The Complexities of Ageing in Cystic Fibrosis

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Sarah L Paterson

School of Biological Sciences

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Abbreviations

AAP	Alanine amino-peptidase
ABPA	Allergic bronchopulmonary aspergillosis
ACR	Albumin creatinine ratio
AHA	American Heart Association
AIx	Augmentation index
AKI	Acute kidney injury
ApoB	Apolipoprotein B
ApoA1	Apolipoprotein A1
AUC	Area under the curve
BAE	Bronchial artery embolisation
BP	Blood pressure
BCC	*Burkholderia cepacia* complex
BMD	Bone mineral density
BMI	Body mass index
CF	Cystic fibrosis
CFA	Cystic fibrosis arthropathy
CFLD	Cystic fibrosis-related liver disease
cfPWV	Carotid-femoral pulse wave velocity
CFRD	Cystic fibrosis-related diabetes mellitus
CFTR	Cystic fibrosis transmembrane receptor (protein)
CG	Cockroft Gault

CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMR	Cardiac magnetic resonance imaging
CRP	C-reactive protein
CT	Computed tomography
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEXA	Dual energy X-ray absorptiometry
DIOS	Distal intestinal obstruction syndrome
DM	Diabetes mellitus
ECMO	Extracorporeal membrane oxygenation
eGFR	Estimated glomerular filtration rate
ECV	Extra-cellular volume
EF	Ejection fraction
ENT	Ear, nose and throat
FE-1	Faecal elastase-1
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GFR	Glomerular filtration rate
GORD	Gastro-oesophageal reflux disease
HDL	High density lipoprotein

ICSI	Intracytoplasmic sperm injection
iLDL	Intermediate density lipoprotein
ITU	Intensive care unit
LDL	Low density lipoprotein
LES	Liverpool epidemic strain
LGE	Late gadolinium enhancement
LVH	Left ventricular hypertrophy
MDRD	Modification of Diet in Renal Disease
MDT	Multidisciplinary team
mGFR	Measured glomerular filtration rate
MRI	Magnetic resonance imaging
MRSA	Methicillin resistant staphylococcus aureus
NAG	N-acetyl-β-D-glucose-aminidase
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NIV	Non-invasive ventilation
NSAIDs	Non-steroidal anti-inflammatory drugs
NTM	Non-tuberculous mycobacteria
PERT	Pancreatic enzyme replacement therapy
PI	Pancreatic insufficiency (exocrine)
PVA	Polyvinyl alcohol (particles)
PWV	Pulse wave velocity

RA	Rheumatoid arthritis
RV	Right ventricle
RVIP	Right ventricular insertion point
SBP	Systolic blood pressure
SLE	Systemic lupus erythematosus
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
vLDL	Very low density lipoprotein

Abstract

Survival in cystic fibrosis (CF) is increasing exponentially and we are now seeing an ageing population. At the Manchester Adult Cystic Fibrosis Centre (MACFC), 20% of the total patient group are aged 40 years and above. Survival is likely to increase further in this exciting era of cystic fibrosis transmembrane conductance regulator protein (CFTR) modulation. Although there has been a paradigm shift in CF management and disease perspectives in recent years, there remains a paucity of research in this important and fascinating older CF patient population, particularly over the last decade.

The CF population aged 40 years and above at MACFC is heterogeneous. There was a high proportion of patients with class I-III CFTR mutations and associated severe phenotypic disease. However, the prevalence of residual function CFTR mutations increased with age and were associated with lower sweat chloride values and later age of CF diagnosis.

Cardiovascular risk exists in this older CF cohort, with 34.9% of patients showing elevated QRisk®3 scores of 10% or greater. The prevalence of cardiovascular disease may increase with an ageing CF population. The recognition and management of cardiovascular risk is essential to reduce further comorbidity in this already complex and challenging disease.

At 3.6%, the prevalence of chronic kidney disease (CKD) was not as high in this older adult CF cohort as perhaps initially predicted. However, the measurement of estimated glomerular filtration rate (GFR) can be inaccurate in CF patients. There may be some justification for the wider use of measured GFR methods, such as iohexol, to improve the reliability of renal monitoring and in the assessment of GFR decline in select phenotypic groups of older CF patients.

Cardiac magnetic resonance imaging (CMR) is feasible and well tolerated in this older adult CF group with a range of pulmonary disease severity. The use of CMR may provide an opportunity to analyse cardiac morphology and function in greater detail in CF than can be done with echocardiography alone, and cardiac imaging may become increasingly relevant in an ageing CF population.

Declaration

No portion of work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning institution.

Copyright statement

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The Author

Dr Paterson achieved her MBChB degree from the University of Cardiff in 2010. Following completion of foundation and core medical training in Wales, and obtaining her MRCP(UK) Diploma in 2013, she moved to the North West in 2014 for specialist registrar training in Respiratory and General Internal Medicine. She worked as a Clinical Fellow at the Manchester Adult Cystic fibrosis Centre from 2018 to 2020, during which she conducted the research formulating this thesis.

Preface

This study was designed to develop knowledge and understanding of older adult CF patients, their clinical characteristics, the complexities of their genetics and disease, and to expose some challenges that may lie ahead as survival continues to improve.

Chapter one provides an introduction to CF, a review of the literature dedicated to ageing in CF and an identification of where some gaps may exist in the research of an older adult CF population. Chapter two outlines the aims and methods of the study. Chapters three to six comprise the results chapters, moving from general demographic analysis of an older CF cohort through to cardiovascular risk, renal pathology and finally cardiac magnetic resonance imaging (CMR).

The results chapters of this thesis are written in journal format. Although each chapter is distinct, the nature of this thesis style and the areas studied confer an inevitable degree of overlap. My hope was that each chapter would introduce the next in some way, providing continuity for the reader.

The limitations of the study are outlined at the end of each results chapter and there is a section dedicated to summation of findings and recommendations for future work.

I have been privileged enough to have presented some of this work at CF conferences in North America and Europe, gaining several subsequent journal publications.

The Complexities of Ageing in Cystic Fibrosis

Chapter One: Introduction and review of literature

Cystic Fibrosis - An overview

Introduction

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive genetic disorder in Caucasians, affecting 1 in 2500-3500 live births in the UK, and with an asymptomatic carrier frequency of 1 in 25(1). However, at a population level CF remains uncommon. There are between 200 and 300 new CF diagnoses in the UK annually. The majority are diagnosed in early life but with an increasing number being diagnosed in adulthood.

1.1.2 Pathophysiology

CF is a complex, multisystem disease caused by mutations in a gene encoding a membrane protein known as the cystic fibrosis transmembrane regulator (CFTR), located on the long arm of chromosome 7. The CFTR protein acts predominantly as a regulator of anion movement across exocrine gland epithelial cell membranes. CFTR is expressed throughout the body and affects secretory function in multiple organs including the lungs, pancreas, gastro-intestinal tract, liver, genitourinary system and sweat glands.

Mutations of the CFTR gene result in abnormal ion movement across epithelial cell membranes. More specifically, this involves chloride and bicarbonate loss from the cell surface. A lack of CFTR inhibition of sodium channels causes movement of sodium ions into cells, enhancing osmotic water resorption. This leads to inadequate hydration of mucous secretions and defective mucociliary clearance. Retained secretions encourage bacterial adherence and a perpetuating cycle of inflammation, infection and tissue destruction(2).

1.1.3 Diagnosis

CF is usually diagnosed in childhood. It is predominantly a clinical diagnosis, supported by a number of clinical tests. Newborn screening was introduced in the 1980s and became UK-wide in 2007. This involves a heel blood spot test in the first week of life for immunoreactive trypsin (IRT), a measure of exocrine pancreatic function. IRT is raised in newborn infants with CF. Given its high sensitivity but lower specificity, a raised IRT necessitates subsequent sweat testing and CFTR genetic studies to confirm the diagnosis of CF(3). Abnormal expression of CFTR in sweat glands causes exaggerated excretion of chloride through the skin surface, forming the basis of the sweat test diagnosis for CF. A positive sweat test (sweat chloride >60mmol) and two functional mutations of the CFTR gene confirms a

diagnosis of CF, alongside typical clinical features which may include recurrent pulmonary infections, failure to thrive, symptoms of malabsorption or meconium ileus.

A minority of CF patients are diagnosed in adulthood. This group may account for less severe genotypes, including those with exocrine pancreatic sufficiency. A milder disease phenotype often accounts for a delay in clinical presentation. Just over 14% of patients in the UK CF Registry in 2017 were diagnosed at the age of 16 years or over(4).

1.1.4 Genetics

Since the discovery of the CFTR gene in 1989, approximately 2017 different mutations of CFTR protein have now been found(5). However, not all abnormal CFTR mutations are clinically relevant, with only around 300 causing phenotypic disease. CF has autosomal recessive inheritance and thus a patient must have a CFTR mutation on each chromosome to exhibit the CF clinical phenotype. CFTR mutations vary according to their effect on CFTR protein production, function, transport or stability(6). Clinical phenotype varies with mutation type and the amount of functional CFTR protein correlates in part with disease severity, particularly the degree of exocrine pancreatic sufficiency. However, genotype correlates less well with lung function and thus environmental and other non-CFTR factors, such as modifier genes, must play a role(7).

There are six established classes of CFTR mutation, which are outlined in the table below (*table 1.1)*. Two CFTR mutant alleles at a single gene locus, i.e. on each chromosome of a pair, are required to cause clinical disease in CF. The presence of a CFTR mutation on only one allele, paired with a normal ("wild-type") CFTR allele, results in heterozygosity and a CF carrier. A patient may have the same CFTR mutation on each allele, such as Phe508del/Phe508del, known as a homozygote, or may have a different disease causing CFTR mutation at each allele, such as Phe508del/Gly551Asp, known as a 'compound heterozygote'. CFTR mutation classes I to III with loss of two functional alleles, resulting in low or absent CFTR activity, in general correspond to the 'classical CF' with the most severe clinical phenotypes. A proportion of CFTR mutations in classes I-III correspond to little or no CFTR protein expression (missense or nonsense CFTR mutations) and are known as *minimal function* (MF) mutations, such as Gly542X and Asn1303Lys(8).

Although there may be considerable phenotypic variability within genetic groups, CFTR mutation classes IV and V have been shown to confer greater residual CFTR protein function, less severe phenotypic disease and 'non-classical' CF. Heterozygotes or homozygotes for these *residual function* (RF) mutations may have less severe CF disease; diagnosed at an older age, more likely to have exocrine pancreatic sufficiency, have better lung function and improved survival(9).

The commonest CFTR mutation in the UK CF population is the Phe508del mutation, a class II mutation, accounting for one or both abnormal CFTR gene alleles in 89.5% of patients in the UK in 2017(4). This is also the commonest CFTR mutation worldwide. The mutation is a deletion of three base pairs encoding phenylalanine at residue 508. This results in abnormal folding of CFTR protein, preventing the correct trafficking of CFTR from the nucleus to the apical membrane. There is little to no normal CFTR activity and, as previously mentioned, homozygous Phe508del CF patients typically have more severe clinical disease. In contrast, Gly551Asp mutations (class III) represent around only 5-6% of the UK population. However, both class II and III mutations have created considerable interest in recent years due to the development of novel CFTR-directed therapies. Ivacaftor was first found to be effective in patients with at least one Gly551Asp mutation(10). Homozygote and heterozygote Phe508del genotypes are responsive to dual CFTR modulation. Minimal function CFTR mutations have no response to CFTR modulation unless paired with a 'responsive' allele such as Phe508del(8). Patients with two minimal function mutations currently have no options for CFTR modulator therapy.

Class of mutation	**Resulting defect in CFTR**	**Genotype**
Class I	Defective protein synthesis, resulting in unstable or no protein expression	Gly542X, 1717-1G>A Arg553X, 621+1G→T Trp1282X
Class II	Defective protein maturation and trafficking	Phe508del, Asn1303Lys AspI507, 3659delC
Class III	Impaired chloride channel activity - a gating mutation, no protein function	Gly551Asp Arg560T, Y569Asp

Class IV	Defective chloride conductance – less protein function	Arg117His Ser549Asn
Class V	Splicing abnormalities resulting in reduction in amount of functional protein	3849+10kb→T Ala445Glu
Class VI	Accelerated turnover – less CFTR stability	1811+11.6kbA>G

Table 1.1 – Classification of CFTR mutations; class I-III (+VI) = severe CFTR mutations, class IV-V = non-severe.

1.1.5 Prognosis

When CF was first discovered as a clinical entity in 1938, survival after birth was rarely in excess of one year. Since the 1950s, developments in CF diagnosis and treatment have led to an exponential increase in survival that continues today. Notably, since the discovery of the CFTR gene in 1989, median predicted survival has increased from the mid 20's to 47 years of age in 2017. Furthermore, Keogh et al in 2018 suggested that CF adults with homozygous Phe508del mutations reaching 30 years or above can now expect to live into their 50's(11). Important developments in CF treatment that most certainly have had a huge impact on overall survival in CF have included, amongst many others, advances in antimicrobial therapy, the use of inhaled mucolytics, pancreatic enzyme supplementation and the development of specialist CF units with multidisciplinary care. In the last decade, the development and use of CFTR modulator therapy represents the potential for a positive impact on survival in CF. These novel therapies will not only modify CFTR-related complications and disease course in CF, but will also have an impact on NHS expenditure, provision of CF care and burden of treatment in an ageing chronic disease population. CFTR modulators will thus be extremely important moving forwards with an ageing CF population.

1.2 Complications of Cystic Fibrosis

1.2.1 Pulmonary exacerbation

Pulmonary manifestations remain the most important complications in terms of morbidity and mortality in the clinical course of CF(12). Viscous pulmonary secretions and deficiency of mucociliary clearance leads to chronic infection of the lungs with pathogenic bacteria,

creating a state of chronic pulmonary sepsis. Persistent infection and inflammation of the lungs lead to progressive destruction of lung parenchyma and bronchiectasis. Recurrent infective exacerbations lead to progressive decline in lung function. Lung function is monitored regularly in both inpatient and outpatient settings, using percentage predicted forced expiratory volume in one second ($ppFEV_1$) and forced vital capacity (FVC) measurements. FEV_1 is also used as a surrogate marker of survival in CF. Chronic symptoms include breathlessness, productive cough, chest pain and intermittent haemoptysis. Patients are also at risk of massive haemoptysis and pneumothorax, requiring urgent intervention. The course of lung disease in CF encompasses a chronic progressive decline in lung function with episodes of acute worsening of symptoms during a pulmonary exacerbation(13). During an exacerbation, there will usually be an acute increase in respiratory symptom burden including worsening cough, breathlessness and change in sputum volume and viscosity. There may also be a reduction in lung function and new radiological changes. A conclusive definition of pulmonary exacerbation has been contentious, however Fuch's criteria is generally used in clinical practice(14). The development of pulmonary exacerbation scoring systems have also been useful, particularly in clinical trials(15). Management is focused on both treating acute infection and suppressing chronic infection to prevent exacerbation but also to delay lung function decline.

1.2.2 Haemoptysis

Haemoptysis is defined as the expectoration of blood from the respiratory tract. The degree of haemoptysis varies depending on the severity of pulmonary disease. Massive haemoptysis is defined as the expectoration of over 240 millilitres (mls) of fresh blood, or any amount leading to airway compromise or haemodynamic instability(16).

In CF patients, haemoptysis is common, is usually mild and is seen most frequently during pulmonary exacerbation. Up to 60% of adults with CF will experience mild haemoptysis(17). Massive haemoptysis can occur in up to 4% of patients, a greater prevalence seen with age and advancing lung disease(18). The cause of haemoptysis in CF is likely to be multifactorial. The lungs have dual blood supply provided by the bronchial arteries and pulmonary vessels. Chronic inflammation and infection of the pulmonary parenchyma leads to altered vascular resistance and blood flow in these vessels, promoting angiogenesis and neovascularisation(19). The resulting collateral bronchial vessels are tortuous, thin-walled

and prone to rupture into the airway, causing haemoptysis(20). Haemoptysis can also be precipitated by inhaled mucolytics and airway clearance techniques due to irritation of the bronchial mucosa.

The management of haemoptysis in CF is based on consensus opinion from expert CF clinicians rather than national guidelines. There are no randomised controlled trials involving the treatment of haemoptysis. Mild to moderate haemoptysis can usually be managed with a combination of antimicrobials and tranexamic acid. Massive haemoptysis is life threatening and requires intensive inpatient management. If haemoptysis fails to subside with intravenous therapy and supportive measures, bronchial artery embolisation (BAE) should be considered. This is a percutaneous procedure whereby, following identification of the culprit vessel using computed tomography (CT) angiography, a catheter is passed into the bronchial circulation via the femoral artery. A range of embolic agents can be used to achieve vascular occlusion depending upon the size and location of the offending vessel, and correct choice is essential for procedure success. In general, polyvinyl alcohol (PVA) particles are most commonly used due to their preferential efficacy and safety profile when compared to both gelatin sponge and coil therapy. Success rate for short term control of haemoptysis is up to 90%(21). However, BAE does not prevent future episodes of haemoptysis and up to 60% of CF patients will have recurrence of significant haemoptysis, often necessitating further procedures(22). BAE is minimally invasive, suitable even for a less stable patient and is considered a relatively safe procedure. Chest discomfort is a frequent complication. Rare major complications can occur with embolisation of a systemic vessel and, since bronchial artery anatomy can frequently be anomalous, the procedure should be undertaken under the supervision of an experienced thoracic interventional radiologist(21).

1.2.3 Allergic bronchopulmonary aspergillosis

Aspergillus fumigatus is a fungal spore ubiquitous in the environment. It is responsible for a spectrum of clinical conditions ranging from asymptomatic airway colonisation to invasive fungal infection. Colonisation of *Aspergillus fumigatus* within CF airways is relatively common and can affect up to 58% of patients(23), often without detrimental clinical impact. However, *Aspergillus fumigatus* exists as a co-pathogen in the sputum of up to 60% of CF

patients with chronic *Pseudomonas aeruginosa*, which can negatively impact lung function(24).

Allergic bronchopulmonary aspergillosis (ABPA) is an immune mediated condition arising from inhalation exposure to *Aspergillus fumigatus* spores following sensitisation in a susceptible individual. Such individuals include those with CF and asthma. An intense humoral response ensues, characterised by the production of polyclonal IgE along with Aspergillus-specific IgE and IgG*,* producing an inflammatory reaction within the airways.

ABPA has a prevalence of between 2 and 14% in CF patients in Europe(25). Often ABPA is challenging to diagnose in CF patients due to ambiguous clinical presentation and lack of screening and diagnostic uniformity. Indicators of aspergillus lung disease may include an increasing requirement for intravenous antibiotics with less therapeutic benefit or an unexplained reduction in spirometry. Physicians follow the 2006 European guidelines by DeBoeck et al (2006)(26) and those more recent by Farrell et al (2017)(27) for diagnosis, which include a combination of unexplained subacute clinical decline, confirmatory aspergillus serology and new and persistent radiological findings(28).

Management goals include control of airway symptoms and prevention of pulmonary exacerbation. Oral corticosteroids are the mainstay of ABPA treatment in CF, with anti-fungal treatment added if there is inadequate clinical response. Anti-fungal therapy reduces fungal burden within the lung and creates a steroid-sparing effect, allowing the dose of corticosteroid to be decreased to minimise side effects. The response to anti-fungal treatment however is variable and side effects are common. Subcutaneous anti-IgE therapy, such as Omalizumab, may also be used in resistant cases to reduce corticosteroid dependency and side effects(29). Environmental or occupational exposures must be sought and minimised where possible.

1.2.4 Pneumothorax

The pleural space exists between the parietal and visceral pleural membranes and, in health, should only contain a small amount of fluid. A pneumothorax is defined as air within the pleural space, occurring due to a breach in both the parietal and visceral pleural membranes. A primary pneumothorax can occur in normal lungs. A secondary pneumothorax occurs in the context of underlying lung disease. In CF, pneumothorax can

occur in up to 3.4% of patients during their lifetime(30). The prevalence increases with age and advancing lung disease with most occurring in adulthood. Several risk factors may contribute including chronic *Pseudomonas aeruginosa* or *Burkholderia cepacia* complex (BCC) infection, an FEV_1 of less than 30%, the presence of ABPA and the use of inhaled therapies such as tobramycin and dornase alfa(17). There is also a risk of pneumothorax with an episode of massive haemoptysis.

The symptoms of pneumothorax may be subtle but new chest pain or worsening breathlessness should prompt investigation. The management of pneumothorax in CF is similar to those without CF, will vary with the size of pneumothorax and should follow the national guidelines(31). Placement of an intercostal drain into the pleural space will usually be required.

The recurrence rate of pneumothorax in a CF patient is between 50 to 90% and may be in the contralateral lung(32). Talc pleurodesis can be performed in a persistent or recurrent pneumothorax, but one must consider future lung transplant and the technical difficulties a pleurodesed lung would present at explantation. The mortality rate for a first pneumothorax may be up to 14% at two years and is higher in those with more severe underlying pulmonary disease(30)(33). Patients must continue with chest clearance as able, although manoeuvres creating positive end expiratory pressure must be avoided. Antibiotics are usually given during treatment of a pneumothorax and will help to prevent pleural infection. CF physicians must work closely with thoracic surgeons and intensive care support may be required in complex cases. Non-invasive ventilation may also be required in cases of advanced lung disease since pneumothorax may worsen or precipitate hypercapnia.

1.2.5 Microbiology

CF microbiology is complex. Airway colonisation changes with age, treatment and development of resistance patterns. The microbiological make-up of a CF lung has huge implications for morbidity and mortality, including response to treatment, rate of lung function decline and suitability for transplant. The most prevalent bacteria in the CF airway include *Staphylococcus aureus* and *Haemophilus influenza* at younger ages, with the prevalence of *Pseudomonas aeruginosa* increasing with time(1). *Figure 1.1* depicts the changing prevalence of bacteria in the CF airway with age.

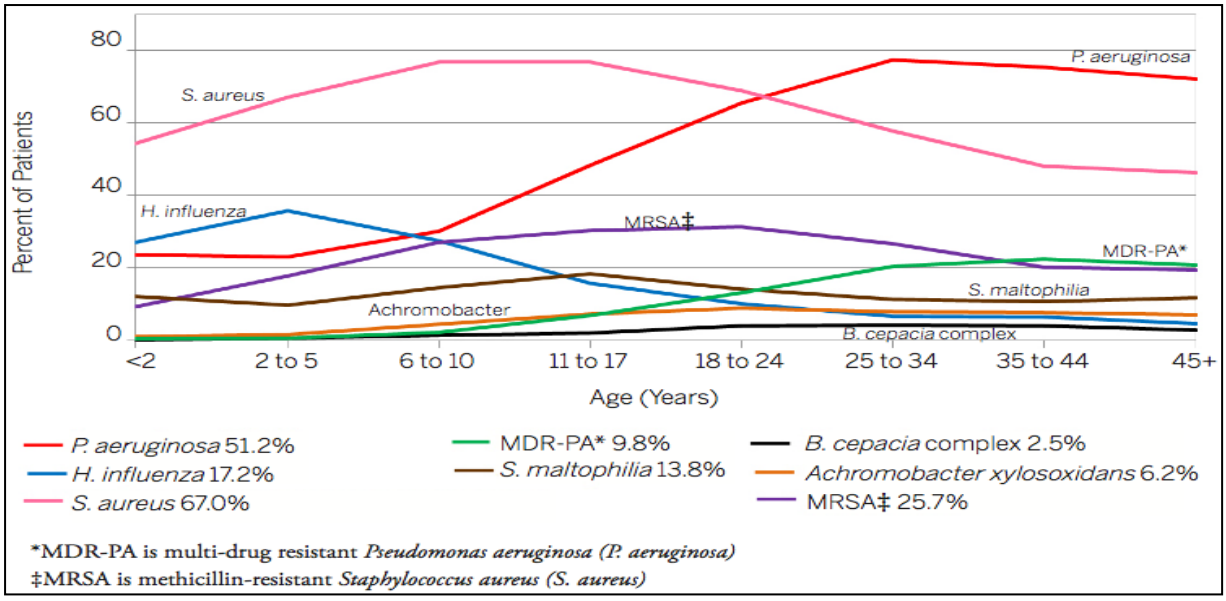


Figure 1.1: The changing prevalence of bacteria in CF airways with age(34)(35).

Treatment of *Pseudomonas aeruginosa* is initially centred on eradication, but inevitably chronic colonisation ensues, and the focus becomes suppression and prevention of pulmonary exacerbation. *Pseudomonas aeruginosa* is one of the most important bacteria in the CF lung in terms of morbidity and decline in lung function. Initially colonising the upper airways in a non-mucoid form, with time there may be progression to colonisation of the lower airways, forming biofilms and chronic infection. The morbidity of chronic *Pseudomonas aeruginosa* colonisation is high, with recurrent exacerbations, declining lung function and the requirement of long term nebulised antibiotic regimens(36). Non-tuberculous mycobacteria, methicillin resistant *Staphylococcus aureus* (MRSA) and *Burkholderia* species are CF pathogens with a lower prevalence but with important morbidity, particularly in an older CF lung. Fungal infection, including *Aspergillus fumigatus*, and respiratory viruses also play a large role in symptomatology, exacerbation rate and pulmonary decline in CF patients.

CF patients are segregated into specific microbiological groups in both outpatient and inpatient settings in order to minimise the spread of transmissible infection. This is particularly important for epidemic strains of *Pseudomonas aeruginosa*, which have been shown to have aerosol spread among CF patients(37) and confer increased resistance patterns and virulence in the host(38). It is also essential to segregate patients with some strains of *Burkholderia* species, particularly *Burkholderia cenocepacia*, and those with

chronic *Mycobacterium abscessus* and MRSA infection, all considered to be highly transmissible. A change in sputum microbiology in a CF patient due to transmissible infection can have drastic consequences for overall survival, including suitability for lung transplantation. *Burkholderia cenocepacia* is a particularly virulent and antibiotic-resistant species of the *Burkholderia cepacia* complex *(BCC).* In CF, chronic infection with this species can cause a significant decline in lung function and risk of 'cepacia syndrome'(39), a life-threatening pneumonic process with systemic infection and few options for treatment. Infection with *Burkholderia cenocepacia* in a CF patient is currently considered a contraindication for lung transplantation due to poor post-transplant survival(40).

1.3 Metabolic complications

1.3.1 Cystic fibrosis related diabetes mellitus

Cystic fibrosis-related diabetes mellitus (CFRD) is the most common extra-pulmonary complication in CF. It is primarily caused by a deficiency of endogenous insulin due to fibrotic destruction of pancreatic islet cells, but insulin resistance also plays a role(41). Although sharing some of their characteristics, it appears to be a distinct entity to type one and type two diabetes mellitus(42). Impaired glucose tolerance is common in CF and may progress to CFRD over time. The prevalence of CFRD increases with age, with around 50% of patients affected by the age of 30 years(43). Its presence is also affected by genotype, exocrine pancreatic insufficiency and gender. Mortality in patients with CFRD remains higher than in those without(44). High glucose load in the airways contributes to mucous retention, pulmonary exacerbation, lung function decline and poor nutritional status. Pulmonary function and nutritional status correlate strongly in CF and both are worse in patients with CFRD than those without(45), hence timely detection and optimal management of CFRD is essential. Annual glucose tolerance tests and selective continuous glucose monitoring is utilised for screening, along with regular monitoring of longer-term glucose control using HbA1c measurements, a measure of glycosylated haemoglobin. Although oral treatments are being investigated and may be adequate in some cases, the mainstay of treatment is subcutaneous insulin therapy. Insulin resistance in CF will fluctuate over time, during pulmonary exacerbation and with oral corticosteroid use(46), thus blood glucose will need to be monitored regularly and insulin dosing adjusted accordingly.

Aggressive treatment of CFRD is also important in order to prevent microvascular complications that develop over time, including retinopathy, nephropathy and peripheral neuropathy(47). It has been thought that these complications may occur with less frequency and severity in the CF population when compared to the general diabetic population. However, increasing age, duration of diabetes mellitus and glycaemic control all play important roles in the development of diabetic complications and subsequent organ dysfunction.

Macrovascular complications are frequent causes of morbidity and mortality in the non-CF diabetic population(48). However, historically these complications are infrequently seen in CFRD. With an ageing CF population, we may begin to see macrovascular complications in CF patients related either to CFRD or to the ageing process itself. Contributing factors may include a long duration of high fat diets, hypercholesterolaemia and systemic hypertension. Renal complications with age may also become more frequent, attributed to a number of causative factors. These areas will be further discussed in subsequent chapters.

1.3.2 Cystic fibrosis-related bone disease

Impaired bone mineral density (BMD) in CF patients is caused by a variety of mechanisms, including malabsorption, vitamin D deficiency, impaired calcium metabolism, delayed puberty and chronic inflammation. In addition, corticosteroid therapy, diabetes mellitus, reduced physical exercise and hypogonadism may contribute. Low BMD in CF patients presents a greater risk of fracture than in the general population(49). Fracture of the ribs and thoracic spine are of particular clinical significance due to impaired chest clearance, predisposing to infection and pulmonary exacerbation.

Osteoporosis often correlates with disease severity and clinical status in CF, including lung function, diabetic control and BMI(50). Those patients with more severe disease may have lower BMD due to poorer nutritional status, less physical activity and perhaps more oral corticosteroid use. The chronic inflammatory state in CF can itself contribute to low BMD. The over-production of pro-inflammatory cytokines TNF alpha, IL-1 and IL-6 in a CF catabolic state can alter bone metabolism and stimulate osteoclast activity, contributing to increased bone resorption and low BMD(51). This process may be accelerated during pulmonary exacerbation. CFTR is expressed in bone cells and thus can have a direct impact on bone metabolism and BMD. BMD in CF patients is monitored regularly using dual energy X-ray

absorptiometry (DEXA). Low BMD is classed as a Z score of less than minus two in selected areas. The management of low BMD and osteoporosis in CF includes vitamin D and calcium supplementation. Bisphosphonate therapy is used if there is significant loss of BMD over time, for treatment of osteoporotic fractures and for pre-lung transplantation optimisation. This should be coupled with regular dietetic and physiotherapy input, including maintenance of optimal BMI and performance of regular weight bearing exercise. BMD status can affect suitability and outcome of lung transplantation. Immunosuppressive therapy following lung transplantation contributes to loss of BMD and bisphosphonate therapy is often required.

Low vitamin D levels may be associated with increased cardiovascular risk, particularly in the setting of chronic kidney disease(52). This may be important in an ageing CF population however, further studies will be required to establish a causal link.

1.4 Gastrointestinal/Nutritional complications

In 1938, Dorothy Andersen first discovered cystic fibrosis as a clinical entity related to disease of the pancreas(53). Although respiratory complications usually dominate and are the main cause of mortality in CF, gastrointestinal complications are often present from early life.

Exocrine pancreatic insufficiency (PI) is common in CF and affects up to 85% of patients(54). Its presence usually correlates with genotypes of CF that are associated with more severe disease phenotypes, and has historically proven to confer survival disadvantage(55). Exocrine pancreatic insufficiency is caused by destruction of pancreatic ducts by loss of adequate pancreatic secretions, leading to gradual fibrotic destruction of the pancreas. This causes pancreatic digestive enzyme deficiency and malabsorption, manifesting clinically as steatorrhoea, failure to thrive and depletion of fat-soluble vitamins (A, D, E, K)(56). Exocrine pancreatic insufficiency is measured clinically by the presence of pancreatic enzymes in spot stool samples. The most commonly used method is faecal elastase-1 (FE-1), a level of <200mg/g being confirmatory(55).

CFTR-related intestinal bile acid dysfunction in CF also leads to fat malabsorption, along with failure of intestinal bicarbonate secretion. Management of malabsorption and subsequent nutritional deficiencies includes implementation of a high fat diet, pancreatic enzyme

replacement therapy (PERT) and vitamin supplementation. Enteral support may be required if body mass index (BMI) is insufficient despite these measures. Optimisation of nutritional status has been shown to improve survival. Low body weight, particularly with advancing age and chronic *Pseudomonas* aeruginosa infection, predicts a greater lung function decline and morbidity in CF(57).

In mice models, high fat diets have been shown to increase adipose tissue deposition, raise total blood cholesterol and lead to fat deposition in the liver. These contribute to a metabolic syndrome of known cardiovascular risk factors(58) and may prove to be important when looking at overall cardiovascular risk in ageing CF patients.

Other gastrointestinal complications in CF include constipation, distal intestinal obstruction (DIOS), gastro-oesophageal reflux disease (GORD) and pancreatitis. DIOS is the older CF patient equivalent of meconium ileus, occurring due to faecal impaction and leading to small bowel obstruction. Management involves rehydration, optimisation of pancreatic enzyme supplementation and oral gastrografin. Increased rates of GORD are seen in CF patients, precipitating cough, aspiration of gastric contents and pulmonary exacerbation. Recurrent episodes of pancreatitis in a pancreatic sufficient patient may eventually lead to exocrine pancreatic insufficiency, which in turn predisposes to CFRD.

1.5 Cancer in CF

As CF patients live longer, the risk of malignancy will increase as with a non-CF ageing population. In transplanted patients, the risk of lymphoma and skin cancer is high due to chronic immunosuppression. In non-transplanted patients, there may be an increased risk of gastrointestinal tract malignancy, including colorectal and pancreas(59). In a United States cohort study in 2013, following patients over a twenty-year period, the risk of colorectal malignancy was found to be higher in CF patients compared with the national average with a higher incidence in the Phe508del genotype group(60). Variation in expression of CFTR protein between organs may influence the corresponding cancer risk. High CFTR expression is maintained throughout the intestinal crypts, pancreas and biliary system(54). Epithelial cell dysfunction and viscous intestinal mucous leads to bacterial overgrowth and may contribute to tumour suppressor gene dysregulation(61). The early detection of colorectal cancer and premalignant adenomatous polyps has been shown to reduce mortality. With colon cancer being the commonest malignancy in CF, with an incidence of up to five times

that of the general population, it is now recommended that CF patients over 40 years of age commence annual colonoscopy screening, even in the absence of bowel symptoms(62).

1.6 Hepatobiliary complications of CF

Gallbladder and liver pathology are common in CF, including cholelithiasis and bile duct abnormalities, hepatic steatosis and cirrhosis, and drug hepatotoxicity. Cystic fibrosis related liver disease (CFLD) tends to be diagnosed in the paediatric and adolescent CF population, and up to 20% of these patients develop liver cirrhosis(63). The underlying histopathological process of CFTR dysfunction is that of defective enterohepatic biliary circulation, plugging of intra-hepatic biliary ducts and focal biliary cirrhosis(64). Those with male gender, exocrine pancreatic insufficiency and more severe CFTR mutations appear more susceptible. Management is based on early detection by annual liver enzyme and ultrasound screening, optimising nutritional status and the use of ursodeoxycholic acid, which may halt disease progression. Patients with CFLD must be followed up regularly, often in conjunction with a liver specialist, to assess progression and development of complications. Portal hypertension in liver cirrhosis can lead to the development of oesophageal varices, associated with gastrointestinal bleeding. Liver failure, independent of lung status, may be seen in CF patients if disease progresses and liver transplantation should be considered(65).

Hepatic steatosis has been recognised in cystic fibrosis, associated with a higher BMI and better preserved lung function, and appears to bear similarities to non-alcoholic fatty liver disease seen in non-CF populations(66). As survival in CF improves, we may see an increasing prevalence of diabetes mellitus, and CFTR modulation may contribute to better pancreatic exocrine function and higher BMI. Consequently, we may see more patients with hepatic steatosis rather than the biliary cirrhosis classically recognised in CFLD, of which the progression to cirrhosis is unknown.

1.7 Renal pathology in CF

CFTR is abundantly expressed in the urinary tract epithelium. Abnormal transmembrane ion movement and abnormalities of calcium metabolism in CF predispose to nephrolithiasis, the development of urinary tract stones. In addition, fat malabsorption leads to hyperoxaluria, predisposing to the development of oxalate stone formation in the renal tract(67). Recurrent antibiotic use may lead to reduction of oxalate-degrading bacteria in the gut, also

predisposing to oxalate stones(68). Renal stones, depending on their size, can cause obstruction of the renal tract leading to hydronephrosis and acute renal failure, requiring percutaneous or surgical intervention. They may also be associated with urinary tract infection. Recurrent renal tract stones may lead to gradual destruction of renal parenchyma and chronic renal failure(69).

CF patients are at risk of acute renal failure from a variety of mechanisms. Pulmonary exacerbation may precipitate septicaemia and hypovolaemia, and enhanced salt-wasting and dehydration are contributory factors. Recurrent pulmonary sepsis with concurrent acute renal injury may contribute to chronic renal failure. During pulmonary exacerbation, exposure to intravenous aminoglycosides, with their effective anti-pseudomonal properties but also nephrotoxicity, has been shown to cause renal impairment. Serum drug levels and renal function must be monitored carefully to minimise this risk. This will be expanded upon in subsequent chapters.

Diabetes mellitus is a major risk factor both for the development of premature cardiovascular disease and chronic renal failure(70). Diabetes mellitus, vascular disease and chronic infection will all accelerate normal renal nephron loss in an ageing kidney and will contribute to the development of chronic renal failure over time(71). In addition, immunosuppressive medication following lung transplantation in CF patients may have consequences for renal function and must be monitored closely in the post-transplant period.

1.8 Treatment of cystic fibrosis

1.8.1 General overview

Traditionally, the management of CF has concentrated on the downstream effects of CFTR dysfunction and organ-specific consequences. Since the discovery of CF as a clinical entity in 1938, treatment has advanced significantly and median survival is increasing each year. Whilst predicted survival of children born with CF in the 1950s was less than one year, the median predicted survival of a child born in 2017 with CF is 47 years and over 60% of patients living with CF in the UK today are adults(4).

Our understanding of the pathophysiology of CF, diagnostic testing and the discovery of new therapies in the last 80 years has progressed such that survival has increased exponentially with time(72).

