04) for day 5 and 2.56 (1.21) for day 12. He reported the mean (SD) FVC (L) for the combination IV group as 2.34 (1.0) for day 1, 2.74 (1.05) for day 5 and 2.93 (1.12) for day 12. Conway reports that there was no statistically significant difference in the FVC between the two groups on day 1, day 5 or day 12.

c. FEV_1/FVC ratio

Conway did not measure or report urinary FEV_1/FVC ratio in this study.

4. Participant-reported QoL scores

Conway did not measure or report participant-reported QoL scores in this study.

5. Adverse effects of treatment

Conway reported that there were 37 reports of adverse effects in 33 participants for the single IV group compared to 37 adverse effects in 26 participants in the combination IV group.

6.5 Discussion

6.5.1 Summary of main findings

Our review so far includes 4 studies looking at different strategies that may prevent kidney injury in CF patients that is caused by intravenous antibiotics. As none of these 4 studies look at the same comparison we could not include them in a meta-analysis. I will now summarize the main findings of my review so far for each comparison.

IV Meropenem & IV Tobramycin versus IV Ceftazidime & IV Tobramycin

Latzin's study which randomised 118 participants failed to demonstrate a superior antibiotic combination for reducing nephrotoxicity. He compared intravenous meropenem plus tobramycin to intravenous ceftazidime and tobramycin but there was no significant difference in serum creatinine levels between the two groups. There was also no significant different in efficacy, lung function or adverse effects between the two groups.

Morning versus evening antibiotic dosing

Prayle's study which randomised 18 participants demonstrated a statistically significant rise in urinary excretion of KIM1 when tobramycin is administered in the evening compared to administration in the morning. Administering antibiotics in the evening may cause an increase in the risk of nephrotoxicity. Glomerular filtration is highest in the daytime compared to the night time which means it has a circadian rhythm.[255] At night time, GFR is reduced meaning that the kidneys are exposed to tobramycin for longer which may cause an increase in proximal tubule damage. However, there was not a statistically significant difference between the two groups in any other urinary biomarkers of proximal tubular toxicity. Prayle was only able to collect urine samples from 14 participants in total and so a limitation of this study was the small sample size. There was a greater median increase in FEV_1 in the evening group but a greater median increase in

FVC in the morning group, however neither of these results were statistically significant. Although Prayle looked at lung function, he did not address effect on PA density, participant reported symptom score or adverse effects. However, as the same dose and antibiotic was used it is unlikely that just by changing the time of the day it is administered that there would be an effect on these outcomes.

Nebulised versus intravenous antibiotics

Al-Aloul's cross-over study which randomised 10 participants to each group originally failed to demonstrate any difference in serum creatinine levels or creatinine levels, when comparing nebulised tobramycin to intravenous tobramycin in an acute exacerbation. Al-Aloul demonstrated a significant increase in urinary excretion of protein in the intravenous tobramycin group compared to the nebulised tobramycin group. He also concluded that there was a significant increase in urinary excretion of three biomarkers of proximal tubular toxicity (NAG, AAP, β2 Microglobulin) in the intravenous group compared to the nebulised group. This suggests that a strategy to reduce nephrotoxicity is to use of nebulised tobramycin in an acute exacerbation rather than intravenous antibiotics. Interestingly, nebulised antibiotics increased lung function and reduced sputum PA density more than the intravenous antibiotics although there was not a significant difference between the two groups. Interestingly though, there was a higher mean participant reported symptom score (participants felt more back to normal) for the intravenous group compared to the nebulised group. There were less adverse effects seen in the intravenous group than the nebulised group, but this was not statistically significant.

Single intravenous antibiotic versus combination intravenous antibiotics

Conway's study which randomised 71 participants reported a statistically significant decrease in creatinine clearance in the combination of intravenous antibiotics group over the course of the treatment. He did not, however, directly compare the creatinine clearance in the single antibiotic group but states there was no significant difference in the change over the course of the treatment. There was no significant difference in serum creatinine levels over the course of the treatment in either the single or combination group. There was no statistical difference between the FEV_1 or FVC between the two groups on day 1, day 5 or day 12.

6.5.2 Overall completeness and applicability of the evidence

At this present time, the evidence for this review is not complete as there may be other studies that can be included in the comparisons, however, we are still waiting for additional information from the authors. Therefore, I will assess the completeness and applicability of the studies we have included so far.

As far as we can tell so far, there is lack of evidence on dosing regimens and lack of studies regarding the use of fluids. There is also only one ongoing study addressing the use of statins. Therefore, the evidence so far does not allow us to address all the strategies that may exist to minimise nephrotoxicity caused by intravenous antibiotics.

Both Al-Aloul and Conway only included adult participants in their study and so we cannot generalise the results of this study to children, as it may only be applicable to adults. Prayle's study only included children, and again we do not know whether his results are applicable to adults. Latzin however included both adults and children in his study and so they are more applicable.

All studies in this review have a small sample size, in particular Prayle's study who only included 18 participants. A small sample size may mean we cannot be certain about the true treatment effects and the results may not be representative.

Although the four studies in this study address nephrotoxicity, they only look at it during or at the end of treatment. There are no studies that address nephrotoxicity as a long-term outcome and therefore the results are only applicable to acute exacerbations.

Although Al-Aloul's trial allowed us to compare the use of nebulised versus intravenous antibiotics in acute exacerbation, it is important to remember that he only looked at tobramycin. Just because nebulised tobramycin seems to reduce risk of nephrotoxicity when compared to intravenous use it does not mean that all nebulised antibiotics would have this same effect.

Results from some of the trials included in this review may be less applicable now as they were conducted over 10 years, particularly Conway's trial that was conducted over 20 years ago.

6.5.3 Quality of the evidence

As we have not yet been able to assess all the evidence as we are awaiting further information from authors, it would be inappropriate at this stage to comment on the overall quality of the evidence for each comparison.

When we have all the evidence for each comparison we will be able to create a summary of findings table for the individual comparisons. We will create the summary of findings table using the GradePro software. For each outcome, I will report the illustrative risk with the intervention and then the risk with the comparison (control or placebo). I will then give the relative effect (RR or MD) with the 95% confidence intervals. I will state

how many studies and participants contributed to this. Where an outcome is not reported, I would specify this in the table with the description, “data not reported”.

I will assess the overall quality of the evidence for each outcome using the Grading of Recommendations Assessment, Development and Evaluation (GRADE).[256] The domains assessed with this tool include risk of bias, inconsistency, indirectness, imprecision, risk of publication bias and other factors such as dose response and large effect. For each outcome we will rank it as high, moderate, low, or very low along with comments explaining our choice. For a problem in any of the five areas assessed, you must class it as ‘serious’ or ‘very serious’. If you class it as ‘serious’, you must downgrade the quality rating by one level, whereas for ‘very serious’, you must downgrade it by two levels.

We will assess risk of bias individually for each outcome by mainly looking at our risk of bias tool. We will assess evidence risk of bias by looking at sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and use of ITT analysis.

We will assess for inconsistency by looking at how much heterogeneity there is between the studies. Clinical heterogeneity is where there are differences between the participants, interventions, or outcomes. Whereas methodological heterogeneity refers to inconsistencies between the study designs. Both types of heterogeneity can then create statistical heterogeneity. We may suspect heterogeneity if different studies report opposite effects of an intervention or different size of effects. We can also use statistical tests to identify heterogeneity.

There are two types of indirectness that we will assess for; indirect comparison and indirect population or intervention or outcome. An indirect comparison is where two interventions you are interested in are both compared to another intervention and so rather

than being able to make a head to head comparison you have to make an indirect comparison. Indirect population may arise if the study only addresses adults, but you were also interested in children. Indirect intervention may arise if they only address a certain dose of a drug and indirect outcomes may occur if they only look at an endpoint rather than a change in an outcome over time.

Imprecision may arise when the sample size is small or there are few dichotomous outcome events or if there are wide confidence intervals around the effect estimate. This can mean that you are less certain of what the exact effect of a treatment is.

We will assess for publication bias by looking at whether there is a difference in effect of treatment in unpublished compared to published work. Publication bias arises when researchers only publish work that shows a significant effect of treatment. Funnel plots can also be used to test for publication bias as I mentioned earlier in this thesis.

6.5.4 Potential biases in the review process

Strengths

Both myself and WC screened the studies, extracted the data, and assessed the risk of bias in the included the studies. By using two authors for screening it reduces the risk of excluding relevant studies.[257]

Myself and WC individually extracted data from the included studies by hand using a data extraction form provided to us by our CRG. I then checked both forms for any differences or any mistakes and then was able to enter this data onto the online data extraction form on Covidence. By checking the data extraction forms it reduces the chance of errors.

Weaknesses

Originally when screening the studies, I was overly inclusive in my approach. For example, if a study compared two types of nebulised treatments but a few participants also required intravenous antibiotics during the course, I included it. Being over inclusive led to a high number of conflicts on Covidence between myself and WC. However, when we met up to discuss the discrepancies WC reminded me that in the protocol we stated that all participants in at least one of the groups must be receiving intravenous antibiotics for it to be included. Even though some patients may be on IV antibiotics and nebulised antibiotics not all patients in one group were on this. Therefore, we would be unable to compare the two groups as they would have had different number of participants on IVs in the two groups and some patients may have had more courses of IV antibiotics throughout the trial than others. Therefore, we decided to exclude any studies looking at long term use of nebulised or oral antibiotics for prophylaxis as intravenous antibiotics may or may not be used in these studies and we cannot control for this.

In the protocol we listed ‘eradication of infection’ as of our outcomes and stated that this would be a dichotomous outcome i.e. was the infection eradicated, yes or no. However, when looking at studies to be included, many of them were simply looking at antibiotic strategies for an acute exacerbation rather than eradication regimes. Therefore, the outcome eradication of infection was technically incorrect. Studies looking at eradication regimens may have looked at eradication of infection as a dichotomous outcome. However, the studies we have included so far look at PA sputum density instead which is a continuous outcome. It may be more appropriate to use the broader term ‘microbiology’ as an outcome instead of ‘eradication of infection’ as this would encompass both eradication and sputum organism density.

6.6 Summary

In this chapter I have discussed my methods, results, and findings of my Cochrane review, 'Strategies to prevent kidney injury from antibiotics in people with cystic fibrosis'. The results of this review so far have suggested that morning dosing of tobramycin rather than evening dosing may help to reduce kidney injury from intravenous antibiotics. It is also possible that using nebulised tobramycin in an acute exacerbation may reduce the risk of kidney injury rather than intravenous tobramycin. When I obtain more information from other study authors it may be possible that identify other effective strategies that can reduce the risk of kidney injury.

In the next chapter, I will discuss the findings of my survey on antibiotic choice for CF patients in the UK.

CHAPTER 7: UK SURVEY TO ASSESS WHICH IV ANTIBIOTICS ARE USED TO TREAT CF RESPIRATORY EXACERBATIONS

7.1 Background to the questionnaire

7.1.1 Introduction

In addition to undertaking our Cochrane Systematic Review, I thought it would be helpful to find out what antibiotics are being used for respiratory exacerbations. I thought this may depend on the organisms that were growing and whether they had ever grown *Pseudomonas aeruginosa* (PA) before. This may vary between hospital to hospital but may also vary between doctor to doctor.

7.1.2 Overview of existing guidelines

First, I looked online for any information in the guidelines regarding what antibiotics should be used. For unidentified organisms, the National Institute for Health and Care Excellence (NICE) guidelines in particular are very vague regarding antibiotic use in respiratory infection. They suggest using broad spectrum oral antibiotics or, if the infection is more severe, intravenous antibiotics, however, they do not specifically state which ones to use. The UK CF Trust lists specific intravenous antibiotics that should be used if an organism is identified and for most organisms a single agent can be used. It also lists specific criteria for when intravenous antibiotics are indicated e.g. fever, increased productive cough, fall in respiratory function or new signs on chest

auscultation. However, again there is no recommendations as to what intravenous antibiotic should be used in an acute exacerbation if the organism is unidentified. Therefore, we designed a questionnaire that would determine the most commonly used antibiotics in practice in this scenario.

There is clear guidance from the CF Trust that in a clinically well patient with a new growth of PA, nebulised colistin and oral ciprofloxacin should be used. They suggest using intravenous anti-pseudomonal antibiotics before commencing eradication therapy if the patient is unwell. NICE in this case suggests using intravenous antibiotics alongside inhaled antibiotics. As there is clear guidance provided by the CF trust on eradication regimes for a new growth of PA that from our knowledge most centres follow, we felt there was no need to investigate this further.

As mentioned above NICE suggests using inhaled antibiotics alongside intravenous antibiotics, however the UK CF trust recognises that most centres stop inhaled antibiotics in these instances. This is because it is thought that although there is little systemic absorption of inhaled aminoglycosides, it may contribute to renal toxicity, but data is conflicting on this issue. There have been cases of acute kidney injury in patients on inhaled aminoglycosides but other studies found no increase in risk of renal toxicity when on them.[187,258] It may have been interesting to see whether centres use inhaled and intravenous aminoglycosides concomitantly for new growths of PA but we will not consider this in our questionnaire as our review is not looking at eradication regimes.

For patients who are chronically infected with PA and that are unwell with an acute exacerbation NICE recommends that they should be treated with an oral antibiotic or two intravenous antibiotics of different classes. The CF Trust recommends for acute exacerbations with PA, a combination of 2 antibiotics with different mechanisms should

be used. A β-lactam (e.g. ceftazidime) or an anti-pseudomonal penicillin (e.g. Piperacillin-Tazobactam) should be used along with an aminoglycoside (e.g. tobramycin). The anti-pseudomonal colistin can be used but this is only usually used in more resistant strains of PA or if tobramycin is contraindicated.[259] The UK CF Trust does not include gentamicin in its list of suitable PA treatments because as we know it is more harmful to the kidneys than tobramycin.[260]

We can take away clear guidance on which specific antibiotics to use for new growth of PA. However, we have no guidance on what to use for unidentified organisms in a respiratory exacerbation. Also, we have a long list of antibiotics that can be used for PA infections (not the first growth) with not two specific ones being identified as best.