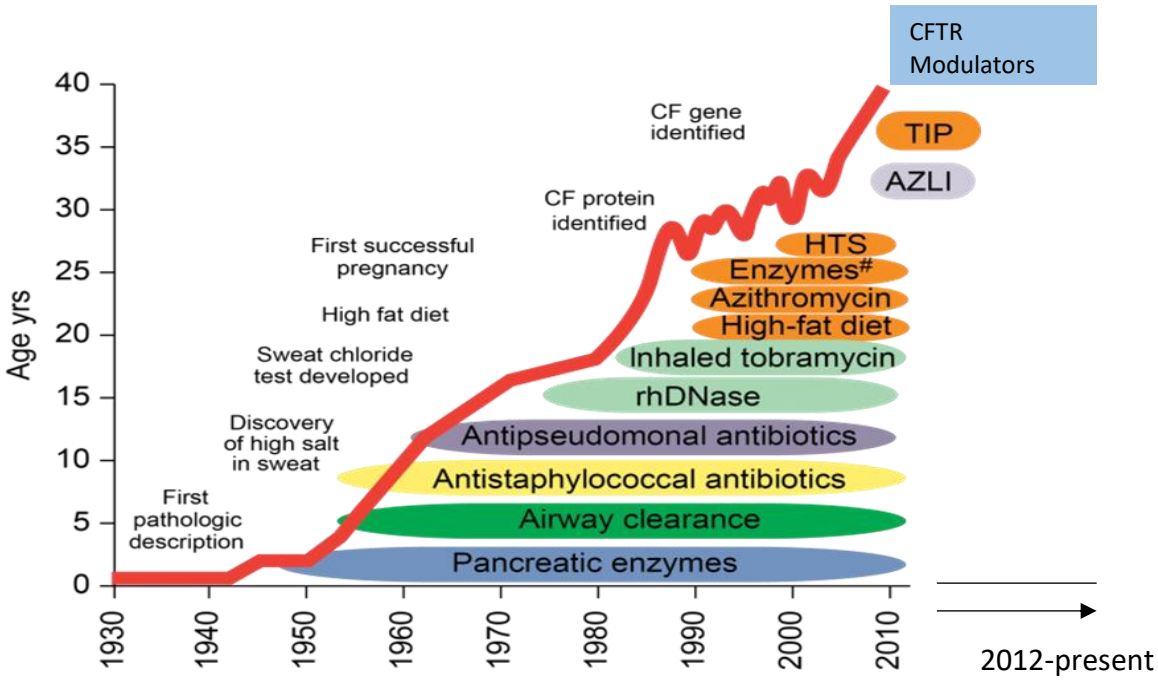


Figure 1.2 –The development of novel CF therapies over time and their influence on survival(72).

The discovery of organism-specific inhaled antibiotics, including those with anti-pseudomonal and anti-staphylococcal properties, revolutionised CF management in the 1950s. Antimicrobials in oral, inhaled and intravenous form are used in chronic and acute management of CF, and regimens are tailored to the individual sputum microbiology and resistance patterns. Those with chronic pulmonary infection, such as *Pseudomonas aeruginosa* colonisation, may be on two different inhaled antibiotics alternating each month. This is done to minimise the development of microbial resistance and to provide continuous antimicrobial cover. Inhaled and oral antimicrobials are also important in eradication regimens following a first growth of *Pseudomonas aeruginosa.* Delay in acquisition of chronic *Pseudomonas aeruginosa* infection may be an important factor in CF survival.

Nutritional status also plays a role in CF longevity. Poor nutritional state correlates with worsening lung function and survival. High metabolic demand and malabsorption in CF

account for malnutrition. Supplemental calorific drinks can be provided along with oral vitamin preparations. Pancreatic enzyme supplementation was introduced in the 1930s and has been developed since then such that fat absorption can now be optimised to enhance growth and normalise body concentrations of fat-soluble vitamins. Diabetic control must be optimised, gastro-oesophageal reflux managed effectively, and pulmonary exacerbations treated aggressively. Nasogastric (NG) and percutaneous endoscopic gastrostomy (PEG) feeding can be performed if BMI remains poor despite the above measures. Regular dietetic input is essential.

Sputum in CF is tenacious and thus inhaled mucolytics such as dornase alfa, hypertonic saline and mannitol are used to aid sputum expectoration, along with intensive physiotherapy techniques for airway clearance. Individual response is variable but evidence has shown a reduction in exacerbation rate and improved lung function with inhaled mucolytic therapy(73), although one does not seem to show superiority over the others. Side effects include bronchoconstriction and cough and thus supervised test doses must be performed to ensure safety and tolerance.

1.8.2 Antimicrobial use in CF

1.8.2.1 Macrolides

Macrolide antibiotics have pleiotropic properties, including anti-microbial, anti-inflammatory and immunomodulatory(74). Long term azithromycin use is currently recommended in the management of adult CF patients. Advantages include delayed decline in lung function along with reduction in pulmonary exacerbation frequency. There are also modest improvements in quality of life and nutritional status(75). The exact mechanism of action of azithromycin is unknown, although *in vitro* it has been shown, in addition to its antimicrobial properties, to reduce neutrophilic inflammation and pro-inflammatory cytokine levels, including IL-8 and TNF alpha(76). The clinical benefit was first shown in patients with bronchiolitis obliterans syndrome(77). Azithromycin has anti-pseudomonal properties but has also been shown to be beneficial in non-pseudomonal CF cohorts(78).

Sensorineural hearing loss has been recognised as an infrequent adverse effect of long-term azithromycin use(79). In the CF community, the risk of adverse events appears to be outweighed by the clinical benefits.

There are concerns about the evolution of macrolide-resistant non-tuberculous mycobacteria (NTM) colonisation with long term azithromycin use. As such, it is recommended that CF patients are screened for NTM prior to commencement of azithromycin and then screened routinely whilst on the drug.

1.8.2.2 Aminoglycosides in CF

Aminoglycosides are bactericidal antimicrobials and their mechanism of action is to inhibit prokaryotic ribosomal bacterial protein synthesis(80). They have been popular historically in the hospital setting due to their rapid efficacy against gram negative bacterial infections and minimal microbial resistance. However, as research into their pharmacokinetics has evolved, there is now a greater awareness of their potential renal and cochleovestibular toxicity profiles.

Aminoglycosides work as concentration-dependent antibiotics, which means that increasing serum concentrations result in increasing bacterial kill. The peak serum concentration (Cmax) and area under the curve (AUC) are important for total antibiotic exposure and resulting efficacy. Aminoglycosides not only have a rapid distribution and achievement of Cmax, but they also have significant post-antibiotic activity (equating to AUC), which will continue even beyond minimal inhibitory concentration (MIC). These properties enable aminoglycosides to have optimal efficacy at a once daily dosing regimen and, when compared to multiple dosing regimens, exhibit less nephrotoxicity(81).

Aminoglycosides have a narrow therapeutic index are excreted exclusively by glomerular filtration in the kidneys. They are dosed according to individual patient weight and renal function. Those patients with abnormal renal function will clear aminoglycosides at a slower rate, leading to a longer duration of drug exposure and greater potential for nephrotoxicity. Renal accumulation of aminoglycoside causes direct damage to renal proximal tubular cells. Dose reductions will be therefore be required in those with renal disease and this may have implications for efficacy. Other factors leading to aminoglycoside nephrotoxicity include advancing age, diuretic use, volume depletion and concomitant use of other nephrotoxic medication.

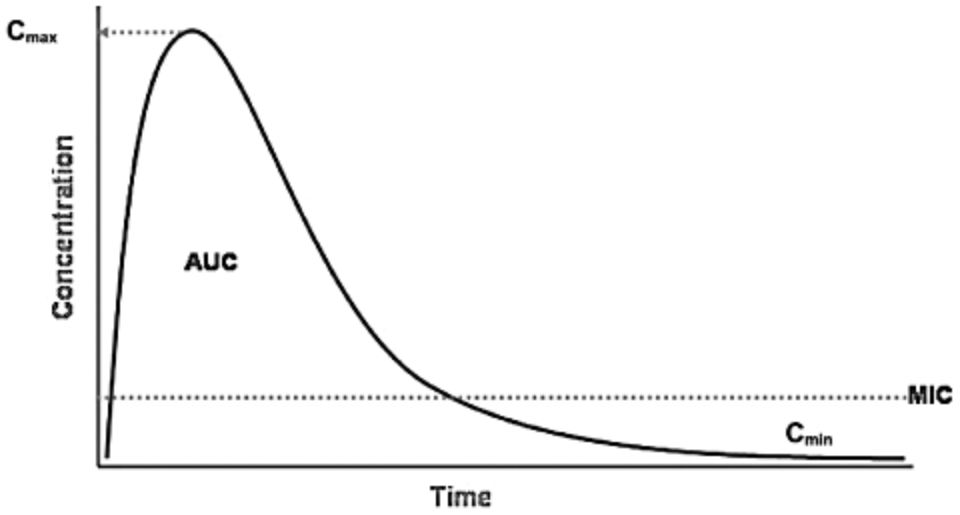


Figure 1.3 – A pharmacodynamic model of aminoglycoside pharmacokinetics(82).

Although intravenous aminoglycosides can be used for empirical treatment for a short duration, there are only a small number of specific indications for extended therapy. These include infections with *Pseudomonas aeruginosa,* streptococcal bacterial endocarditis and infections resistant to other antimicrobials. Intravenous and nebulised aminoglycosides are important treatment options in CF due to their anti-pseudomonal and anti-mycobacterial properties. They are used for the treatment of pulmonary exacerbation in intravenous form, and for anti-pseudomonal maintenance and eradication therapy in inhaled form(83). They are usually used in synergy with other antimicrobials, such as beta lactams(80). Due to repetitive pulmonary exacerbations dominating the clinical course of most CF patients, the lifetime cumulative aminoglycoside exposure can be high. This will be of particular importance as median age of CF survival continues to increase. The pharmacokinetics of aminoglycosides in the CF population is different to a non-CF population. CF patients require higher doses for the desired therapeutic effect due to larger volumes of tissue distribution and faster renal clearance. However, clearance of aminoglycosides may be slower in the adult CF population compared with paediatric(84). Aminoglycosides are associated with important adverse effects, including ototoxicity, vestibular toxicity and nephrotoxicity. Tobramycin is predominantly used in CF, in preference to other aminoglycosides, due to its lower nephrotoxic profile.

1.8.2.3 Nephrotoxicity

There are several factors contributing to both acute and chronic renal failure in CF. Repetitive use of aminoglycosides has been shown to contribute to renal dysfunction in CF.

Aminoglycosides induce proximal renal tubular cell apoptosis and necrosis. Progressive renal tubular damage increases resistance in the renal vascular bed and causes a reduction in glomerular and renal blood flow(85).

Creatinine clearance has been shown to decline with increasing courses of aminoglycosides(86). Gentamicin appears to be more nephrotoxic than tobramycin or amikacin, hence it is avoided in CF. In the paediatric CF population, aminoglycosides have been shown to cause acute renal tubular injury following two weeks of intravenous treatment(87). Tobramycin is recommended as a once-daily dosing regimen with careful drug level monitoring in order to minimise the risk of nephrotoxicity and maximise therapeutic benefit.

1.8.3 Gene Therapy in CF

The first CF gene was cloned in 1989 and led to the development of pulmonary gene therapy in CF. Phase one trials, around 20 to date, were designed to transfer a normal CFTR gene carried on an adenovirus vector onto the epithelial lung surfaces via inhalation. Adequate uptake of the corrected CFTR gene by 'CF cells' was then thought to have the ability to modify epithelial CFTR expression and correct chloride ion transport, with a hope to normalise organ function(88). Although modification of abnormal CFTR expression on large airway epithelium seemed reasonable via inhalation, smaller airways were more difficult to reach and other barriers to efficacy such as mucous retention and chronic bacterial infection presented problems. Non-viral gene therapy was studied by Alton et al in 2015, studying lung transfer of plasmid DNA encoding the CFTR gene contained within a cationic liposome, showing this to be feasible and with a modest increase in lung function at one year(89). Research into gene therapy in CF is ongoing.

1.8.4 CFTR modulators in CF

Until recently, treatment of CF could only focus on symptom control and slowing lung function decline. However, the introduction of CFTR modulating therapies has changed the landscape of CF treatment over the last decade. Correction of abnormalities in CFTR protein function and expression are now the targets of therapy rather than the consequent downstream organ dysfunction. The CFTR genotype is of particular relevance in the era of CFTR modulation since class determines patient eligibility for certain modulators.

Ivacaftor (Kalydeco®) was the first CFTR modulator developed and was approved for clinical use in 2012. As a CFTR potentiator, it increases the time of opening of the CFTR channel and thus augments epithelial chloride transport. It is designed for CFTR 'gating mutations' with abnormal CFTR channel opening, the commonest of which is Gly551Asp. This genotype however only accounts for a minority of CF patients in the UK. The STRIVE and ENVISION trials(10) showed ivacaftor, compared with placebo, to have statistically significant improvement in lung function and BMI at 48 weeks, as well as a reduction in pulmonary exacerbations and sweat chloride levels. The open label PERSIST trial(90) showed this benefit to be sustained after 144 weeks. These trials included only those patients with better lung function and excluded those with an FEV_1 of below 40% predicted. A subsequent trial by Barry et al in 2014 showed ivacaftor to be just as effective in CF patients with more severe lung disease(91).

Many abnormal CFTR genes have been shown to cause clinical CF and abnormal CFTR shows great "allelic diversity"(92). Around 85-90% of Caucasian CF patients carry either one or two copies of the Phe508del mutation, a class II mutation with reduced functional CFTR protein at the epithelial cell surface. In the last few years, there have been exciting advances in CFTR modulation for Phe508del mutations and hence potentially life-altering treatment in a significant proportion of CF patients. Ivacaftor/Lumacaftor (Orkambi®) is a combination CFTR corrector (lumacaftor) and CFTR potentiator (ivacaftor) modulator therapy used for class II CFTR mutations. Ivacaftor and lumacaftor work synergistically to correct CFTR protein misfolding and increase its delivery to the cell surface, thus improving chloride transport and modifying downstream organ dysfunction. Phase three clinical trials conducted in 2013 and 2014, the TRAFFIC and TRANSPORT studies(93), showed sustained clinical benefit over a 24-week period with reduced pulmonary exacerbation rate, improved lung function and BMI, and improved quality of life scores. In some cases, FEV_1 improved up to 10% compared with placebo. Importantly, clinical benefit spanned a wide range of baseline lung function, including those with an FEV_1 of less than 40% predicted. The greatest clinical benefit was seen in patients with homozygous Phe508del mutations. Chest tightness may be a barrier to the clinical efficacy of Orkambi®, which is usually self-limiting after a few weeks. In addition, liver function must be closely monitored and Orkambi® is contraindicated in those with significantly abnormal baseline liver function. In-patient

commencement of Orkambi® can be done with close lung function monitoring, in patients with homozygous phe508del mutations and with a baseline FEV_1 of less than 40% predicted. Orkambi® is now rarely used in adult CF patients and has been superseded by triple CFTR modulation therapy.

Following the introduction of Orkambi® into CF management, a further combination therapy of Tezacaftor and Ivacaftor (Symdeko®) was been subsequently developed and may have an advantageous adverse event profile as compared with Orkambi®. It has also shown *in vivo* efficacy in patients with residual function mutations, thus may benefit a wider range of CFTR mutation groups(94). In current clinical practice in the UK, Symdeko® may be used as an alternative in homozygous Phe508del patients who cannot tolerate Orkambi®. In addition, the recent emergence of 'triple' CFTR modulation therapy has been an exciting development in CF treatment. Kaftrio® is now licensed for use in the UK in Phe508del homozygotes, and Phe508del heterozygotes with either a minimal or residual function CFTR mutation. Phase 3 trials in patients aged 12 years and above, with $ppFEV_1$ of between 40 and 90%, have shown tremendous reductions in sweat chloride levels (over 40mmol/L), an increase in $ppFEV_1$ of up to 14.3% and reduction in pulmonary exacerbation rate by 63% over a 28 day period(95)(96). Further clinical data is eagerly anticipated and the longer term efficacy of triple therapy will undoubtedly impact survival in CF.

1.8.5 Oxygen, NIV and transplant in CF

The typical CF disease course usually is one of progressive respiratory failure and end stage lung disease. Domiciliary oxygen therapy and non-invasive ventilation (NIV) are strategies that have been adopted in routine CF care, either as palliative interventions or as a bridge to lung transplantation. After years of experimental work, the first human lung transplant was performed by James Hardy in the USA in 1963. Although initial survival was poor, the development of effective immunosuppressive therapies has meant that the median survival following double lung transplantation is now around 8.9 years for CF patients(97). Patients undergoing lung transplantation for CF, although numbers are lower, have better outcomes than patients with other transplantable chronic lung diseases.

1.8.6 The MDT in CF

Patients with CF require regular contact with healthcare professionals in both their acute and chronic disease course. The development of multidisciplinary team (MDT) working,

specialist clinics, strict infection control practices and patient segregation are invaluable interventions and have all heavily contributed to increased survival in CF. All are now included in the European Cystic Fibrosis Society Best Practice guidelines(98). MDT models in CF care are also of vital importance in the transition of patients from paediatric to adult care.

Increasing survival of the CF patient will present further challenges to the CF MDT. The demographics of survival will have an impact on clinical manifestations of CF in the future. Ageing in CF will likely present further complexities in management, with an increase in treatment burden and the emergence of microbial resistance. Age-prevalent CF complications with become an increasing concern, including CFRD, osteoporosis and malignancy. General age-related complications may also have significant impact on management and survival, including cardiovascular disease and renal disease, potentially compounded by conventional CF treatment strategies.

1.9 Ageing in general population

The UK population is ageing. In 2017, 18.2% of the UK population were over 65 years of age, compared with 15.9% in 2007. In 2027, this figure is predicted to increase to 20.7%(99). Ageing in humans is a process that involves gradual physiological and functional decline over time. At a cellular level, reactive oxygen and nitrogen species accumulate over time and cause oxidative stress to tissues, progressing to organ dysfunction(100). The rate of biological ageing depends on both environmental and genetic factors and will vary between individuals. Ageing is a risk factor for cardiovascular disease, chronic renal failure, chronic lung pathology, lung function decline and malignancy.

1.9.1 Cardiovascular disease and age

Cardiovascular disease (CVD) remains the most common cause of death in the developed world. In 2015, CVD was the second commonest cause of death in the UK(101). Although overall mortality has decreased in recent years in the UK, CVD morbidity remains prevalent. Research in the twentieth century in this area has focused on increasing our understanding of cardiovascular disease and its risk factors, with a view to both primary and secondary prevention. The Framingham Heart Study began in 1948 and is ongoing(102). It is the largest epidemiological cohort study of its kind and has identified the cardiovascular 'risk factors' we refer to in practice today. Indeed, the Framingham risk scoring system was derived from this research and enables clinicians to calculate individual cardiovascular risk from multiple clinical and epidemiological parameters. Cardiovascular disease includes ischaemic heart disease, cerebrovascular accident (CVA/'stroke'), peripheral vascular disease and heart failure. Risk factors for CVD can be separated into modifiable and non-modifiable. Modifiable factors include smoking, obesity, systemic hypertension, hyperlipidaemia and diabetes mellitus. Non-modifiable risk factors include age, sex, ethnicity and genetic susceptibility. In addition, low socioeconomic status is associated with a higher risk of CVD.

The hallmark of vascular disease is the development of atherosclerosis. The atherosclerotic process involves the formation of fat cells in the intimal layer of the arterial wall due to infiltration by lipoprotein particles. This stimulates endothelial cell activation and the release of cytokines, which results in the formation of an atheroma. Smooth muscle cell proliferation and migration to the atheromatous area leads to atherosclerotic plaque

development. Blood flow is disrupted in the area of atherosclerosis and predisposes to thrombus formation(103). Atherosclerotic plaque is more prone to developing at areas of high flow velocity within the artery. Atherosclerosis is inevitable with ageing but conditions predisposing to its premature development include systemic hypertension, dyslipidaemia, diabetes mellitus, smoking, obesity and genetics.

1.9.2 Hypercholesterolaemia

Cholesterol is an essential structural component of cell membranes and its metabolism is regulated by the liver and gastrointestinal tract. It is transported in low density and high density lipoprotein molecules. Cholesterol is a major component of atherosclerotic plaque formation and, in high quantities, can contribute to premature CVD. Risk factors for high serum cholesterol include poorly controlled diabetes mellitus, high fat diet, obesity, smoking and high levels of alcohol consumption. In addition, liver disease and nephrotic syndrome are associated with secondary dyslipidaemia. In clinical practice, routine serum lipid measurements include total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels. More specific profiles may also include apolipoprotein measurements. Normal lipid profile parameters and definitions of hypercholesterolaemia will vary slightly between centres but will be based on a combination of raised serum total cholesterol, LDL-C levels, LDL/HDL-C ratio and triglycerides.

Familial hypercholesterolaemia is a genetic cause of high cholesterol and of premature cardiovascular disease. A significantly raised cholesterol level with a suggestive family history should prompt further investigation.

Multiple large multicentre primary and secondary prevention studies over the last five decades have shown that a reduction in cholesterol results in a significant reduction in ischaemic heart disease and subsequent mortality. These include the Helsinki Heart Study(104), the Lipid Research Programme(105) and the Coronary Drug Project Research Group(106). This has led to the widespread use of lipid lowering medication, the commonest of which is the statin group. Statins act to drive LDL-C particles out of circulation via inhibition of the HMG-CoA reductase pathway(107). Current NICE guidance recommends the use of both lifestyle modification and lipid lowering therapies in the management of hyperlipidaemia, following individual cardiovascular risk prediction scoring. For primary CVD

prevention, if lifestyle modifications fail to lower cholesterol and there is a 10% or greater risk of CVD within 10 years, patients are offered a low dose statin. In secondary prevention for those who have already sustained a cardiovascular event, high dose statin therapy is recommended(108).

LDL-C is the most atherogenic component of cholesterol and increased LDL-C levels correlate with a greater risk of cardiovascular disease, particularly coronary artery disease. LDL-C particles become oxidised within the intimal layer of the vessel wall and are taken up by macrophages to produce foam cells. These cells drive the atherosclerotic process(109). It is important to note that LDL-C is a derived measure using the *Friedewald formula*(110) and cannot be measured directly;

***Friedewald formula: LDL-C** (mg/dL) = TC (mg/dL) − **HDL-C** (mg/dL) − TG (mg/dL)/5.*

Friedewald's calculation may be inaccurate in the setting of high TG levels (>4mmol/L), giving rise to the development of other LDL-C equations and novel markers of serum cholesterol burden, such as apolipoproteins, over recent decades. Despite this, Friedewald's calculation remains the most widely used in clinical practice.

Studies have shown a positive correlation between total cholesterol, LDL cholesterol and apolipoprotein B with the presence of atherosclerotic plaque(111). Conversely, HDL cholesterol has been shown an inverse relationship to cardiovascular disease and is thought to be protective. The components of HDL-C function to reduce inflammation and oxidative stress to encourage cholesterol efflux from the atheromatous lesion, hence reversing the atherosclerotic process(112).

Apolipoprotein B (ApoB) is the main structural component of LDL-C. It exists as ApoB 100, synthesised in the liver, and ApoB 48, synthesised in the small intestine. ApoB 100 makes up 95% of apolipoproteins in the circulation and forms the structural stability for the LDL-C molecule. All low-density lipoprotein cholesterol entities, including LDL, very low-density lipoprotein (vLDL) and intermediate density lipoprotein (iLDL) contain ApoB and are capable of atheroma formation, although LDL-C is the most abundant in fasting blood(113). Apolipoproteins, unlike LDL-C, can be directly measured in the blood, and have several other advantages over LDL-C measurements. These include reliable non-fasting results,

standardised reference ranges and lack of influence by metabolic syndrome, where LDL-C may be falsely low. ApoB can also retain reliable CV risk prediction in patients already on lipid lowering therapy(114).

In the AMORIS study (2001), serum ApoB levels were shown to be a more sensitive predictor of risk for ischaemic heart disease than LDL-C, and indeed levels may be raised despite a normal LDL-C result(115). It is also a useful measure of risk if the patient is already on lipid lowering therapy. The Quebec Cardiovascular Study (1996) showed that raised ApoB levels are an independent risk factor for ischaemic heart disease and as such should be included in CV risk scoring(116). The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) guidelines now recommend that ApoB measurements be used as a primary target for lipid lowering therapy along with other serum cholesterol markers and have set a parameter of less than 90 milligrams per litre (g/L) as the clinical goal(117).

Apolipoprotein AI (ApoA1) is the major protein component of HDL-C. The ratio of ApoB and ApoA1 may be used to predict individual CV risk and may well be superior to LDL-C, HDL-C and total cholesterol parameters(118). Given the correlation with ApoA1 and HDL-C, and their positive effect on atherosclerosis, some groups have been testing the outcomes of ApoA1 compounds in animal models. Interestingly they have shown reduction in myocardial infarct size and damage, perhaps representing action against ischaemia-reperfusion injury(119). HDL cholesterol levels may have a correlation with high sensitivity C-reactive protein (hsCRP), a measure of inflammation, and cystatin C, a maker of glomerular filtration rate and renal function(120).

Inflammation is a major component of the process of atherosclerosis. CRP is an acute phase protein originating in the liver. It is a marker of inflammation and is produced following stimulation by pro-inflammatory cytokines. The production of pro-inflammatory cytokines, evidenced by raised CRP, in chronic inflammatory disease has been shown to accelerate atherosclerosis(121). As such, high sensitivity CRP measurements are considered to be a useful risk and prognosis prediction marker in cardiovascular disease. In CF, patients suffer from a chronic inflammatory state whereby serum markers of inflammation such as CRP may never reach a normal level. Although interpretation of CRP in relation to CVD in this

patient group may be challenging, it may also have an important role as a predictor of cardiovascular risk in the ageing CF patient.

1.9.3 Hypertension

Systemic hypertension can be defined as primary (essential) or secondary. Essential hypertension is common and is associated with age and increased cardiovascular risk. Normal, or 'optimal', blood pressure (BP) is defined as a systolic blood pressure (SBP) of ≤120mmHg and a diastolic blood pressure (DBP) of ≤90mmHg. In clinical practice, systemic hypertension is broadly defined as SBP consistently above 140mmHg and DBP of above 90mmHg(122). Beyond this, hypertension can be graded according to degree of SBP and DBP (*table 1.2*). ADD normal BP line

Category of blood pressure	**SBP (mmHg)**	**DBP (mmHg)**
Normal - 'optimal'	<120	<80
Normal	120-129	80-84
High-normal	130-139	85-89
Grade I	140-159	90-99
Grade II	160-179	100-109
Grade III	≥180	≥110
Isolated systolic hypertension	>140	<90

Table 1.2: Classification of systemic hypertension in current clinical practice(123).

A diagnosis of systemic hypertension cannot be made on the basis of an isolated elevated blood pressure recording, but rather a sustained elevation of blood pressure over a period of time with several recordings. This may be carried out over a number of outpatient clinic attendances or with the assistance of domiciliary ambulatory blood pressure monitoring.

Once a diagnosis of systemic hypertension is made, investigations should be carried out for evidence of end organ dysfunction. This may include electrocardiography to assess for left ventricular hypertrophy, urine dipstick for proteinuria to assess for renal dysfunction and ophthalmologic assessment to exclude hypertensive retinopathy. Anti-hypertensive treatment is guided by age, cardiovascular risk scoring, evidence of end organ damage and

severity of hypertension. Thresholds for treatment are lower in those with established cardiovascular disease, diabetes mellitus and renal disease.

Both diastolic and systolic blood pressure increase with age. Cardiovascular morbidity increases as blood pressure rises, and each incremental rise of 10-20mmHg may as much as double cardiovascular risk, independent of other risk factors(124). Historically, diastolic hypertension has been associated with the majority of hypertension-related end organ damage and cardiovascular morbidity(125). However, isolated systolic hypertension is becoming increasingly prevalent in an elderly population. Although systolic and diastolic blood pressure may rise in concurrence, isolated systolic hypertension has been shown to contribute to atherogenesis, cardiovascular risk and renal disease, thus its treatment may warrant increasing scrutiny in an ageing population. In the Framingham heart study, a change in systolic blood pressure of around 35mmHg in women and 25mmHg in men over four decades was seen. This can be attributed to structural vascular changes and declining arterial compliance with age, cellular oxidative stress and dysregulation of cellular ion homeostasis, including sodium, calcium and magnesium(126). There is also an increase in alpha-adrenergic sympathetic drive, causing arterial vasoconstriction and subsequent hypertension.

High blood pressure contributes to cardiac remodelling and left ventricular hypertrophy (LVH). Cardiovascular events have been shown to occur more frequently in patients with LVH than without(127). Hypertension also accelerates the atherosclerotic process, inducing arterial wall remodelling and increasing vascular resistance(128). This increases the risk of ischaemic coronary and cerebrovascular disease.

Systemic hypertension is confounded by obesity and hypercholesterolaemia and is associated with a state of peripheral insulin resistance and hyperglycaemia. Control of metabolic disturbances such as hyperglycaemia often results in blood pressure reduction. Caution must be taken however when lowering blood pressure(129) in patients with significant coronary artery disease. Since coronary arteries are perfused in diastole, lowering diastolic blood pressure may lead to under-perfusion and may exacerbate cardiac ischaemia.

1.9.4 Diabetes mellitus

The pathophysiology of type one diabetes mellitus (T1DM) is autoimmune destruction of pancreatic Islet of Langerhan cells, causing an absolute insulin deficiency. Hyperglycaemia ensues following glucose loading due to inadequate glucose regulation through lack of endogenous insulin. Exogenous insulin treatment is essential to control its symptoms and secondary complications.

Type two diabetes mellitus (T2DM) is a different entity to T1DM in that, rather than a deficiency of insulin production, there is a state of insulin resistance. This results firstly in postprandial hyperglycaemia and impaired glucose tolerance, progressing to overt diabetes mellitus(130). Genetically predisposed individuals are at risk, confounded by obesity and hypertension, both of which produce states of insulin resistance. Importantly in a respiratory disease patient population, corticosteroid therapy can induce or exacerbate hyperglycaemia due to effects on insulin resistance. Long term oral corticosteroid therapy can affect the adrenal hormonal axis.

Hyperglycaemia exerts negative effects on the vascular endothelium, contributing to thrombosis and atherosclerosis. Despite treatment, microvascular disease is common in diabetes mellitus, including retinopathy, nephropathy and peripheral neuropathy. Macrovascular complications such as coronary artery disease, peripheral vascular disease and cerebrovascular disease are becoming more prevalent as we see an increase in T2DM in association with an ageing population and increasing prevalence of obesity in a sedentary Western world.

Given the higher cardiovascular risk associated with both T1DM and T2DM, anti-hypertensive and lipid lowering medication is usually considered at a younger age than the general population and is essential in those with evidence of diabetic vascular complications.

1.9.5 Obesity

Obesity is defined as an excess of body fat that presents a risk to health(131). Obesity is usually measured by using body mass index (BMI) calculations. Cardiovascular disease has been shown to be more prevalent with obesity, particularly in those with a central deposition of adiposity. In addition, obesity is associated with systemic hypertension,

dyslipidaemia, hyperglycaemia and elevated CRP, all of which positively correlate with cardiovascular risk. Obesity may become an issue with CF patients in the future due to CFTR modulation and the potential for weight gain on these therapies. Phase three trials of CFTR modulators have shown an increase in BMI for the majority of patients. Ivacaftor has been shown to normalise gastrointestinal pH, thus improving absorption and nutritional status(132). In longitudinal analysis of these patients, improved survival and weight gain may have consequences for cholesterol and cardiovascular risk.

1.9.6 The 'metabolic syndrome'

The 'metabolic syndrome' has been defined as a collection of metabolic abnormalities associated with insulin resistance. These include visceral obesity, dyslipidaemia, hyperglycaemia and systemic hypertension. All separately and synergistically are strongly associated with cardiovascular disease. Indeed, up to 80% of patients with T2DM will die of cardiovascular disease(133). Women with T2DM may be more prone to cardiovascular disease with ageing due to the loss of oestrogen protective effects in menopause.

1.9.7 Smoking

Cigarette smoking, amongst many other detrimental health impacts, notably increases the risk of cardiovascular events. This risk was seen to at least double compared with the non-smoking population in the Framingham Study data in the 1980s(102). The pathophysiology behind this has long been debated but seems to favour endothelial cell dysfunction and oxidative stress, leading to accelerated atherosclerosis and eventually thrombosis(134). Cigarette smoking increases the risk of coronary artery disease, stroke, heart failure and peripheral vascular disease. A significant amount of NHS resources in recent years has been dedicated to the development of smoking cessation services and promoting healthier lifestyles.

1.9.8 Non-atherosclerotic vascular disease

We know that vascular atherosclerosis is in large part an inflammatory process and is related to oxidative stress and endothelial cell dysfunction. As such, inflammatory markers such as CRP can be used as an independent predictor of cardiovascular mortality(135). This may be of particular relevance in inflammatory conditions such as rheumatoid arthritis and systemic vasculitis. Inflammatory conditions have been shown to be independent risk

factors for cardiovascular disease(136). It is therefore possible that there may be relevance to an ageing CF population, with a lifelong, high inflammatory burden.

However, more recently emphasis has been placed on the role of central arterial stiffness in cardiovascular disease. As we age, arteries undergo a process called arteriosclerosis. This involves calcification of the vessel wall and alteration of elastin, collagen and smooth muscle cell balance resulting in increased arterial stiffness(137). As a consequence, vascular impedance is affected, altering the relationship between blood pressure and flow. This process of vascular ageing may be accelerated by conditions such as diabetes mellitus, renal disease and systemic hypertension, in which inflammation and oxidative stress contribute to accelerated vascular stiffening. There is also a positive correlation between arterial stiffness and atherosclerosis(138). Systolic blood pressure and pulse pressure both increase with age, and correlate with arterial stiffness(139). Pressure of blood flow and arterial stiffness in the aorta are surrogate markers of coronary blood flow and left ventricular workload. As such, if aortic stiffness increases, the resulting rise in systolic blood pressure will increase ventricular load and myocardial oxygen demand, compromising coronary circulation and contributing to left ventricular hypertrophy (LVH). Aortic stiffness is thus associated with systolic hypertension, coronary artery disease, stroke, heart failure and atrial fibrillation. Markers of arterial stiffness can also be performed using cardiac magnetic resonance imaging (MRI), in addition to assessing biventricular function.

1.9.9 Arterial stiffness measurements

Early detection of arterial stiffness and changes to vascular structure may help to predict premature vascular disease, allowing treatment strategies for primary cardiovascular prevention. Indices of large artery stiffness such as pulse wave velocity (PWV) and augmentation index (AIx) have been shown to predict cardiovascular risk in the general population(140) as well as specific patient groups, such as those with systemic hypertension(141) and chronic kidney disease(142). Moreover, PWV is independently associated with increased cardiovascular risk when adjusted for standard cardiovascular risk factors(143). Inclusion of carotid femoral PWV (cfPWV) in cardiovascular risk prediction scoring has been shown to increase classification accuracy in low and intermediate risk groups by up to 13%(144).

1.9.10 Pulse wave velocity

Non-invasive PWV is the gold standard for direct measurements of arterial stiffness as set out by the American Heart Association (AHA) guidelines in 2010(145). PWV is essentially the velocity of the pulse wave travelling through a measured segment of an artery. The pulse wave is reflected off the aortic bifurcation and these retrograde waves are recorded using pulse wave analysis. The higher the arterial stiffness, the faster these pulse waves will travel, resulting in a higher PWV. PWV measurements are derived using concepts from the Moens-Korteweg equation, which first described the speed of pulse wave propagation as inversely proportional to the distensibility of the vessel in large arteries(146). Central arterial stiffness measurements, such as the aorta, are preferentially used over peripheral arterial parameters due to their higher reliability particularly in chronic kidney disease cohorts in which the predictive value of brachial PWV for cardiovascular risk, as compared with aortic PWV, is suboptimal(147). Aortic PWV (or carotid-femoral, cfPWV) can be calculated using several non-invasive arterial tonometry devices using a calculation of the distance between carotid and femoral pulses divided by the transit time of the pulse wave(148).

In normal physiology, there is a 'stiffness gradient' in the arterial tree, with PWV increasing relative to distance from the proximal aorta. In addition, PWV values vary according to age, gender, ethnicity and blood pressure. Although a quick and non-invasive technique, the use of PWV measurement in routine cardiovascular risk prediction has been limited due to the lack of normative reference ranges. The European Society of Hypertension guidelines (2007) suggested a normal PWV should be less than 12 metres per second (m/s)(145). A study by Laurent et al (2001) suggested an association between PWV of >13.5m/s and high cardiovascular risk(141). However, a subsequent document published in 2010 by the Arterial Stiffness Collaboration based on population data from a large multicentre European study, suggests more accurate reference values for PWV should be categorised according to age and degree of hypertension. Mean PWV values according to blood pressure and age from this study are shown below, along with the application of example equations for PWV derived from linear regression models(149).

BP category:	Optimal	Normal	High normal	Grade I hypertension	Grade II hypertension
Age (years)	←		PWV (as mean +/- 2SD)		→
<30	6.1 (4.6-7.5)	6.6 (4.9-8.2)	6.8 (5.1-8.5)	7.4 (4.6-10.1)	7.7 (4.4-11)
30-39	6.6 (4.4-8.9)	6.8 (4.2-9.4)	7.1 (4.5-9.7)	7.3 (4-10.7)	8.2 (3.3-13)
40-49	7 (4.5-9.6)	7.4 (5.1-10)	7.9 (5.2-10.7)	8.6 (5.1-12)	9.8 (3.8-15.7)
50-59	7.6 (4.8-10.5)	8.4 (5.1-11.7)	8.8 (4.8-12.8)	9.6 (4.9-14.3)	10.5 (4.1-16.8)
60-69	9.1 (5.2-12.9)	9.7 (5.7-13.6)	10.3 (5.5-15.1)	11.1 (6.1-16.2)	12.2 (5.7-18.6)
≥70	10.4 (5.2-15.6)	11.7 (6-17.5)	11.8 (5.7-17.9)	12.9 (6.9-18.9)	14 (7.4-20.6)

Table 1.3: Recommended reference ranges for PWV according to age and degree of systemic hypertension(149). *SD = standard deviation, PWV = pulse wave velocity (m/s)*

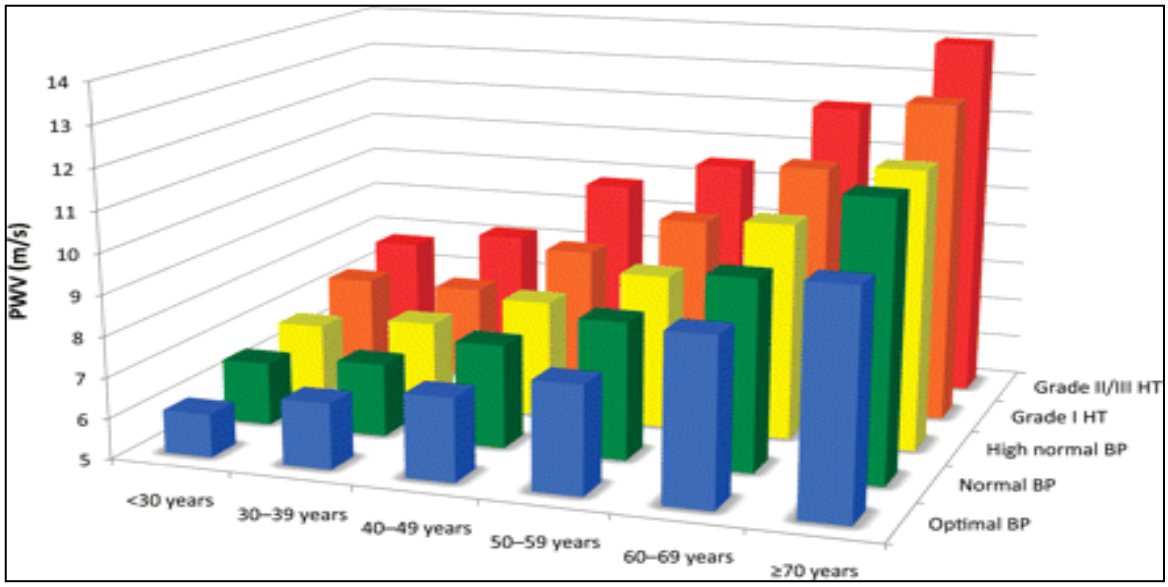


Figure 1.4: PWV reference values according to blood pressure and age.