We know from anecdotal reports that some centres are using renal toxic antibiotics (Ceftazidime and Tobramycin) as a standard IV regimen for all CF respiratory exacerbations, not just those associated with PA. We wanted to establish how widespread this practice was. Therefore, we thought it would be important to find out the UK practice on which specific intravenous antibiotics are used in different circumstances and if they use anything else alongside this. We were interested to see if any centres used any therapies that may protect the kidneys or any therapies that may increase the risk of kidney during intravenous antibiotic. To investigate this, I designed two online surveys that were sent out to paediatric and adult CF centres in UK centres. I will now go on to describe the methods I used to produce and distribute the survey and the results that were found.

7.2 Methods

7.2.1 Audience

Our target audience for the survey was the main adult and paediatric CF centres in the UK. The centres were listed on the CF Trust directory in the annual report and from this we were able to construct a list with their names and then later added their email addresses. There are 25 adult centres and 27 paediatric centres providing specialist care for CF according to the 2016 annual data report.[1]

7.2.2 Designing the questions

PA is the most common organism causing chronic infection and definitions have been invented based on infection status with this organism that are used to categorise patients.[261] I designed the questionnaire based on these definitions which I have listed below.

Definitions[262]

Never – PA never cultured from sputum or cough swab.

Free of infection – No growth of PA during the previous twelve months, having previously had a positive culture for PA.

Intermittent infection – When 50% or less of months, when samples had been taken, were PA culture positive

Chronic infection – When more than 50% of months, when samples had been taken, were PA culture positive.

My questions were based on a short case vignette with similar scenarios but with patients of different ages in the adults one compared to the paediatric one. Case vignettes were used as FG advised me that it was easier to get across how unwell the patient was, and he thought it would make it more interesting for the doctors to read. The scenario in both

surveys highlighted that the patient would need intravenous antibiotics due to an unresolving respiratory exacerbation.

I asked which antibiotics the centres would prescribe if the patient was in the 'never' category, 'free of infection' category and the 'chronic infection' category. I was interested to see whether some centres would start patients on anti-pseudomonal agents even though the patient had never grown PA before. I was also interested in the 'free of infection' category as to whether the time since the patient last grew PA would affect what they treated the patient with. Perhaps the more recently the patient grew PA, the more likely they would be to give anti-pseudomonal agents.

For each of the three categories ('never', 'free of infection' and 'chronic infection') I asked if they would add in any other medication or therapies alongside the intravenous antibiotics. This question was non-specific as I wanted to know whether they added in any other antibiotics (oral or nebulised) or if they added in any renal protective agents e.g. fluids or statins.

Next, we asked about renal function; whether they monitored it, how they monitored it and when. I asked if they would monitor it when on intravenous antibiotics rather than asking if they monitored it while on aminoglycosides. This is because they may not have given any answers saying aminoglycosides and I did not want this to lead them to go back and change their answers. Finally, we asked about whether they monitor hearing function and when. Please see appendix 1 and 2 for the paediatric and adult surveys, respectively.

For the questions asking about what intravenous antibiotics they used we decided to use a text entry style format with an essay box. We chose this over multiple-choice answers as there are many different antibiotics that can be used and in many different combinations. We thought that if we wrote all these possible combinations out then the

list would be so long that they may struggle to find their answer. We discussed this at length and FG and myself decided that it would be much easier and quicker to give them an essay text box where they can write as much or as little as they like.

For the question regarding what other medications or therapies they would add in we also chose to use a text box entry. This is because we did not want them to see our suggestions such as adding in statins or adding in fluids because this may create response bias with them answering how they think we want them to answer.

For questions like do you test renal function we thought it would be much simpler to give them multiple choice options, for example ‘yes’ or ‘no’. As there are only two answers it is quick and easy to answer by simply clicking on their chosen option.

7.2.3 Qualtrics software

I created the surveys on the Qualtrics software which is a website that I was shown how to use by FG. The software has many different useful features which I will now go on to describe.

First, I used the ‘Look & Feel’ icon which brought up the text box as seen in Figure 7.1. I was able to change the survey so that it only displayed one question per page. FG suggested this as he thought some doctors may answer the questionnaire on their mobile phones. By changing it so that there is one question per page, it prevents the need to scroll down the page and hopefully reduces the chance of people missing questions by mistake. On the ‘Look & Feel’ icon I was also able to change the text font, font size and colour to a style of my choice. I was also able to add in a back button which I thought would be useful in case the doctors had forgotten their previous answer.

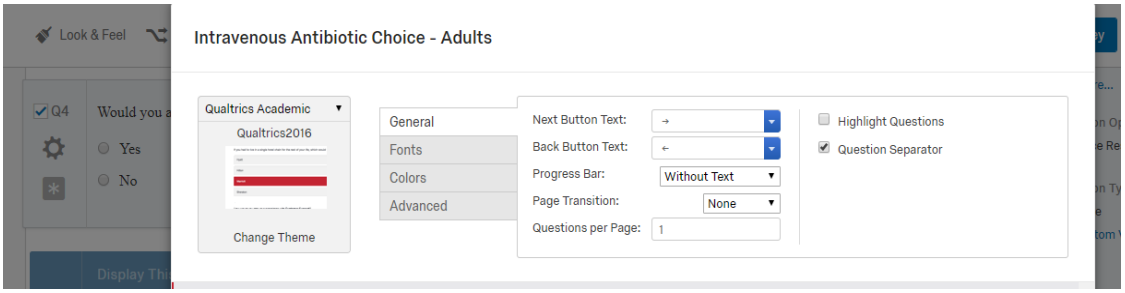

Figure 7.1 Screenshot of 'Look & Feel' feature on Qualtrics

When you highlight each individual question, there is an option that you can tick called 'force response'. If this button is ticked like as seen in Figure 7.2, then it ensures the respondent answers that question before they can move on to the next question. I chose to have turn on 'force response' for all my questions. I thought this would prevent respondents accidentally skipping a question or skipping questions they did not want to answer.

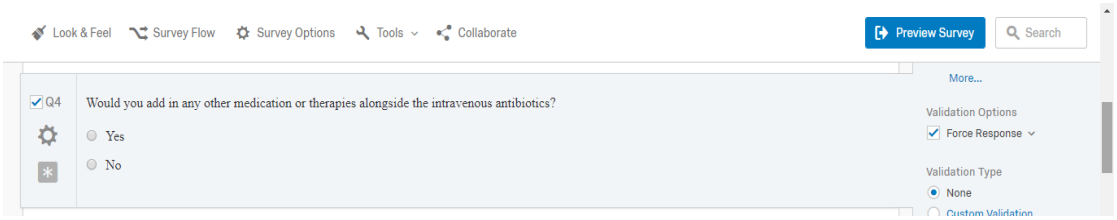

Figure 7.2 – Screenshot of 'Force Response' feature on Qualtrics

'Display Logic' feature allows you to only show certain questions to respondents who answer a previous question in a certain way. For example, for my question 'Would you add in any other medications or therapies alongside the intravenous antibiotics?', I set it up so that only if the respondent answered 'Yes' would they see the next question regarding what they would add in. If they answered 'No' then it would not show the respondent this second question as it was irrelevant to them and so would go straight to the next question after that. To do this I highlighted the question and simply clicked 'Add Display Logic'. I then asked it to only display Q5 if they answered yes to Q4 as seen below in Figure 7.3