<30 PWV = 0.0472 × MBP + 2.20 0.26
30–39 PWV = 0.0423 × MBP + 2.20 0.13
40–49 PWV = 0.0646 × MBP + 1.41 0.23
50–59 PWV = 0.0731 × MBP + 1.35 0.17
60–69 PWV = 0.0715 × MBP + 3.16 0.13
≥70 PWV = 0.0676 × MBP + 5.46 0.07

Figure 1.5: equations for PWV derived from linear regression.

The majority of PWV reference ranges have been studied in patient cohorts with CKD or end-stage renal disease (ESRD). Blacher and colleagues(150) showed a significant increase in both all-cause and CVD mortality in an ESRD population with aortic PWV >12m/s. A study by London and colleagues in 2001 suggests an optimal "cut-off value" for PWV of 11.3m/s for CVD mortality and 11.5m/s for all-cause mortality, and hence a normative value of PWV <11m/s may be acceptable(151).

1.9.11 Augmentation Index

Augmentation index (AIx) is another marker of arterial wall elasticity. It is measured by pulse wave analysis (PWA), using similar processes of arterial tonometry. AIx is expressed as a percentage (%) and represents the degree by which the central pulse pressure is augmented by the reflected pulse wave. The AIx is measured by analysing a double-peaked waveform, with the first peak (P1) representing the left ventricular ejection and the second (P2)

representing the reflected pulse wave. The difference between these two peaks reflects the degree of central pressure augmentation and therefore the AIx. An example of this waveform and calculation of radial AIx is shown below(152).

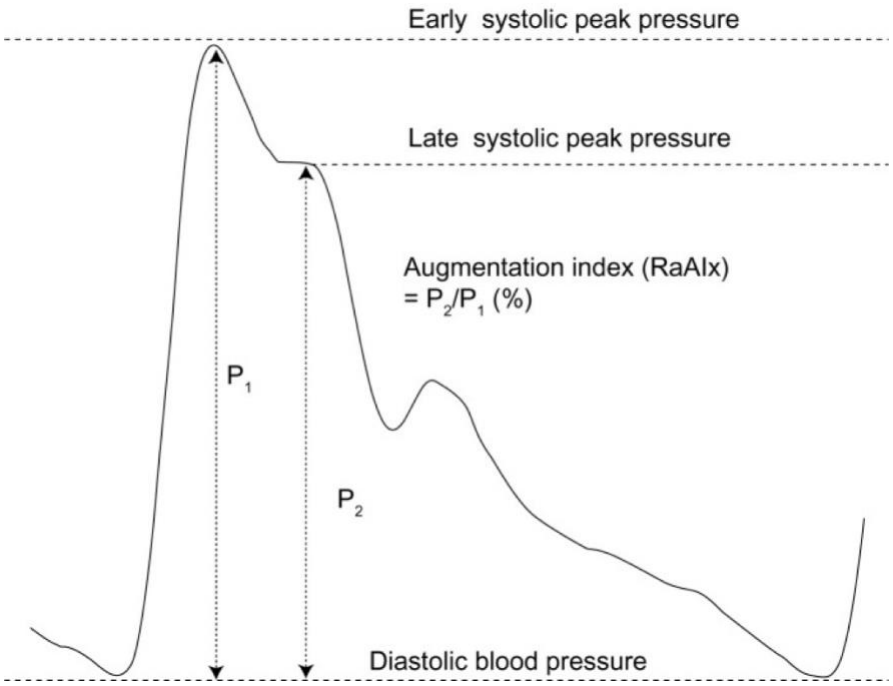


Figure 1.6: AIx waveform as calculated from pulse wave analysis(152)

In similarity to PWV, normative reference ranges for AIx are lacking, most likely due to the considerable variations of AIx with age, gender and heart rate. However, London and colleagues (2001) observed an increase in CVD mortality with 10% incremental increases of AIx and suggest an optimal normative cut-off value of 25%(151). Increasing AIx has been shown to correlate with increasing arterial stiffness and CV morbidity(153). However, due to its limitations, PWV remains the preferred measure of arterial stiffness in clinical practice. In addition, a high PWV in the presence of normal AIx can be an indication of diffuse vessel calcification rather than localised atheroma and may intimate underlying renal pathology.

Arterial stiffness determines the blood flow, pressure and changes in vessel diameter during each cardiac cycle. The elasticity of the vessel determines arterial stiffness and is regulated by collagen and elastin within the vessel wall as well as sympathetic tone and genetic factors. Arterial stiffness can be affected by oxidative stress and inflammation, and can be seen to increase with both disease and age(143). In an ageing population, there is an

increasing overlap between arterial stiffness and the process of endothelial cell dysfunction in atherosclerosis. Arterial stiffness parameters can be measured effectively and non-invasively in large populations, hence should be used as a predicting tool for cardiovascular risk where relevant. Indeed, central arterial stiffness has been measured as part of a risk scoring system in the chronic renal failure population with good prediction of cardiovascular disease(154).

Strategies to combat accelerated arterial stiffness in susceptible populations have been postulated but thus far do not provide sufficient evidence to sustain use in routine clinical practice. For inflammatory conditions such as chronic renal disease, anti-inflammatory drugs may have roles in reducing arterial stiffness via vascular endothelin receptor antagonism and reducing circulating inflammatory cytokines contributing to arterial wall stiffness, thus reducing cardiovascular morbidity(155). However, their use is limited due to potential detrimental effects on renal function. Research surrounding the benefits of anti-inflammatory medication in CFTR-mediated chronic lung inflammation in CF is promising and this may become more relevant in terms of arterial stiffness and cardiovascular risk in an ageing CF population.

Antihypertensives have the ability to reduce arterial stiffness by lowering arterial wall pressure. Angiotensin converting enzyme inhibitors (ACEi) yield potential to reduce arterial stiffness and cardiovascular mortality, independent of their blood pressure lowering effect. Statin therapy and vitamin D may also have an anti-inflammatory role and vascular-protective properties via endothelin modification(155).

1.9.12 Genetics and cardiovascular risk

Although environmental influences play a huge role in cardiovascular risk, genetic factors also have an important part in the development of premature cardiovascular disease. Familial hypercholesterolaemia is an inherited, autosomal dominant LDL receptor mutation, predisposing to cholesterol levels often twice as high as in the unaffected population of the same age and sex. This, compounded by a Western diet, increases the risk of premature coronary artery disease and stroke. In addition, familial mutations in the ApoB gene result in a seven-fold increased risk of CVD in this population(156). Genetics also play a role in hypertension, T2DM and congenital heart disease(157), all predisposing to cardiovascular morbidity.

1.9.13 Cardiovascular risk scoring

Cardiovascular disease is the leading cause of mortality worldwide and accounts for almost one third of deaths in the UK per annum(158). Cardiovascular morbidity is also a significant annual NHS expense. As such, it is of utmost importance to detect cardiovascular disease early and treat it aggressively, particularly in high risk patient groups. Cardiovascular risk factors are grouped into non-modifiable including age, family history, ethnic background and sex, and modifiable risk factors including obesity, systemic hypertension, high cholesterol and smoking.

In addition to patients with significant modifiable cardiovascular risk factors, patients with chronic inflammatory disease also remain at higher risk of CVD. The majority of cardiovascular risk scores are calculated in primary care and require the relevant clinical and epidemiological data to be inputted via computer into a scoring system, which will then generate a ten-year cardiovascular risk score for the individual patient. Treatment strategies for primary prevention will then be utilised according to the calculated level of risk, including anti-hypertensive and lipid lowering therapy.

Although there have been several scoring systems used in the past, including the *Framingham Risk Score,* the *HEARTScore* and the *QRisk®2* score(159), the most recent score is now the *QRisk®3* score, developed in 2017. It is yet to be utilised in primary care and current NICE guidance still recommends the use of the *QRisk®2* in CVD risk assessment. The *QRisk®* scores are derived from primary care data from almost eight million cardiovascular naïve patients(160). They have been developed and validated only for use in the UK population. The *QRisk®3* is an updated version of the *QRisk2* score and has the addition of several new cardiovascular risk parameters. *QRisk®* scores are updated annually and data is stored within the *QResearch* database.

The *QRisk®3* score calculates ten-year cardiovascular risk for patients between 25 and 84 years of age. Cardiovascular risk must be assessed regularly in patients over the age of 40 years. The score, amongst other parameters, includes demographic data as well as smoking status, the presence of diabetes mellitus, the presence of chronic kidney disease (CKD) stages three to five (III to V) and inflammatory conditions such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Interestingly, a diagnosis of severe mental illness

and the use of anti-psychotic medication is now included in scoring since there appears to be a modestly increased cardiovascular risk in this patient cohort. Both corticosteroids and second-generation anti-psychotics are associated with an increase in serum lipid levels(108)(161).

Importantly, the score is only relevant in those without a pre-existing diagnosis of cardiovascular disease. In addition, those with familial hypercholesterolaemia should not be included. Scores must be interpreted with appropriate clinical judgement and may be underestimated in younger patients. In conjunction with NICE guidance (2014), treatment should be offered to those patients with a calculated ten-year cardiovascular risk score of 10% or greater(108). This should include a combination of anti-hypertensive medication, lipid lowering therapy and lifestyle modification.

An example of the *QRisk®3* scoring system is shown in figure 1.7 (appendix 1). The modifications from *QRisk®2* are marked by as asterisk (*)(162).

1.10 Renal function

1.10.1 Renal function and age

Renal function rises to optimal levels after the second year of birth and remains so until the fourth decade of life. Renal function is measured by glomerular filtration rate (GFR) and usually peaks at between 120 and 140ml/min/1.73m^2. After the fourth decade of life, GFR declines by around 8ml/min/1.73m^2 each subsequent decade(163). Reduction in renal function with age is extremely variable between individuals and can be attributed to several causative factors including loss of renal mass, renovascular changes with reduction in renal blood flow and tubular-interstitial changes, with renal hypofiltration, disruption of the renin-angiotensin-aldosterone axis and subsequent cellular osmotic and ionic homeostasis. Declining renal function due to age is distinct from chronic renal failure. In an ageing kidney, in the absence of chronic renal failure, one would expect to see the preservation of normal urea and creatinine, erythropoietin production and urinalysis(164).

1.10.2 Chronic kidney Disease

Chronic renal failure, also known as chronic kidney disease (CKD), is defined as a persistent reduction in renal function (as measured by glomerular filtration rate, GFR) over a period of three months or more(165). There are five stages of CKD, each determined by the degree of

loss of GFR. An estimated GFR (eGFR) of >90ml/min/1.73m^2 is considered normal renal function. However, an eGFR of >90ml/min/1.73m^2 in the presence of renal disease is classed as CKD grade I. Similarly, an eGFR of >60ml/min/1.73m^2 may be appropriate for age in some patients. However, in the presence of renal disease, an eGFR of between 60 and 90ml/min/1.73m^2 is classed as CKD grade II. Renal disease may include haematuria, proteinuria, structural kidney abnormalities, genetic kidney syndromes, renal tubular disorders and history of renal transplantation.

Grade of Chronic kidney disease (CKD)	**Glomerular filtration rate (ml/min/1.73m^2)**
I	>90*
II	60-90*
IIIa	45-59
IIIb	30-44
IV	15-29
V	<15

Table 1.4: Grades of chronic kidney disease, Prayle et al 2010(166)(167).**With the presence of renal disease.*

Clinical symptoms are not usually evident until stage IV and above. When GFR falls to less than 60ml/min/1.73m^2, complications of CKD become more prevalent. Management of CKD focuses on symptomatic relief, treatment of reversible causes, electrolyte and fluid abnormalities and managing cardiovascular risk. Stage V CKD necessitates the consideration of renal replacement therapy and possible transplant in eligible patients.

There are many causes of chronic renal failure. In the ageing CF population, diabetic nephropathy and systemic hypertension seem particularly relevant. Diabetes mellitus is an age prevalent condition in CF and, as mentioned previously, usually requires treatment with insulin therapy. In insulin dependent diabetes mellitus, diabetic nephropathy is common and is characterised by a clinical spectrum of renal disease starting with microalbuminuria and progressing to overt proteinuria and chronic renal failure. Urine albumin excretion can be measured using a urine albumin creatinine ratio (ACR), which is performed regularly in routine follow-up of diabetic patients. Microalbuminuria is the first sign of diabetic nephropathy. It can also be raised with poor glycaemic control, systemic hypertension and

non-diabetic renal diseases. Since there is a significant risk of cardiovascular disease in chronic renal failure, early detection of diabetic nephropathy is essential to instigate preventative management of cardiovascular morbidity.

An ageing kidney does not necessarily lead to chronic renal failure. However, accelerated loss of renal function may occur with hypertension, dyslipidaemia, obesity, male gender, smoking, the presence of inflammatory markers, atherosclerotic disease and diabetes mellitus(168).

1.10.3 Acute kidney injury

Acute renal failure, now known as acute kidney injury (AKI), is defined as an acute deterioration in renal function over hours or days. Oliguria is common and there will be a rise in serum urea and creatinine. AKI can be classified into three groups(169).

1. Pre-renal AKI accounts for the majority of cases. The commonest cause is reduced renal perfusion due to sepsis or hypovolaemia. Other causes include cardiac and liver failure, medication affecting renal blood flow, such as non-steroidal anti-inflammatories (NSAIDs), and reno-vascular disease.
2. Renal AKI is due to intrinsic renal pathology including glomerulonephropathies, renal tubular pathology, radiological contrast and nephrotoxic medication.
3. Post-renal AKI is caused by intrinsic or extrinsic obstruction of any part of the renal tract.

Management of AKI includes identification and treatment of the precipitating cause. An ageing kidney may be more susceptible to acute insults due to lack of adequate compensatory mechanisms as seen in a younger kidney. In particular, circulatory volume changes(170), sodium and potassium transport, and renal metabolism of medication may be affected. Recurrent episodes of acute renal failure may be a risk factor for the development of chronic renal failure. This may be of particular relevance in our ageing CF population, particularly those with recurrent pulmonary sepsis with concurrent acute renal impairment, as well as cumulative intravenous aminoglycoside exposure. Patients with CF are also susceptible to renal calculi and obstructive renal failure.

1.10.4 Measuring glomerular filtration rate (GFR)

Normal kidney function varies between individuals and depends upon kidney size and total body surface area. A surface area of 1.73m^2 is taken as the normal mean value of a young adult and is a continuous variable. Body surface area must be considered when interpreting 'normal' renal function. The GFR is widely accepted as the most reliable measure of renal function. It can be measured directly via urinary or plasma clearance measurements of an exogenous renal marker or via estimations using endogenous serum markers within biochemical equations. Accurate measurements of GFR are important in order to adequately diagnose those with chronic kidney disease, accurately adjust medications with narrow therapeutic indices in light of renal dysfunction and to risk stratify for cardiovascular disease and end stage renal failure(171).

1.10.5 Exogenous renal markers and *measured* GFR

'Clearance' is defined as "*the volume of plasma from which a substance is completely removed, per unit time,"* measured in millilitres per minute (ml/min)(172). The gold standard for measuring GFR is through clearance of an 'ideal' filtration marker such as inulin, iohexol or iothalamate(173). These exogenous compounds are exclusively filtered and excreted by the kidneys. This method would directly measure GFR (mGFR) and hence is the most accurate way to calculate renal function. However, these measurements would necessitate intravenous infusion of the filtration marker along with timed collection of urine and serum samples to determine renal clearance, making this technique both cumbersome and expensive in clinical practice, and as such is not used routinely.

The first technique of measuring GFR using inulin was in the 1950s. Inulin is a derivative of fructose, is expensive in purified form and, during continuous intravenous infusion, multiple samples of serum and urine inulin are required to measure GFR. Both expense and lack of practicality limit its use in clinical practice and as such it is now only used in research arenas when very accurate GFR needs to be obtained.

Radioisotope methods were developed in the 1960s and include iothalamate. The calculation of mGFR via the clearance of iothalamate correlates well with inulin and only a single intravenous dose is required. However, the proper handling and disposing of radioactive materials in clinical environments limits its use. Radioisotope measurements of mGFR are again used mostly in the renal transplant setting if accurate GFR has important

implications for clinical decision-making. Both inulin and iothalamate clearance require both urine and serum samples post-infusion over an increasing time interval with declining GFR, such that those with advanced CKD may require samples 24 hours after initial infusion to assess the terminal elimination phase. This obviates further clinical impracticality.

Iohexol clearance GFR is becoming increasingly popular in renal research centres. Iohexol clearance has been shown to highly correlate with radioisotope mGFR(174) as well as inulin clearance(175). As such, iohexol may be the most suitable alternative to inulin given its equivocal exclusive renal excretion and plasma stability coupled with lower cost and more practical administration technique. Plasma clearance measurements are preferred over urine samples and single sample protocols have been developed, increasing patient convenience. Iohexol mGFR has been shown to be superior in measuring accurate renal function in multiple settings when compared with creatinine-based estimated GFR calculations(176). However, the logistics of use including timing, equipment and expense currently limit its use in standard clinical care.

1.10.6 Endogenous renal markers and *estimated* GFR

In current clinical practice, endogenous glomerular filtration markers such as creatinine, are used to make an *estimation* of GFR (eGFR) by calculating serum creatinine alone or by using measurements of creatinine clearance via a variety of biochemical models. Creatinine is an amino acid and is a product of creatine breakdown in muscle. It is produced at a fairly constant rate, excreted via glomerular filtration with minimal tubular reabsorption and can be measured in both serum and urine. Estimated GFR based on creatinine in serum or urine is quick and cost-effective and is preferred in routine clinical practice to the 'gold standard,' more time-consuming method of inulin or iohexol clearance mGFR.

Measured creatinine will vary depending on its metabolism within muscles and its degree of renal tubular secretion(177). Factors affecting these variables include muscle mass, age, gender and race. This results in different 'normal' values between patient groups and must be considered when interpreting results. Due to the variation in creatinine between patient groups and laboratory reference ranges, there may be a discrepancy between paired eGFR and creatinine levels. A significant decline in eGFR may be seen prior to creatinine reaching abnormal levels.

To improve accuracy of eGFR measurements, the Kidney Disease Improving Global Outcomes (KDIGO) recommends eGFR calculation derived from equations that utilise serum creatinine levels in combination with age, gender, weight and race(178). The accuracy of these equations has improved in recent decades. It is important to note that these equations are only validated for use in CKD, not acute renal impairment.

The Cockcroft-Gault (CG) equation was developed in 1973(179). The eGFR is derived from the below calculation;

C_{Cr}=*{((140-age) x weight)/(72* S_{Cr}*)} x 0.85 if female (mL/min)*

This calculation is not adjusted for body surface area and has been superseded by the Modification of Diet in Renal Disease Study formula (MDRD or 4-v MDRD)(180) in 1999. The most recent version of MDRD is adjusted for body surface area along with age, sex and race, thus is thought to be more accurate than CG. The MDRD equation is shown below;

GFR = 175 x (Standardized S_{Cr}*)*$^{-1.154}$ *x (age)*$^{-0.203}$ *x (0.742 if female) x (1.210 if African American) (mL/min/1.73m*^{2}*)*

The MDRD equation was developed using data from patients with chronic renal disease and thus may underestimate GFR in healthy populations(181). Notably, equations to measure eGFR from serum creatinine seem to be less accurate for eGFR levels of over 60ml/min/1.73m^2. The relationship between GFR and creatinine is disproportionate and a much greater decline in eGFR will be seen for a similar decline in creatinine for patients with better preserved renal function than those without(182).

Serum creatinine may be unreliable in a 'non-steady' clinical state or those with nutritional deficiency. Patients with chronic disease may have reduced muscle mass and thus creatinine-based calculations may underestimate GFR. This is of particular importance in CF patients due to chronic malabsorption, high metabolic rate and caloric requirement, relative under-nutrition and characteristic low muscle mass.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations were developed in the early 2000s and have been recognised in KDIGO guidelines since 2012.

However, they are not yet being used in preference to MDRD in calculating eGFR in all secondary care centres. CKD-EPI calculations have three forms to include a creatinine-based equation, a cystatin C-based equation and a combination of the two. All adjust for the same four variables as in MDRD calculations. The CKD-EPI creatinine and the CKD-EPI creatinine-cystatin C calculations have been shown to have improved reliability when compared with MDRD, particularly in the elderly and those in higher GFR ranges(183)(184).

The inaccuracies of creatinine-based eGFR equations have led to the development of calculations using other endogenous biomarkers. Cystatin C is a protease inhibitor present in all nucleated cells. It is filtered by the glomerulus and then reabsorbed by renal tubular epithelial cells and thus only small amounts are excreted in the urine. Only serum levels therefore can be measured. Serum cystatin C levels in health remain fairly constant and have been shown to vary less with body mass, age and gender(185). Blood concentration of cystatin C rises in response to decline in renal function. Studies have shown serum cystatin C to better predict GFR than serum creatinine(186). In addition, cystatin C has been shown to be superior to creatinine-based MDRD calculations in measuring eGFR in patients with lower GFR ranges when compared directly to the gold standard of renal iohexol clearance(187). Cystatin C may also be superior to creatinine clearance in diabetic populations when calculating eGFR and also early renal impairment in the presence of normal serum creatinine(188). Cystatin C has also been shown to be a more accurate measure of GFR in CF patients, compared to methods using serum creatinine and creatinine clearance(189), and is a more accurate predictor of CV risk than creatinine in a CKD population. Accurate GFR measurements in the CF population are important both for optimal medication dosing and early detection of renal disease. A deficiency in availability of laboratory cystatin C assay and cost has impacted on this test being used in routine clinical practice for populations where creatinine based eGFR may less inaccurate.

Urinary creatinine clearance may be a more accurate marker of eGFR in populations with nutritional impairment and fluctuating clinical stability in chronic disease, in the absence of cystatin C measurements. Urinary creatinine clearance historically has been considered the ‘gold standard’ for eGFR measurements and its calculation requires a 24-hour urine collection for creatinine along with urine volume and a single serum creatinine sample. Information is then inputted into the equation below;

Creatinine clearance (CrCl) ml/min = (urine creatinine x volume) / serum creatinine.

However, the technique is limited by patient compliance and timing of collection. In addition, since creatinine is secreted by the renal tubules, GFR is often over-estimated, particularly in patients with CKD. For these reasons, the measurement of eGFR from urine creatinine clearance has largely been replaced by the above serum creatinine eGFR formulas. Decline in urinary creatinine clearance may be useful in CKD patients as a prediction tool for renal decline and mortality(190).

1.10.7 Proteinuria

Urinary albumin creatinine ratio (ACR) is a marker of renal protein loss. Raised urinary protein and ACR may be the first indicator of early renal dysfunction and may be seen in patients with diabetes mellitus, systemic hypertension and intrinsic renal pathology. Microalbuminuria has been found to be a surrogate marker of enhanced cardiovascular risk. Spot urine ACR measurements are routinely performed in diabetic clinic and are utilised in annual assessments of diabetic CF patients to screen for early diabetic nephropathy. Urine ACR is also used to detect renal decline in CKD populations and in hypertensive populations to monitor the effects of treatment. The presence of proteinuria in hypertensive patients is associated with a greater risk of cardiovascular disease and mortality(191). In CF cohorts, urine ACR may be useful in the detection of renal dysfunction in both non-diabetic and diabetic groups(192). A 24-hour urine sample can also be measured for protein, however a spot morning ACR sample is both easier and has equivocal accuracy as a measure of microalbuminuria.

1.11 Ageing in Cystic Fibrosis

1.11.1 Overview of ageing in CF

Following the discovery of CF as a clinical entity by Dorothy Andersen in 1938 the prognosis was extremely poor, evidenced by the famous quotation; “The child will soon die whose brow tastes salty when kissed”(193).

As understanding, screening and treatment options for the disease have advanced, there has been an exponential increase in survival. The predicted median age of survival for a CF patient born in the UK today is 47 years(4). This is compared to a survival of less than one year around four decades previously. Historically, CF has been a childhood disease with early mortality however, the UK CF Registry reported in 2017 that over 60% of patients were adults, reflecting the changing demographics of the disease.

Factors associated with worse survival in CF include female gender, ethnicity, lower lung function, sputum microbiology and poor nutritional status(194). Although there remains a cohort of CF patients with aggressive disease who die at younger ages, CF centres around the world are now seeing an ageing CF population. Some influencing factors on increasing survival rate no doubt include the introduction of anti-pseudomonal antibiotics, nebulised mucolytics and pancreatic enzyme replacement. In addition, the development of individualised physiotherapy regimens, with emphasis on airway clearance(195), and multidisciplinary (MDT) services have revolutionised CF care. The shift of CF management to specialist regional centres has proven to be beneficial for patient outcomes, including improved BMI and lung function(196). Recognition of transmissible infections and patient segregation in clinic and ward environments have contributed, and more recently CFTR modulating therapies offer an exciting new era of treatment.

Although newborn CF screening is now routine in most UK centres, over 70% of CF patients in 2017 were diagnosed following recognition of classic CF symptoms rather than screening(4). Diagnostic techniques for CF have certainly advanced over the years and patients may now be diagnosed with increasing genotypic clarity. There are now over 2000 recognised CFTR mutations, with approximately 300 disease-causing mutations(197). These are separated into seven functional classes depending on the degree of CFTR function. The diversity of genotypic classes now includes those patients with residual function CFTR

mutations conferring atypical or 'non-classical' CF. In the absence of typical CF clinical features, these patients may be diagnosed later in life, often in adulthood, and such genotypes may correspond to less severe phenotypic disease. This may include exocrine pancreatic sufficiency, less prevalent chronic *Pseudomonas aeruginosa* infection and higher lung function(198). It has been hypothesised that previous CF survival data may be distorted by these "less severe" disease genotypes and are not a true reflection of improving survival in 'classical' CF(199). However, a case series published by Simmonds et al in 2009, describing a cohort of CF patients aged 40 years and above, has shown that a significant proportion of their ageing CF population had 'classical' CF, with 30% homozygous for Phe508del mutation and over 80% with exocrine pancreatic insufficiency(200).

Although the CF story over the last few decades has been one of success, we must recognise the potential problems associated with an ageing CF population and increasing numbers of patients with chronic illness. This will inevitably place strain on healthcare resources as treatment burden continues to increase in relation to direct CF complications as well as complications from longevity of treatment and non-CF ageing comorbidities. A study by Burgel et al in early 2018 has shown that, in Western European societies, the total number of CF patients is likely to rise by approximately 50% by 2025(201), and over 75% of this increase will be in CF adults. If service capacity does not expand along with demand, this may lead to a concerning deficiency in future CF resources. The burden of treatment for CF patients in the UK remains high, with over 50% of adult patients requiring at least one course of intravenous antibiotics and with 20% taking three or more inhaled medications in 2017(4).

Historically, the goal of CF treatment was to manage progressive lung disease and the majority of patients would succumb to end stage lung disease and respiratory failure. Although mortality in CF is predominantly related to pulmonary complications, with an ageing population CF management is evolving to recognise and treat emerging age-related comorbidities. These may be direct complications of CFTR dysfunction with age, CF treatment-related complications or comorbidities related to a general ageing process.

1.12 Age prevalent CFTR-related complications

1.12.1 Pulmonary complications

Pulmonary disease is CF is the predominant cause of morbidity and mortality. Pulmonary exacerbations are relentless with time and negatively correlate with both lung function and survival(202). As lung disease progresses there is also a greater incidence of both haemoptysis and pneumothorax with increasing age, both impacting survival. CF patients experience a progressive decline in lung function with advancing age and annual $ppFEV_1$ acts as a marker for clinical deterioration, risk of death and suitability for lung transplantation. However, with advances in treatment and increasing numbers of adult patients, we may be observing a shift in lung function trends. Inhaled mucolytics and antimicrobials as well as long term macrolide therapy have contributed positively to reduction of pulmonary exacerbation rates and resulting decline in $ppFEV_1$. A study by Que et al in 2006 presented a decreasing annual FEV_1 decline among a cohort of young adults with CF over a 40-year period,(203) correlating with the improving survival being observed in CF populations today.

Patients with chronic *Pseudomonas aeruginosa* infection show a greater rate of lung function decline than those without. This reflects the importance of CF sputum microbiology which changes with age and represents major prognostic significance. The prevalence of chronic infection within *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex (BCC) and non-tuberculous *mycobacteria* (NTM) increases with age. Although the incidence of BCC in UK CF patients has shown a mild reduction in the last few years, in part as a result of improving patient segregation practices, it remains challenging to treat. *Burkholderia cenocepacia*, a prominent member of the BCC family, in particular shows increased virulence and antimicrobial resistance compared with other species. It is associated with accelerated FEV_1 decline, increased pulmonary exacerbation rate and in some cases 'cepacia syndrome,' a rapidly progressive pneumonia with high mortality(204). There is a high risk of post-transplant sepsis with *Burkholderia cenocepacia* infection and thus patients with chronic infection are deemed unsuitable candidates for lung transplantation. However, patients with other species of BCC may still be considered suitable for lung transplantation due to evidence that they have improved post-transplant survival as compared to those with *Burkholderia cenocepacia*(205).

As survival in CF populations improves, NTM infection is becoming more prevalent and may be as high as 13% in some cohorts(206). It has been hypothesised that the widespread use of long-term macrolide therapy has contributed to the rise in chronic NTM infection(207). The emergence of NTM brings new treatment challenges. NTM infection is diverse with a wide spectrum of clinical phenotypes and resistance patterns, often necessitating multiple antibiotics regimens for control. Chronic NTM infection can cause a similar clinical impact as BCC, with more rapid progression of lung disease and accelerated lung function decline. The commonest NTM infection in CF is *Mycobacterium avium complex (*MAC) but chronic *Mycobacterium abscessus* infection is also increasingly prevalent. *Mycobacterium abscessus,* a fast-growing NTM*,* is of particular importance due to increased morbidity and worse clinical outcomes when compared with other NTM species. It is also causative of poor lung transplantation outcomes, attributed to disseminated infection following the commencement of immunosuppression(202).

In addition, emerging pathogens such as *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* are becoming more prevalent in an ageing CF population. In a recent Canadian study, chronic *Stenotrophomonas maltophilia* infection, although not independently associated with decline in FEV_1, showed a higher rate of pulmonary exacerbation requiring inpatient treatment(208).

Not only does the pathogenic make-up of the CF lung change over time, the resistance patterns of said pathogens also change, making management more complex. The prolonged and recurrent use of antimicrobials certainly plays a role in alterations in antimicrobial resistance patterns with time, particularly with chronic *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections.

With improving CF survival, end-stage lung disease and respiratory failure is now becoming increasingly an adult phenomenon. FEV_1 of 30% of predicted or less indicates advanced lung disease and historically this parameter has been the most reliable predictor of mortality in CF(209). Amongst other variables, this is an indication for lung transplant referral. However, many patients are now surviving for longer into adulthood with low lung function. In 2008, Hodson and colleagues analysed a cohort of 366 CF patients over several CF centres worldwide, showing that those patients reaching the age of 40 years and above showed

stabilisation of lung function and BMI, and had a median survival of 53 years(210). George et al (2011) showed improved survival in CF patients with low lung function, perhaps partly as a result of increased use of nebulised mucolytic therapy(211). Ramos et al (2017) observed CF patients with FEV_1 <30% predicted to exceed survival expectations, with perhaps increasing pulmonary exacerbation frequency and the use of supplemental oxygen use to be stronger predictors of mortality in CF(212).

Non-invasive ventilation and domiciliary oxygen therapy are used in CF patients with respiratory failure. NIV is indicated for both palliation and as a bridge to lung transplantation. Increasing survival in CF will influence lung transplant numbers, and indeed the CF Registry reported a 9.8% increase in CF lung transplants 2017 than in the previous year. With increasing numbers of patients on the transplant waiting list comes a greater demand for organs which may vastly exceed supply in an ageing CF population. Optimal timing of lung transplantation in the CF disease course is challenging and relies on 'stability' of severe lung disease along with optimal BMI, adequate liver, renal and cardiac function as well as psychological stability. As survival in CF increases and comorbidities emerge, we may see 'ageing' organs and their subsequent dysfunction become a barrier to lung transplantation. Since CFTR-related complications continue to occur in other native organs post lung transplant, optimal pre-transplant care should remain the main goal in the quest for longevity in CF.

Invasive ventilation in an intensive care setting can be used in selected CF patients and tends to be reserved for those with acute, reversible pulmonary complications such as haemoptysis and pneumothorax, with generally a good prognosis. However invasive ventilation in the event of NIV failure in end stage disease or during deterioration whilst awaiting lung transplantation carries a poor prognosis. In some transplant centres extra-corporeal membrane oxygenation (ECMO) has been used in a minority of CF patients as a bridge to lung transplant. Patients with refractory hypercapnic respiratory failure may benefit from a limited time on veno-venous (VV) ECMO whilst awaiting transplantation. 'Awake' ECMO allows the patient to be assisted with airway clearance and maintain a degree of functional limb strength before heading into transplantation, potentially producing better outcomes. With this being said, ECMO is still considered a contraindication to lung transplant in many centres. Outcomes of CF patients on ECMO remain poor(213) but

optimal ECMO strategies and intensive care input may prove increasingly necessary in an ageing CF population with a greater proportion of patients reaching end stage lung disease and its associated complications(214).

1.12.2 Extra-pulmonary complications

A number of extra-pulmonary comorbidities in CF are more prevalent with age. These include bone disease, CF-related diabetes mellitus (CFRD), CF arthropathy and gastrointestinal malignancy.

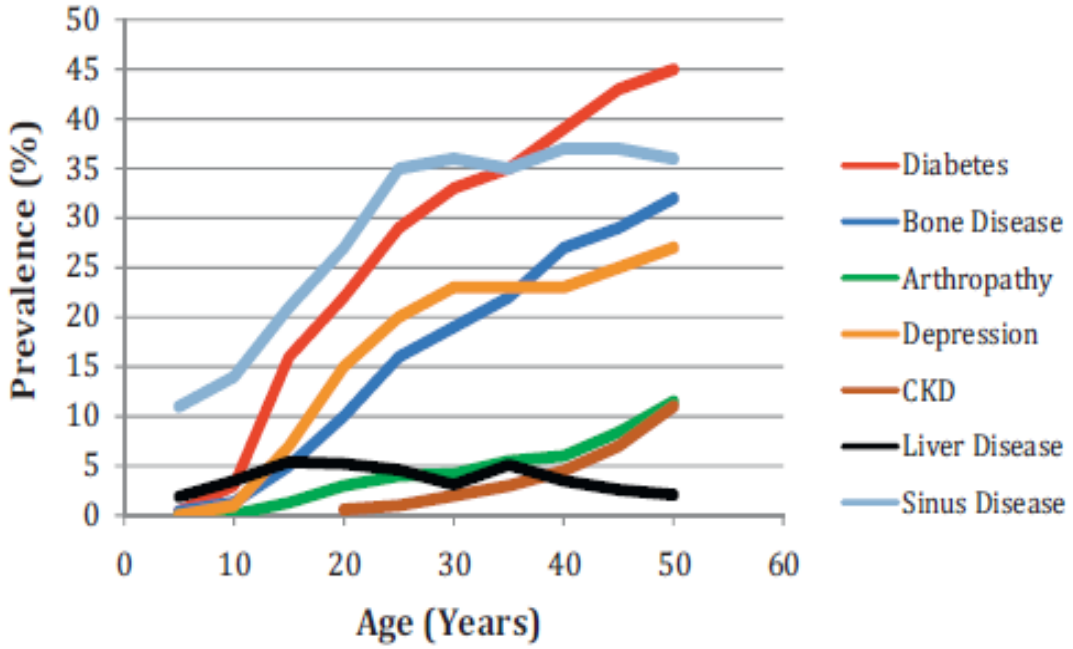


Figure 1.8: The prevalence of extra-pulmonary manifestations of CF with age – from Quon et al, 2012(202).

Specifically, macrovascular disease and renal dysfunction may become more common in an ageing CF population.

1.12.2.1 Bone disease

Osteoporosis and osteopenia are more prevalent with age in CF. Low bone mineral density (BMD) in CF is the result of a multitude of factors, including malabsorption, vitamin K and D deficiency, malnutrition, chronic pulmonary infection and reduced physical activity. Corticosteroid therapy and hormonal imbalance including delayed puberty also contribute. Some studies have shown a lower BMD in those with homozygous Phe508del mutations than in other CFTR mutation classes(215). A direct CFTR effect on bone interferes with osteoclast and osteoblast function. Loss of BMD may be increased by inflammatory cytokine release during pulmonary exacerbation. Fractures associated with low BMD generally occur

in adulthood, of which the commonest sites are ribs and thoracic vertebrae. Low BMD has implications for morbidity and is closely related to lung function(216). Optimising BMD is also important for transplant eligibility. In an ageing CF population with increasing risk of BMD and fracture, we may see increasing prevalence of bisphosphonate use.

1.12.2.2 Diabetes mellitus

CF related diabetes mellitus (CFRD) is the commonest extra-pulmonary comorbidity in CF and is a distinct entity from T1DM and T2DM. There is an increasing insulin deficiency over time due to pancreatic fibrosis and islet cell destruction. There is also fluctuating peripheral insulin resistance with age, malabsorption and pulmonary exacerbation. Hyperglycaemia in CF includes a clinical spectrum ranging from impaired glucose tolerance to overt diabetes, with or without fasting hyperglycaemia. The incidence of fasting hyperglycaemia amongst CFRD patients increases with age(45).

CFRD is an age prevalent condition, with the majority of patients being diagnosed in adulthood. In some studies, this is as high as 52% of patients over the age of 40 years(217). The prevalence of CFRD is also influenced by exocrine pancreatic status, gender, CFTR class and oral corticosteroid use. Lung function, nutritional status and CFRD are intimately linked(218), and optimal treatment of CFRD is essential to prevent sustained clinical decline. CFRD screening is performed annually using glucose tolerance testing or continuous glucose monitoring. Mortality is higher in CF patients with CFRD and microvascular complications seem to accumulate with time following established fasting hyperglycaemia in those with poorer glycaemic control(219). Microalbuminuria is common in CFRD and is often the first sign of diabetic nephropathy. CFRD may predispose both chronic renal impairment(220) and antibiotic-related acute renal injury(221).

1.12.2.3 Macrovascular disease and arterial stiffness

Macrovascular disease is thought to be rare in the CF population as compared with the general population. However, the incidence of macrovascular disease, as with non-CF diabetics, increases with duration of hyperglycaemia and thus is likely to become more significant with an ageing CF population. As survival in CF continues to increase, the prevalence of other cardiovascular risk factors may also increase along with CFRD, including systemic hypertension, dyslipidaemia and obesity. A study by Skolnik and colleagues in 2016 showed a CFRD prevalence of 56% and a dyslipidaemia prevalence of 69% in a small CF

population over 40 years of age(222). High fat diets and high inflammatory burden in CF contribute to cardiovascular risk. Those on long term oral corticosteroid therapy will be more prone to dyslipidaemia and systemic hypertension. CFTR modulating therapy, in correcting CFTR-related malabsorption and nutritional deficits, may indeed contribute to hypercholesterolaemia and weight gain with increased CF survival. Ivacaftor has been shown to normalise gastrointestinal pH and raise BMI in as little as four weeks(223). Longitudinal data on this topic is yet to be explored but may prove insightful.