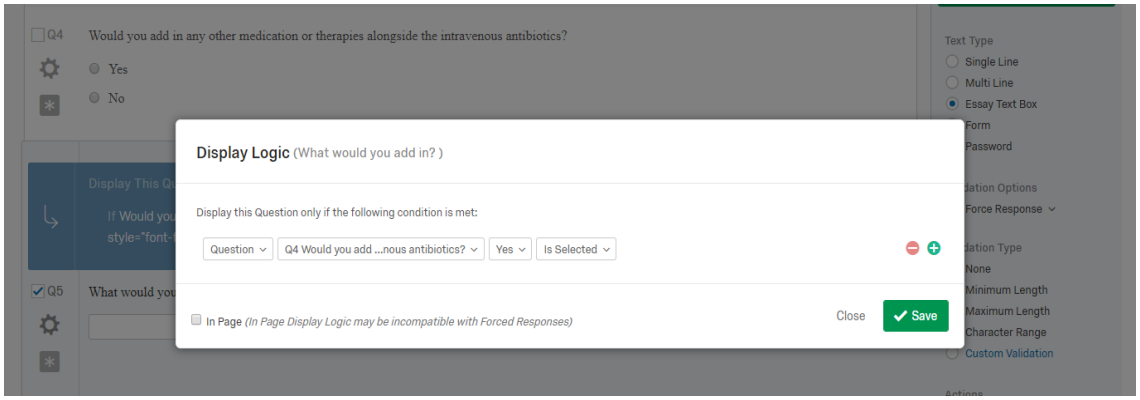

Figure 7.3 – Screenshot showing the 'Display Logic' feature on Qualtrics

7.2.4 Survey distribution

Qualtrics provided us a link for each of the two surveys which were emailed to the lead clinicians at each of the 27 paediatric and 25 adult centres. The results of the completed survey were recorded on Qualtrics. We emailed the survey on the 17/04/2018 to all centres and sent reminder emails on 14/05/2018 to those who had not completed the survey.

7.2.5 Data collection

On 07/06/2018 I exported the survey responses from Qualtrics into spreadsheets on Microsoft Excel. I used Microsoft Excel to collate the answers and analyse the results. We obtained responses from 23/27 paediatric centres and 15/25 of the adult centres.

7.3 Results

7.3.1 Data analysis

The choice of IV antibiotics used by paediatric and adult CF centres in patients who had never grown PA is shown in Figure 7.4. 9/23 (39%) paediatric centres and 6/15 (40%) reported using tobramycin and ceftazidime in combination.

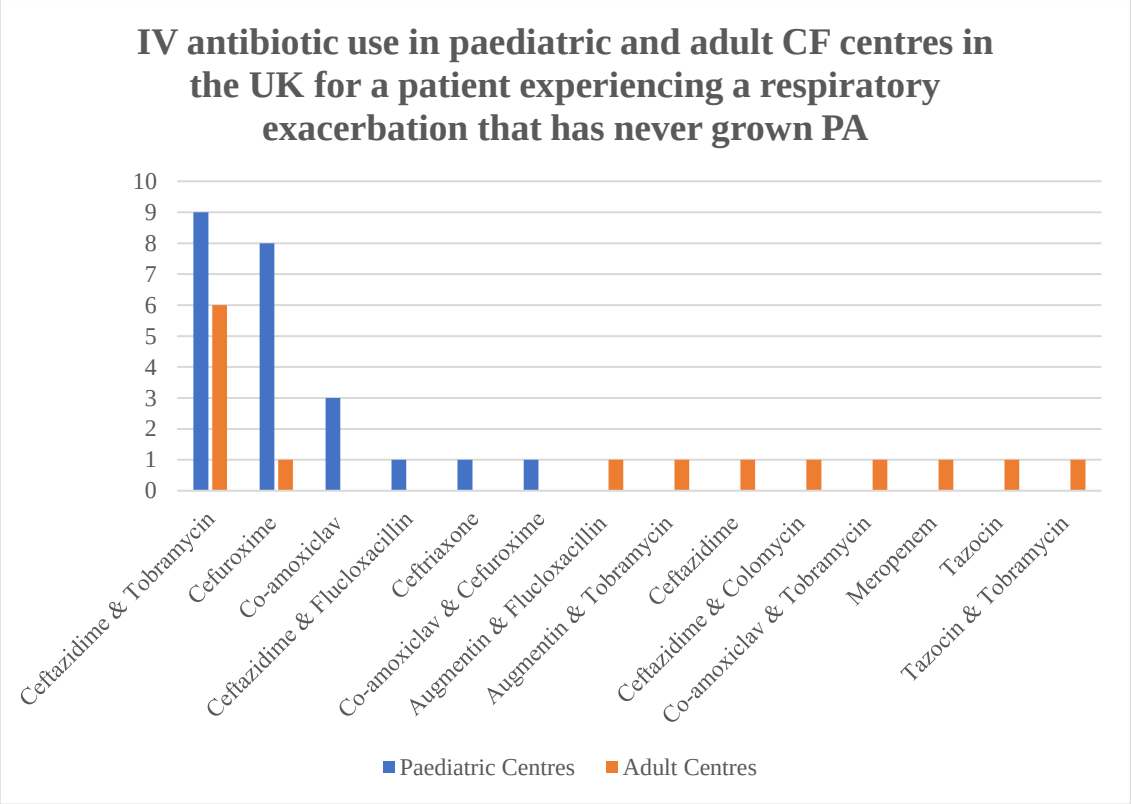

Figure 7.4 – Bar chart to show IV antibiotic use in paediatric and adult CF centres in the UK for a patient experiencing a respiratory exacerbation that has never grown Pseudomonas aeruginosa

The choice of IV antibiotics used by paediatric and adult CF Centres in patients who had isolated PA from a cough swab 18 months ago but received eradication therapy and has

been free from PA ever is shown in Figure 7.5. 13/23 (57%) paediatric centres and 10/15 (67%) reported using tobramycin and ceftazidime in combination.

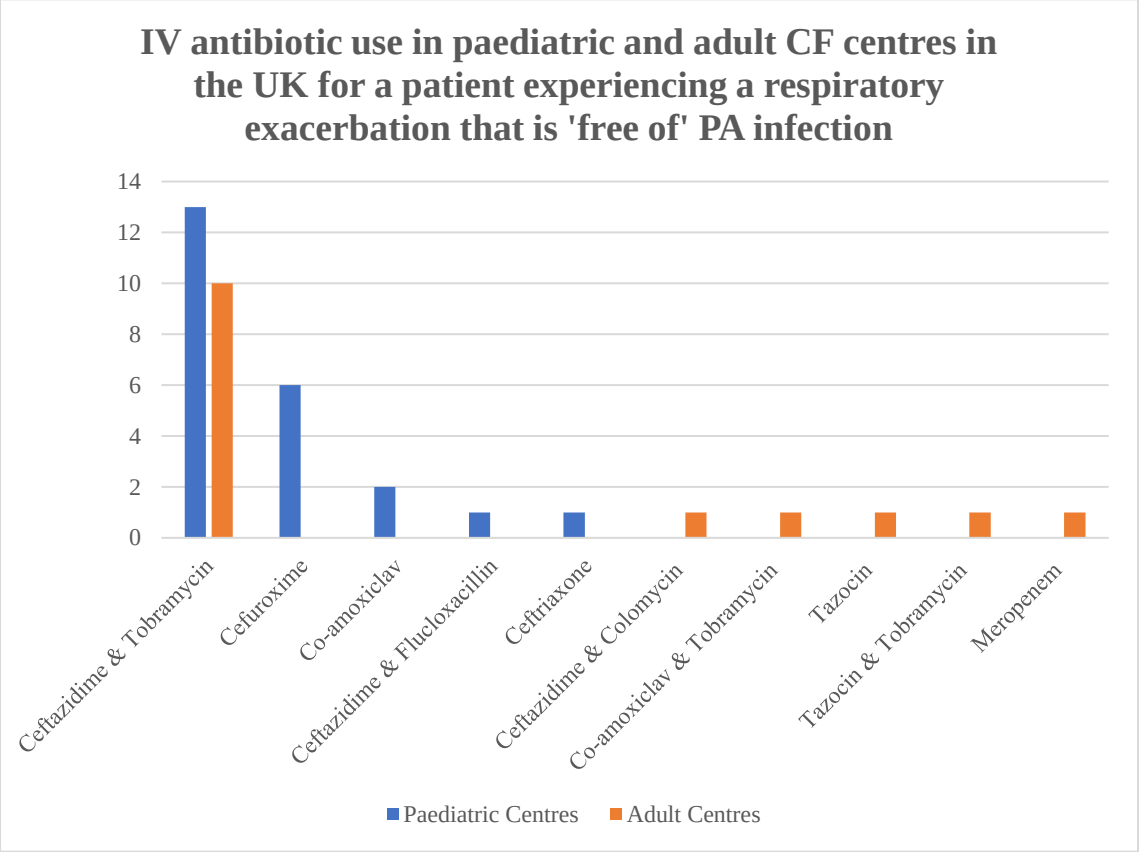

Figure 7.5 - Bar chart to show IV antibiotic use in paediatric and adult CF centres in the UK for a patient experiencing a respiratory exacerbation that is 'free of' Pseudomonas aeruginosa infection

When asked if time since the last isolate of PA would affect whether what antibiotic they were to use, 13/23 said paediatric centres said 'Yes' and 6/15 adult centres said 'Yes'. Out of paediatric centres that said 'Yes', over 50% of these described how if the last isolate was less than 12 months ago they would be more likely to presume it was PA growing again and so would treat with anti-pseudomonal antibiotics. Other centres referred to time since last isolate of PA as a factor influencing their decision on what intravenous antibiotics to use with 6 months and 2 years being other cut off points that

were mentioned. Other centres did not refer to a cut off time but for example said 'More recent growth- more inclined for antipseudomonal antibiotics'.

The choice of IV antibiotics used by paediatric and adult CF centres in patients who have chronic PA infection is shown in Figure 7.6. 23/23 (100%) paediatric centres and 12/15 (80%) reported using tobramycin and ceftazidime in combination.

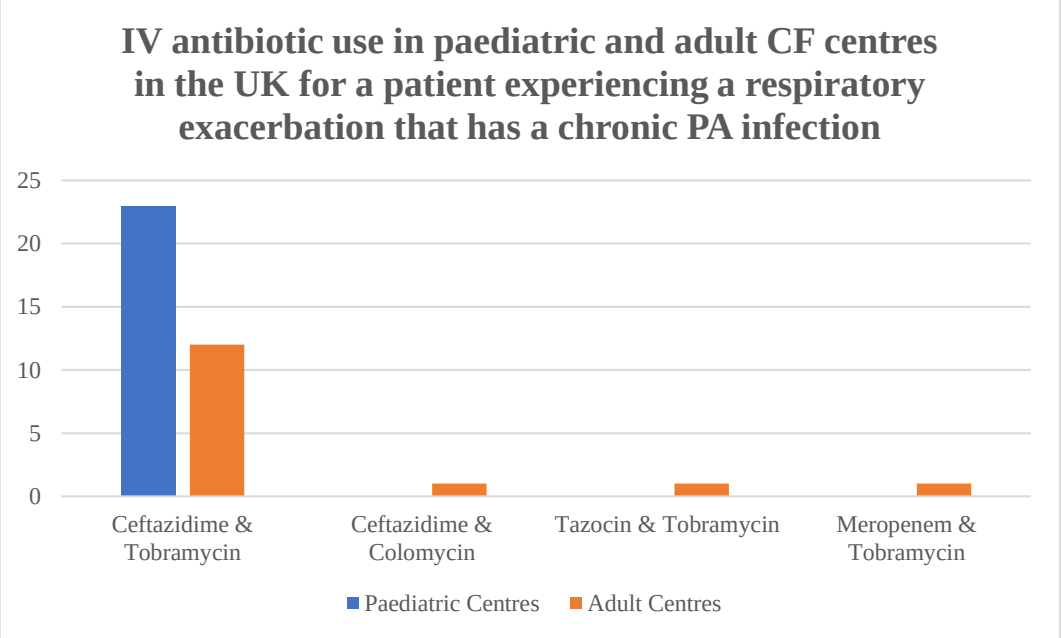

Figure 7.6 - Bar chart to show IV antibiotic use in paediatric and adult CF centres in the UK for a patient experiencing a respiratory exacerbation that has a chronic Pseudomonas aeruginosa infection

There were a range of answers given when asking centres what they would add on to the intravenous antibiotics. The centres that chose to add something on to the intravenous antibiotics tended to add the same things on regardless of whether they fit into the 'never', 'free of' or 'chronic' category of PA growth. The most common thing the paediatric centres chose to add on was mucolytics, with both DNase and hypertonic saline being mentioned. Other things that were mentioned by the paediatric centres include bronchodilators, oxygen, oral flucloxacillin and azithromycin. Similarly, the adult centres also chose to add on mucolytics most commonly, but equally they mentioned

physiotherapy. Other adult centres mentioned exercise, paracetamol, overnight fluids, and aminophylline. 4 paediatric centres mentioned that they would add in colistin nebulisers if they weren't already on them. One adult centre said they would remove nebulised and oral antibiotics while the patient was on intravenous antibiotic and another said they would review nebulised antibiotics.

19/22 (86%) paediatric centres said they monitor renal function in those on intravenous antibiotics compared to 15/15 (100%) adult centres. In all CF centres that monitor renal function, they do so by monitoring serum urea and creatinine. 14/19 (74%) paediatric centres also mentioned looking at tobramycin levels compared to only 4/15 (27%) adult centres. Out of the centres that monitor renal function 11/19 (58%) and 8/15 (53%) of them do it at baseline, before starting intravenous antibiotics.

18/22 (82%) of the paediatric centres assess hearing in those on intravenous antibiotics with 10 out of the 18 centres assessing it annually. In those that monitor it but do not assess it annually, they suggested they would only test hearing in those that receive frequent IVs, those about to begin NTM eradication, those with high serum aminoglycoside levels or those with symptoms. Only 4/15 (27%) of the adult centres that responded to the questionnaire test for hearing. Out of these 4, one centre test it annually and the other 3 centres test it if symptoms appear.