Coronary artery disease is rare in CF but has been seen in a handful of cases in the pre-transplant setting, one reported case with significant multi-vessel disease in the absence of CFRD(224). Aortic wall stiffness increases as part of the normal vascular ageing process and has been shown to be an independent risk factor for coronary artery disease. Central arterial (aortic) stiffness can be measured non-invasively using carotid-femoral pulse wave analysis, calculating measurements of AIx and PWV. PWV is a direct measure of arterial stiffness and is the most reliable in adult populations. AIx is a surrogate measure of arterial stiffness and may be influenced by left ventricular function, heart rate and blood pressure.

The chronic systemic inflammation occurring in CF increases arterial stiffness and endothelial cell dysfunction, creating a risk factor for premature cardiovascular disease. AIx appears to correlate with blood markers of inflammation, C-reactive protein (CRP), in CF patients when adjusted for heart rate, but PWV does not. Hull et al (2010) showed that using a short course of intravenous antibiotics to reduce systemic inflammatory levels in a small number of adult CF patients resulted in no change in AIx or PWV, indicating that other factors must contribute to arterial stiffness in CF(225).

AIx shows more sensitivity to change at younger ages and has been used to determine arterial stiffness in the paediatric CF population. Buehler et al (2011) showed that arterial stiffness is higher in children with CF than those without and is higher in those with chronic *Pseudomonas aeruginosa* infection. It does not however seem to correlate with diabetic status(226). Conversely, Hull et al (2009) showed that both PWV and AIx are higher in adults with CFRD than those without. The lack of correlation with CFRD and arterial stiffness in paediatric CF patients may indicate the importance of duration of hyperglycaemia in CF vascular dynamics. Arterial stiffness in an ageing CF population is likely to play a role in

cardiovascular risk and this may be independent of the presence of CFRD or other cardiovascular risk factors(227).

Structural vascular changes in CF may occur as early as childhood. It is hypothesised that CF patients therefore suffer from premature vascular ageing as a result of their chronic inflammatory process and this process may be accelerated in those with CFRD(199). A similar process occurs in chronic obstructive pulmonary disease and non-CF bronchiectasis given the chronic inflammatory burden and recurrent infection(228). Research into arterial stiffness specifically in an ageing CF population will help to provide evidence of cardiovascular risk in this group and may have implications for future management as survival continues to increase.

There is currently no guidance in the CF population regarding specific assessment of cardiovascular risk. It is feasible that, as CF survival improves, utilising primary care cardiovascular risk scores such as the *QRisk®2/3* as in the general population, would be appropriate in this setting. It may then help to stratify those older CF patients at higher risk of cardiovascular disease. Total cholesterol and LDL cholesterol increase with advancing age and raised blood levels can be seen in 24-43% in adult CF patients, depending on exocrine pancreatic status(229). Cardiovascular risk scoring in CF patients aged of 40 years and above, and those with traditional cardiovascular risk factors, will aid decision making regarding the introduction of cholesterol lowering medication, anti-hypertensives and performing further investigation. The assessment of cardiac function in more detail using cardiac MRI may be useful in those with higher cardiovascular risk, particularly given that advancing pulmonary disease can lead to pulmonary hypertension, right ventricular dysfunction, left ventricular diastolic dysfunction and cardiac failure(230).

The inclusion of CFRD into cardiovascular risk scoring will be challenging due to the lack of ability to confidently categorise it into a distinct T1DM or T2DM class. The difference in calculated risk between these two entities in CFRD may be insightful and worth further exploration. Cardiovascular risk scoring in CF may also prompt closer attention to family history and genetic factors, which will have bearing on the risk of premature CVD. In addition, the use of ApoB measurements in the general population has been shown in the

literature to be a more sensitive marker of hypercholesterolaemia and cardiovascular risk prediction than LDL cholesterol(231). This is yet to be investigated in an older CF population.

1.12.2.4 Renal disease in CF

Both chronic and acute renal disease is seen in the CF patient. The majority of this is secondary in nature with primary renal pathology remaining rare. Current data suggests that CKD of a moderate to severe degree is seen in around 2% of the adult CF population, with significant risk in the CFRD cohort(220). Alongside renal nephron loss and decline in renal function with general ageing, the prevalence of CKD in CF seems to increase with age to a greater degree than in the general population. Renal nephron loss is accelerated by diabetes mellitus, vascular disease and chronic infection, all of which pertain to the ageing CF patient. In addition, renal risk factors in CF include recurrent sepsis, nephrolithiasis and the recurrent use of nephrotoxic medication such as aminoglycosides, non-steroidal anti-inflammatories (NSAIDs) and post-transplant immunosuppressive agents.

As CF survival increases, a greater number may receive lung transplantation and at older ages. Immunosuppressive therapy such as ciclosporin is a risk factor for renal failure. A study by Ishani et al found that renal dysfunction was present in up to 90% of patients following lung transplantation with around 7% eventually developing end stage renal failure. However, the risk in CF patients did not seem to be higher than in other recipients. The risk for post-transplant renal dysfunction seems to be higher in females and those with a lower eGFR in the pre-transplant period(232).

1.12.2.5 Measuring renal function in CF

As survival continues to increase in the CF population, renal failure will become an increasing concern and it is essential to find an optimal clinical tool for monitoring and diagnosing renal dysfunction in these patients. Measuring renal function accurately in CF patients is challenging. Frequent fluctuations of creatinine with clinical status means that creatinine-based eGFR equations may often be inaccurate. In addition, the MDRD and CG formulas, although widely used, have not been validated in CF populations. CKD-EPI equations are increasingly being used and may prove the most reliable serum creatinine measurement of eGFR. Although urinary creatinine clearance has long been established in clinical practice as an adequate marker of eGFR in CF, its measurement requires accurate

urine collection over a 24-hour period and is subject to error. Literature suggests that creatinine-based eGFR calculations tend to over-estimate renal function in CF patients and thus may miss the subtleties of early renal disease(71). A study by Al-Aloul in 2007 showed that CG and MDRD formulas overestimate renal function by over 18 and 15ml/min respectively in adult CF patients with measured urinary creatinine clearance of less than 80ml/min(233). In addition, serum creatinine may not rise above normal levels until up to 60% of GFR has been lost(234). Hence it is vital that, when creatinine is within normal range, accurate GFR calculation is sought to optimally assess renal function.

Extensive research in non-CF populations has previously shown the most accurate GFR measurement to be obtained by serial serum and urine clearance measurements of exogenous compounds filtered exclusively by the glomerulus, including inulin, iothalamate and iohexol. A study by Novel-Caitin et al in 2017 compared iohexol and inulin mGFR to MDRD and CG eGFR in 21 CF patients with a median age of 31 years awaiting lung transplantation, finding a lower mGFR than eGFR in 9.5% of patients(235). However, the expense and impracticality of their use in standard clinical practice has influenced research into other urine and serum endogenous biomarkers as measurements of renal function in CF.

Cystatin C is a useful serum endogenous renal marker since it is exclusively filtered by the glomerulus with minimal tubular reabsorption. Its incorporation into recent CKD-EPI eGFR calculations have shown promise, although more research needs to be done in its use in a large CF population. A study by Beringer and colleagues in 2009 showed cystatin C to be a more sensitive marker of eGFR in CF patients than serum creatinine-based equations when compared to iothalamate GFR(189). A study by Halacova et al showed Cystatin C to be a better indicator of eGFR in CF patients being treated with IV amikacin than both serum creatinine and creatinine clearance(236). Cystatin C eGFR calculations have also shown promise in monitoring renal function decline in diabetic populations when compared to iothalamate clearance, which may be extremely relevant in a comparable, ageing diabetic CF population(237).In addition, urine biomarkers of renal tubular damage may have a role in early renal disease screening in CF, including N-acetyl-β-D-glucose-aminidase (NAG) and alanine amino-peptidase (AAP). A study by Etherington et al has shown urinary NAG levels

to be raised in CF patients undergoing intravenous aminoglycoside treatment in the acute setting and, although this appeared to normalise within a few months in most, patients with CFRD had raised urinary NAG at baseline and follow-up indicating a degree of sustained renal impairment(221).

The use of intravenous aminoglycoside therapy for the treatment of pulmonary exacerbation is a necessity in a large number of patients due to its rapid distribution, high efficacy and anti-pseudomonal activity. The most prevalent strain of *Pseudomonas aeruginosa* in the UK is the Liverpool epidemic strain (LES), most sensitive in vitro to tobramycin. However, aminoglycosides such as tobramycin are nephrotoxic and cause direct proximal renal tubular cell damage in a dose-dependent manner. Due to alterations in renal pharmacokinetics in CF patients(238), high doses are often required to achieve target therapeutic levels thus increasing risk of renal damage. Aminoglycoside therapy has been shown, as early as the 1980s, to contribute to both acute renal impairment and cumulative renal injury predisposing the CF patient to chronic renal failure later in life(239).

In 2005, Al-Aloul and colleagues examined a CF cohort for renal damage from cumulative aminoglycoside use, using urinary creatinine clearance and CG estimations of GFR. They showed that 42% of patients with normal baseline creatinine had renal impairment of less than 80ml/min/1.73m^2, appearing to correlate with cumulative intravenous aminoglycoside use with a median number of patient antibiotic courses of 40(86). If aminoglycosides cannot be avoided, a once daily dosing strategy is used to reduce the risk of renal toxicity with similar therapeutic outcomes to multiple dosing regimens, as shown in the TOPIC study in 2005(240). Alternative routes of aminoglycoside administration have been studied, showing less renal damage with the nebulised than with the intravenous route, however further studies are needed prior to its replacement in standard clinical practice. In an ageing CF patient, recurrent and cumulative use of intravenous aminoglycosides may be a causative factor in the prevalence of renal disease, and this has not yet specifically been studied in the over 40-year old population. Further work is needed to establish the most accurate measure of renal function in CF patients as a whole as well as establishing the causative factors and prevalence of renal dysfunction in older CF patients.

Nephrolithiasis in CF is caused primarily by electrolyte imbalance from malabsorption and dehydration, resulting in hyperoxaluria and hypercalciuria(241). Diuretics and corticosteroid treatment can exacerbate abnormalities in calcium metabolism. Recurrent antibiotic use alters the function of oxalate-degrading bacteria in the gastro-intestinal tract, predisposing to renal oxalate stones(242). Cumulative antibiotic use with age may therefore exacerbate this pathology.

Although primary renal pathology is rare in CF, amyloidosis and IgA nephropathy are increasingly being recognised. Secondary amyloidosis is a result of chronic inflammation in CF, can cause nephrotic syndrome and has a poor prognosis(243). IgA nephropathy is the commonest glomerulonephritis reported in CF patients(244). High IgA levels and associated renal deposition is associated with autoimmune disease but can also be seen in the chronic inflammatory state created by CF. It too may carry a poor prognosis.

1.12.2.6 Psychosocial complications

An ageing CF population inevitably will alter psychosocial aspects of the disease. With survival historically being poor, options for employment and having families were limited. As survival increases, more patients are entering and sustaining full or part-time employment. It has also been shown that CF is not necessarily a barrier to career success and progression(245). The number of successful CF pregnancies is rising and the CF Registry reported increasing numbers of female CF patients having children in 2017(4). Pregnancy can sometimes be associated with decline in overall clinical status so must be monitored closely by CF and obstetric teams. Goss and colleagues in 2003 showed that pregnancy in CF is not associated with a survival disadvantage in the longer term(246). With the advent of intracytoplasmic sperm injection, increasing numbers of male CF patients are also becoming fathers. This perhaps evidences the shift in attitudes towards CF and disease longevity. Patients are 'living with' CF rather than suffering its consequences. Qualitative research into this area may reveal new patient perspectives of ageing with CF and will be an exciting area for research.

Improving health-related quality of life in CF is becoming increasingly important in an ageing population, on which mental illness has a large impact. Anxiety and depression are up to 30% more common in CF patients than the general population and are associated with

poorer quality of life and, in some cases, lower lung function(247). Questionnaires and scoring systems are used frequently in CF communities to monitor psychosocial health and most MDT services include dedicated psychology and social work teams. Some themes contributing to mental health issues in CF may include high treatment burden, family and employment life balance, difficulties in self-management and maintenance of independence, and premature death. Insomnia is also common in CF patients and may contribute to mental health issues(248). CF clinicians may see an increasing prevalence of anxiety and depression with advancing disease in an ageing CF population, adding to complexities of multidisciplinary management.

1.12.2.7 Complications of treatment with age

As already discussed, aminoglycosides contribute significantly to renal toxicity in CF. Since aminoglycosides remain important in CF care, clinicians will utilise the least nephrotoxic aminoglycoside agent, monitor drug levels closely and adjust dosing according to target drug levels and renal function. Tobramycin is less nephrotoxic than gentamicin. Concomitant nephrotoxic medication such as nonsteroidal anti-inflammatories should be avoided. A strategy using additional drugs to compete for renal binding sites, and therefore reducing tubular reabsorption of aminoglycoside, may decrease renal toxicity. Fosfomycin is such a drug, with good anti-pseudomonal properties and which has been shown to be well tolerated in patients when used in combination with standard antibiotics during treatment of pulmonary exacerbation(249). However, Fosfomycin is not routinely used in intravenous form for the treatment of pulmonary exacerbation in CF patients in the UK. This may become more relevant in an ageing CF population, with the emergence of bacterial resistance to antibiotic therapy and deteriorating renal function with cumulative aminoglycoside use.

Inhaled antibiotic therapy is widely used in CF due to the high sputum distribution, ease of self-administration and efficacy against chronic *Pseudomonas aeruginosa* infection. Their regular use may reduce the requirement for intravenous therapy and, given the low systemic absorption of nebulised tobramycin, there is less potential for renal toxicity(98).

Anti-inflammatory therapies in CF remain contentious due to side effects, particularly in an older age group. NSAIDs have been shown to benefit lung function in a paediatric population(250) but their use is limited in adult populations due to the potential for

gastrointestinal and renal complications. Oral corticosteroids are only recommended for chronic use in ABPA given their negative long-term effects on diabetes mellitus, lipid control and BMD. Azithromycin is widely used in CF due to its anti-inflammatory and antimicrobial properties. Chronic use has been shown to improve lung function and reduce pulmonary exacerbations(251). Concerns with long term use spanning into an ageing adult population include emergence of drug-resistant pathogens, including NTM.

1.12.2.8 Genotype and survival

The effect of CFTR genotype and resulting clinical disease expression is variable in CF. The phenotypic variability between genetic groups will depend upon the interplay between the degree of CFTR activity, modifier genes contributing to non-CFTR specific organ dysfunction and environmental factors. However, in general terms, the greater the loss of CFTR activity the more significant the clinical disease. CFTR class I to III mutations confer more severe disease in CF, usually accounting for exocrine pancreatic insufficiency, greater lung function decline and higher rates of chronic *Pseudomonas aeruginosa* infection. In 1996, Kerem et al showed that CF patients homozygous for Phe508del, a class II mutation, were diagnosed earlier in life, were exocrine pancreatic insufficient, had worse lung disease and a lower BMI than those with heterozygous Phe508del or no Phe508del mutations(252). Class IV and V CFTR mutations confer residual function of CFTR protein and usually less severe disease. A study by McKone et al in 2006 showed that groups with these "low risk," residual CFTR function mutations are diagnosed at an older age, have lower rates of chronic *Pseudomonas aeruginosa* infection and lower mortality when compared with classes II and III(253). In addition, higher sweat chloride levels correlate with greater loss of CFTR activity, lower lung function, lower BMI and worse survival(254). When assessing the genetics and sweat chloride levels in our older CF population, we may see a high proportion of low risk CFTR mutations accounting for their survival advantage. Simmonds et al in 2009 showed that 37% of their over 40 CF cohort were Phe508del homozygous, the rest being in Phe508del heterozygous or other mutation groups(200). It is worth noting however that class IV and V CFTR mutations can still cause severe disease and premature mortality. Lung function decline is inevitable across all genotype classes, however the rate of decline may appear to be slower in those with heterozygous Phe508del mutations with greater residual CFTR function(255). The rate of decline in lung function may be seen to 'even out' between

genotypes with advancing age, given that the more severe genotypes confer lower lung function and premature mortality. In addition, age of diagnosis and gender has an influence on long term survival. Females diagnosed with CF in childhood have a worse prognosis than males, although there is no such survival disadvantage in adult-diagnosed females. Patients diagnosed in adulthood may have non-classical genotypes, a delay in pulmonary disease, higher baseline lung function and slower rate decline. However, those who survive beyond 40 years of age will see similar rate of FEV_1 decline irrespective of genotype and age at diagnosis(256).

1.12.2.9 CFTR modulators

The importance of CF genotype cannot be understated in modern CF treatment and there has been a significant shift in management over the last decade to include CFTR mutation-specific therapies. The defect in CFTR protein quantity or function determines eligibility for specific therapies. CFTR mutation classes, CFTR abnormality and potential targets for CFTR modulation are shown below.

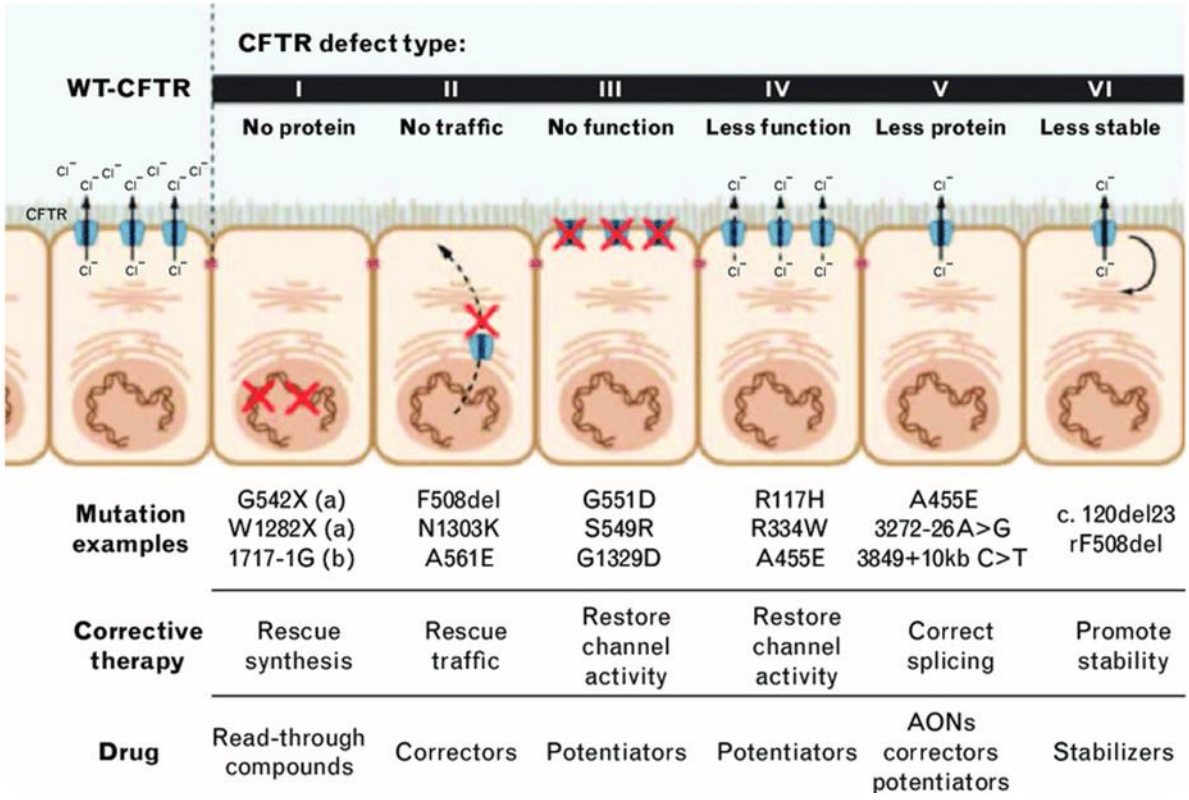


Figure 1.9: CFTR classes and options for CFTR modulator therapy. Adapted from Moss et al, 2015(257) *(WT=wild type {normal}).*

Ivacaftor (Kalydeco®) monotherapy has shown clinical benefit in CFTR class III and IV gating mutations, which represent a relatively small number of CF patients. However, in combination with lumacaftor or tezacaftor, ivacaftor has shown clinical benefit in class II Phe508del homozygotes by improving lung function, exacerbation rates, BMI and quality of life. Tezacaftor/ivacaftor (Symdeko®) has also shown benefit in heterozygous Phe508del and CFTR residual function mutations(258), creating potential treatment options for a larger number of CF patients. The recent emergence of triple combination CFTR modulators will have benefit to a wider range of CFTR genotypes moving forwards, including those with residual and minimal function CFTR mutations(95)(96). Survival in CF will undoubtedly be positively influenced by the introduction of these medications. Longitudinal data regarding adverse effects of these medications will be important to examine in an ageing CF population as survival continues to increase.

1.13 Conclusion

The advances in diagnosis and management of CF and the resulting impact on survival is one of the success stories of modern medicine. As survival rates in the CF population continue to increase and more patients survive into their fifth and sixth decades, and even beyond, there is no doubt that the complexities of CF disease and associated comorbidities will make management increasingly more challenging.

We may see an evolving complex interplay between metabolic factors, renal disease, conventional cardiovascular risk and inflammatory arteriopathy. This will predispose an ageing CF patient to premature vascular ageing and atherosclerosis, increasing their risk of cardiovascular morbidity and mortality. Cumulative doses of nephrotoxic medication will impact comorbid burden and treatment course. Survival advantage conferred by the development of CFTR modulation therapy and continual advances in CF MDT care may be complicated by the emergence of ageing, CFTR-related and treatment-related comorbidities. This will inevitably result in higher treatment burden for older CF patients as well as placing strain on specialist CF services. Although recent literature has contextualised the older CF patient and developing complications, the true impact of renal disease, cardiovascular morbidity and ototoxicity is unknown in the CF population over 40 years of age. Therefore, we must consider some important research questions;

1. How prevalent is cardiovascular disease in the CF population over 40 years of age and how can we better stratify risk in order to positively influence management decisions?

2. How prevalent is renal disease and what is the most accurate measure of renal function in these patients?

3. Are there ways to protect older CF patients from the risks of ageing and optimise management of the comorbidities associated with this process?

I will be undertaking an observational, cross-sectional, prospective study of CF patients aged 40 years and above currently under the care of the Manchester Adult Cystic Fibrosis Centre (MACFC). My research aims to further analyse and advance knowledge in the area of ageing in CF, closely observing the cardiovascular and renal comorbidities in this patient cohort. In

addition, treatment complications with age such as aminoglycoside nephrotoxicity will be analysed. We hope to provide further insight into the ageing CF patient in order to aid clinical decision-making and management of this group, but of greater importance to understand the process of ageing in CF and how we can work together to maintain stability and quality of life in CF patients as survival continues to increase.

The Complexities of Ageing in Cystic Fibrosis

Chapter Two: Study aims and methods

2.0 The Complexities of Ageing in Cystic Fibrosis Study (COA-CF)

2.1 Hypothesis

Survival in CF is improving exponentially and with this brings an ageing CF population. Along with CFTR-mediated disease progression over time, patients will begin to develop age-related comorbidities similar to an ageing general population. I postulate that these comorbidities may develop to a greater extent in older CF patients compared with the general population and will increase the complexity of their management.

2.2 Study aims and objectives

1. To characterise the demographics of an older adult cystic fibrosis population, specifically those aged 40 years of age and above.
2. To assess the cardiovascular risk and contributory factors in this cohort, including the prevalence of systemic hypertension, hypercholesterolaemia and CF-related diabetes mellitus (CFRD).
3. To assess arterial stiffness parameters in the older adult CF patient, whether this is augmented in CF when compared with the general population, and a chronic kidney disease (CKD) cohort, and thus if CF patients are at risk of premature vascular ageing as part of their disease process.
4. To assess the prevalence and causative factors of renal disease in an older adult CF population.
5. To better define the most accurate method of calculating renal function (using glomerular filtration rate, GFR) in order to diagnose early renal disease in higher risk groups, such as those with CFRD.
6. To investigate cardiac structure, function and the presence of myocardial fibrosis in older adult CF patients.

Our overall aim is to better understand an ageing CF population and the complexities that will inevitably arise with improving survival. These complexities will include age-related, treatment-related and CFTR-related comorbidities. Median age of survival is increasing exponentially and factors contributing to survival are plentiful. With the advent of CFTR

modulator therapies and the ever-changing landscape of CF treatment, patients will continue to gain survival benefit giving rise to age-related comorbidities not commonplace in previous CF management. It will be of utmost importance to recognise and manage these emerging complications in order to optimise future CF care.

2.3 Study Methods

2.3.1 Study Overview

This was a prospective, cross-sectional, observational, cohort study of CF patients at the Manchester Adult Cystic Fibrosis Centre (MACFC) aged 40 years and above. We aimed to recruit from a total of 92 eligible patients within our centre, selected on the basis of inclusion criteria listed below.

Ethical review was undertaken and approved by The Health Research Authority (HRA) and Health and Care Research Wales (HCRW) Ethics committee (reference 19/EM/0067). This work was conducted according to the Helsinki Declaration.

The study took place between August 2018 and August 2020.

2.3.2 Participants

Inclusion criteria

- Diagnosis of CF and under the care of MACFC
- Aged 40 years and above
- Able to provide informed consent

Exclusion criteria

- Current pulmonary exacerbation
- Lung transplant recipient
- Pregnant
- Under the age of 40 years

Eligible patients were recruited during one of their routine clinic visits, preferably at their annual assessment due to the necessity of a fasting blood sample, which is performed as part of the CF annual assessment. Collating the annual visit and study visit aimed to optimise patient convenience.

Patients were provided with a patient information sheet (PIS) via post at least 24 hours prior to recruitment, providing the opportunity to ask questions and discuss the study in greater detail if needed.

2.3.3 Data collection

1. Patient questionnaires were completed by the research team with each patient during study visit one for epidemiological and clinical data collection (appendix 2).
2. Supplementary retrospective data collection was performed from patient case notes.
3. Intravenous aminoglycoside days were calculated for the preceding ten-year period using specific case notes containing detailed records of pulmonary exacerbations and treatment for each study patient for the duration of their care at MACFC to date.
4. Study participants then had the following prospective data collected;
    a. Central systolic blood pressure (CSBP) using Vicorder® analysis (Skidmore Medical).
    b. Arterial stiffness measurements using Vicorder® analysis (Skidmore Medical), to include pulse wave velocity (PWV) and augmentation index (AIx). A carotid/femoral approach was used in order to measure central aortic PWV (cfPWV) and AIx.
    c. Sweat testing was performed using the protocol outlined by Manchester University NHS Foundation Trust, MFT (Wescor macroduct™ iontophoresis); to assess sweat chloride concentration (appendix 4). Pre-modulator sweat chloride results were used for patients already commenced on CFTR modulation at the time of the study.
    d. Blood sampling was undertaken as follows;
        - High sensitivity C-reactive protein (hsCRP).
        - Standard renal profile (sodium, potassium, urea, creatinine) and derivations of estimated glomerular filtration rate (eGFR) were made using Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulae.

- Cystatin C; as a measure of renal function and derived eGFR using CKD-EPI equations.
- Lipid profile to include LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG) and HDL-C/TC ratios.
- Samples for apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and ApoB/A1 ratios (samples sent to Sheffield).
- Iohexol clearance GFR – blood sampling was performed at 0, 30, 120, 180, 240 minutes following iohexol injection (see protocol; appendix 3).

e. Urine sampling was performed to include:
   - Albumin-creatinine ratio (ACR).
   - 24-hour urinary creatinine clearance.

f. Cardiac magnetic resonance imaging (CMR) was performed in a select group of patients to assess cardiac structure and function.

Although most data were collected prospectively, we recognise the limitations of retrospective data collection (bias and systematic error) and this may be particularly relevant to the calculation of intravenous aminoglycoside days from case records.

2.3.4 Study visit schedule

The majority of visit data collection for each patient was performed at their routine outpatient clinic visit. A minority of patients attended for an additional 'study-specific' appointment at their request. The study visit schedule is shown below (figure 2.1).

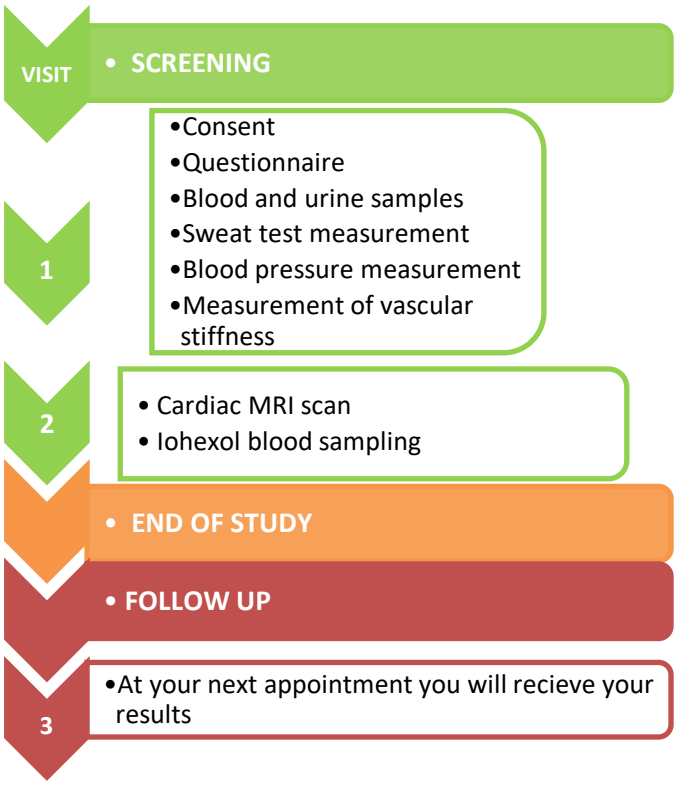


Figure 2.1: Study visit flow diagram

Visit one

This visit lasted approximately 1.5 to 2 hours. Informed written consent was taken by myself, or my supervising consultants in my absence, as per the study delegation log. A questionnaire was completed with each patient to collect epidemiological and clinical data (appendix 2). Data collection for this visit also included;

- Measurements of CSBP, AIx and carotid femoral PWV (cfPWV) using the Vicorder® device (Skidmore Medical).
- A sweat test.
- Blood sampling for the biochemical tests as outlined above. The patient received instructions to be fasted for ten hours prior to this visit (if possible). If the first study visit coincided with the patient's annual assessment, they were fasted as per usual annual visit protocol.
- Urine sampling was taken for the biochemical tests as outlined above.

Visit two

Selection of participants for the second study visit was largely determined from the outcome of visit one. Although recruitment for visit one included all eligible patients (n=92), the nature of visit two required recruitment of a smaller patient numbers with an aim to include 10 to 20 patients for each test.

- Eligible patients attended for CMR, chosen based on lack of contraindications and willingness to undertake cardiac MRI.
- Iohexol blood sampling was be undertaken over a four-hour period according to the derived protocol (appendix 3).

Visit three

The third study visit was optional, following on from the study data collection performed in the first two visits. Study results and feedback, according to patient wishes, were disseminated at their next routine outpatient clinic visit at MACFC.

2.3.5 Sampling methods

2.3.5.1 Blood sampling

Patients were invited to fast for ten hours prior to a morning study visit to enable all initial study blood biomarkers to be collected within one visit.

Blood sampling was undertaken via a single venepuncture procedure where possible by myself or the specialist CF clinic nurses. A total of three serum blood tubes were filled.

Samples were transferred directly from MACFC to the biochemical laboratory at Wythenshawe Hospital. Blood samples were then centrifuged and split into appropriate aliquots for further processing. All samples aside from the non-routine apolipoprotein and cystatin C were processed and stored at Wythenshawe hospital, according to standard MFT laboratory protocol.

Cystatin C samples were transported via internal sample transport to the biochemistry laboratory at Manchester Royal Infirmary (MRI), where samples were processed and stored according to standard MFT laboratory protocols.

The apolipoprotein A1 and B samples were transported by first class Royal Mail delivery to the Immunology department at Sheffield Teaching Hospitals NHS Trust. Samples were processed and stored according to local laboratory protocols, under the supervision of Jonathan Aldis, Immunology Laboratory Manager. Results were sent directly back to MACFC for collation and analysis.

Details of laboratory processing procedures are shown in the tables below. Normal reference values for clinical biochemistry tests will vary between laboratories and the quoted reference ranges are applied to interpretation of results within the stated laboratory trusts.

Biochemical test	**Sample assay**	**Assay frequency**	**Reference range and source**	**Processing method**	**Storage information**
hsCRP	Serum, lithium heparin plasma or EDTA plasma.	Daily	<5 mg/L (Roche kit insert)	Particle enhanced immunoturbidimetry (Roche)	4 C for 4 days
Creatinine (serum)	Serum, lithium heparin plasma or EDTA plasma.	Daily	59-104 µmol/L male 45-84 µmol/L female (Roche kit insert)	Enzymatic (Roche)	4 C* for 4 days
Urea	Serum, lithium heparin plasma or	Daily	2.5-7.8 mmol/L (Pathology harmony)	Enzymatic (Roche)	4 C for 4 days

	EDTA plasma.				
Triglycerides (fasting)	Serum, lithium heparin plasma or EDTA plasma.	Daily	<1.7 mmol/L (Roche kit insert)	Enzymatic (Roche)	4C for 4 days Used to calculate LDL-cholesterol (Freidewald equation) Fasting sample required.
HDL-cholesterol (HDL)	Serum, lithium heparin plasma or EDTA plasma.	Daily	Male >1.0 mmol/L Female >1.2 mmol/L (JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice)	Enzymatic cholesterol esterase (Roche)	4C for 4 days Total cholesterol: HDL ratio used to calculate cardiovascular risk in primary prevention
Cholesterol (total)	Serum, lithium heparin plasma or EDTA plasma.	Daily	Ideal cholesterol ≤5.0 mmol/l See risk factor tables in BNF for primary prevention	Enzymatic, colorimetric (Roche)	4 days at 4C
Cystatin C	Serum, lithium heparin plasma.	Daily	Age-related: 18-50 years: 0.56-0.98 >50 years: 0.61-1.40	Particle enhanced immunoturbidimetric method (Roche Diagnostics c702 analyser)	At 4C until testing

Apolipoprotein A1, B and B/A1 ratio	Serum, lithium heparin plasma or EDTA plasma	Daily	Apolipoprotein A1 = 1.30 g/L (1.10 - 2.05) Apolipoprotein B = 0.65 g/L (0.55 - 1.40) Apolipoprotein B/A1 Ratio = 0.50 (0.35 - 1.00)	Siemens BNII nephelometer (method principle = nephelometry	At 4C until testing

*Table 2.1: Laboratory analysis methods for serum cholesterol sampling (*4C = 4 degrees centigrade).*

2.3.5.2 Measurements of renal function

Estimated glomerular filtration rate (eGFR) is a derived result from serum creatinine and cystatin C measurements. The following equations for eGFR calculation were utilised in this study, taken from The International Society of Nephrology(259);

1. Modification of Diet in Renal Disease (MDRD)

   GFR = 175 x (Standardized SCr)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.212 if African American) (mL/min/1.73m^2)

eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2, S_{cr} (standardized serum creatinine) = mg/dL, age = years

2. Chronic Kidney Disease Epidemiology Collaboration - CKD-EPI
   a. CKD-EPI creatinine (2009):

      eGFR = 141 x min(SCr/κ, 1)$^{\alpha}$ x max(SCr /κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if Black]

κ = 0.7 (females) or 0.9 (males), α = -0.329 (females) or -0.411 (males), min = indicates the minimum of S_{Cr}/κ or 1, max = indicates the maximum of S_{Cr}/κ or 1

   b. CKD-EPI cystatin C (2012):

eGFR = 133 x $\min(Scys/0.8, 1)^{-0.499}$ x $\max(Scys/0.8, 1)^{-1.328}$ x 0.996^{Age} x 0.932 [if female]

Scys (standardized serum cystatin C) = mg/l, min = indicates the minimum of Scys/0.8 or 1, max = indicates the maximum of Scys/0.8 or 1.

c. CKD-EPI cystatin C creatinine (2012):

eGFR = $135 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-0.601} \times \min(S_{cys}/0.8, 1)^{-0.375} \times \max(S_{cys}/0.8, 1)^{-0.711} \times 0.995^{Age} \times 0.969$ [if female] × 1.08 [if black]

α = -0.248 (females) or -0.207 (males).

Definitions used to classify chronic kidney disease (CKD) and proteinuria (as measured by ACR) were taken from The Kidney Disease Improving Global Outcomes collaboration (KDIGO) guidelines, 2012(259). For study purposes, GFR of >90 ml/min/1.73m^2 and ACR <3 mg/mmol were classed as normal. As per guidelines, a GFR of <60 ml/min/1.73m^2 (G3a, G3b, G4 and G5) was classed as CKD. G1 and G2 were classed as abnormal renal function if there was evidence of proteinuria (and thus renal damage).

GFR category	GFR (ml/min/1.73m^2)	Terms
G1	>90	Normal or high*
G2	60-89	Mildly decreased*
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

*Table 2.2: Categories of CKD according to GFR. *In the absence of evidence of renal damage, G1 and G2 do not constitute CKD.*

Category	Albumin excretion rate (AER) mg/24hr	ACR mg/mmol	Terms
A1	<30	<3	Normal to mildly increased
A2	30-300	3-30	Moderately increased

A3	>300	>30	Severely increased

*Table 2.3: Albuminuria categories in CKD according to KDIGO. (ACR=albumin creatinine ratio). *Presence of renal disease to include haematuria, proteinuria, structural kidney abnormalities, genetic kidney syndromes, renal tubular disorders and history of renal transplantation.*

2.3.5.3 Iohexol protocol

Accurate (measured) GFR was calculated using iohexol clearance. This was then used as a reference for identifying the most accurate eGFR method outlined above.

Iohexol GFR was calculated using a multi-sample method, with blood sampling undertaken at 0, 30, 120, 180 and 240 mins following iohexol infusion (see appendix 3).

2.3.5.4 Iohexol laboratory processing and interpretation of mGFR

Iohexol is stable at room temperature and therefore iohexol clearance measurements can be performed in most clinical settings. In addition, samples may be taken to the laboratory for storage and processing without the need for dry ice transportation. Urine sampling for iohexol clearance can be used, however plasma sampling is more accurate and clinically feasible.

We used liquid chromatography-tandem mass spectrometry (LC-MS) for iohexol sample analysis(260). The most commonly used method for iohexol analysis in European studies is high performance liquid chromatography with ultraviolet detection (HPLC-UV) due to its lower cost and easier technique, however the higher sensitivity and specificity of LC-MS makes this the preferred method where available(261).