7.4 Discussion

7.4.1 Main findings

It was interesting that many centres chose to prescribe anti-pseudomonal antibiotics to the patient in the 'never' category even though there was no evidence of PA infection. As discussed, tobramycin is associated with kidney injury and cumulative lifetime dose

is associated with risk of renal disease. It seems more reasonable to use tobramycin and ceftazidime in adult centres, as the older the patient the more likely they are to grow PA. As well as this if this is their first growth of PA they are unlikely to have received many courses of anti-pseudomonals and so their cumulative lifetime dose is low. There was much more variation in the antibiotics selected by the adult CF centres compared to the paediatric centres.

There was an increase in the use of tobramycin and ceftazidime in combination for the patient that was 'free of infection' compared to the patient in the 'never' category. This is likely since the patient has grown PA before, the doctors are presuming it is PA again. However, the use of tobramycin if it is not PA may be harmful and unnecessary.

Interestingly for the patient who had chronic growth of PA experiencing an acute respiratory exacerbation all paediatric centres indicated that they would use intravenous tobramycin and ceftazidime in combination. This complies with the guidelines set out by NICE and CF Trust which suggest using combination of 2 intravenous antibiotics, one being an aminoglycoside and the other being a β-lactam (e.g. ceftazidime) or an anti-pseudomonal penicillin (e.g. Piperacillin-Tazobactam). There was more variation in antibiotic combinations used in adult centres for chronic PA infections which can be seen in the bar charts above.

One centre mentioned using overnight fluids when patients are on intravenous antibiotics and we wondered whether they used the overnight fluids as a strategy to minimise the risk of nephrotoxicity or if there was another reason behind this and so I aimed to contact them for more information. There were conflicting views as to whether nebulised antibiotics should be continued or withdrawn while patients are receiving intravenous antibiotics. The CF trust recognises that most centres stop nebulised antibiotics while

using intravenous antibiotics due to the risk of nephrotoxicity however the questionnaires demonstrates a variety of practices. This highlights why our Cochrane review is so important in order to determine whether nebulised antibiotics can be used safely alongside intravenous antibiotics.

I was surprised by the three paediatric centres that said they did not monitor renal function in patients on intravenous antibiotics because they all mentioned tobramycin in at least one of their previous answers. The CF Trust describes in its guidelines that those on tobramycin should have their renal function checked at the start and states that tobramycin can cause nephrotoxicity. With most antibiotics the guidance recommends reducing the dose in renal impairment and these centres may be missing this by not checking.

All centres that monitored renal function did so by looking at U&E. Some centres also mentioned measuring tobramycin levels. The paediatric centres commonly mentioned taking the U&Es at the same time as the tobramycin levels, probably to decrease the number of times the child would have to be exposed to a needle. The CF trust states in the guidelines that renal function should be assessed at baseline in those about receive a course of intravenous tobramycin. In the paediatric centres that monitor renal function there was a variety of monitoring regimes with some centres only assessing it once overall throughout the course of antibiotics all the way up to some centres monitoring it twice weekly. Similarly, in the adult centres, there was no concordance being monitoring renal function with it ranging from weekly to three times weekly.

There is no mention in the guidelines as when or how to test hearing, but it does clearly state that ototoxicity is a side effect of aminoglycoside. Therefore, I would have thought that all centres screened for it but was particularly surprised by how few adult centres

monitored for this given they are likely to have been exposed to a higher number of IV anti-pseudomonals over their lifetime.

7.4.2 Strengths of the survey

One of the strengths of the surveys was we got a high response rate in particular from the paediatric centres. The paediatric response rate was 85% compared to the adult survey which was only 60%. High response rates reduce the risk of non-response bias and so the answers are more likely to be valid and representative of the population.

A further strength of our survey was that it was relatively quick to answer. Qualtrics provided an estimation time of 5 minutes to answer but we also tested the questionnaire ourselves to make sure it was not too long. By making the questionnaire as short as possible it increases the likelihood that the doctors will complete it.

Another strength of our survey was that we used clinical cases. This made it more interesting for the doctors to read and also made it more relatable. If the doctors find the questionnaire interesting they are more likely to complete it and so helps maximise the response rate.

In the questionnaire I used to free text box rather than ticking answers that I had provided. I think this is better as if I had provided the answers, I may have missed off a combination that is used somewhere. Therefore, we get a truer picture of what antibiotics they actually use and so it is more accurate.

7.4.3 Weaknesses of the survey

Unfortunately, we did not get responses from all centres. For the paediatric centre survey, we did not receive responses from Bristol, Aberdeen, Wishaw, or Tayside. In

the adult centre survey, we did not receive results from Frimley, St James', St Bartholomew's, Kings college, Norwich, Nottingham, Southampton, York, Belfast and UHNM. This means that the results are more difficult to interpret.

Another weakness of the survey is that it was only conducted in the UK. Therefore, these results are only representative of the UK as the rest of the world may have completely different practices when it comes to prescribing antibiotics. By only looking at the UK it also means there is a fairly small sample size as there are only limited number of CF centres in the UK.

A disadvantage of using the free text boxes was that people were able to write long answers that sometimes included many different antibiotics that they would use in different scenarios. Therefore, for some centres we did not necessarily which one they would use first line in the given scenario.

The question asking if they would add anything else alongside the intravenous antibiotics was very broad. Therefore, it was hard to know whether we got all the answers that we wanted or whether they did not think to tell us, for example, about the statins they use. However, if we were to have asked a few more narrower questions e.g. did you add in statins if using an aminoglycoside or did you give nebulised antibiotics as well, then they may have uncovered the aims of the study. This would give biased results as they may give answers that they think you want to hear.

7.5 Conclusions

Antibiotic prescribing depends on national/local guidelines, antibiotic sensitivities, availability, and cost. There is a lack of guidance particularly in those that have never grown PA. We identified high use of nephrotoxic anti-pseudomonals (tobramycin) in

patients who had never grown PA. As discussed, cumulative lifetime aminoglycoside dose correlates with risk of kidney disease. These patients are therefore being put at an unnecessary risk of future renal disease.

There was a wide variation in the time reported since last PA isolate that would affect whether centres chose to use anti-pseudomonals or not. The definition of 'free from' uses the time point of 12 months which was the most commonly reported time. There needs to be clear guidance on this which would require further research.

From the results of the survey we can see that although there is guidance provided by the CF Trust that renal function should be monitored, there are still 3 paediatric centres in the UK that do not monitor renal function. The CF Trust also recommends that this renal function is monitored at baseline, however around half of the centres do not measure it then. This highlights a need for more education to doctors regarding monitoring renal function in those on intravenous antibiotics. Other than measuring renal function at baseline the CF Trust does not highlight need to monitor renal function at any other time. By measuring renal function at baseline, you can identify pre-existing renal problems which may affect which antibiotics you give the patient. However, I also think that it should be recommended that renal function is monitored throughout treatment to check that function is not deteriorating. There needs to be more guidance on this so that doctors know when to monitor it. By identifying deteriorating renal function early, the antibiotics can be stopped, and it may reduce the risk of acute kidney injury.