There are considerations for intra-individual variation of mGFR using the iohexol method and a coefficient of variation between 4.2 and 10% is deemed acceptable, independent of the analytical method used. The accuracy of one method results within 10% of another study method results is considered excellent agreement. Our samples were validated using comparison between MFT analytical method and that of a Belgian group studying iohexol GFR in CF(262).

The accuracy of mGFR to eGFR within 10 to 30% (P10 and P30) is frequently used in study analysis and will be referenced in subsequent results.

The level of estimated GFR determines the timing of iohexol sampling, and a longer duration of sampling is required to improve accuracy of measured iohexol GFR in those with lower baseline renal function. Although this is not relevant to the majority of our CF patients, the lack of validity of single-sample iohexol protocols necessitated the use of a multi-sample method in our study group.

Although sampling was performed at 30 minutes following iohexol injection as a measure of iohexol distribution (fast) phase, this was not included in final analysis. Sampling at least two hours following iohexol injection, and a one compartment clearance model in the elimination phase of iohexol clearance, was utilised here.

Measured GFR was calculated from iohexol plasma clearance over time, using a one compartment model and corrected using the Bröchner-Mortensen factor to account for lack of multiple sampling in the initial iohexol distribution (fast) phase.

The resulting 'slope-intercept' measured GFR as a derivative of iohexol clearance was calculated using the method below(262)(263);

- The slope-intercept GFR (mL/min) = k × Iohexol dose (µg)/C_0 (µg/mL)

  k = slope of the (neperian) semi-logarithmic plot of plasma iohexol concentration, C_0 is the calculated iohexol concentration at time zero (intercept),

- This value was multiplied by 1.73 and divided by the body surface area (BSA, calculated from the equation: BSA = 0.20247 × height (metres)$^{0.725}$ × weight (kg)$^{0.425}$ (DuBois equation).
- The BSA-slope intercept GFR value (ml/min/1.73 m^2) was corrected by the Bröchner-Mortensen correction factor = (0.990778 × GFR) - (0.001218 × GFR^2), producing a measured iohexol GFR value (ml/min/1.73 m^2).

A single sample method using the Jacobssen formula(261) can be used in preference to a multi-sample method, although extremes of weight and the required calculation of extracellular volume are limiting factors in this method, hence we have not used this method here.

Biochemical test	Sample assay	Assay frequency	Reference range and source	Processing method	Storage information
Iohexol GFR	LC-MS	As needed	LC-MS validation against ioversal (HPC-UV to LC-MS method)(260) Validated for iohexol concentrations between 6.8 and 250µg/ml	Deuterated iohexol, 400 acetonitrile Vortex centrifuge Waters TQS Micro mass spectrometer	-80 C

Table 2.4: Iohexol laboratory processing method using LC-MS at MFT.

2.3.5.5 Urine sampling

Urine samples were sent to the Wythenshawe biochemistry laboratory for processing, including albumin-creatinine ratio (ACR) and 24-hour urine samples for creatinine clearance (where possible). These tests are done routinely in CF outpatient care.

Biochemical test	Sample assay	Assay frequency	Reference range and source	Processing method	Storage information
Creatinine (Urine) 24 hr or random urine, (24 hr for creatinine clearance)	Plain urine preferred, samples acidified with HCl or preserved with boric acid are also acceptable.	Daily	Male 9-21 mmol/24 hrs Female 7-14 mmol/24hrs(264) Normal creatinine clearance GFR=66–143 ml/min	Enzymatic (Roche)	4 C for 4 days
Albumin/ACR (urine)	Plain urine	Daily	In context of ACR, ≤2.9mg/mmol is normal	Colorimetric Bromocresol purple (Roche)	4 C for 4 days

Table 2.5: Laboratory processing procedure for urine samples.

Creatinine clearance and urine ACR are derived measurements. ACR, a strong marker of prognosis and cardiovascular risk in CKD populations, is calculated from urinary creatinine and urinary albumin. Normal ACR is ≤2.9mg/mmol.

The 24-hour urinary creatinine clearance is calculated from the following equation;

Creatinine clearance (CrCl) ml/min = (urine creatinine x volume) / serum creatinine

The normative reference values for urinary creatinine clearance at MFT is currently quoted at 66–143 ml/min, with no variation for gender(264). Reduced urinary creatinine clearance was defined as <80ml/min/1.73m^2 in line with a similar study in CF(86).

All laboratory samples will be stored for no greater than one week and, following processing, will be destroyed within this time frame according to local trust policy.

2.3.5.6 Sweat testing

In the years preceding the discovery of the CFTR gene in CF, diagnosis was made on the basis of clinical symptoms and sweat testing. Patients with CFTR dysfunction consistent with phenotypic CF classically have an elevated sweat chloride and this can be measured via a simple non-invasive method. The later introductions of new-born screening and gene testing, alongside sweat collection, form the foundation of definitive CF diagnosis. If new-born screening suggests a diagnosis of CF, sweat chloride measurement is the gold standard confirmatory test, usually done prior to genetic testing(265).

The sweat test can be undertaken in patients suspected of having CF from as early as two weeks after birth. Furthermore, sweat chloride levels can also be used to monitor therapeutic effect of and patient compliance to CFTR modulator therapy.

Sweat chloride levels of >30mmol/L are considered abnormal based on current guidelines, with 30-59 being an intermediate (equivocal) result(27). However, to make a diagnosis of CF, sweat chloride concentration must be ≥60mmol/L(265). Details of the sweat test laboratory processing at MFT are shown below (table 2.6). It should be noted that MFT uses an equivocal sweat chloride concentration of between 40 and 60mmol/L, slightly different to current guidelines.

Biochemical test	Sample assay	Assay frequency	Reference range and source	Processing method	Storage information
Sweat test	Sweat (plain tube)	Weekly	Cl <40 mmol/L conductivity (CF foundation) <50 NaCl equivalents Cl >60 mmol/L - abnormal, consistent with cystic fibrosis Cl 40-60 mmol/L - equivocal results CL <40 mmol/L - normal	Conductivity-argentimetry Sweat chloride concentration-colourometry (Sherwood 926 chloride meter) Chloride - Indirect ISE (Roche)	7 days at 4C

Table 2.6: Sweat test analysis method at MFT

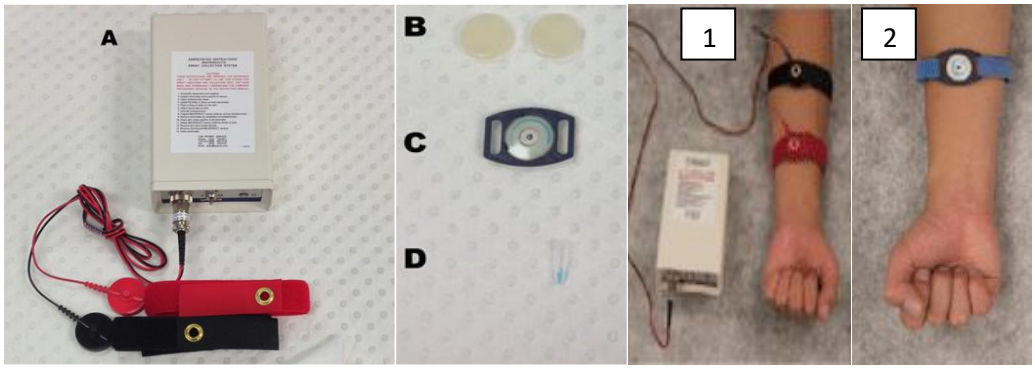
The sweat test procedure takes around 45 minutes to complete. The sweat collection method at MACFC uses the Wescor Macroduct™ (Logan, Utah 1983) system. The Wescor Macroduct™ system is suggested in the guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis in the UK (BI-X-MISC-10) as being an appropriate system for sweat collection(266).

The Wescor Macroduct™ system uses iontophoresis. This is a process by which topical pilocarpine (within gel discs) is propelled transdermally using a small electrical current (2.5-4mA for five minutes), causing stimulation of muscarinic receptors in eccrine sweat glands to produce sweat. The sweat is collected into a narrow plastic tubing forming part of the macroduct system.

The sweat test procedure

The test was performed by a qualified nurse or doctor in MACFC, who has been appropriately trained and in whom competency in the procedure has been established and regularly reviewed.

The full Wescor Macroduct™ sweat collection kit is required is shown in figure 2.2. The procedure is outlined in appendix 4.



A – Sweat inducer component – electrical current/positive and negative electrodes/straps

B – Pilocarpine infused gel discs

C – Collecting disc/coil

1 – Iontophoresis

2 – Sweat collection

Figure 2.2 – sweat test equipment and procedure steps(267)

In an older CF patient cohort, sweat testing will have pre-dated both new-born screening and genetic testing for diagnosis. Genotype-phenotype studies in CF show correlations between CFTR mutation class and disease severity(268). In addition, sweat chloride as a measure of CFTR activity shows variations between CFTR mutation classes and phenotypic disease expression(269). Given the predicted clinical diversity of the over-40 years age group of CF patients at MACFC, the relationship between sweat chloride, genotype and clinical disease has added an interesting and informative element to the study.

2.3.5.7 Arterial stiffness measurements

Multiple devices are available in the clinical setting for the non-invasive measurement of arterial stiffness. The Vicorder® device (Skidmore medical, Bristol, UK) was chosen for this study due to MACFC links with the reno-vascular research teams at Salford Royal Foundation Trust (SRFT) and availability of software.

The Vicorder® system (Skidmore Medical) is an instrument used for measurement of PWV and AIx, along with a variety of other measurements of vascular stiffness and arterial pulse wave analysis. The process by which the Vicorder® analyses arterial pulse waves uses applanation tonometry. The Vicorder® generates highly reproducible PWV measurements,

which are comparable to those generated by the SphygmCor® (AtCor Medical), an alternative device. Aortic (central) AIx and PWV are most frequently measured and bear the most relevance to CV morbidity and mortality.

The equipment required for the Vicorder® method of pulse wave analysis (PWA)(270);

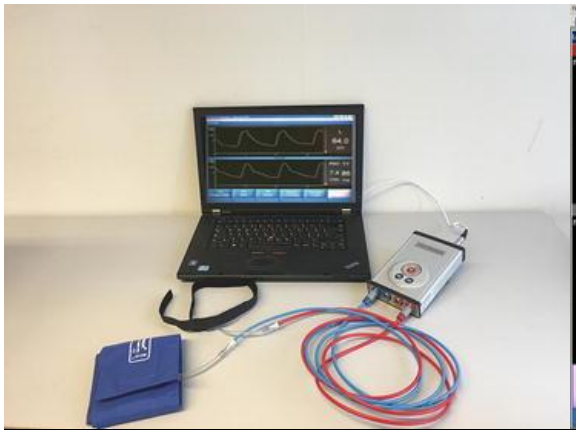


Figure 2.3: Vicorder® transducer device with brachial/femoral blood pressure cuff, carotid cuff with photoplethysmographic sensor and PWA recording via computer software.

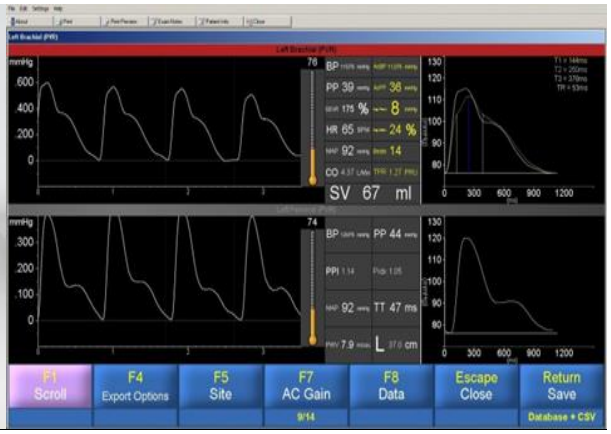


Figure 2.4: An example of PWA on Vicorder® computer software.

Vicorder® procedure

In order to perform the Vicorder® (Skidmore Medical) measurements the patient is settled into a quiet room for approximately ten minutes to ensure that he/she is relaxed and calm. The patient is asked to lay semi-recumbent on their back with the head supported and arms by their side. By using the appropriate size cuff and Korotkov method two brachial blood pressure readings are obtained manually using a Timesco Sapphire sphygmomanometer. A third blood pressure reading is obtained after a five minute interval if there is a discrepancy of more than 5mmHg in the initial two blood pressure recordings. The data are entered into the computer software connected to the Vicorder® device.

A 100mm wide blood pressure cuff (Hokanson Inc. Bellevue, WA, USA) is placed around the upper thigh to measure the femoral pulse and a 30mm partial cuff (Hokanson Inc. Bellevue, WA, USA), which is equipped with a photoplethysmographic sensor at the level of the carotid artery, is placed around the neck in order to record the carotid pressure. The cuffs are inflated to 90mmHg and high-quality waveforms are recorded simultaneously for three seconds with the subject in the supine position.

Path length (between carotid and femoral cuffs) is the length obtained following the subtraction of proximal from distal measurements. In order to determine the path length, using a tape measure, the distance from the sternal notch to the site of the probe on the carotid artery is recorded in millimetres. This is labelled as the proximal measurement. Then the distance from the sternal notch to the groin crease at the point where the femoral artery is studied is measured and recorded in millimetres. This is the distal measurement. These measurements are entered into the software and the path length is automatically calculated by subtracting the proximal from the distal measurement. The Vicorder® measurements are repeated twice for each patient for accuracy.

The software displays pulse waveforms in real-time and calculates measurements of PWV and AIx, which are automatically saved onto a laptop with links to an excel sheet format such that data can easily be extracted for analysis purposes.

Reference ranges for PWV and AIx are contentious due to variations of normative values with advancing age and degree of systemic hypertension. For the purposes of this study we will be referring to guidelines published in current literature(149)(151).

2.3.5.8 Cardiovascular risk scoring

Cardiovascular disease (CVD) is the leading cause of premature death in the UK. Multiple cardiovascular (CV) risk factors influence the prevalence of CVD, including systemic hypertension, hypercholesterolaemia and diabetes mellitus (DM). CF-related diabetes mellitus (CFRD) is an age prevalent condition in CF with 40-50% of adults being affected(271). In the general population, cardiovascular risk scoring is applicable to patients over the age of 40 years. Several CVD risk scoring systems have been developed and, although QRisk®3 is the most recent and recommended by NICE (2017), the QRisk®2 is still the most widely utilised within primary care. These CVD scoring systems are computerised methods that have been developed using data from large CVD-naive general population studies(160). Patients undergoing cardiovascular risk scoring must have no previous diagnosis of CVD in order to be scored accurately. Evidence has shown that early treatment with lipid lowering therapy and anti-hypertensives for primary CVD prevention can substantially lower the risk of CVD, forming the clinical evidence for CVD risk scoring(272).

In CF management, most care is delivered in a specialist cystic fibrosis centre and patients rarely utilise primary care. As a consequence, cardiovascular risk scoring in an older CF population may be overlooked. We developed a questionnaire that would enable specific clinical and epidemiological data to be collected in our over-40 population to apply to standard QRisk®2 and QRisk®3 cardiovascular risk scoring systems. It must be highlighted that the inclusion of DM within CV risk scoring necessitates a distinction between type 1 and type 2 classes of DM. No such distinction can be applied to CFRD, with the pathophysiological process including both insulin deficiency and insulin resistance. This could be a limitation to CV risk scoring within CF, however we thought it interesting to establish any significant difference between diabetic subtypes and CV risk in this cohort.

Questionnaires were completed with the patient during the first study visit. This data was then transferred onto a spreadsheet and subsequently inputted into computerised QRisk®2 and QRisk®3 scores(162)(273). These algorithms automatically generated a percentage risk score of sustaining a cardiovascular event over the forthcoming ten years, i.e. a ten-year CVD risk prediction. The QRisk®3 score is shown in appendix 1.

2.3.5.9 Cardiac magnetic resonance imaging (CMR)

CFTR is expressed in myocardial cells. The cardiac pathological process in CF is classically right ventricular (RV) hypertrophy and dilatation, manifesting as cor pulmonale, as a result of chronic hypoxia and progressive lung disease(274). Although there have been a few case reports in the literature, primary cardiac disease in CF is seen infrequently, particularly ischaemic heart disease and left ventricular pathology. This may be due simply to the fact that CF patients have historically not survived long enough to develop the atherosclerotic cardiovascular disease seen with ageing and cardiovascular comorbidity in the general population. Cardiac disease may become more relevant in CF as survival continues to improve and may have implications for both morbidity and mortality as well as organ transplantation suitability in an ageing CF cohort.

The role of magnetic resonance imaging (MRI) in CF clinical practice has largely been focused on the study of lung pathology and ventilation. Cardiac MRI (CMR) allows us to image the CF heart and great vessels in detail, assessing atrial and ventricular morphology and dynamics as well as myocardial structure and pathology.

CMR enables highly specific, three dimensional cardiac images to be obtained with great clarity regardless of body habitus and without the use of ionising radiation, hence being advantageous over echocardiography and cardiac CT. In addition, the accuracy and reproducibility of CMR means that relatively small sample sizes are often sufficient, hence our small sample size of ten patients.

Ten patients for cardiac MRI were prospectively selected following visit one and represented a variety of cardiovascular risk factors/scores and CF clinical disease severity. No patients were identified as having a diagnosis of CVD during screening and hence none were excluded from this part of the study. CMR-specific exclusion criteria also included claustrophobia, allergy to gadolinium-based contrast agents (GBCA) and eGFR < 40ml/min/1.73m^2.

Protocol for cardiac MRI

The CMR scan was performed by Lenin Francis, CMR radiographer (Alliance Medical) using a specific CMR protocol, designed in conjunction with Dr Christopher Miller (Consultant Cardiologist). The 1.5 T Tesla MR scanner at Wythenshawe Hospital (Avanto, Siemens Medical Imaging). The scan time was around 45 minutes and the CMR protocol used is shown in appendix 5.

Scans were supervised and initial image analysis performed by Dr Christopher Orsborne (Cardiology Clinical Research Fellow).

Parameters of measurement included left and right ventricle mass, volume and ejection fraction, atrial area and myocardial tissue characterisation. Important techniques used are T1 mapping, late gadolinium enhancement (LGE) and extracellular volume (ECV) fraction calculation. LGE imaging began at six minutes following gadolinium bolus to assess for focal myocardial fibrosis. T1 mapping (by Modified Look-Looker Inversion Recover (MOLLI)) at basal and mid left ventricular short axis level before and 15 minutes following the final bolus of gadolinium provided evaluation of myocardial fibrosis and oedema.

T1 time is longitudinal (spin lattice) relaxation time of the myocardium. T1 time is an important indicator of myocardial disease and, in the absence of contrast, may be increased in myocardial oedema and fibrosis and shortened in excess lipid or iron deposition. In the

presence of contrast (LGE), T1 mapping can be used to calculate the ECV fraction, which is an important marker of myocardial tissue remodelling and fibrosis(275). LGE with T1 mapping can assess for localised myocardial scar and fibrosis. The combination of LGE, T1 mapping and ECV fraction calculation can assess for more diffuse myocardial fibrosis.

LGE and both native and post-contrast T1 mapping were performed to assess for localised myocardial fibrosis. ECV fractions, based on native and post-contrast T1 values of blood and myocardium, were calculated to evaluate the presence of diffuse myocardial fibrosis. ECV fraction estimates are calculated using pre and post-contrast T1 mapping and patient haematocrit value(276) (shown below).

$$ECV = (1 - hct)\frac{1}{post\ contrast\ T1\ myo} - \frac{1}{native\ T1\ myo} \div \frac{1}{post\ contrast\ T1\ blood} - \frac{1}{native\ T1\ blood}$$

Analysis and interpretation of cardiac MRI data was performed using CVI Circle Imaging™, with the assistance of Dr Christopher Miller and Dr Christopher Orsborne.

2.4 Statistical analysis

- *Characterising the demographics of an adult cystic fibrosis population, specifically aged 40 years and above.*

Data were collected prospectively during study visits using a formulated questionnaire (appendix 2). Epidemiological and clinical demographic data of this population was supplemented by review of patient case notes. We aimed to investigate the overall genetic and phenotypic characteristics of this group. We also aimed to investigate specific correlations between patient clinical characteristics, genetic CFTR classification and sweat chloride.

Data were analysed, represented and discussed using descriptive statistics, to include tabular numerical summaries and relevant graphical methods. Group comparison was represented using one-way ANOVA, Student's T-test and Chi squared testing. Non-parametric equivalents were used for skewed data.

- *To assess the cardiovascular risk and contributory factors in an older adult CF cohort, including the prevalence of hypertension, hypercholesterolemia and CF-related diabetes mellitus (CFRD).*

Observational data collected from study visits provided insight into the prevalence of cardiovascular risk factors in this older CF population. Blood sampling for the described tests provided cholesterol parameters and an idea of the degree of inflammatory burden (hsCRP), contributing to altered arterial dynamics. Data were used to assess demographics and comorbidities in this population, to specifically include the presence of CFRD, systemic hypertension and hyperlipidaemia. Cardiovascular risk scoring was calculated using methods described and were used to define cardiovascular risk in our older CF cohort. Patients were recruited for further assessment of cardiovascular morbidity using cardiac MRI (CMR). CMR enabled us to evaluate cardiac morphology and function in greater detail as well as providing further insight into central arterial vascular integrity.

- *To assess arterial stiffness parameters in the older adult CF patient*

Arterial stiffness (cfPWV and AIx) and central systolic blood pressure (CSBP) measurements provided information regarding the prevalence of systemic hypertension and arterial stiffness in this cohort. Data were compared to a matched data from CKD cohort (Salford reno-vascular research group) to assess for trends and significant differences between groups. Inferential statistical analysis was used, including appropriate combinations of student's T-test, one-way ANOVA, Bonferroni, Chi squared and correlations to analyse, display and discuss the data. Linear regression models were used to make comparisons between groups, adjusting for confounding variables, particularly in the absence of matched data.

- *To assess the prevalence and causative factors of renal disease in an older adult CF population.*

Blood and urine sampling were performed in order to collect information on renal status for each patient. Methods to obtain estimated measurements of renal function were utilised to include MDRD, CKD-EPI and creatinine clearance. Urinary ACR measurements were used to detect any microalbuminuria which may indicate early renal disease, particularly in patients with CFRD.

- *To better define the most accurate method of calculating renal function (using glomerular filtration rate, GFR) in older adult CF patients.*

Estimated GFR (eGFR) was calculated using several formulae as outlined previously. Inaccuracies in measuring eGFR using creatinine-based equations in CF patients exist and more novel biomarkers such as cystatin C may improve reliability of renal function measurements in this population group. Gold standard iohexol-measured GFR can be used as a comparator to assess validity of each derived eGFR value and was performed in a small number of patients. Comparisons between GFR methods were performed using assessments of agreement and bias with Bland Altman plots, and accuracy between methods (P10 and P30).

- *To investigate cardiac function, structure and presence of myocardial fibrosis in older adult CF patients.*

A specifically developed cardiac MRI (CMR) protocol (appendix 5) using late gadolinium enhancement (LGE) and T1 mapping was used to investigate cardiac structure, function and

volumetrics in nine patients in this cohort. Data for LV/RV mass, left ventricular ejection fraction (LVEF), extracellular volume (ECV) fraction and presence/absence of myocardial fibrosis was recorded for each patient. Data were analysed using descriptive methods and comparison between severe ($ppFEV_1$ <40%) and non-severe ($ppFEV_1$ >40%) lung disease groups was undertaken using student's T testing.

All statistical analysis was performed using SPSS® (IBM®, version 25.0), with two-tailed p values of <0.05 deemed statistically significant.

2.5 Individual contributions to study

The research in this thesis was conducted at the Manchester Adult Cystic Fibrosis Centre, in collaboration with several other internal and external institutions. The author of this thesis (Sarah Paterson) was the study principle investigator, supervised by Professor Andrew Jones and Dr Rowland Bright-Thomas.

- Dr Sarah Paterson, Respiratory ST7 and Research Fellow - consent, data collection, data analysis, write-up.
- Professor Andrew Jones (MD) and Dr Rowland Bright-Thomas (PhD), Consultant respiratory and CF physicians – consent, assistance with analysis and write-up.
- Dr Darren Green (PhD), Consultant renal and acute medicine physician at SRFT – provision of Vicorder® machine, assistance with analysis of PWV and AIx data (to include control CKD data and statistical analysis) and assistance with completion of successful Kidneys UK research grant for iohexol study arm.
- Dr Anne-Marie Kelly, Consultant physician/Biochemist at Manchester NHS Foundation Trust (MFT) – assistance in lipid profile/QRisk® analysis and interpretation.
- Dr Christopher Miller (PhD), Consultant cardiologist at MFT - development of CMR study protocol and interpretation of CMR data
- Dr Christopher Orsborne, Cardiology ST4 and Research Fellow at MFT – assistance with development of CMR study protocol, supervision of CMR scans, analysis of images and interpretation of results.
- Professor Brian Keevil – Consultant biochemist at MFT. Processing and interpretation of iohexol samples and mGFR calculations.
- Mr David Marshall – Senior biochemist at MFT. Assistance with processing iohexol samples and mGFR calculations.
- Mr Jonathan Aldis – Chief Biochemist, Sheffield Teaching Hospitals. Processing of apolipoprotein samples.
- Mrs Karen Morris and Mrs Sherly George, Biochemists at MFT - assistance with laboratory protocols, transport and processing of blood and urine samples.
- Mr John Belcher, Chief Statistician at MFT – assistance with statistical analysis of data.

The Complexities of Ageing in Cystic fibrosis

3.0 Chapter 3: The demographics of an older cystic fibrosis cohort

3.1 Abstract

3.1.1 Introduction

Survival in cystic fibrosis (CF) is increasing exponentially. A child born with CF today has a median survival into their fifth decade and there are now more adults than children living with CF in the UK. An insight into the older CF cohort and exploration of reasons for longevity is crucial in order to understand this fascinating population, the complexities of their disease as they age and to target potential new areas for management.

3.1.2 Methods

Patients aged 40 years and above, attending Manchester Adult Cystic fibrosis Centre (MACFC), underwent data collection between August 2018 and August 2020. Study visits incorporated data collection for general demographics and disease comorbidities using a formulated questionnaire. Serum high sensitivity C-reactive protein (hsCRP) and total intravenous (IV) aminoglycoside days over a preceding ten-year period were measured as markers of disease severity. Data collation was supplemented by retrospective case note review. Sweat testing was performed to assess for trends in genotype, sweat chloride and disease phenotype. Severity of disease in this group was classified by percent predicted forced expiratory volume in one second ($ppFEV_1$) and total intravenous (IV) antibiotic days. Statistical analysis was performed using SPSS (IBM), with $p<0.05$ used for statistical significance.

3.1.3 Results

A total of 85 patients were studied from 92 eligible at the time of data collection. This group accounted for 20% of the total CF patient cohort at MACFC. Mean($\pm$SD) age of this older cohort was 48.6($\pm$7.70) years and 65.9% were male. Mean $ppFEV_1$ was 52.9($\pm$23.3)%, indicating a cohort with plentiful lung disease. 72.9% had severe cystic fibrosis transmembrane regulator gene (CFTR) mutations and 65.9% had chronic *Pseudomonas aeruginosa* (PsA) infection. A high proportion had CF-related diabetes mellitus (CFRD); 49.4% (n=42). Median age of CF diagnosis was 1.5 (IQR; 19, range 0-72) years and 83.5%

were exocrine pancreatic insufficient. Median sweat chloride was 97.0 (IQR; 35.8, range; 23-135) mmol/L. Median hsCRP was 5.0(IQR 8.0, range;1-42)mg/L and median IV antibiotic use was 88.0 (IQR 188) days. 55.3% of patients were in full or part-time employment and 34.1% had biological children.

Analysis between age deciles and between late and early diagnosis groups observed statistically significant trends towards lower sweat chloride levels ($p=0.039$), higher prevalence of exocrine pancreatic sufficiency ($p=0.02$) and less severe CFTR mutation classes ($p<0.001$) with advancing age. However, there were no significant differences in pulmonary and nutritional parameters between age groups. Variation in phenotype was seen throughout age groups and within CFTR mutation classes. There also exists a degree of cardiovascular risk in this older CF cohort, with 18.8% and 15.5% with systemic hypertension and hyperlipidaemia respectively. This will be an important area for future research.

3.1.4 Conclusion

Patients aged 40 years and above constitute a significant proportion of the total patient cohort at MACFC. This is a heterogeneous group and, although a proportion have late diagnoses of CF and milder clinical disease, many have severe CFTR genotypes with exocrine pancreatic insufficiency, chronic *Pseudomonas aeruginosa* infection and classical CF phenotypic disease. However, the demographics of an ageing CF population is likely to change over time, particularly in the era of CFTR modulation therapy.

In the older CF population, we may observe end organ damage from the general ageing process as well as CFTR dysfunction and, with advancing pulmonary disease, more older patients may progress to lung transplantation. Further areas for research should logically include cardiovascular risk in this ageing CF population, the effect of CFTR dysfunction on the ageing CF myocardium and the impact of chronic CF therapies on renal function. Predicted future increases in CF survival, particularly in the era of CFTR modulation, makes this research into ageing in CF both timely and essential, and will aid in understanding and management of an ever-challenging and complex disease.

3.2 Introduction

Cystic fibrosis (CF) is an autosomal recessive, multi-system, life-limiting genetic disease seen mainly in Caucasian populations. The underlying pathological process stems from abnormal cystic fibrosis-related transmembrane conductance regulator (CFTR) gene, encoding for an anion channel in the membrane of epithelial cells. The corresponding clinical manifestations involve the accumulation of viscous secretions in multiple organs expressing abnormal epithelial cell CFTR, including the lungs, gastrointestinal tract, pancreas and liver(2).

When CF was first diagnosed as a clinical entity by Dorothy Anderson in 1938, survival rarely surpassed one year(53). However, the 20th century has seen disease perspectives and treatment modalities for CF develop dramatically, such that the 21st century CF community is now beginning to see an ageing CF population. There are now a greater proportion of adult than paediatric CF patients in the UK(4). The median age of survival of a child born with CF today is into their fifth decade(11) and this is predicted to rise further, particularly in the era of disease-modifying CFTR therapy.

The rapid development and subsequent success of CF treatment over the last 80 years has undoubtedly contributed to survival advantages. However, there may be many more factors contributing to improving survival in CF. What does an ageing CF population look like now? It is likely that they will incorporate a vast spectrum of phenotypic disease, influenced not only by genetics but also by environmental and socioeconomic factors. Although CF patients with milder disease and later age of diagnosis may have a survival advantage, the demographics of an ageing CF population are likely to change over time with an increasing proportion of patients with 'classical' CF reaching older ages, particularly in the era of CFTR modulation. It is necessary to develop our knowledge of this older CF population in order to enhance our understanding of the complexities of older CF phenotypes. Ageing complications may be predominantly related to the longevity of CFTR organ dysfunction, however non-CFTR related complications such as the long term effects of chronic therapies and of the general ageing process itself are likely to become important factors in future CF management. An ageing CF population will bring novel challenges to the CF multidisciplinary team and, as with the general ageing population, new demands on resources.

Less severe CF phenotypes, with residual CFTR function, may correlate with a delayed or adult diagnosis. However, we know from previous studies that older CF populations still include a high proportion of patients with early diagnosis and severe 'classical' disease, with a high prevalence of exocrine pancreatic insufficiency and chronic *Pseudomonas aeruginosa* infection(200). However, this study is over a decade old and the demographics of long term CF survivors may have changed over time. The heterogeneity of an older CF group exemplifies the challenges of analysing survival advantage and predicting emerging comorbidities in an ageing CF population.

Within each CFTR mutation class exists a spectrum of clinical disease(268). Studies analysing the genotype-phenotype relationship within CF have postulated that a milder CFTR genotype (i.e. non-severe CFTR mutation classes IV and V) may confer a less severe, "non-classical" CF phenotype when compared with severe CFTR mutations. Clinical phenotype depends upon the degree of preservation of normal CFTR protein translation and function. The genotype-phenotype correlation of CFTR function and its relationship to CF clinical disease manifestation is illustrated in figure 3.1. Although this categorisation of CFTR function and resulting phenotypic expression may be accurate in the majority of patients, making definitive conclusions about genotype-phenotype correlation is challenging due to considerable variability of phenotypic expression within CFTR mutation classes(253)(199). An adult diagnosis of CF often accompanies a less severe CFTR genotype. Although still experiencing disease progression, this patient group is likely to be prevalent in an older CF cohort due to less severe disease and subsequent perceived survival advantage.

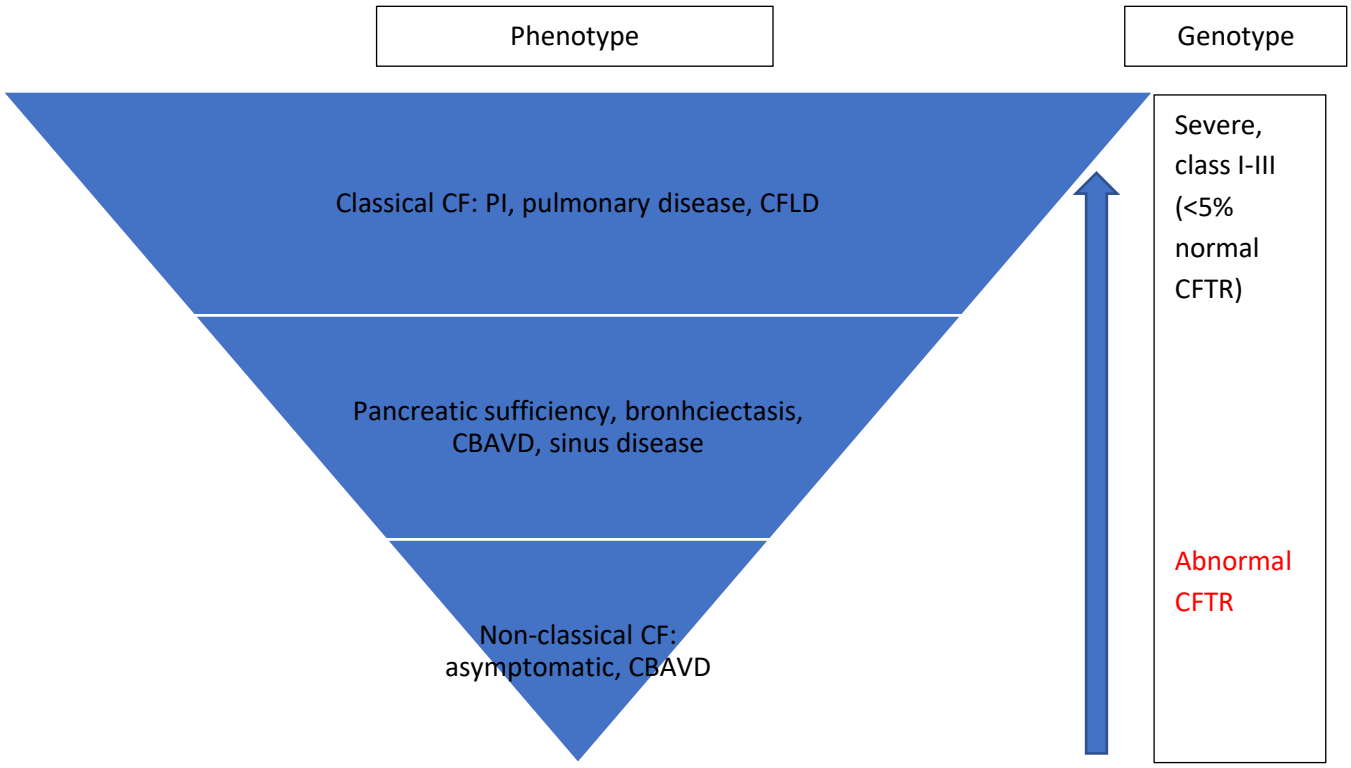


Figure 3.1: Illustration of genotype-phenotype correlation in CF (CFLD=CF-related liver disease, PI=exocrine pancreatic insufficiency, CBAVD-congenital bilateral absence of vas deferens).

Only a few studies to date have analysed the demographics of older CF cohorts. Simmonds and colleagues (2010) explored factors for longevity in CF by studying a CF patient group aged 40 years and above with a birth date-matched control group of CF patients with mortality below the age of 30 years(277). Although a late diagnosis group was not studied and the patterns of disease phenotypes in early diagnosis patients were variable, survival advantage could be explained, and not unexpectedly, by higher ppFEV$_1$ at transition to adult services, absence of chronic *Pseudomonas aeruginosa* infection and higher body mass index (BMI). Rodman and colleagues analysed an older CF population in terms of both early and late diagnosis groups, observing that in patients with late diagnoses (>24 years) there existed a trend towards lower sweat chloride and higher ppFEV$_1$. In addition, and as expected, the frequency of the Phe508del mutation was lower in the late diagnosis group, corresponding clinically as increased prevalence of exocrine pancreatic sufficiency, higher body mass index (BMI) and lower prevalence of CF-related diabetes mellitus (CFRD)(199).

Although there was a lower rate of chronic *Pseudomonas aeruginosa* infection in the late diagnosis group, there was a higher prevalence of non-tuberculous bacteria (NTM).

Increasing survival in CF will be accompanied by an increasing burden of chronic therapies, microbial resistance and progressive organ dysfunction. CFTR-related ageing will also predispose to CF-arthropathy, metabolic bone disease and CFRD. Lung transplantation may become relevant for a larger number of older patients and CFTR modulation is likely to alter the landscape of CF disease as a whole. Pulmonary decline may still be inevitable despite CFTR modulation and the effects of residual CFTR function on progression of disease is unknown, making the future of CF unpredictable. The introduction of triple CFTR modulation therapy (Kaftrio®) is an exciting recent development in CF treatment and is likely to further improve survival. Middleton et al (2019) report Kaftrio®, a triple CFTR modulator (elexacaftor, tezacaftor and ivacaftor) to produce a 14.3% increase in $ppFEV_1$, 63% reduction in pulmonary exacerbation rate and 41.8mmol/L reduction in sweat chloride concentration at 28 days in CF patients aged 12 years and above with one Phe508del and one minimal function CFTR mutation(95). Heijerman et al (2019) show similar benefit in Phe508del homozygotes with Kaftrio®, reporting a 10% increase in $ppFEV_1$, a 45.1mmol/L reduction in sweat chloride and an increase of 17.4 points in the CF-questionnaire respiratory domain (CFQ-R) quality of life index at 28 days in those aged 12 years and above(96). These results are outstanding and kaftrio will change the landscape of CF dramatically in years to come.

A paucity of data in older CF populations highlights the importance of further studies aimed at developing our knowledge of emerging complications with CF longevity and to develop more definitive conclusions regarding optimal future management of these complex patients.

3.3 Methods

A full description of methodology for this study is outlined in chapter two. The age of 40 years and above was used to define an 'ageing' CF cohort. This age group has been used as a milestone in previous studies to define longer term survival in CF(278)(199).

Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) Ethics committee (reference 19/EM/0067).

In brief, a database of all CF patients at MACFC aged 40 years and above was created in order to assess trends in their demographics, predict emerging complications and identify further areas for research. An observational, cross-sectional cohort study was then performed from August 2018 to August 2020 at MACFC. Eligible patients were consented and recruited for a study visit during a time of clinical stability, defined as the absence of pulmonary exacerbation requiring oral or intravenous antibiotics within the preceding four-week period(279). Lung transplantation recipients were excluded.

3.3.1 Demographic data

Data were collated for demographic data at the first study visit using a formulated study questionnaire (appendix 2). Supplementary demographic data were collected retrospectively from patient case notes. Data not obtained at the time of study visit were collected from within the previous 12 month period, either at latest clinical review or from annual assessment results. Demographic data collected during study visits included; genotype, age at CF diagnosis, lung function parameters (percent of predicted forced expiratory volume in one second {ppFEV$_1$} and forced vital capacity {FVC}), total intravenous (IV) aminoglycoside days within the preceding ten-year period, sputum microbiology (previous three samples or predominant organism in last 24-month period), exocrine pancreatic status, body mass index (BMI), presence of CF-related diabetes mellitus (CFRD) and the presence of other organ involvement (to include liver, renal, bone disease, gastro-oesophageal reflux disease {GORD}, malignancy and arthropathy). Employment, social and family history information was also collected.

The presence of CFRD was confirmed by previous diagnostic OGTT and/or continuous glucose monitoring. Exocrine pancreatic status was defined from historical faecal elastase

measurements and/or the use of pancreatic enzyme supplementation. Disease severity was defined in terms of ppFEV$_1$ (</≥40%), high sensitivity C-reactive protein (hsCRP), (>5mg/L) and BMI (<18.5kg/m^2). Total intravenous aminoglycoside days over the preceding ten year period were calculated from detailed clinical records of pulmonary exacerbations for each patient. Most patients received a dual combination of intravenous antibiotic treatment during pulmonary exacerbation, however only intravenous aminoglycoside days were recorded for the study.

Demographic data from this cohort were analysed together and then with reference to differences between three distinct age groups; 40-49, 50-59 and ≥60 years of age.

3.3.2 Blood sampling

Blood sampling was performed using venepuncture at the patient study visit. Measured biomarkers included renal function, hsCRP, lipid profiles and HbA1c. The analysis of hsCRP in the context of disease severity is described in this chapter. The other blood parameters are analysed further in future chapters.

3.3.3 Sweat testing

Prospective sweat testing was performed for the majority of patients using the Wescor macroduct® iontophoresis method(280)(appendix 4). For patients already commenced on CFTR modulation (and thus have an expected sweat chloride reduction), the pre-initiation sweat chloride level was used. For those patients with a recent diagnosis of CF (within the last ten years), the sweat chloride value obtained during initial diagnostic work-up was used in analysis. Age of CF diagnosis, sweat chloride concentration (colourometry), CFTR genotype and other demographic data were then analysed in early diagnosis (<16 years) and late diagnosis (≥16 years) comparator groups. CFTR mutation classes were divided into severe (class I-III) and non-severe (class IV-V). Rarer class VI CFTR mutations were included as severe.

3.3.4 Data analysis

Data analysis was performed using SPSS® statistical software (IBM®, version 25.0). Data were analysed for normality using the Kolmogorov-Smirnov method. Independent T-test, one-way ANOVA and Pearson correlations were performed to display normally distributed

data. Logarithmic transformation was performed prior to analysis of skewed data and results displayed as geometric mean/SD or back-transformed mean/CI as applicable. For data without normal distribution on logarithmic transformation, non-parametric testing was used for analysis. Categorical data were analysed using Chi-squared testing. Univariate and multivariate regression models were also used to determine relationships between dependent and independent variables. Two-tailed p values of <0.05 were deemed statistically significant.

3.4 Results

From the total number of CF patients at MACFC, 92 patients were identified as being aged 40 years or above at the time of data collection, between August 2018 and August 2020. 85 of these patients went on to participate in prospective study investigations and this group represents 20% of the total CF patient population at MACFC.

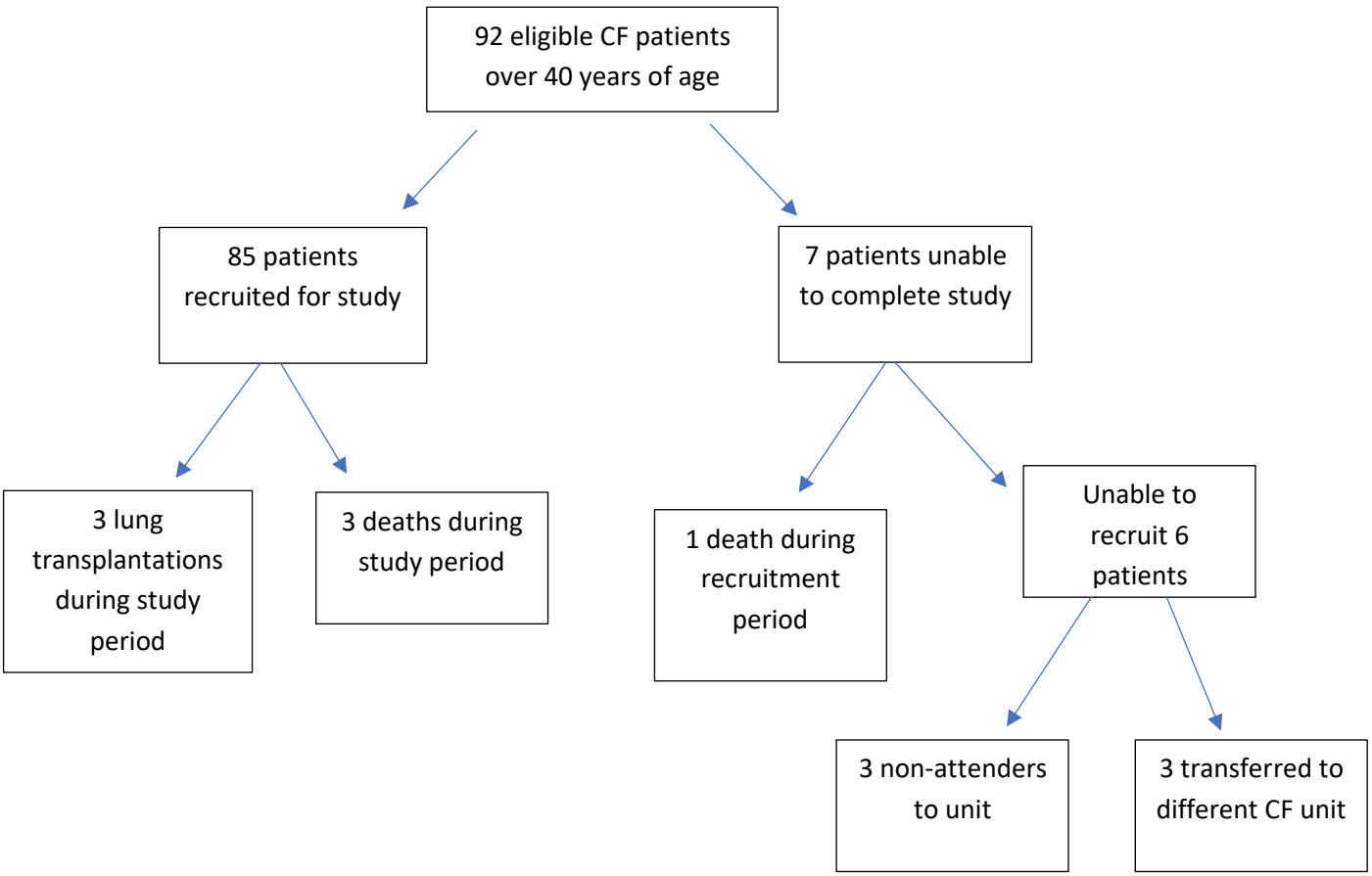


Figure 3.2: A consort flow diagram of patient recruitment.

3.4.1 Demographics of study cohort

Full demographic data for all 85 study participants are shown in table 3.1 (appendix 6). The mean (±SD) age of the study cohort was 48.6 (±7.70) years. 65.9% are male. 72.9% of patients had a severe CFTR genotype. There was a high prevalence of exocrine pancreatic insufficiency; 83.5% and chronic *Pseudomonas aeruginosa* colonisation; 65.9%. Mean $ppFEV_1$ was 52.9(±23.3)%, mean BMI was 23.9(±3.13)kg/m^2 and 49.4% had CFRD. 72.9% of patients were on regular nebulised antibiotic treatment and 63.5% on nebulised mucolytic therapy.

Three patients received a double lung transplantation during the study period (patient 36, 37 and 56, see table 3.1, appendix 6). All transplant recipients had a $ppFEV_1$ of less than 30%, chronic *Pseudomonas aeruginosa* infection and exocrine pancreatic insufficiency. There were three deaths (patient 1, 20 and 60, see table 3.1, appendix 6), all from recalcitrant pulmonary exacerbation, providing a two-year mortality rate of 3.3% in our older cohort. The mean $ppFEV_1$ of this group was 30.7(±9.7)%, two had chronic *Pseudomonas aeruginosa* infection, all were exocrine pancreatic insufficient and all had CFRD.

3.4.2 Analysis of groups by age range

Data were categorised into age groups; 40-49 years, 50-59 years and ≥60 years to assess for demographic trends with advancing age. Statistically significant differences were shown between groups for exocrine pancreatic status (p=0.022), CFTR mutation class severity (p=0.005), median age at diagnosis (p<0.001) and median sweat chloride (p=0.017) using one-way ANOVA/Kruskal-Wallis testing. There was also a statistically significant difference in *Pseudomonas aeruginosa* prevalence between groups. There was a greater prevalence of systemic hypertension and hyperlipidaemia (total cholesterol >5mmol/L) with increasing age. No significant difference was seen in either $ppFEV_1$ or BMI between groups (table 3.2).

Age range	**Total**	**40-49**	**50-59**	**≥ 60**	**P value**
Number of patients (n)	85	57	20	8	-
%male	65.9%	70.2%	50.0%	75.0%	0.228
Median age at Diagnosis (IQR, range) (years)	88.0 (188)	1.5 (5, 0-40)	4.5 (25, 0-55)	52.5 (54, 0-72)	**<0.017***
Mean $ppFEV_1$ (%)(SD)	52.9 (23.3) %	53.0 (22.2) %	50.3 (24.6) %	58.0 (29.7) %	0.731
Mean BMI (kg/m^2) (SD)	23.9 (3.13)	24.2 (3.24)	23.2 (2.41)	24.2 (3.99)	0.450
% with CFTR class I-III (n)	72.9% (62)	78.9% (45)	75.0% (15)	25.0% (2)	**0.005**

% PI (n)	83.5% (71)	85.9% (49)	90.0% (18)	50.0% (4)	**0.022**
% chronic PsA infection (n)	65.9% (56)	73.7% (42)	65.0% (13)	12.5% (1)	**0.003**
% chronic BCC infection (n)	15.3% (13)	17.5% (10)	15.0% (3)	0	0.434
% CFRD (n)	49.4% (42)	52.6% (30)	55.0% (11)	12.5% (1)	0.089
% HTN (n)	18.8% (16)	15.8% (9)	20.0% (4)	37.5% (3)	**0.034**
% Hyperlipidaemia (n)	15.5% (13)	7.02% (4)	25.0% (5)	37.5% (3)	**0.007**

*Table 3.2: Demographic analysis by age group of study population, *Kruskal-Wallis used. (HTN=systemic hypertension, BCC=Burkholderia cepacia complex, PsA=Pseudomonas aeruginosa).*

3.4.3 Disease severity

Total intravenous (IV) antibiotic days over the preceding ten-year period, baseline high sensitivity C-reactive protein (hsCRP), $ppFEV_1$ and BMI were used as markers of disease severity. In the total cohort, mean $ppFEV_1$ was 52.9($\pm$23.3) %, showing quite significant lung function impairment in the study group. Median hsCRP was 5.0(IQR 8.0, range;1-42)mg/L and mean BMI 23.9($\pm$3.13) kg/m^2. There was no statistically significant difference in these parameters between age groups. The median total IV aminoglycoside days over ten years for the cohort was 88.0 (IQR 188, range;0-562) days. There was no significant difference in IV antibiotic days between the defined age groups (p=0.146). However, unsurprisingly, there was a statistically significant negative correlation between total IV aminoglycoside days and $ppFEV_1$ in the total over 40 year old cohort (r=-0.526, p<0.001). Linear regression analysis suggests that $ppFEV_1$ decreases by 0.084% for every additional IV day (r^2=0.276, p<0.001). As expected, there was a strong correlation between $ppFEV_1$ and BMI; r=0.436, p=<0.001 (figure 3.3). There were also statistically significant correlations between IV days and baseline hsCRP (r=0.454, p<0.001), the presence of CFRD and BMI (r= -0.224, p=0.04) and $ppFEV_1$ and hsCRP (r= -0.515, p<0.001).

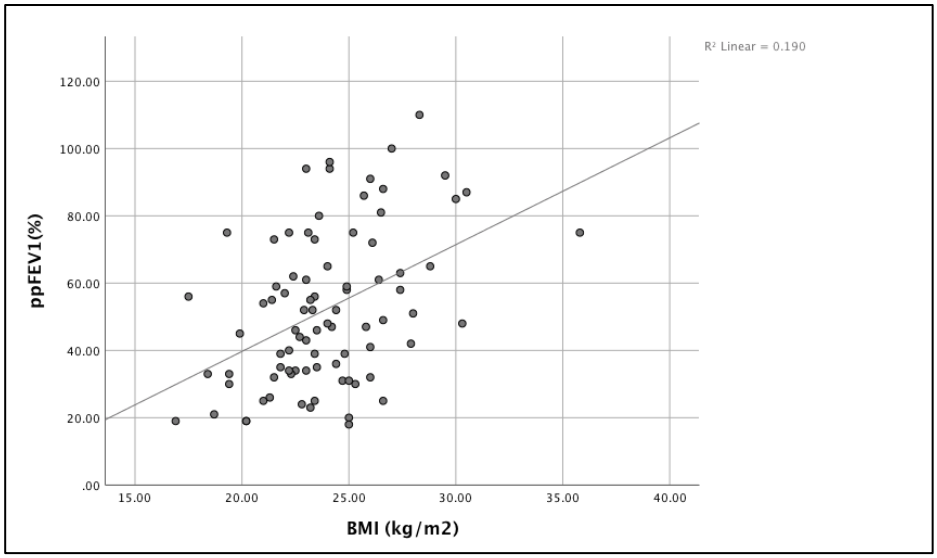


Figure 3.3: A scatterplot showing the correlation between ppFEV$_1$ and BMI (r=0.436, p<0.001).

3.4.4 Gender and disease severity

The majority of patients in our older CF cohort were male (65.9%). This gender disparity seems to decrease between the ages of 50 and 59, with 43.5% of this group being female. In addition, all 15% (n=3) of the patients in this age group (50-59 years) chronically colonised with *Burkholderia cenocepacia* are female, with relatively preserved lung function (mean ppFEV$_1$ 57.7%).

Analysis of gender and ppFEV$_1$, when categorised into severe (<40%) and less severe (≥40%) groups, show no statistical difference using Fishers Exact test; p=0.075. The relationship between gender and hsCRP (<5 and ≥5mg/L) similarly shows no statistical significance using Fishers exact test; p=0.408.

3.4.5 CF-related diabetes mellitus (CFRD)

In our cohort, 49.4% had a diagnosis of CFRD. There was a statistically significant difference in the prevalence of CFRD between severe and non-severe CFTR class groups (p=0.008) and also between early and late diagnosis groups (p=0.024). A higher prevalence of CFRD was seen in the severe CFTR mutation and early diagnosis group; 56 vs 20%.

There was a significant statistical difference in ppFEV$_1$ (p=0.040) and BMI (p=0.041) between those with CFRD and those without (Chi squared analysis; table 3.3).

Variable	CFRD	Non-CFRD	P value
Patient number (n)	43	42	-
Mean ppFEV$_1$ % (SD)	47.6 (21.4)	57.9 (24.2)	**0.040**
Mean BMI kg/m^2 (SD)	23.3 (2.79)	24.6 (3.33)	**0.041**

Table 3.3: Differences in disease parameters for CFRD and non-CFRD groups.

3.4.6 Sputum microbiology

The most prevalent pathogen isolated in sputa of our older CF cohort was *Pseudomonas aeruginosa* (table 3.4).

Organism in sputum	n (% of group)	Male vs female (%)
Pseudomonas aeruginosa (PsA)	56 (65.9%)	41.2 vs 24.7 %
Burkholderia cepacia complex (BCC)	13 (15.3%) *B.multivorans*; n= 6 *B.cenocepacia*; n=7	9.4 vs 5.9 %
MRSA	6 (7.1%)	↔ 3.5 %
Stenotrophomonas maltophilia	2 (2.4%)	↔ 1.2 %
NTM	3 (3.3%) *M.abscessus*; n=1 MAC n=2	-

Table 3.4: Chronic microbial sputum colonisation in our older CF cohort (MRSA=methicillin resistant staphylococcus aureus, NTM-non-tuberculous mycobacteria, MAC=mycobacterium avium complex).

Our cohort showed a lower ppFEV$_1$ in patients chronically colonised with *Pseudomonas aeruginosa*; 47.4 vs 63.4% (Student's T-test; p=0.008). No such trend existed for chronic BCC infection (p=0.287).

Three patients (3.3%) had isolated non-tuberculous mycobacteria (NTM) within the 12 months preceding this study, one with *Mycobacterium abscessus* and two with *Mycobacterium avium* complex (MAC).

3.4.7 Cardiovascular morbidity

One patient in the late diagnosis group has a history of a cerebrovascular accident (CVA) preceding his CF diagnosis. 15.5% of our patients aged 40 years or above have been diagnosed with hypercholesterolaemia and 18.8% are on anti-hypertensive therapies. We note that some patients may have been started on angiotensin converting enzyme inhibitors (ACEi) for microalbuminuria. The prevalence of systemic hypertension and hyperlipidaemia increased with advancing age in our cohort (one way ANOVA; p=0.034 and 0.007 respectively). Although no patients in this group have a diagnosis of cardiovascular disease (CVD), a significant family history of cardiovascular disease (first degree relative with cardiovascular event $\leq$60 years), determined from the study questionnaire, was found in 11.8% of patients. The impact of CFRD and duration of dysregulated glycaemic control in ageing CF patients is likely to have an impact on the prevalence of macrovascular disease, as is true for the general population. Cardiovascular risk will be discussed further chapter four.

3.4.8 Analysis of other comorbidities in an ageing CF population

One patient is currently being treated for rectal adenocarcinoma. One patient has previously had thyroid papillary carcinoma with successful surgical resection and radiotherapy.

One patient has had a staggered renal and pancreatic transplant for refractory hypertension and CFRD. 20% have CF-related liver disease. 14.1% have CF-related arthropathy and 24.7% had evidence of low bone mineral density on DEXA imaging. 61.2% of patients were on treatment for chronic gastro-oesophageal reflux disease (GORD), although a number were noted to be on proton pump inhibitors for optimisation of gastrointestinal absorption with creon use, with two under regular endoscopic surveillance for Barrett's oesophagus. 8% of our older patients are currently being treated for depression.

3.4.9 Sweat chloride analysis

A sweat chloride result of >60mmol/L is diagnostic of CF. A level of 40-60mmol/L was classed as an equivocal result and <40mmol/L is normal at MFT. It should again be noted

that this slightly differs to diagnostic measurements set out in consensus CF guidelines by Farrel et al, in which <30mmol/L is a normal result(27).

Sweat chloride analysis data was available for 59 patients. Both sweat chloride concentration (colourometry) and conductivity were measured, the former being the most important and used in analysis. Prospective sweat testing was performed at the study visit of 45 patients. Retrospective sweat chloride results were analysed for a further 14 patients, with pre-modulator (n=11) or recent diagnostic sweat chloride levels (n=3). The demographics of the sweat chloride group are shown in table 3.5 (see appendix 7).

54 patients had an elevated sweat chloride, four had normal sweat chloride and one had an equivocal result. All normal or equivocal results were seen in patients aged ≥50 years. Median sweat chloride levels were higher in males than females; 100 (IQR 34.5) vs 92 (IQR 39)mmol/L, p=0.700. Mean baseline ppFEV$_1$ is also higher in males; 54.9(±23.7) vs 48.9(±22.5)%, p=0.732.

Comparison between age groups revealed a statistically significant difference in sweat chloride values using one way ANOVA testing, showing lower sweat chloride with advancing age (table 3.6).

Age (years)	**Total (n=59)**	**Age 40-49 (n=38)**	**Age 50-59 (n=15)**	**Age ≥60 (n=6)**	**p value**
Sweat chloride Median (IQR) {range} (mmol/L)	97.0 (35.8) {23-135}	100 (23.3) {30-135}	94.5 (63.3) {23-117}	67 (44) {26-103}	**0.039***

*Table 3.6: Sweat chloride comparison between age groups (*Kruskal-Wallis test)*

3.4.10 Analysis of early and late diagnosis groups

3.4.10.1 Total cohort analysis

Our older CF cohort was divided into early and late diagnosis groups. The early diagnosis (ED) group was defined as CF diagnosis aged <16 years, and late diagnosis (LD) as ≥16 years.

The median age of diagnosis of the total patient group was 1.5 (19, range 0-72) years. From the 77 patients with a confirmed age of diagnosis, 57 (74%) were classified as ED and 20 (26%) as LD. The median age of diagnosis was 1.0 (IQR 3, range 0-12) year in the ED group and 36.5 (IQR 29, range 16-72) years in the LD group (Mann-Whitney U test; p<0.001). Figure 3.4 represents the differences between current mean age and median age at diagnosis between ED and LD groups.

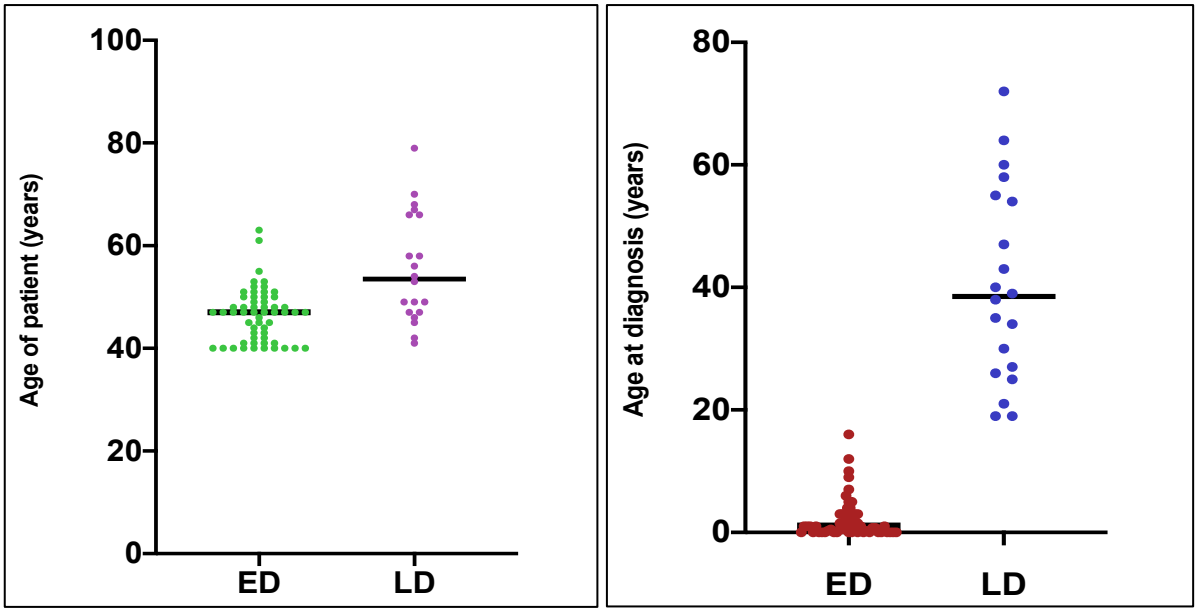


Figure 3.4: Age of patient and age of CF diagnosis as a comparison between ED and LD groups.

Comparison of clinical parameters between ED and LD groups using independent sample T-testing shows a significant difference, with a lower ppFEV$_1$ (47.8±17.6 vs 63.4±28.8%, p<0.001) and BMI (23.8±2.6 vs 24.5±4.5 kg/m^2, p=0.005) in the ED group.

Logistic regression shows the relative risk of being diagnosed with CFRD to be higher in the ED group, along with a higher probability of *Pseudomonas aeruginosa* infection and exocrine pancreatic insufficiency (table 3.7).

Comorbidity	ED group	LD group	OR	95% CI	p value
CFRD (n)	33 (57)	6 (20)	3.35	1.12, 10.0	**0.024**
PsA n	45 (57)	6 (20)	9.55	2.99, 30.5	**<0.001**
PI (n)	55 (57)	1 (20)	67.2	7.71, 585.8	**<0.001**

Table 3.7: Regression analysis of ED and LD groups with regards to markers of severe disease. PsA – Pseudomonas aeruginosa, PI= exocrine pancreatic insufficiency, CFRD= Cystic fibrosis-related diabetes mellitus, OR=odds ratio, CI= confidence interval.

From the three bilateral lung transplantation recipients, two were from the ED and one from the LD group. There were three deaths during the study period from recalcitrant pulmonary exacerbation, all from the ED group.

Analysis of sweat chloride and age of CF diagnosis was undertaken (figure 3.5), showing a significantly higher sweat chloride level in the ED group when compared with the LD group.

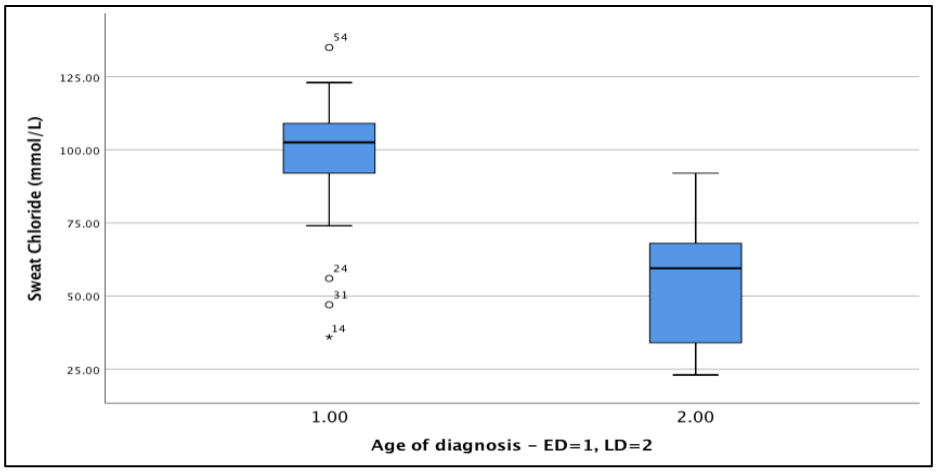


Figure 3.5: Boxplot analysis of differences in sweat chloride between ED and LD group.

There is a negative Spearman correlation between sweat chloride and age of diagnosis with a lower sweat chloride for those patients diagnosed at an older age; r=-0.662, p<0.001 (figure 3.6). A statistically significant correlation also exists between increasing sweat chloride and lower ppFEV$_1$ (r=-0.250, p<0.046).

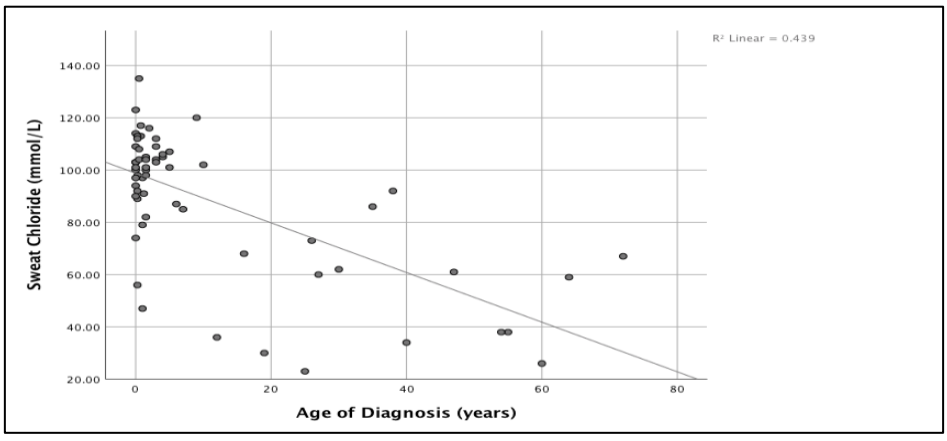


*Figure 3.6: Correlation between sweat chloride and age of CF diagnosis (*r=-0.662, p<0.001).

3.4.10.2 LD group analysis - demographics

For further analysis, the characteristics of the LD group are described in table 3.8 (appendix 8). There were 20 patients in the late diagnosis group, 23.5% of the total cohort. Median age of diagnosis was 36.5 (IQR 29, range 16-72) years and 65% of these patients had preserved exocrine pancreatic function. Although there is a relatively high prevalence of chronic *Pseudomonas aeruginosa* colonisation within the LD group, the majority of patients are exocrine pancreatic sufficient. Mean ppFEV$_1$ in the LD group is 65.4($\pm$29.4)%, compared with 47.8 ($\pm$17.6)% in the ED group ($p<0.001$). We observed a high prevalence of Phe508del heterozygotes in the LD group (n=17).

3.4.10.3 LD analysis – sweat chloride

For those with available sweat test data, five patients had elevated sweat chloride levels. However, a significant number were noted to have normal (n=4) or equivocal (n=1) sweat chloride. The median sweat chloride in the LD group is 59.5 (IQR 36.3, range 23-92) mmol/L, which is significantly lower than for the ED group; 102.5 (IQR 17.3, range 36-135) mmol/L, Mann-Whitney U test; $p<0.001$.

3.4.10.4 Residual function CFTR mutations

There was also a high frequency of residual function mutations in the LD group, with Arg117His being the commonest (n=8). From these, two patients were heterozygote for Phe508del/Arg117His-5T and four for Phe508del/Arg117His-7T. Two patients were homozygote for Arg117His-7T. Interestingly, one patient in the group was a Phe508del homozygote with a ppFEV$_1$ <40%, exocrine pancreatic insufficiency and an atypical late diagnosis aged 43 years.

3.4.11 Genotype-phenotype correlation

Similar to data presented by Simmonds et al in 2009(200), our older CF study population showed a high prevalence of Phe508del homozygotes at 37.6% (n=32), with the majority being in the 40-49 years of age cohort (n=24, 42% of this age group). There were no Phe508del homozygotes in the $\geq$60 year age group. Compound Phe508del heterozygotes were prevalent in all age groups and the highest rate of Gly551Asp (heterozygote or homozygote) mutation was seen between 50 and 59 years (shown together with Phe508del heterozygotes and as a separate bar in figures below). Five out of eight patients in the $\geq$60 year age group had either Arg117His-5T, Asp1152His or TG12-5T residual function

mutations accompanying Phe508del. Genotype proportions of the total group and then within age groups are shown below (figures 3.7-3.10).

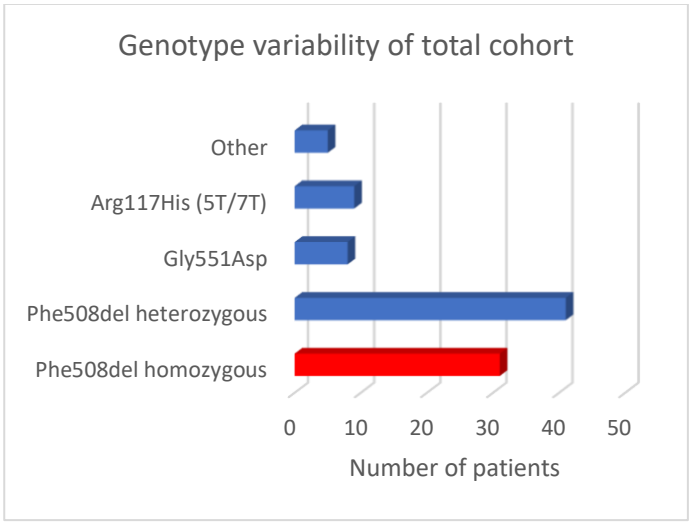


Figure 3.7: Genotype distribution in total cohort

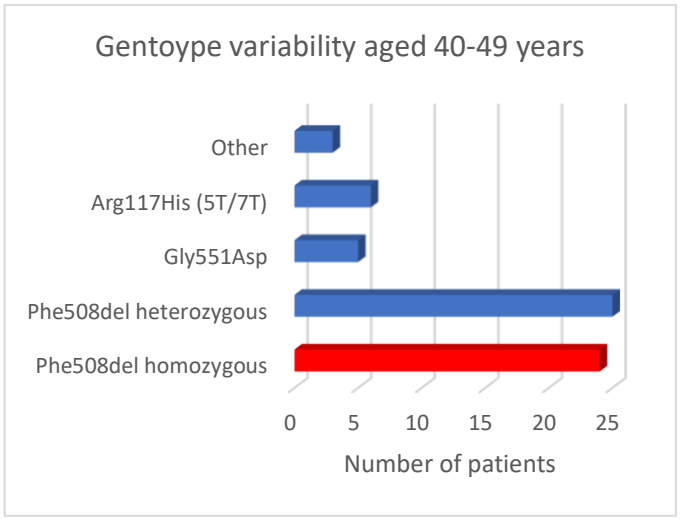


Figure 3.8: Genotype distribution in the 40-49 year age group

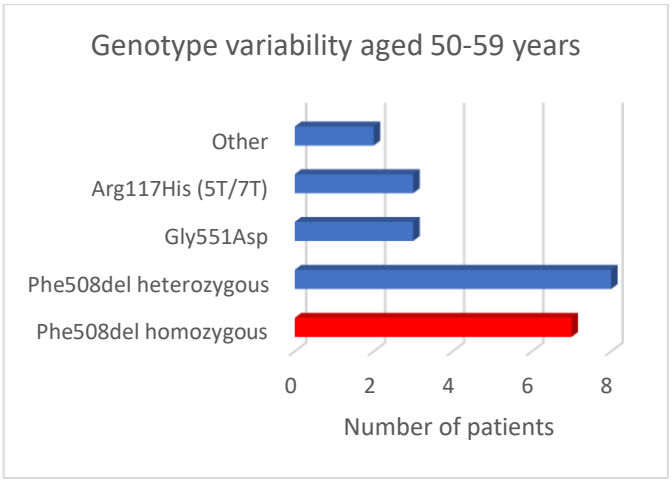


Figure 3.9: *Genotype distribution in the 50-59 year age group*

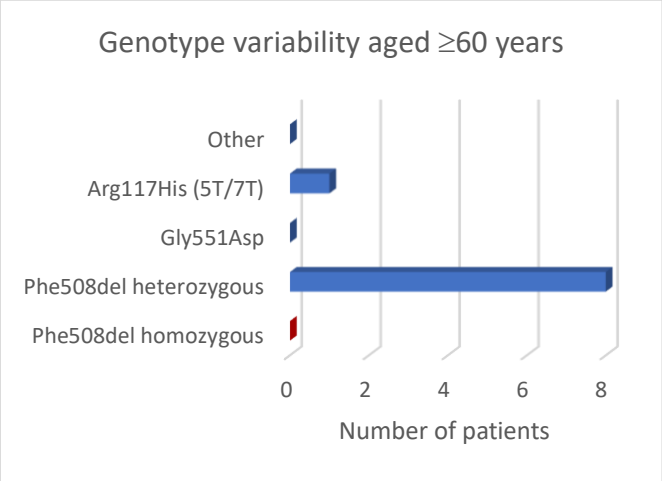


Figure 3.10: *Genotype distribution in the ≥60 year age group*

Data were then categorised into severe (class I-III) and non-severe (class IV-V) CFTR mutation groups for further analysis (table 3.9). All patients included in the study had two confirmed CFTR mutations.

Mutation class	**Severe vs non-severe**	**Number of patients n (%)**
CFTR class I-III: 1st allele: • Phe508del • Gly551Asp • Stop codon 2nd allele: • Phe508del • Gly551Asp • Stop codon • Other class I-III	Severe	62 (72.9% of total group) *51.6% Phe508del homozygous, n=32* *41.9% Phe508del heterozygotes, n=26* *6.5% other, n=4*
CFTR class IV-V (residual function, RF) At least one class IV-V mutation: e.g. • Arg117His(5T/7T) • Other class IV-V	Non-severe	23 (27% of total cohort) *39.1% Arg117His(5T/7T), n=9*

Table 3.9: Proportions of severe and non-severe CFTR mutations in our cohort.

Patient	Allele 1	Allele 2	Phenotype	Disease features
13	Phe508del	Arg117His-7T	Non-classical	CBAVD, sinus disease
22	Phe508del	Arg117His-5T	Classical	PsA, ppFEV$_1$ 56%, PS
27	Phe508del	Arg117His-5T	Classical	MRSA, BCC, ppFEV$_1$ 26%, PI
34*	Phe508del	Arg117His-7T	Non-classical	CBAVD, CFRD
38**	Arg117His-7T	Arg117His-7T	Asymptomatic	CBAVD
49**	Arg117His-7T	Ar117His-7T	Non-classical/ ?asymptomatic	Joint disease only
59*	Phe508del	Arg117His-7T	Non-classical	CBAVD, CFRD, pulmonary disease
82	Phe508del	Arg117His-7T	Asymptomatic	CBAVD
85	Phe508del	Arg117His-7T	Non-classical	CBAVD, pulmonary disease, PsA

*Table 3.10: Disease characteristics and phenotypic variability of the Arg117His cohort in our older CF population (patient numbers referenced from table 3.1) {*sibling pair, **sibling pair}*

Table 3.10 highlights the variability in disease expression within the residual function Arg117His-5T/7T genotype group. More severe, classical disease is seen with Arg117His-5T, although both patients had a late CF diagnosis with a mean age of 32 years. Asymptomatic and non-classical disease is seen with Arg117His-7T. All patients in this cohort had an adult CF diagnosis, median age 36.5 (IQR 29) years. Differences in disease expression is seen in patient 34 and 59 despite being siblings with the same CFTR genotype.

Genotype analysis revealed a greater prevalence of non-severe CFTR mutations with increasing age, with a statistically significant difference between the 40-59 and ≥60 year age groups on Chi squared testing (p=0.002). Although there were outlying results, the median age of CF diagnosis was significantly lower in the severe CFTR group compared to the non-severe group; 1.0 (IQR 3, range 0-47) vs 30.0 (IQR 37, range 0-72) years, p<0.001 (figure 3.11).