Relating this survey back to our Cochrane review we identified one centre that used fluids overnight while patients are receiving intravenous antibiotics. This is a strategy we said we would look at to see if reduces nephrotoxicity but currently there are no studies addressing this. The survey revealed conflicting views on the use of inhaled and oral

antibiotics alongside intravenous antibiotics. Although the CF trust identifies that many centres stop nebulised antibiotics, NICE advises to keep them going. Our review is important in determining whether the use of inhaled and oral antibiotics alongside intravenous antibiotics is safe so that appropriate guidelines can be produced.

CHAPTER 8: CONCLUSIONS

8.1 Main findings from my thesis

On writing my thesis it has become evident to me that CF is becoming a condition where individuals die in their adult life as oppose to during their childhood. There is a vast amount of research regarding the management of pulmonary complications in CF. With better management of respiratory complications, life expectancy is increasing year upon year. However, it is important to remember that some of the treatments that we use can have detrimental effects on other organs. As individuals with CF are now surviving into their 40's, it is important to address long term consequences of these treatments and to minimise them where possible. In my thesis, I discussed how the kidneys are injured in CF patients, most frequently by intravenous antibiotics. Kidney injury can progress to chronic kidney disease which can then have many negative implications on the individual's heath. The objectives of my thesis included performing a Cochrane review examining strategies that may reduce the risk of kidney injury caused by intravenous antibiotics and to conduct a survey to look at the use of intravenous antibiotics in the UK.

From the search there seems to be many studies that compare different combinations of different antibiotics although we have not yet been able to analyse this data. The Cochrane review revealed a lack of evidence for other strategies that we considered that may reduce kidney injury. For example, at the moment we have not yet identified any studies that look at the use of fluids alongside antibiotics. The review as yet is not complete as we are still awaiting further information from authors, however, at this stage we have been able to include four studies in our review.

There was evidence that administering tobramycin intravenously in the morning in children reduced the urinary excretion of a biomarker KIM1 compared to administering it in the evening. This may suggest that evening dosing of tobramycin may increase the risk of nephrotoxicity and therefore a strategy to minimise kidney injury would be to always administer intravenous antibiotics in the morning.

Al-Aloul's study demonstrated that using nebulised tobramycin for an acute exacerbation reduced the urinary excretion of protein and biomarkers NAG, AAP and β2 Microglobulin compared to when using intravenous antibiotics. This suggests that using nebulised antibiotics instead of an intravenous antibiotic for an acute respiratory exacerbation may reduce the risk of kidney injury.

The survey demonstrated a lack of evidence on consensus of what antibiotic should be used for an unidentified infection in a CF patient who has never grown PA. There was no consensus on how long since last PA isolation that it is appropriate to not use anti-pseudomonals. Other than one adult centre that used overnight fluids when patients are on intravenous antibiotics there was no other use of fluids or statins to minimise nephrotoxicity. There was some use of nebulised antibiotics alongside intravenous antibiotics whereas some centres stopped oral and nebulised antibiotics. This highlighted the importance of my Cochrane review in determining whether using nebulised antibiotics alongside intravenous antibiotics may contribute to nephrotoxicity.

8.2 Implications for future practice

Although Prayle's study size had a very small sample size, it may be appropriate for us to suggest use of tobramycin in the morning rather than the evening. It is unlikely that by

doing this we would cause any harm as they are still receiving the same antibiotic and same dose.

In future we may be able to recommend using nebulised tobramycin over intravenous tobramycin to reduce nephrotoxicity. However, the study was only small and did not hold enough power to recommend this. Although it suggested that nebulised tobramycin is as effective at resolving the respiratory exacerbation, we cannot be sure due to the small sample size and it could be dangerous if the results weren't correct. It is important that when looking at strategies that may reduce nephrotoxicity, the strategy must be as successful at treating the respiratory exacerbation.

8.3 Implications for future research

There is a lack of studies looking at adjuvant medication such as fluids or statins alongside the intravenous antibiotic. More research is needed to look at these potential strategies to minimise nephrotoxicity.

Although this review so far has highlighted that morning dose of tobramycin may be better for the kidneys than an evening dose, a limitation of this study was that it had a small sample size. A further limitation was that it only addressed children and not adults. Before guidance can advise the use of morning dosing a much larger randomised controlled trial is needed that also includes adults. As well as this the effect seen may only be limited to tobramycin and so further randomised controlled trials are needed to see if other antibiotics have the same effect.

Similarly, before we can advise using nebulised tobramycin instead of intravenous tobramycin a larger randomised controlled trial is required that also looks at children.

This trial only addresses tobramycin and so further research is required looking at other antibiotics administered by nebuliser rather than intravenously.

Ideally further research should be placebo-controlled randomised trials that have large sample sizes hence the evidence is more robust.

8.4 Reflections on intercalating

The most difficult aspect of my masters this year has been timing. I found it frustrating that I was often waiting around before I could begin the next part of the Cochrane review. For example, I waited for a long time before the protocol was published. This prevented me from running the searches and beginning data extraction. While I was awaiting publication, I spent this time focusing on my questionnaire. I worried about sending reminder emails asking if tasks were completed. On reflection, if tasks had been rushed then they may not have been completed to the same high quality. By the end of the year I started worrying less about the Cochrane review being completed as I did not want to compromise on quality. In conclusion, I learnt that research can take time and is not something that can be rushed. Careful planning is required so that there are other tasks that you can be on with while waiting for other people to complete their tasks.

Before starting my masters, I was already keen to pursue a career in paediatrics. This year has confirmed that desire further. I have thoroughly enjoyed working with the CF team at University Hospital of North Midlands. I particularly enjoyed seeing patients more than once and building up rapport with them. I was happy to see increases in lung function and less symptoms from one clinic to the next in some patient. Being able to treat patients and increase their life expectancy to beyond what was ever imaginable reminded me how important Medicine as a subject is. In conclusion, I am certain I would like to pursue a

career in paediatrics, particularly in a sub speciality that allows me to work with chronic conditions.

Before beginning this year, I was always intrigued by research but was unsure whether I would enjoy it or not. I have always been the medical student that would ask 'why?'. However, I did not predict that I would enjoy research as much as I did. I have found myself submerged in topic areas at times during the year. On reflection, this has prompted me to think about a career in research. I have decided I should apply for the academic foundation programme. I am also keen to get involved in more research projects in the near future.

Being an undergraduate student, I had no previous experience in systematic reviews, meta-analysis, or scientific writing. This year has been a very steep learning curve for myself, but I have enjoyed it and I know these skills will be valuable for years to come. At times I have been overwhelmed by the enormity of the task, particularly when the search returned over 6000 results that I had to screen. The Cochrane review training course was helpful, but it was not until I started working on my own Cochrane review that the knowledge was cemented. I asked countless questions over the course of the year to my supervisors, WC and FG and also to the support team at Cochrane, NJ, TR, and AK. I have learnt a great deal about critical appraisal of studies and my statistical knowledge has vastly improved. I now feel able to not only look at a study and interpret the results but also to be critical of it and highlight potential problems with it. I am now able to produce, distribute and analyse the results of a survey.