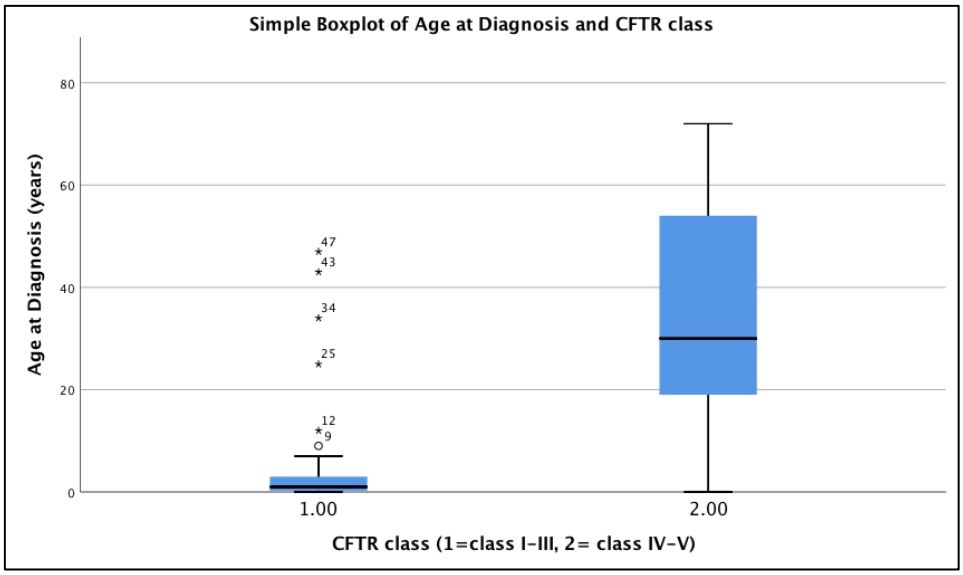


Figure 3.11: Boxplot of age at diagnosis vs CFTR class.

Chi squared testing also revealed a significantly higher prevalence of CFRD, exocrine pancreatic insufficiency and chronic *Pseudomonas aeruginosa* infection in patients with two severe CFTR mutations. Binary logistic regression did not maintain this significance for the presence of exocrine pancreatic insufficiency and CFRD between classes, however it did show a higher rate of both clinical parameters in the severe CFTR mutation group as expected (table 3.11).

Clinical parameter	CFTR class I-III	CFTR class IV-V	OR	95% CI	P value (LR)	P value (Chi squared)
CFRD (n)	36 (62)	6 (23)	1.47	0.26, 8.26	0.662	**0.008**
PI (n)	60 (62)	11 (23)	3.02	0.30, 30.1	0.347	**<0.001**
PsA (n)	50 (62)	6 (23)	6.14	1.29, 29.3	**0.023**	**<0.001**

Table 3.11: Analysis of clinical severity parameters against CFTR class.

CFTR class showed no relationship to gender in our cohort using Chi squared testing; p=0.566. There was a lower baseline $ppFEV_1$ in patients with severe CFTR genotypes as compared to those without (Students T-test; p<0.001). However, there was no significant difference in BMI between CFTR mutation groups.

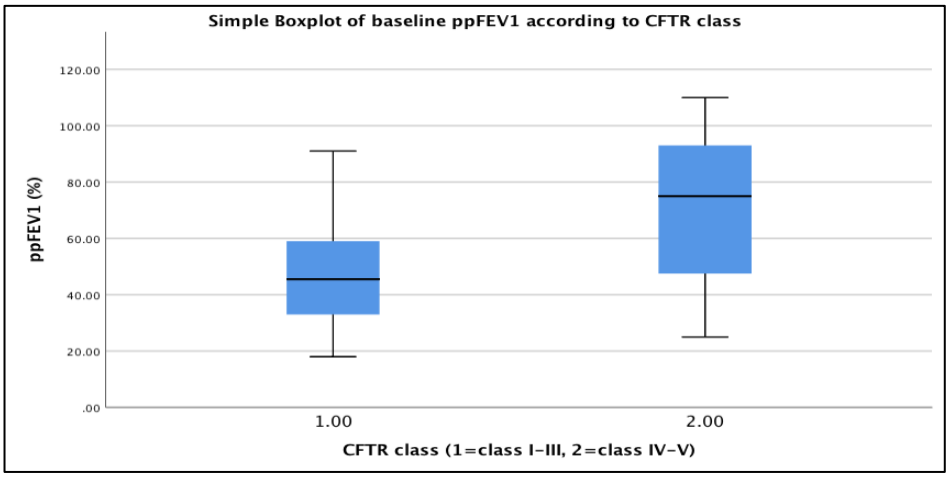


Figure 3.12: Boxplot showing the difference in $ppFEV_1$ between severe and non-severe CFTR mutation groups.

There was a significantly higher baseline sweat chloride level in the severe (class I-III) CFTR mutation group compared to the non-severe (class IV-V) group (Mann Whitney U test; p=0.001).

CFTR class	CFTR class I-III	CFTR class IV-V	P value
Sweat chloride value median (IQR) {range} (mmol/L)	101 (19.5) {23-135}	67 (58.5) {26-123}	**0.001***

*Table 3.12: Sweat chloride values by CFTR mutation class (IQR=interquartile range), *Mann Whitney U analysis.*

We also observed a lower sweat chloride with higher $ppFEV_1$; Spearman rank correlation; r=-0.250, p=0.046.

3.4.12 Social analysis

55.3% of our older CF cohort (n=47) are currently in part time or full time employment; 48.2% (n=13) of all women and 60.7% (n=34) of all men. Those with more severe pulmonary disease ($ppFEV_1$ <40%) expectedly had a lower rate of employment than those without

(42.9% vs 74.5%, Chi squared; $p<0.001$). Five patients from our older CF cohort were retired, two of whom have severe disease and three of whom have residual function CFTR mutations.

34.1% of all patients (n=29) had biological children, ten of whom are CF mothers; 34.5% of all women and 33.9% of all men. Similarly, more patients in the preserved lung function group had children; 36.4% vs 16.7% ($p=0.046$).

3.5 Discussion

3.5.1 General discussion

The aim of this study was to explore the demographics of a current older cohort of CF patients, to stimulate discussion and to identify areas for further research. Our study represents one of the largest cross-sectional cohort studies of CF patients over 40 years of age thus far in the UK. This is a diverse cohort with a spectrum of CFTR genotypes and phenotypic disease severity. We observed a relatively high prevalence of class I-III CFTR genotypes and a 37.6% prevalence of Phe508del homozygosity, similar to results reported by Simmonds and colleagues in 2009. There was also a high rate of CFRD (49.4%), chronic *Pseudomonas aeruginosa* colonisation (65.9%) and exocrine pancreatic insufficiency (83.5%). An increasing prevalence of non-severe CFTR mutations conferring residual CFTR function was seen with increasing age. In particular, for those aged 60 years and above, only a minority had severe CFTR genotypes. There was an increasing prevalence of systemic hypertension (15.8% aged 40-49 years and 37.5% aged ≥60 years) and hyperlipidaemia (7% aged 40-49 years and 37.5% aged ≥60 years) with increasing age, the analysis of which will be expanded on in chapter four. These patients exhibit a spectrum of functional status, with a large proportion in part or full time employment (55.3%), and an increasing number with biological children (34.1%). Although it is challenging to predict emerging comorbidities in an older CF cohort with such a range of genotypes and phenotypic diversity, there is a signal that indicates the need to develop our knowledge of long term survivors, particularly in the era of CFTR modulation, and how the complexities of CF ageing will impact future management.

3.5.2 Disease severity

In our cohort, 36.1% (n=30) had severe pulmonary disease as measured by $ppFEV_1$ <40%. Ten (33.3%) of these patients had a $ppFEV_1$ of <30%, three of whom were transplanted during the study period and one who was on the active lung transplantation list. Lung function decline (as measured by $ppFEV_1$) is the most robust marker of survival in CF(281). Recurrent pulmonary exacerbations, particularly those requiring intravenous antibiotics, contribute to significant disease morbidity. A higher pulmonary exacerbation rate correlates with a more rapid $ppFEV_1$ decline and a higher mortality rate(202). In our cohort, the median total IV antibiotic use over the preceding ten year period was 88.0 (IQR 188) days. A

higher rate of IV aminoglycoside use was seen in patients with lower baseline $ppFEV_1$ ($p<0.001$). There was a high prevalence of both nebulised antibiotics (72.9%) and mucolytics (dornase alfa and 7% sodium chloride; 63.5%), evidencing a high chronic respiratory therapy burden in this group. The relatively high prevalence of severe lung function impairment with a wide range of IV aminoglycoside use in our older CF adults further highlights the diversity of this group and that the problems associated with classical CF are still frequently encountered in older patients.

Although median age of survival in CF is increasing, progressive loss of lung function is seen in all age and genetic cohorts. Corey et al (1997) reported exocrine pancreatic sufficiency, non-Phe508del CFTR mutations and male gender to be associated with a slower rate of pulmonary decline(255). However, Desai et al (2019) showed a proportion of an adult-diagnosis (>18 years) CF cohort with a high prevalence of residual function CFTR mutations to still have significant annual lung function deterioration(202)(282). Interestingly, the International Study of Ageing in CF observed 'relative stability' in cross sectional lung function data for patients over the age of 35 years, with a mean annual $ppFEV_1$ decline of only 0.4%, and around 12% higher mean predicted $ppFEV_1$ in exocrine pancreatic sufficient patients(210). These studies highlight the variability of phenotypic expression in the older CF population.

3.6% (n=3) of patients had a BMI of $<18.5 kg/m^2$. However, the majority (61.3%) of our older CF cohort had a normal BMI between 18.5 and 24.9, with a mean of 23.9 (±3.13) kg/m^2. Interestingly, 28.6% were overweight (n=24) and 4.8% (n=4) were classed as obese. From the overweight group, four patients were on CFTR modulation (three on ivacaftor, one on Symkevi®) and five were in non-severe CFTR mutation groups. One patient in the obese group had a severe CFTR genotype (Phe508del/Phe508del). Nutritional status in CF, expressed in terms of context of body mass index (BMI), is one of the most important factors in CF survival, closely correlating with pulmonary severity(196). This is true of our older CF cohort, showing a statistically significant positive correlation between $ppFEV_1$ and BMI ($r=0.436$, $p<0.001$). BMI also correlates with exocrine pancreatic function and CFRD status. Exocrine pancreatic status has the strongest genotype-phenotype correlation, with exocrine pancreatic insufficiency manifesting almost universally in patients with severe CFTR mutations(283). A raised BMI was associated predominantly with less severe CFTR

genotypes in our cohort, however interestingly we observed the majority of overweight and obese patients to have exocrine pancreatic insufficiency; n=22 (91.6%) and n=3 (75%) respectively. CFTR modulators such as ivacaftor have been shown to be associated with weight gain(284) and hence BMI must be monitored closely in an older CF population with other emerging cardiovascular risk factors, particularly with the increasing availability of CFTR modulation.

High sensitivity C-reactive protein (hsCRP) is a sensitive biomarker of systemic inflammation and can be used to both confirm and measure response to treatment during pulmonary exacerbation(285). CF patients with more severe underlying disease and a higher degree of chronic systemic inflammation, as evidenced by elevated levels of pro-inflammatory cytokines, may have a raised baseline hsCRP. In our older cohort, 46.4% of patients had a raised baseline hsCRP (>5mg/L). In our cohort, raised baseline hsCRP was seen in patients with lower $ppFEV_1$ ($r= -0.515$, $p<0.001$) and a higher IV aminoglycoside requirement ($r^2=0.276$, $p<0.001$). Matouk and colleagues (2014) also observed a higher risk of pulmonary exacerbation in a high baseline hsCRP patient group (>5.2mg/L), noting a higher degree of correlation in patients with lower lung function(279).

Female survival disadvantage seems evident in our older CF cohort with the majority of patients being male (65.9%). Simmonds and colleagues also observed a male predominance in their study cohort at 57%(200). Our male survivors also surpass those of a study by Nick et al (2010), showing a 53.6% male predominance in a large US registry study(286). However, Nick et al also observed the majority of long term CF survivors with an *adult* diagnosis to be female and, upon reaching aged 40 years and above, females had a distinct survival advantage compared to their male counterparts(286). However, our study shows a male predominance again in our late diagnosis group at 60%, highlighting a female disadvantage with delayed CF diagnosis. Could this difference be explained by female under-representation in milder CF disease? Interestingly the gender disparity in our study seems to decrease between the ages of 50 and 59 years, with a more equitable male to female ratio in this age group (56.5% male). Female CF patients have consistently been observed to have lower predicted median survival than males and the causes of this gender gap are largely uncertain. Speculated reasons include the effects of oestrogen on bronchial epithelial mucous viscosity and the acquisition of chronic *Pseudomonas aeruginosa* infection at an

earlier age(287). As survival increases, more women will reach menopause, and this may also have a detrimental impact on lung function and other parameters of health status in CF. The study of hormonal changes and menopause in ageing female CF patients would be insightful.

3.5.3 Other predictors of survival

Reaching the age of 40 years and above defines long term survival in CF(278). Since ageing in CF is a relatively novel concept when considering the natural history of disease, the effects of the ageing process on CFTR dysfunction and clinical phenotype, and vice versa, are largely unknown. As survival continues to increase, it is essential to understand more about the ageing CF patient and how this process alters and complicates disease.

Ageing comorbidities in CF may be classed as CFTR-related and non-CFTR related. CFTR related ageing complications are defined by longevity of CFTR organ dysfunction. Non-CFTR related complications may include the processes of general organ and vascular ageing as well as side effects of chronic therapies. The relationship between CFTR-related and non-CFTR related ageing complications will be challenging to decipher and together add further layers of disease complexity to the ageing CF patient.

3.5.3.1 CF-related diabetes mellitus

CF-related diabetes mellitus (CFRD) is an age prevalent complication of CF, with increasing risk of dysglycaemia with advancing age. In our cohort, 49.4% had a diagnosis of CFRD, which is in keeping with the overall UK prevalence of CFRD in patients over 40 years of age(43). Chi squared testing showed a significantly higher prevalence of CFRD in the severe CFTR mutation group compared to the non-severe groups (p=0.008) and in the early diagnosis compared to late diagnosis group (p=0.024). A diagnosis of CFRD has significant impact on morbidity and mortality in CF, contributing to poorer nutritional status and more rapid decline in lung function(288). In our cohort, we observed lower mean ppFEV$_1$ (47.6 vs 57.9%, p=0.040) and lower BMI (23.3 vs 24.6 kg/m^2, p=0.041) in the CFRD group compared to the non-CFRD group.

Complications of CFRD are particularly important in older CF patients in whom the risk of developing microvascular complications is proportional to the duration of hyperglycaemia. Although there have been only a handful of case reports of macrovascular complications in

CF to date(224)(289), these may well become more relevant with an ageing population and particularly in the context of conventional cardiovascular risk factors associated with general ageing, such as systemic hypertension and hypercholesterolaemia. CFRD-related renal disease may also become more prevalent with increasing survival. These areas will be discussed in more detail in subsequent chapters. Having observed over 30% of our older CF population with a raised BMI, obesity is also likely to have an impact on the development of dysglycaemia in an ageing CF population. The resulting metabolic syndrome will perhaps confer a different diabetic phenotype in some patients than classically seen with CF-related diabetes mellitus and may in turn require different treatment strategies.

3.5.3.2 Sputum microbiology

A significant proportion of patients in our older adult cohort had chronic *Pseudomonas aeruginosa* infection (65.9%). Higher morbidity and mortality have been shown in CF patients with earlier acquisition of chronic *Pseudomonas aeruginosa* infection, with a higher frequency of pulmonary exacerbation and antimicrobial treatment burden, more rapid decline in pulmonary function and a lower median survival(290) than their non-*Pseudomonas aeruginosa* counterparts. Just over 15% (n=13) of our older cohort had chronic infection with *Burkholderia cepacia* complex (BCC), seven patients with *Burkholderia cenocepacia* and the remainder with *Burkholderia multivorans*. Chronic BCC infection is classically associated with poor prognosis in CF and can lead to fulminant systemic 'cepacia' infection in up to 30% of patients(291). In addition, chronic infection with *Burkholderia cenocepacia* has adverse impacts for both suitability and outcomes of lung transplantation(292). Over the last few decades, the majority of patients with *Burkholderia cenocepacia* in our unit have died, the remaining few comprising only around 1% of the total population at MACFC. Interestingly, severe lung function impairment was not significantly related to BCC infection in our cohort. The discordancy between the prevalence of BCC infection and severe clinical phenotype further highlights the diversity of this older CF population and implies a plethora of different factors influencing survival.

A small proportion of our older cohort have had recent isolation of non-tuberculous mycobacteria (NTM) in sputum analysis. Two patients had isolated *Mycobacterium avium* complex (MAC) within the previous 12 months, and one had chronic *Mycobacterium abscessus* infection. This is perhaps less prevalent than expected, particularly given the

findings by Rodman et al in their late diagnosis CF survivors(199). The low prevalence of NTM infection in our centre is challenging to explain, however some geographical variation may be relevant. The prevalence of chronic NTM infection increases in an age-dependent manner in CF, with Olivier and colleagues observing older age to be the most significant predictor of NTM isolation in CF, particularly the MAC subgroup(293). Longevity of azithromycin use in CF patients has been considered a risk for the development of macrolide-resistant NTM infection over time and may become more relevant in an ageing population(294). Infection with multi-resistant NTM strains such as *Mycobacterium abscessus* are difficult to treat and can have adverse implications for lung transplantation(295).

3.5.4 Organ transplantation

Three of our patients received double lung transplantation during the study period. Increasing survival in CF may lead to older age at lung transplantation. US data from 2010 shows 18.8% of CF lung transplant recipients were older than 40 years of age(198). One patient in our cohort was a pancreas and renal transplant recipient for refractory CFRD and systemic hypertension. It is thus worth noting that, with an increasing number of patients aged ≥40 years, we may also see a higher prevalence of solid organ transplantation in older CF, which will impact clinical disease course. There is likely to be an accumulation of age-prevalent comorbidities as survival in CF increases, including CFRD, and with an increasing age at transplantation we may also see emerging renal and cardiovascular disease, rendering post-transplant management ever more complex.

3.5.5 Malignancy

One patient in our study group was undergoing treatment for rectosigmoid adenocarcinoma (stage T3N0M1). Increasing survival is likely to have an impact on the prevalence of malignancy in CF, similar to that seen in the general population. However, an inherent carcinogenic risk may exist from CFTR dysfunction itself. Studies have found an increased frequency of gastrointestinal adenocarcinoma in CF cohorts and have suggested a link between CFTR-influenced microbial gut dysregulation and the development of malignancy(59)(296). There is particular concern regarding the existence of a ‘colon cancer syndrome’ in CF. Niccum and colleagues(62) observed an increased incidence of pre-malignant adenomatous polyps and colorectal cancer in CF patients compared to the

general population, particularly those over 40 years of age. It is therefore recommended that regular colonoscopic review be carried out for CF patients aged 40 year and above, even if asymptomatic, ten years earlier than colorectal screening in the general population. It is noteworthy that one patient from our cohort is currently being treated for rectal adenocarcinoma. In a study of long term survivors by Simmonds and colleagues (median age 43.1 years), one patient had died of metastatic pancreatic carcinoma(200). There is also a risk of malignancy, including skin and haematological, following organ transplantation and subsequent long term immunosuppression(297). One patient in our cohort was a renal and pancreatic transplant recipient and three had bilateral lung transplantations during the study period. In an older CF population with the potential for an increasing incidence of organ transplantation, we may see an associated rise in malignancy.

3.5.6 Cardiovascular risk

One late diagnosis patient in our cohort had had a previous stroke. None had a history of cardiovascular disease, although the prevalence of systemic hypertension and hyperlipidaemia was 18.8 and 15.5% respectively, increasing with age. Historically CF patients were presumed to have low risk of cardiovascular disease due to exocrine pancreatic insufficiency, lipid malabsorption and low serum cholesterol levels(298). A lifetime of high fat diets, an increasing prevalence of CFRD and the nutritional impacts of CFTR modulation may change that perspective as CF survival improves.

Hypertriglyceridaemia has been shown to be prevalent in CF patients, independent of CFRD status(299). There may be a higher prevalence of residual CFTR function in an older CF cohort, and Rhodes and colleagues observed higher levels of total cholesterol and LDL-cholesterol in patients with exocrine pancreatic sufficiency and higher BMI(300). This particular CF group may therefore harbour a higher level of cardiovascular risk, and results of my current study corroborate this. Skolnik and colleagues observed 69% of their CF cohort over 40 years of age to have evidence of dyslipidaemia(222), which is significantly higher than my study. However, this was a small study (n=25) concentrating on lipid status during lung transplantation assessment. Despite this, evidence shows must not overlook the importance of lipid monitoring and adequate management of hyperlipidaemia in an ageing CF population.

CFTR modulation may also have an impact on cholesterol metabolism. Longitudinal ivacaftor studies have observed alterations in gastrointestinal absorption, leading to weight gain in CF children(284)(301). Four patients in our study group were overweight and on CFTR modulation. Could this elude to the potential for weight gain and altered lipid metabolism with CFTR modulator longevity in CF adults? The study of cholesterol trends and CFTR modulation would be informative.

C-reactive protein (CRP) and systemic inflammatory burden in CF may also have an association with cardiovascular risk, independent of other cardiovascular risk factors. We have seen that a high proportion of our patients (46.4%) have raised baseline hsCRP during periods of clinical stability. The effects of enduring systemic inflammation in CF could be similar to those seen in other chronic lung conditions such as chronic obstructive pulmonary disease (COPD), where airflow limitation is a risk factor for cardiovascular disease and systemic inflammation may correlate with the development of atherosclerosis(302).

Non-CFTR related complications of ageing such as atherosclerosis, systemic hypertension and hypercholesterolaemia will be discussed further in subsequent chapters, where I will also explore arterial stiffness, central systolic blood pressure, cholesterol biomarkers and QRisk® scores as predictors of cardiovascular disease in CF.

3.5.7 Genotype phenotype correlation

Defining genetics and markers of disease severity in CF has never been as important as in this present era of CFTR modulation, influencing initial patient selection and resulting clinical efficacy. In the UK CF population, the most common CFTR mutation is Phe508del, with 49.3% of patients homozygous for Phe508del and 40.5% compound heterozygotes(4). 37.6% of our long term CF survivors were Phe508del homozygotes and 72.9% had severe class I-III CFTR mutations, resulting in severe CF disease manifestations. However, I did see an increasing prevalence of 'residual function' CFTR mutations with advancing age in the study group, often associated with an adult CF diagnosis. Less severe CFTR mutations may be an important factor in long term CF survival however, as CF survival increases, we could observe a rising prevalence of severe CFTR genotypes in an older CF population.

3.5.7.1 Age of CF diagnosis

Genetic and clinical advances over the last eight decades have led to a greater understanding of the vast spectrum of CF disease and a greater number of adult diagnoses over time. In fact, 8.5% of CF diagnoses made on the UK in 2018 were in patients aged 16 years or above(4). In our older group, 27.3% of patients had an adult CF diagnosis, with a median age of diagnosis of 36.5 (IQR 29) years in this late diagnosis group. It should be noted that the current study cohort were all born before initiation of newborn CF screening.

Our late diagnosis group shows a trend towards lower sweat chloride and exocrine pancreatic sufficiency than in the early diagnosis group. However, a significant proportion have chronic *Pseudomonas aeruginosa* infection and severe lung function impairment. The prevalence of residual function CFTR mutations was high in the late diagnosis group, with Arg117His-5T or 7T at 45% being the most prevalent CFTR mutation seen. This is similar to McCloskey and colleague's findings in a late diagnosis CF group in Northern Ireland(303). As expected, our late diagnosis group had a significantly higher $ppFEV_1$ ($p<0.001$) and BMI ($p=0.005$) than the early diagnosis group, and a lower prevalence of CFRD.

The older adult CF population is diverse with a variety of genetic and phenotypic disease expression. It has been observed that a late diagnosis adult CF group has a trend towards higher prevalence of exocrine pancreatic sufficiency, better preserved lung function and less severe CFTR genotypes(199). However, our study group consists of both classical CF patients with severe CFTR mutations and those with later CF diagnosis and less severe genotypes. Defining potential survival advantages in older CF patients and exploring the relationship between genotype and phenotype in this population may provide insight into the complexities of their disease and develop avenues for management optimisation as survival continues to increase. The genotype-phenotype correlation in CF is notoriously challenging to define, with wide phenotypic disease expression within CFTR mutation classes.

Several studies have analysed the ageing population and survival in CF in the context of early and late diagnosis, showing similar results to our study. Gan and colleagues (1995) compared LD and ED groups, with late diagnosis defined as $\geq$16 years, showing higher baseline $ppFEV_1$, a greater prevalence of exocrine pancreatic sufficiency and lower rates of chronic *Pseudomonas aeruginosa* infection in the LD group(304). Rodman and colleagues (2005), using $\leq$15 years as early diagnosis and >24 years as late diagnosis, observed a higher

prevalence of exocrine pancreatic insufficiency and lower BMI in the ED group(199). Widerman and colleagues (2000) explored a patient group with an adult CF diagnosis (≥18 years), exhibiting significantly less hospital admissions and intravenous antibiotic use alongside a lower prevalence of Phe508del homozygotes and exocrine pancreatic insufficiency(305). This study also showed a higher proportion of women in the late diagnosis group, in contrast to our cohort in which males outnumbered females 3:2. Durno and colleagues observed exocrine pancreatic sufficiency to be related to increased survival in CF and has a greater prevalence in milder CF genotypes and in a late diagnosis group(283). Although, as with similar studies, our older CF cohort contains a high prevalence of severe disease phenotypes, late diagnosis patients with a greater degree of residual CFTR function may introduce bias into CF survival statistics.

The high proportion of severe phenotypic disease expression in our cohort provides further insight into the heterogeneity of the older CF population. This diversity also correlates with a similar study by Simmonds et al(200). One of the oldest patients in our study (aged 71 years), a heterozygote for Phe508del/Arg117His-5T, with severe clinical disease, exocrine pancreatic insufficiency, a baseline ppFEV$_1$ of <40% and chronic infection with both *Pseudomonas aeruginosa* and MRSA, was diagnosed with CF at aged 38 years. Three patients in our study group, heterozygote for Phe508del and a residual CFTR mutation and with preserved exocrine pancreatic function, had early diagnosis and have severe lung function impairment. These patients highlight disease diversity and relative inaccuracy of assessing older CF cohorts in terms of age at diagnosis.

3.5.7.2 Residual function CFTR genotypes

The term ‘residual function’ in CF genetics refers to the degree of preservation of normal CFTR protein expression and function(252). The majority of patients have “classical CF,” caused by severe CFTR class I-III mutations accounting for both CFTR gene alleles, resulting in little or no CFTR protein function. Severe CFTR genotypes are associated with exocrine pancreatic insufficiency, severe lung disease and multiorgan involvement. However, around 5% of the CF population have ‘residual function’ CFTR mutations. Corresponding genotypes are within the less severe CFTR mutation classes IV and V, including Arg117His(5T/7T) and TG12-5T, exhibiting variable preservation of chloride channel gating and function. Residual function CFTR mutations confer a less severe CF phenotype and clinically these patients

usually have exocrine pancreatic sufficiency and less severe disease manifestations, often termed “non-classical” CF(6). Definitions of residual CFTR function for clinical trial purposes include a sweat chloride of less than 86mmol/L, and thus evidence of residual chloride ion transport, and a low incidence of exocrine pancreatic insufficiency (<50%)(306). Homozygotes for residual function CFTR mutations may be asymptomatic, with congenital bilateral absence of the vas deferens (CBVD) and infertility being the only clinical sign in affected males(307). Some examples are shown in table 3.13.

Allele 1	**Allele 2**	**Phenotype**
Severe	Severe	Classical
Arg117His-5T	Severe	Non-classical
Arg117-His-7T	Non-severe	Asymptomatic female or CBVD
TG12-5T	Severe	Non-classical
TG12-5T	Non-severe	Asymptomatic female or CBVD
Arg117His-7T or 9T	Arg117His-7T or 9T	Asymptomatic

Table 3.13: Examples of residual function CFTR mutations.(CBVD=congenital absence of vas deferens, severe = class I-III mutations, non-severe = class IV-V mutations).

The variable penetrance of Arg117His depends upon the number of poly-thymidine repeats on intron 9 (5T, 7T or 9T), caused by abnormal RNA splicing, resulting in interruption of normal CFTR protein development(308). Arg117His-5T is the only variant associated with classical CF disease when paired with a severe CFTR mutation.

Despite these classifications, variability of disease severity exists within CFTR genotype groups, highlighting the complexity of the genotype-phenotype relationship in CF. Variability in pulmonary disease severity between severe and non-severe CFTR mutation groups are well defined. Hubert and colleagues observed more rapid lung function decline, lower arterial oxygen levels and higher chronic *Pseudomonas aeruginosa* infection rates in

patients with two severe CFTR mutations(309). In a large US CF Registry study, McKone and colleagues showed survival disadvantage in patients with high risk CFTR mutations (class I-III), with the median age of death around 13 years lower compared with the lower risk CFTR mutation classes (IV-V). However, it was noted that differences in mortality could not be completely explained by clinical disease severity and variability of phenotypic expression within groups exists(268).

Less severe, residual function CFTR mutations have been shown to correlate with a later age of diagnosis and exocrine pancreatic sufficiency. Although less severe CFTR mutations are usually associated with improved survival, patients will still experience lung function decline and pulmonary disease progression that may in fact just be delayed rather than reduced. DeBoek and colleagues (2017) showed decline in lung function in patients with at least one non-severe CFTR mutation and those with higher baseline ppFEV$_1$ (and less severe disease) had the highest annual rate of decline(310). Wagener and colleagues (2016) observed lung function deterioration in patients with residual function mutations over a four year period and, although delayed when compared to Phe508del homozygotes, the rate of decline becomes similar to homozygotes with advancing age and may well be more significant in those with higher baseline values(311).

Our late diagnosis cohort displays a high prevalence of Arg117His mutations; four patients heterozygous for Arg117His-7T, three heterozygous for Arg117His-5T and two homozygous for Arg117His-7T. We observed a diversity of phenotypic expression within this group. This may be explained by the variable penetrance of residual function CFTR mutations, but also by variation in environmental factors and genetic polymorphisms between individuals. In addition, although our data shows a relationship between severe (class I-III) CFTR mutations and more severe phenotypic disease, there still remains some disease variability and later diagnosis in this group. CFTR genotype-phenotype studies have postulated various factors contributing to this discrepancy. Burke and colleagues observed disease variability in CF siblings with genetically identical CF gene loci, identifying that environmental factors may influence CF phenotype(312). Collaco and colleagues showed an equal contribution of environment and genetics on disease variability between CF twins and siblings(7). Some environmental factors contributing to this phenotypic difference may include socioeconomic status and exposure to both tobacco smoke and air pollution(202).

The variability of genotype-phenotype relationship in CF patients may also be explained by differences in the expression of "modifier" genes between patients of identical CFTR alleles, affecting CF phenotype independent of CFTR function. Gu and colleagues identified variable expression of IFRD1, a transcriptional regulator of neutrophil differentiation, between CF siblings, influencing inter-subject levels of pulmonary inflammation(313). In addition, they observed variation in bacterial airway clearance and neutrophilic inflammation in mice models. Genetic polymorphisms in transforming growth factor β1 (TGFβ1), a cytokine involved in cell growth, have been shown to affect pulmonary disease severity in Phe508del homozygotes(314).

The study of genotype and phenotype relationship has also observed a disparity in organ 'dysfunction thresholds' and response to abnormal CFTR, resulting in variable disease expression(315). Although inconsistent in its degree of abnormality, a sweat chloride level of >60mmol/L is a universal feature of clinical CF, independent of genotype. However, elevated sweat chloride alone does not cause phenotypic disease. The most reliable clinical manifestation of abnormal CFTR is male infertility through congenital bilateral absence of the vas deferens (CBAVD)(316). Other organ involvement has been shown to vary greatly between genotype groups and tissue sensitivity to abnormal CFTR. Exocrine pancreatic insufficiency often presents in early diagnosis, 'classical' CF since the pancreas requires a high degree of CFTR dysfunction in order to manifest disease.

Nine patients in my study group had Arg117His mutations and two had TG12-5T. The CFTR mutation Arg117His (5T or 7T) is classically associated with less severe CF clinical disease. However, diversity in clinical phenotype with residual function CFTR mutations have been observed. This variable penetrance of less severe CFTR mutations in CF populations has been reported by Thauvin-Robinet and colleagues, who observed only a 3.1% prevalence of clinical CF in those with at least one Arg117His mutation(307). A high proportion of patients with Arg117His-7T present with infertility due to CBAVD with a low prevalence of other CFTR-related disease, hence these patients have a late age of diagnosis that may constitute a 'CF-related disorder' rather than true CF. It is likely that there are a proportion of people carrying two copies of the Arg117His gene and who are asymptomatic, likely never to experience pulmonary disease. Nasal potential difference may be a useful diagnostic adjunct in less classical CF presentations, particularly in the setting of a normal or equivocal sweat

chloride level(317). The diagnosis of CF in Arg117His-7T homozygotes is contentious and their inclusion in our study may skew our long term survival figures.

However, in a recent publication by Keogh et al (2020), the proportion of CF patients aged 40 years and above at our centre appears reflective of that of the UK CF population as a whole based on 2017 UK registry figures(318).

Diagnosis and follow-up remains important in residual function CFTR mutations due to the possibility of developing pulmonary disease later in life, particularly with TG12-5T homozygotes, and also for appropriate genetic counselling.

Waller and colleagues observed variable phenotypic expression of Arg117His-7T in male monozygotic twins, one with an equivocal sweat chloride level, CBAVD and a diagnosis of CF-related disorder and the other, a biological father, with raised sweat chloride but the absence of any clinical manifestations of CF-related disease(319). Picci et al describe phenotypic disparity for TG12-5T in monozygotic twins, both with exocrine pancreatic sufficiency but differing pulmonary involvement(320).

Although Arg117His-7T is historically associated with less severe disease than Arg117His-5T, Keenan and colleagues observed a cohort of Arg117His-7T patients with similarities in disease severity to those with Arg117His-5T, highlighting the need for diagnosis and monitoring in these patients(321). Patients with residual function mutations such as Arg117His-7T and single organ CFTR dysfunction, such as sinonasal disease or obstructive azoospermia, can be challenging to define in current CF classification, and could represent CFTR-related disorders rather than distinct clinical CF. This is exemplified by two siblings in my study, one male and one female, homozygous for Arg117His-7T. The male patient had isolated CBAVD in the absence of other clinical disease, and the female patient had significant inflammatory arthropathy but no obvious CFTR-specific disease manifestations, having been diagnosed only through genetic screening following her brother's infertility diagnosis.

There is a consensus European and US guideline regarding CF diagnosis, including diagnostic algorithm and sweat chloride threshold(27). For patients with CFTR dysfunction, who do not fall into the category of classical CF, there is a lack of longitudinal data regarding disease

progression. However, studies have observed significant morbidity and clinical decline over time in patients with milder CFTR genotypes, with premature mortality compared to the general population(310)(311). Defining disease in these less severe genotypic cohorts will have implications for prognosis and treatment options, particularly in the era of CFTR modulation. Regular multidisciplinary review remains essential given the uncertainty of their disease course and their potential for greater treatment requirements with time.

3.5.7.3 Future survival in CF

Estimating future survival in CF is complex. Further advancement of treatment, the impact of CFTR modulation and the unpredictability of disease in some genetic cohorts must all be considered. The inference of a constant reduction in annual mortality rate also limits reliability. Taking these factors into account, Keogh and colleagues (2018) performed a large UK CF cohort study analysing predicted median survival from birth in various genetic groups, using flexible parametric survival modelling(11). CF survival was analysed based on gender, age of diagnosis and the condition of reaching certain age deciles, showing survival advantages in male Phe508del homozygotes and heterozygotes, and in Phe508del heterozygotes with an older age of diagnosis. Extrapolation of survival curves for Phe508del homozygotes postulates a median age of survival into the sixth decade if survival to 30 years of age had been achieved. The highest estimated survival rate was seen in the male Phe508del heterozygote group with a later age of diagnosis (>5 years)(11).

These estimates indicate a continued upward trend in median survival in CF over the coming years and, with the impact of CFTR modulation, an ageing CF population seems inevitable. As such, the study of ageing comorbidity in CF is of paramount importance in the current CF research climate to ensure optimal clinical care in the immediate and future care of an ageing CF population.

3.5.8 Sweat chloride and CFTR modulation therapy

CFTR genotype is essential for determination of individual eligibility for novel CFTR modulation. These compounds target abnormal CFTR at different stages in its formation, from production at RNA level, trafficking throughout the epithelial cell to its transport across epithelial cell membranes. The efficacy of CFTR modulation is relative to the preservation and degree of normal CFTR function and expression. Although it is too early to assess the

long term impact of CFTR modulation on survival in CF, it is predicted to change the landscape of the CF phenotype and clinical management moving forwards.

The efficacy of CFTR modulation in clinical practice has been assessed by improvements in clinical parameters such as CF Questionnaire-Respiratory symptom (CFQ-R) scores, BMI and $ppFEV_1$. The degree of sweat chloride reduction has also been an important parameter in measuring treatment success. Initial ivacaftor studies showed significant reductions in sweat chloride, indicating the extent of CFTR response at cellular level. The measurement of sweat chloride can also give us an indication of disease severity in CF. As such, we analysed the relationship of sweat chloride to genotype and phenotype in our older CF population.

In our cohort we observed significantly higher sweat chloride levels in the severe (class I-III) CFTR mutation group compared to the non-severe (class IV-V) group. Similar results were shown by Wilschanski and colleagues (1995) in a large paediatric CF cohort, using the initial diagnostic sweat chloride result(269). McKone and colleagues (2003) also showed lower mean sweat chloride levels in patients with class IV-V CFTR mutations, along with later age of diagnosis and higher $ppFEV_1$(268). However, the relationship between sweat chloride level (as a marker of residual CFTR function) and survival in CF is unclear. A study by Simmonds and colleagues (2011) in fact demonstrated no link between long term survival (>38 years) and sweat chloride levels taken later in life(322). In addition, sweat chloride concentration as a marker of CFTR dysfunction and clinical response to modulation therapy may be reliable on a population level but not necessarily on an individual one(323).

Sweat chloride and CFTR genotype analysis are important tools, however neither must be used alone to navigate to a diagnosis of CF, and variability in sweat chloride levels exists within CFTR genotype and phenotypic groups. There is a poor correlation between sweat chloride and clinical phenotype in Arg117His-7T mutations(307) and sweat chloride is not necessarily predictive of severity of CF lung disease(324). Ratkeiwicz and colleagues (2017) showed intermediate sweat chloride results in patients with two identified CFTR mutations, and elevated sweat chloride levels in patients with no CFTR mutations found(325). Patients with intermediate sweat chloride levels and two CFTR mutations are more likely to have CFTR dysfunction and have been shown to be a distinct phenotypic group when compared to both ‘non-classical’ CF with exocrine pancreatic sufficiency and to ‘classical’ CF with

exocrine pancreatic insufficiency(326). Mickle and colleagues reported a rare heterozygote CFTR mutation in a mother and daughter with isolated elevation of sweat chloride but no clinical CF(327). This validates the importance of CFTR mutation analysis, sweat chloride and clinical phenotypic expression together in the CF diagnostic algorithm.

The measurement of sweat chloride in CFTR modulation has become an important factor in determining efficacy of treatment and as a measure of CFTR response. Since sweat glands are not affected by the inflammatory response seen in other CF organ involvement, sweat chloride analysis is an accurate biomarker of CFTR dysfunction and hence of CFTR modulator response. The variation in sweat chloride response renders this test of modulator efficacy in the older CF population challenging, particularly in those with previously borderline sweat chloride levels.