To conclude, I have gained a lot of experience from doing an MPhil and have enjoyed it. I have learnt many skills that I hope to use in the future. I am proud to have been involved in a Cochrane review that will hopefully contribute to research that can help to prevent

kidney disease from antibiotics in CF patients. I have been inspired to get involved in more research and I am certain I would like to pursue a career in Paediatrics.

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APPENDICES

Appendix 1: Paediatric centre survey to assess which IV antibiotics are used to treat CF respiratory exacerbations

Intravenous Antibiotic Choice - Paeds

Q1 Many thanks for taking the time to complete this short survey.

Our team is currently undertaking a Cochrane Systematic Review on optimal antibiotic strategies for respiratory infections in individuals with Cystic Fibrosis. To complement this, we thought it would be useful to ascertain what the current UK practice is for the treatment of respiratory infections with intravenous antibiotics.

The survey is based around short case vignettes and should only take around 5 minutes to compete.

Q2 Which centre are you from?

_____

Q3 Tommy is a 6 year old boy with Cystic Fibrosis. He has developed a nasty wet cough that did not respond to a two week course of oral antibiotics. Although he sounds productive, he does not expectorate sputum as he swallows it. Cough swabs have only isolated normal respiratory tract flora. He is saturating at 93% and has a temperature of 37.8. You decide to start him on intravenous antibiotics. He has no known drug allergies.

Please list the intravenous antibiotic(s) you would start him on in the following scenarios.

Q4 Scenario 1
Tommy has never grown *Pseudomonas aeruginosa* before.

Please list the intravenous antibiotic(s) that you would start him on for his current infective exacerbation.

_____

_____

_____

_____

_____

Q5 Would you add in any other medication or therapies alongside the intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Would you add in any other medication or therapies alongside the intravenous antibiotics? = Yes

Q6 What would you add in?

_____

_____

_____

_____

_____

Q7 Scenario 2
Tommy isolated *Pseudomonas aeruginosa* from a cough swab 18 months ago. He received eradication therapy for this and subsequent cough swabs (10 in total) have been negative.

Please list the intravenous antibiotic(s) that you would start him on for his current infective exacerbation.

_____

_____

_____

_____

_____

Q8 Would you add in any other medication or therapies alongside the intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Would you add in any other medication or therapies alongside the intravenous antibiotics? = Yes

Q9 What would you add in?

_____

_____

_____

_____

_____

Q10 Does the time duration since his last isolate of *Pseudomonas aeruginosa* influence your choice of intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Does the time duration since his last isolate of Pseudomonas aeruginosa influence your choice of... = Yes

Q11 Please explain how time duration since last isolate of *Pseudomonas aeruginosa* influences your choice of intravenous antibiotics.

_____

_____

_____

_____

_____

Q12 Scenario 3

Tommy has chronic *Pseudomonas aeruginosa* growth with a fully sensitive organism.

Please list the intravenous antibiotic(s) that you would initially start him on.

_____

_____

_____

_____

_____

Q13 Would you add in any other medications or therapies alongside the intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Would you add in any other medications or therapies alongside the intravenous antibiotics? = Yes

Q14 What would you add in?

_____

_____

_____

_____

_____

Q15 Do you monitor renal function in those on intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Do you monitor renal function in those on intravenous antibiotics? = Yes

Q16 How do you do this?

_____

_____

_____

_____

_____

Display This Question:

If Do you monitor renal function in those on intravenous antibiotics? = Yes

Q17 When do you do this?

_____

_____

_____

_____

_____

Q18 Do you monitor hearing in those on intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Do you monitor hearing in those on intravenous antibiotics? = Yes

Q19 When do you monitor for this?

_____

_____

_____

_____

_____

Q20 Many thanks for taking the time to complete this survey.

Appendix 2: Adult centre survey to assess which IV antibiotics are used to treat CF respiratory exacerbations

Intravenous Antibiotic Choice - Adults

Q1 Many thanks for taking the time to complete this short survey.

Our team is currently undertaking a Cochrane Systematic Review on optimal antibiotic strategies for respiratory infections in individuals with Cystic Fibrosis. To complement this, we thought it would be useful to ascertain what the current UK practice is for the treatment of respiratory infections with intravenous antibiotics.

The survey is based around short case vignettes and should only take around 5 minutes to compete.

Q2 Which centre are you from?

_____

Q3 Jenny is a 29 year old female with Cystic Fibrosis. She has developed a nasty wet cough that did not respond to a two week course of oral antibiotics. She expectorates sputum but the sputum cultures have only isolated normal respiratory tract flora. She is saturating at 93% and has a temperature of 37.8. You decide to start her on intravenous antibiotics. She has no known drug allergies.

Please list the intravenous antibiotic(s) you would start her on in the following scenarios.

Q4 Scenario 1
Jenny has never grown *Pseudomonas aeruginosa* before.

Please list the intravenous antibiotic(s) that you would start her on for her current infective exacerbation.

_____

_____

_____

_____

_____

Q5 Would you add in any other medication or therapies alongside the intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Would you add in any other medication or therapies alongside the intravenous antibiotics? = Yes

Q6 What would you add in?

_____

_____

_____

_____

_____

Q7 Scenario 2

Jenny isolated *Pseudomonas aeruginosa* from a cough swab 18 months ago. She received eradication therapy for this and subsequent cough swabs (10 in total) have been negative.

Please list the intravenous antibiotic(s) that you would start her on for her current infective exacerbation.

_____

_____

_____

_____

_____

Q8 Would you add in any other medication or therapies alongside the intravenous antibiotics?

- Yes (1)
- No (2)

Display This Question:

If Would you add in any other medication or therapies alongside the intravenous antibiotics? = Yes

Q9 What would you add in?

_____

_____

_____

_____

_____

Q10 Does the time duration since her last isolate of *Pseudomonas aeruginosa* influence your choice of intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Does the time duration since her last isolate of Pseudomonas aeruginosa influence your choice of... = Yes

Q11 Please explain how time duration since last isolate of *Pseudomonas aeruginos*a influences your choice of intravenous antibiotics.

_____

_____

_____

_____

_____

Q12 Scenario 3

Jenny has chronic *Pseudomonas aeruginosa* growth with a fully sensitive organism.

Please list the intravenous antibiotic(s) that you would initially start her on.

_____

_____

_____

_____

_____

Q13 Would you add in any other medications or therapies alongside the intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Would you add in any other medications or therapies alongside the intravenous antibiotics? = Yes

Q14 What would you add in?

_____

_____

_____

_____

_____

Q15 Do you monitor renal function in those on intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Do you monitor renal function in those on intravenous antibiotics? = Yes

Q16 How do you do this?

_____

_____

_____

_____

_____

Display This Question:

If Do you monitor renal function in those on intravenous antibiotics? = Yes

Q17 When do you do this?

_____

_____

_____

_____

_____

Q18 Do you monitor hearing in those on intravenous antibiotics?

- ○ Yes (1)
- ○ No (2)

Display This Question:

If Do you monitor hearing in those on intravenous antibiotics? = Yes

Q19 When do you monitor for this?

_____

_____

_____

_____

_____

Q20 Many thanks for taking the time to complete this survey.

Appendix 3: Protocol for Cochrane Review - Strategies to prevent kidney injury from antibiotics in people with cystic fibrosis

The protocol can be accessed online at:

https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013032/full