Just under 30% of our older adult CF patients had already been commenced on CFTR modulation therapy during their participation in the study, some on a compassionate use basis. Since then, Symkevi® is now being funded by NHS England for patients with eligible genotypes. In addition, it has recently been announced that Kaftrio® (triple therapy) will be funded through the NHS, offering CFTR modulation to a greater number of patients. These developments have meant that a much larger number of our older CF cohort are now on CFTR modulation than at the start of this study. Interestingly, two patients in our group have been temporarily removed from the active lung transplant list due to the degree of improvement in both symptoms and lung function on Kaftrio®. Longer term CFTR modulation in the CF population as a whole is expected to impact survival and change the landscape of CF management, particularly in younger patients. However, it will be interesting to assess the benefit of CFTR modulation in an older CF cohort, the majority of whom will already have established organ disease.

3.5.9 Psychosocial factors

A significant proportion of our older patient cohort were in part or full time employment at the time of study (55.3%). In addition, 34.1% of patients had biological children. These results are similar to those found in the study of long term CF survivors by Simmonds et al in 2009(200). Unsurprisingly, patients with more advanced lung function had a lower rate of employment. There are increasing numbers of CF patients having families and entering regular employment. Indeed, five of the older CF patients in our study are now in

retirement. For younger patients, improving survival predictions will undoubtedly influence perspectives regarding employment and family planning. However, along with fertility challenges, there will be implications for both physical health and psychological wellbeing during parenthood. We have already seen a significant number of our older patients suffering with mental health issues and contributing themes in the context of family and work life may include a continuing high treatment burden, challenges with work-life balance, difficulties with self-management and maintenance of independence, and premature death. Insomnia is also common in CF patients and may contribute to both physical and psychological health issues.

Nevertheless, an increasing notion of achievable family and work life in CF perhaps represents a shift in attitudes towards CF and disease longevity. Patients are 'living with' CF rather than suffering its consequences. Particularly with increasing availability of CFTR modulation, patients will need to consider developing skills and making plans early in adulthood to prepare them for employment, family life and possibly even retirement as survival continues to improve.

It is also important to remember that a number of patients in our older CF cohort were born at a time when prognosis was still extremely poor. Having observed dramatic treatment development during their lifetimes, their perspectives of disease and survival in CF will have been greatly influenced, adding a level of cognitive depth to this already complex group. Qualitative research into this area may reveal new patient perspectives of ageing in CF and will be an exciting area for future research.

3.6 Limitations

A number of limitations to this study should be recognised. This is a cross sectional analysis of an older CF cohort and therefore assumptions regarding factors contributing to disease longevity must be interpreted with caution. We were unable to analyse sweat chloride levels in a small number of patients due to their pre-existing commencement of CFTR modulation, without availability of pre-modulator levels. Analysis of early and late diagnosis groups was challenging due to a lack of consensus for age parameters. Longer term survival in CF has been defined as aged 40 years and above, as in similar studies, however the

targets for “old age” in CF is likely to change over time. The influence of CFTR modulation on survival in CF may mean that defining an older CF population will be different in years to come. Certainly, the CF population aged 40 years and above will continue to evolve, and a greater number of patients with severe CFTR mutations and classical CF disease will reach longer term survival. Given the rarity of disease, the study of CF cohorts in general, particularly with reference to older age, is accompanied by the inevitability of small sample sizes. Larger, multicentre epidemiological studies are required to analyse and interpret survival influences in the older CF community.

3.7 Conclusion

At 20%, a significant proportion of our adult CF centre comprises patients aged 40 years and above. This is likely to increase on an annual basis for the foreseeable future, particularly in this new era of CFTR modulation therapy.

The older CF patient cohort is a diverse, heterogeneous and fascinating population. There exists a spectrum of CFTR genotype and phenotypic disease and this study demonstrates poor correlation between the two. Although a percentage have less severe CFTR genotypes and milder clinical disease in this population, it is important to recognise that a large proportion of our older CF patients have severe CFTR mutations with classical disease. The reasons for variability between genotype and phenotypic expression are diverse and largely unproven, although the expression of modifier genes and environmental factors are likely to play a large part in the clinical diversity between genetically identical patients.

Older patients with a later age of diagnosis, a milder genotype and less severe disease phenotype may skew CF survival figures, given their anticipated survival advantage. However, this group may still have significant morbidity, a less predictable disease course and a greater degree of uncertainty regarding future disease complications(328). Perhaps the survival gap between severe and less severe genotypes will be narrowed by CFTR modulation and as such we are likely to see median CF survival continue to improve.

CF survival is predicted to increase in each birth cohort over time and the demographics of the older CF population will continue to evolve. This highlights the timeliness of our research into this heterogeneous group, and why further work is essential in order to

identify emerging ageing comorbidities and to prepare for future challenges of large numbers of CF patients with complex, multisystem CFTR-related and comorbid disease.

The Complexities of Ageing in Cystic Fibrosis

4.0 Chapter 4: Cardiovascular risk in an older cystic fibrosis cohort

4.1 Abstract

4.1.1 Introduction

As survival in cystic fibrosis (CF) increases, addressing ageing comorbidities will become an important part of patient management. Cardiovascular disease is the leading cause of mortality in the general population worldwide, accounting for around 30% of all deaths(329). The risk of sustaining a cardiovascular event increases with age and the presence of various risk factors including systemic hypertension, diabetes mellitus and hyperlipidaemia, all of which may become relevant in an ageing CF population. This paper aims to explore cardiovascular risk in an older CF cohort, to analyse CFTR-related and non-CFTR related influences on this risk and to identify areas for closer scrutiny as more patients achieve longer term survival.

4.1.2 Methods

A cross-sectional, observational study was carried out for CF patients aged 40 years and above attending MACFC between August 2018 and August 2020, during a period of clinical stability. Data were collected for disease demographics using a formulated questionnaire and review of case notes. Measurements of central (aortic) arterial stiffness (pulse wave velocity {PWV} and augmentation index {AIx}) and central systolic blood pressure (CSBP) were taken using a Vicorder® (Skidmore Medical). Blood sampling for high sensitivity C-reactive protein (hsCRP), lipid profiles and apolipoprotein markers was performed using venepuncture. QRisk® scoring was used to calculate a ten-year cardiovascular risk for each patient, with ≥10% deemed significant. Comparison of our older CF cohort with a smaller, age and gender matched chronic kidney disease (CKD) cohort was undertaken for arterial stiffness measurements. Statistical analysis was performed using SPSS (IBM), with $p<0.05$ used for statistical significance.

4.1.3 Results

Data from 85 CF patients were analysed. There were a high prevalence of cardiovascular risk factors identified; 18.8% had systemic hypertension, 15.5% had hyperlipidaemia and 11.8% had a significant family history of cardiovascular disease. Patients with exocrine pancreatic sufficiency had higher total cholesterol levels than those without (p=0.037). QRisk® scores of ≥10% were seen in 34.9% of patients. Mean QRisk® scores increased with age and were higher for non-severe CFTR mutation and CFRD groups (p<0.001). Raised AIx (>25%) was seen in 44.7% of patients and raised PWV (>11m/s) seen in 7.1% and, after adjustment for BMI, the odds ratio (OR) for raised AIx in the CF group compared to a matched CKD group was 2.95 (95% CI 1.03-8.45).

4.1.4 Conclusion

This paper highlights the emerging cardiovascular risk in an ageing CF cohort. This is the first study to analyse arterial stiffness, QRisk® scores and apolipoprotein levels as part of cardiovascular risk assessment in CF patients aged 40 years and above. Risk factors for cardiovascular disease in CF may include exocrine pancreatic sufficiency, non-severe CFTR mutations and CFRD. The advantage of apolipoprotein analysis in the assessment of cardiovascular risk in CF is uncertain. QRisk® scoring may be helpful in assessing cardiovascular risk in CF patients aged 40 years and above. Arterial stiffness increases with age and central systolic blood pressure (CSBP) in CF but is not related to raised hsCRP. The lack of significant difference between AIx and PWV parameters between CF and CKD groups may highlight an inherent cardiovascular risk in CF, as seen in other chronic respiratory disease. Improving survival in CF patients necessitates further research into evolving cardiovascular risk and contributing factors, so that we can effectively manage additional vascular comorbidity in an already complex and challenging multisystem disease.

4.2 Introduction

Survival in CF is improving exponentially, and we are now seeing an ageing population. When CF was first discovered as a clinical entity in 1938(53), survival rarely surpassed one year. This is in stark contrast to children born with CF today, who's predicted median survival is now into their fifth decade(11).

Although the CF story over the last eight decades has been one of success, we must recognise the potential problems associated with an ageing population and increasing numbers of patients with chronic illness. Disease longevity in CF will undoubtedly be accompanied by further complexities of disease and will present new challenges to the CF multidisciplinary team (MDT). Although mortality in CF is predominantly related to pulmonary disease, emerging age-related comorbidities will become ever more prevalent. These will include direct complications of CFTR dysfunction with age as well as non-CFTR related comorbidities including those sustained with chronic treatment longevity and the general ageing process itself.

Historically, poor survival in CF has meant that established cardiovascular disease is rare and has only been described in case reports. However, as the leading causes of mortality in the general population worldwide, cardiovascular disease is a significant comorbidity to consider as CF survival increases. The hallmark of vascular disease is atherosclerosis, an inevitability as part of the general ageing process. This process can be accelerated by various conditions, enhancing cardiovascular risk and the development of premature cardiovascular disease. Cardiovascular risk varies between individuals and risk factors can be separated into modifiable and non-modifiable. Non-modifiable risk factors include genetic susceptibility, ethnicity and gender. Modifiable risk factors include smoking, obesity, systemic hypertension, hyperlipidaemia and diabetes mellitus, the majority of which may be relevant to CF patients as they age. Understanding and minimising cardiovascular risk in CF is of paramount importance as survival continues to increase. This raises some important questions for the CF MDT;

1. What are the effects of CFTR dysfunction on the development of cardiovascular disease?

2. Does CFTR dysfunction accelerate cardiovascular ageing and are CF patients at heightened risk of cardiovascular disease compared with the general population?
3. What non-CFTR related factors contribute to cardiovascular risk in CF?

This paper will begin to explore some of these areas.

4.2.1 Systemic hypertension

Salt depletion and osmotic imbalance in CF has historically been associated with low systolic blood pressure (SBP) and an assumed degree of cardiovascular protection(330). However, as CF patients age, attention must be paid to the development of systemic hypertension particularly in those with milder CFTR mutations, exocrine pancreatic sufficiency and a higher BMI. Essential (primary) systemic hypertension is associated with advancing age and increased cardiovascular risk. Historically, diastolic blood pressure rise has been associated with the majority of end organ damage in hypertension. However, isolated systolic hypertension and raised pulse pressure are becoming increasingly relevant in an ageing population and have been shown to correlate with atherogenesis and cardiovascular disease(331). The increasing prevalence of CF-related diabetes mellitus (CFRD) with age may influence the development of systemic hypertension. Both CFRD and hypertension can contribute to and accelerate chronic renal disease(332). Blood pressure monitoring and adequate management of systemic hypertension in CF will thus be important not only to reduce cardiovascular risk but also to minimise the risk of renal dysfunction, of particular importance when considering potential lung transplantation and chronic exposure to nephrotoxic medication. It may be worth considering that CFTR modulation, restoring a degree of normal CFTR protein function, may affect salt imbalance and enhance cardiovascular risk in ageing CF patients.

4.2.2 Hyperlipidaemia in CF

The majority of patients with CF will have exocrine pancreatic insufficiency, causing fat malabsorption and nutritional deficiencies. Reduced dietary lipid absorption and altered cholesterol metabolism lead to lower total cholesterol levels in CF patients when compared to the general population(298). Dietary advice for CF patients has changed significantly over the years, particularly with the introduction of pancreatic enzyme replacement therapy (PERT). However, for those with exocrine pancreatic insufficiency, a high fat, high calorie

diet still forms an integral part of CF management. Over time, this dietary trajectory may cause dyslipidaemia and contribute to cardiovascular risk, as is seen with high fat diets in the general population. Skolnik and colleagues reported 69% of a small CF cohort over 40 years of age, with advanced pulmonary disease, to have evidence of dyslipidaemia(222). Those on long term oral corticosteroids will also be at risk of hyperlipidaemia and systemic hypertension. Given the link between dyslipidaemia and diabetes mellitus in the general population(333), an increasing prevalence of CFRD with age may also influence the presence of hyperlipidaemia and cardiovascular risk in CF.

CF patients with exocrine pancreatic sufficiency, although nutritionally replete, may also be at risk of hyperlipidaemia as they age. Rhodes and colleagues observed an increase in total cholesterol levels in patients with preserved exocrine pancreatic function, and a trend towards higher cholesterol and triglycerides with increasing age and BMI in CF, independent of exocrine pancreatic and diabetic status. Indeed, 24-43% of CF patients, dependent on exocrine pancreatic status, were shown to have a raised total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) with advancing age(300). CFTR modulation may also contribute to raised BMI and dyslipidaemia over time, as exemplified by the evidence of improved gastrointestinal absorption and weight gain in longitudinal data of patients with a gating CFTR mutation taking ivacaftor(334)(301).

Dyslipidaemia has been shown in the general population to contribute significantly to atherosclerosis and cardiovascular morbidity. Ischaemic heart disease in CF has only been reported in a small number of case studies thus far(224)(289) but is likely to become more relevant as survival in CF continues to improve. The typical atherogenic triad of hyperlipidaemia consists of elevated LDL-C, low high density lipoprotein cholesterol (HDL-C) and high triglycerides (TG). In CF clinical practice, these fasting parameters are measured at annual assessment. Within the last decade, several studies have shown the benefit of measuring apolipoprotein levels, which form the structural ‘envelope’ of lipoprotein molecules(335). ApoB corresponds to LDL-C, ApoA1 to HDL-C and ApoB/A1 equivalent to the TC/HDL ratio. They can be measured directly in the blood, are reliable in non-fasting states and retain reliable cardiovascular risk prediction in those already on lipid lowering medication. Studies have shown superiority of serum ApoB monitoring as a marker of cardiovascular risk than LDL-C(115). Although there has been extensive study of

apolipoproteins in the general population, there has been no such research in CF to date. This study will represent the first in apolipoprotein analysis in CF patients aged 40 years and above.

4.2.3 CF-related diabetes mellitus

CFRD is diagnosed in up to 50% of adults with CF and the risk of developing impaired glucose tolerance and CFRD increases with age(271). CFRD appears to be a distinct entity from type one and type two diabetes mellitus, combining both relative insulin deficiency, due to progressive pancreatic fibrosis and destruction of β cells, and insulin resistance. CFRD is commoner in patients with severe CFTR genotypes and exocrine pancreatic insufficiency. Historically, CFRD has been associated with more rapid pulmonary decline, poor nutritional status and increased mortality. However, developments in treatment over time has seen the mortality gap between CF diabetics and non-diabetics significantly decrease.

The risk of microvascular disease in diabetes mellitus correlates with the duration of fasting hyperglycaemia in the general population and similarly this risk will become more relevant in CFRD with longevity of disease. In addition, it would seem likely that CF diabetics will also have an increased risk of macrovascular disease over time. CF patients may also be at risk of metabolic syndrome, a triad of hyperglycaemia, increased BMI and hyperlipidaemia. This may be particularly relevant for those with milder CFTR genotypes and exocrine pancreatic sufficiency. Could hyperglycaemia in an exocrine pancreatic sufficient cohort with residual CFTR function in fact represent a non-CF-related diabetic phenotype?

In the general population, cardiovascular risk is higher in those with diabetes mellitus and hence lipid-lowering and antihypertensive therapies are commenced at lower thresholds. This may also be relevant for our ageing CF population, with an increased risk of impaired glycaemic control and CFRD over time. Should we be assessing cardiovascular risk at earlier ages in CF?

4.2.4 Arterial stiffness and non-atherosclerotic risk in CF

When considering the pathophysiology of vascular disease, priority is often given to atherosclerosis, a process by which inflammation, endothelial cell dysfunction and lipid accumulation within the intimal layer of the arterial wall causes intra-arterial plaque and thrombus formation. In contrast, arteriosclerosis predominantly affects the medial layer of

the arterial wall and is a dominant process in vascular ageing. Arteriosclerosis causes vascular calcification, leading to a reduction in compliance and distensibility of the arterial wall, resulting in arterial 'stiffening'(336). Although this is an ageing phenomenon occurring in healthy individuals, this process can be accelerated in the presence of certain comorbidities such as diabetes mellitus, chronic kidney disease (CKD) and systemic hypertension(337).

Arterial wall stiffening affects vascular impedance, altering the relationship between blood pressure and flow. Central (aortic) arterial stiffening alters cardiac dynamics and contributes to raised pulse pressure, which has been shown to increase cardiovascular morbidity and mortality, particularly coronary artery disease(338). The presence of vascular calcification and arterial stiffness assists to explain the heightened cardiovascular risk found in certain patient groups, such as those with CKD and end stage renal disease (ESRD), independent of atherosclerotic risk factors. Arteriosclerosis in these populations have been extensively investigated using non-invasive arterial stiffness methods. Raised pulse pressure has been found to be one of the highest predictors of mortality in patients with ESRD, with raised large artery wall stiffness associated with increased cardiovascular morbidity and mortality in both ESRD and hypertensive groups(151)(150). Early detection of arterial stiffening may enable clinicians to identify and manage cardiovascular risk in a primary prevention setting, but also may allow adoption of 'de-stiffening' treatment strategies for specific patient groups. Examples of de-stiffening strategies, studied in CKD and rheumatoid arthritis (RA) populations, would be the use of angiotensin converting enzyme (ACE) inhibitors and non-steroidal anti-inflammatories (NSAIDs)(339)(340).

Arterial stiffness can be measured non-invasively using applanation tonometry and pulse wave analysis. Carotid-femoral pulse wave velocity (cfPWV) is the gold standard method used to measure aortic (central) arterial stiffness. The stiffer the artery, the faster the flow of blood, the reflected pulse wave and subsequent PWV. PWV must be interpreted in the context of central systolic blood pressure (CSBP), which is simultaneously recorded with this technique(341). Aortic PWV has been shown to be an independent risk factor for cardiovascular disease and its inclusion in cardiovascular risk scoring has been shown to increase the accuracy of predictive risk classification(149). Augmentation index (AIx) can also be used as a measure of arterial stiffness using the percentage of 'augmentation' of the

central pulse pressure by the reflected pulse wave. The higher the value, the stiffer the artery. This has less appeal for use in clinical practice as a measure of arterial stiffness due to its considerable variation with other physiological parameters, such as pulse rate, and its lower reliability in patients of older ages(153).

4.2.5 Chronic inflammation and arterial stiffening

Chronic inflammation has been shown to contribute to arterial stiffness, affecting endothelial cell function and vascular wall integrity. This is thought, in part, to be related to increased levels of circulating pro-inflammatory cytokines such as TNF alpha(342). Anti-TNF therapy in rheumatoid arthritis patients has been shown to cause a reduction in AIx and PWV(343). Chronic inflammatory conditions such as rheumatoid arthritis (RA) carry an increased cardiovascular risk and as such are included in cardiovascular risk prediction tools(162). Diabetes mellitus and CKD also create pro-inflammatory states, which contribute to arterial stiffness and cardiovascular risk(344)(345). CF pulmonary disease is characterised by a complex relationship between chronic infection and a predominantly neutrophil-mediated inflammatory response within the lungs, with an elevated presence of pro-inflammatory cytokines found in both sputum and bronchoalveolar lavage (BAL) samples(346). C-reactive protein (CRP) is an acute phase protein and marker of systemic inflammation. Patients with CF will commonly have raised baseline CRP levels during times of clinical stability. The chronic systemic inflammatory state in CF is caused by circulating pro-inflammatory cytokines, such as IL-6 and TNF alpha, which have been shown to be present at higher concentrations in CF patients compared to healthy controls(347).

The chronic systemic inflammation observed in CF patients, along with an impending cardiovascular risk from CFRD, systemic hypertension and hyperlipidaemia, may deem patients to be at higher risk of premature arterial stiffness, accelerated vascular ageing and cardiovascular disease(348). Indeed, patients with CF have been shown to have abnormal vascular dynamics as a consequence of their disease(349). This may be accentuated in those with CFRD and has been shown to manifest as a raised augmentation index at rest, which can increase during exercise(350).

Studies evaluating the efficacy of anti-inflammatory agents in CF, such as azithromycin, have shown improvement in pulmonary outcomes along with reduction of plasma CRP(346). Intravenous antibiotics have been shown to decrease systemic inflammation in CF during

pulmonary exacerbations and systemic biomarkers of inflammation often guide response to acute therapy(351). Although the relationship between biomarkers of systemic inflammation and cardiovascular risk may be challenging to define, chronic inflammation may have an important role in cardiovascular risk prediction in CF.

4.2.6 The QRisk® score

The QRisk® scoring system allows physicians in primary care to calculate the risk of a patient sustaining a cardiovascular event within the subsequent ten-year period. It is not applicable to those with a preceding history of cardiovascular disease. A QRisk® calculation is generated by inputting demographic and biomarker data into a pre-existing online calculator, producing a percentage score(352). Scores of ≥10% are used as the threshold for significance and may warrant further attention, including lifestyle modification and optimal management of specific cardiovascular risk factors such as systemic hypertension and diabetes mellitus. A score of >20% is deemed high risk and warrants commencement of primary cardiovascular prevention therapy as a first line strategy, including lipid lowering treatment(353)(108) Of course, all scores must be interpreted with appropriate clinical judgment, and may be underestimated in those already established on preventative treatment.

The QRisk® scoring system has replaced the Framingham cardiovascular risk score and is recommended by NICE for use in primary care. The QRisk® 3 score has recently superseded QRisk® 2(353). A QRisk® cardiovascular risk calculation is offered to those aged 40 years and above in the general population. A high burden of specialist appointments for CF patients means they are likely to have low primary care attendance. Could we therefore be missing opportunities to assess cardiovascular risk and implementing primary cardiovascular preventative measures in our older CF patients?

Cardiovascular risk scoring in CF may also prompt closer attention to family history and genetic factors, which will influence the risk of premature cardiovascular disease. The inclusion of CFRD into cardiovascular risk scoring is challenging due to the inability to categorise into distinct type one and type two diabetes mellitus (T1DM, T2DM) classes. The difference in calculated risk between CFRD when classified as T1DM or T2DM may be insightful and worth further exploration.

As more patients reach 40 years and above in CF, cardiovascular risk scoring will become ever more relevant as the prevalence of cardiovascular disease increases. Therefore, should formal cardiovascular risk assessment form part of routine CF clinical management?

4.3 Methods

A full description of methods used are outlined in chapter two. In brief, a cross sectional analysis of our cohort of 85 CF patients aged 40 years and above attending MACFC was conducted to assess factors associated with cardiovascular risk. Study investigations were performed during periods of clinical stability, defined as the absence of pulmonary exacerbation requiring oral or intravenous antibiotics within the preceding four-week period(279).

Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) Ethics committee (reference 19/EM/0067).

1. Prospective data collection was performed for demographic, social and family history.
2. Blood testing was performed via venepuncture to collect biomarkers of cardiovascular risk, including HbA1c, high sensitivity C-reactive protein (hsCRP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and apolipoproteins A1 and B (ApoB and ApoA1).
3. Pulse wave velocity (PWV), augmentation index (AIx) and central systolic blood pressure (CSBP) recordings were performed on all patients using non-invasive applanation tonometry via a Vicorder® (Skidmore Medical). Results were analysed and compared to a cohort of chronic kidney disease patients with retrospective Vicorder® data (Salford Renovascular Research Group).
4. QRisk® scores were calculated for all patients using QRisk®2 and QRisk®3 calculators to define cardiovascular risk in this cohort. Patients with CFRD were then analysed as a separate group to establish the variation in scores dependent upon classification of CFRD as T1DM or T2DM.

A positive family history of cardiovascular disease was defined as a first degree relative sustaining a cardiovascular event aged 60 years or below. Raised baseline hsCRP was defined as $\geq$5 mg/L. Raised AIx was defined as >25% and raised PWV as $>$11m/s as per

recommended guidelines(354). Normal cholesterol parameters were referenced according to local trust guidelines (TC <5 mmol/L, TG <1.6 mmol/L, HDL-C >1 mmol/L, LDL-C <3 mmol/L, TC/HDL ratio <4, ApoB 0.55-1.40 g/L, ApoA1 1.10-2.05 g/L and ApoB/A1 ratio 0.35-1). A QRisk® score of ≥10% was deemed significant, according to national guidelines(272).

Data were analysed as a total cohort, in age groups; 40-49 years, 50-59 years and ≥60 years, and then grouped for other predictor variables. Data were also compared to a chronic kidney disease (CKD) cohort from Salford Royal Foundation Trust (Renovascular Research Group), matched for age and gender. Independent T-test, one-way ANOVA and Pearson correlations were performed to display normally distributed data. Logarithmic transformation was performed prior to analysis of data without normal distribution and results displayed as geometric mean/SD or back-transformed mean/CI as applicable. For data without normal distribution on logarithmic transformation, non-parametric testing was used for analysis. Categorical data were analysed using Chi-squared tests. Univariate and multivariate regression models were also used to determine relationships between dependent and independent variables. SPSS® (IBM®, version 25.0) was used for analysis of data and two-tailed p values of <0.05 were deemed statistically significant.

4.4 Results

4.4.1 Demographics

General demographics of the total cohort are shown in table 4.1.

Demographic	Mean ±SD
Age (years)	48.6 (7.70)
Gender % male (n)	65.9% (56)
BMI (kg/m^2)	23.9 (3.13)
ppFEV$_1$ (%)	52.9 (23.3) %
PI % (n)	83.5% (71)
CFRD % (n)	49.4% (42)
PsA % (n)	65.9% (56)
CFTR class I-III (severe) %	72.9% (62)
Smoking status	20.2% ex-smokers 2.4% current smokers
Family history of cardiovascular disease (%)	11.9% (10)

Table 4.1: Demographics of the total study cohort. PsA-Pseudomonas aeruginosa (sputum colonisation), BMI= body mass index, PI=exocrine pancreatic insufficiency, CFTR – Cystic fibrosis transmembrane conductance regulator protein.

We analysed data from 85 (out of 92) eligible patients aged 40 years and above, representing 20% of the total CF cohort at MACFC. 16 patients (18.8%) were found to have central systolic hypertension, with 10 of these patients already on anti-hypertensive treatment. 13 patients (15.5%) had raised total cholesterol levels. Ten patients (11.9%) had a positive family history of cardiovascular disease. 49.4% had a diagnosis of CFRD with a mean(±SD) HbA1c of 44.1(±8.76)mmol/L.

4.4.2 Cholesterol and apolipoprotein analysis

Data for cholesterol parameters including apolipoproteins are shown in the table 4.2 and 4.3.

Age (years)	**Total**	**40-49**	**50-59**	**≥60**	**P value**
Number of patients (n)	84	54	21	9	-
TC (mmol/L) Mean (SD)	4.06 (0.89)	3.86 (0.77)	4.41 (0.87)	4.67 (1.31)	**0.008**
TG (mmol/L) Mean (SD)	1.08 (0.48)	1.07 (0.46)	1.01 (0.43)	1.26 (0.73)	0.449
HDL-C (mmol/L) Mean (SD)	1.52 (0.47)	1.44 (0.37)	1.66 (0.60)	1.70 (0.65)	0.111
*LDL-C (mmol/L) Mean (CI)	1.93 (1.78, 2.08)	1.82 (1.65, 1.99)	2.15 (1.81, 2.55)	2.25 (1.65, 3.06)	0.098
TC/HDL-C Mean (SD)	2.83 (0.86)	2.80 (0.76)	2.88 (1.06)	2.93 (1.07)	0.901
*ApoB (g/L) Mean (CI	0.70 (0.65, 0.75)	0.66 (0.62, 0.72)	0.78 (0.64, 0.95)	0.79 (0.60, 1.03)	0.099
ApoA1(g/L) Mean (SD)	1.53 (0.35)	1.46 (0.31)	1.67 (0.37)	1.69 (0.47)	**0.034**
ApoB/A1 Mean (SD)	0.49 (0.18)	0.49 (0.17)	0.50 (0.24)	0.50 (0.15)	0.990

*Table 4.2: Cholesterol parameters for total cohort and per age group. Above normal values analysis: ApoB >1.4mg/L, ApoA1 <1.1mg/L, ApoA1/B >1, TC>5 mmol/L, TG>1.6 mmol/L, HDL <1mmol/L, LDL >3mmol/L, TC/HDL >4.(*Logarithmic transformation used; expressed as back-transformed mean/CI).*

% Raised levels (n)	Total	40-49 (years)	50-59 (years)	≥60 (years)	P value
Number of patients (n)	84	54	21	9	-
TC (mmol/L)	15.5% (13)	7.4% (4)	28.6% (6)	33.3% (3)	**0.007**
TG (mmol/L)	14.3% (12)	13.0% (7)	14.3% (3)	22.2% (2)	0.615
HDL-C (mmol/L)	10.7% (9)	13.0% (7)	9.5% (2)	0	0.575
*LDL-C (mmol/L)	11.9% (10)	9.3% (4)	19.0 (4)	22.2% (2)	0.127
TC/HDL-C	8.3% (7)	9.3% (5)	4.8% (1)	11.1% (1)	0.806
*ApoB (g/L)	1.2% (1)	0	4.8% (1)	0	0.177
ApoA1(g/L)	9.5% (8)	13.0% (7)	4.8% (1)	0	0.418
ApoB/A1	2.4% (2)	1.9% (1)	4.8% (1)	0	0.616

Table 4.3: Total number of raised cholesterol markers (% and n) for study cohort.

Due to the small patient number in the ≥60 years age group, we analysed the data between 40-49 and ≥50 year age groups as a separate sub-analysis. This showed significantly higher TC (p=0.002), HDL-C (p=0.036) and ApoA1 levels (p=0.009) with increasing age.

Comparison between cholesterol biomarkers for CFRD and non-CFRD groups are shown in table 4.4.

Cholesterol biomarker	CFRD	Non-CFRD	P value
*Apo-B (g/L) Mean (CI)	0.64 (0.59, 0.70)	0.76 (0.67, 0.87)	**0.017**
ApoA1 (g/L) Mean (SD)	1.53 (0.37)	1.53 (0.32)	0.949
ApoB/A1 Mean (SD)	0.46 (0.17)	0.52 (0.19)	0.128
*LDL-C (mmol/L) Mean (CI)	1.88 (0.61)	2.25 (0.86)	**0.033**
HDL (mmol/L) Mean (SD)	1.53 (0.48)	1.51 (0.45)	0.849
TC (mmol/L) Mean (SD)	3.90 (0.81)	4.24 (0.95)	0.087
TC/HDL Mean (SD)	2.70 (0.73)	2.98 (0.97)	0.126
TG (mmol/L) Mean (SD)	1.10 (0.51)	1.05 (0.45)	0.652

*Table 4.4: Cholesterol parameters for CFRD and non-CFRD groups. Above normal values analysis: ApoB >1.4mg/L, ApoA1 <1.1mg/L, Apo-A1/B >1, TC>5 mmol/L, TG>1.6 mmol/L, HDL <1mmol/L, LDL >3mmol/L, TC/HDL >4.(*Logarithmic transformation used; expressed as back-transformed mean with CI).*

Ten patients (11.9%) had raised LDL-C. TC/HDL ratio was raised in 8.3% of patients. TG level was raised in 14.3% of patients. 15.5% had raised TC (>5 mmol/L), with a mean of 4.06(±0.89)mmol/L for the whole group. Age groups analysis revealed significantly higher TC with increasing age (p=0.008) and ApoA1 (p=0.034) with increasing age. Lower levels of LDL-C and TC were seen in the non-CFRD group compared to the CFRD group, with $\log_{10}$LDL-C difference showing statistical significance on T-testing (p=0.03).

There was a strong positive correlation between ApoB/A1 and TC/HDL ratios (r=0.931, p<0.001). Strong correlations were also seen for LDL-C and ApoB (r=0.559, p<0.001) and

ApoA1 with HDL-C (r=0.796, p<0.001). Despite this, only one patient from the cohort had raised Apo-B levels compared to ten with raised LDL-C. However, interestingly this patient did not have concurrently raised LDL-C or TC levels. There were eight patients with reduced ApoA1 levels and nine patients with HDL-C levels lower than normal. Only two patients out of the eight with reduced ApoA1 did not have concurrently reduced HDL-C levels, showing better clinical correlation. ApoB/A1 ratio, thought to be a more sensitive marker of cardiovascular risk than TC/HDL, was raised in only 2.4% of patients as compared to 8.3% with raised TC/HDL ratio. 11.8% of patients were on statin therapy for a diagnosis of hyperlipidaemia prior to study participation, which may be a limitation to apolipoprotein analysis. One patient had low ApoA1 in the presence of normal HDL-C. Four patients in the statin group remained with TC of >5mmol/L (all with raised LDL-C), but with normal apolipoprotein parameters.

Using analysis of log-transformed data, there is a significant difference in both $\log_{10}$LDL-C and $\log_{10}$ApoB between CFRD and non-CFRD groups, with a trend to higher levels in patients without CFRD; p=0.033 and p=0.017 respectively (table 4.4).

There was a trend towards higher TC, LDL-C, TC-HDL ratio and TG in patients with exocrine pancreatic sufficiency, although this was not statistically significant. Exocrine pancreatic status was significant as a predictor of raised TC on univariate regression analysis (p=0.003, r^2=0.106). In multivariate regression analysis, BMI, exocrine pancreatic status and CFRD status together showed statistical significance as predictors of raised total cholesterol level (p=0.03, r^2 = 0.107). Patients with exocrine pancreatic sufficiency are more likely to have a raised TC (>5mmol/L) than those without (p=0.037). Patients in a less severe CFTR class (IV-V) are more likely to have a raised LDL-C (>3mmol/L) than those in severe groups (p=0.006). Data therefore suggests a relationship between exocrine pancreatic sufficiency and raised cholesterol.

A positive correlation was seen between BMI and TG levels (r=0.284, p=0.009). Although the mean TG concentration was slightly higher in patients with CFRD than those without (1.10±0.45 vs 1.05±0.51mmol/L), this result was not statistically significant.

4.4.3 Blood pressure, PWV and AIx analysis

Age (years)	Total	40-49	50-59	≥60	P value
Number of patients (n)	85	54	22	9	-
SBP mmHg Mean (SD)	128.5 (13.3)	126.5 (12.4)	130.6 (14.6)	138.1 (13.1)	**0.049**
DBP mmHg Mean (SD)	71.8 (8.29)	71.8 (8.36)	71.6 (8.38)	72.2 (8.65)	0.987
PWV m/s Mean (SD)	8.76 (1.35)	8.45 (1.02)	9.24 (1.73)	9.81 (1.59)	**0.004**
AIx % Mean (SD)	24.1 (8.56)	23.3 (8.06)	24.9 (10.2)	28.4 (7.01)	0.249
*hsCRP mmol/L Mean (CI)	4.44 (3.49, 5.64)	4.57 (3.41, 6.12)	4.90 (2.74, 8.79)	2.82 (1.49, 5.36)	0.465

*Table 4.5: Arterial stiffness, BP and hsCRP measurements for the total cohort and by age group.*Logarithmic transformation used; expressed as back-transformed mean with CI.*

16 patients (18.8%) had raised central systolic BP (CSBP) (>140mmHg) using Vicorder® analysis, of whom ten were already on anti-hypertensive treatment in the form of calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors or a combination of both. Seven patients with pre-existing systemic hypertension had hyperlipidaemia and two had a positive family history of cardiovascular disease. 12 patients in the hypertensive group had exocrine pancreatic insufficiency, nine had CFRD and four had a history of renal dysfunction; two with renal stones, one with diabetic nephropathy and one with refractory hypertension/CFRD with a previous renal and pancreatic transplantation.

The mean PWV and AIx for the total group was 8.76(±1.35)m/s and 24.1(±8.56)% respectively. As expected, both PWV and AIx increased with age, showing statistical significance using Pearson correlation; $r=0.382$, $p<0.001$ and $r=0.260$, $p=0.016$ respectively.

Although median PWV was higher in males than females (9.39 vs 8.37 m/s), this was not statistically significant. Six patients (7.1%) had a PWV of >11m/s. A larger proportion had an increased AIx, with 38 patients (44.7%) having a mean AIx recording of >25%. Mean CSBP was 128.5($\pm$13.3)mmHg.

One way ANOVA testing shows a significant difference in both CSBP and PWV between age groups, observing higher parameters with advancing age (p=0.049, p=0.004 respectively). On post hoc analysis (Tukey's) this significance was held for PWV, with a significantly higher PWV between age groups one (40-49 years) and three ($\geq$60 years); p=0.016.

There were positive correlations between both CSBP and PWV (r=0.533, p<0.001), with regression analysis showing statistical significance between these two variables (adjusted r^2 0.271, p<0.001).

There were no significant differences seen in mean AIx and PWV between non-CFRD and CFRD groups; 25.7 vs 22.6% (p=0.969) and 8.77 vs 8.75m/s (p=0.650) respectively. There were significant correlations between both TC and $\log_{10}$LDL with AIx (r=0.255, p=0.019 and r=0.266, p=0.015 respectively), however neither retained significance as a predictor of increased AIx on linear regression.

46.4% of patients had a raised baseline hsCRP (>5mg/L). No significant relationship was found between $\log_{10}$CRP and AIx or PWV. There was also no significant relationship between $ppFEV_1$ and arterial stiffness parameters in this cohort. Analysis of other markers of disease severity using binary logistic regression showed the absence of *Pseudomonas aeruginosa* to significantly increase the probability of obtaining a PWV of >11m/s; odd ratio (OR) 15.0, p=0.029 and of obtaining an AIx of greater than 25%; OR 3.43, p=0.037 (table 4.6).

Although PWV and AIx showed a trend towards higher values in those with a non-severe CFTR mutation class, this was not statistically significant on chi squared or regression analysis.

Variables	Number of patients	OR	CI	P value
PsA (-ve)	29	15.0	1.32, 170.9	0.029
CFTR class (severe)	62	0.82	0.06, 11.2	0.883
$ppFEV_1$ <40%	30	3.84	0.48, 31.1	0.207
PI	71	2.85	0.17, 47.2	0.465
CFRD	44	0.61	0.07, 5.01	0.642

Table 4.6: Regression analysis of disease severity and raised arterial stiffness. (OR=odds ratio, CI=confidence interval, severe CFTR class=I-III).

4.4.4 CKD comparison

31 matched pairs (62 patients) were studied with a mean age of 53.5 and 53.3 years in the CF and CKD cohorts respectively.

Variable	CF cohort	CKD cohort	P value
Number of patients	31	31	-
Mean age (SD) Years	53.5 (9.9)	53.3 (11.2)	0.931
Mean BMI (SD) kg/m^2	23.9 (3.83)	27.4 (5.65)	**0.006**
Mean eGFR (SD) $ml/min/1.73m^2$	89.9 (12.0)	57.4 (10.7)	**<0.001**
Mean central systolic BP (SD) mmHg	128.5 (13.3)	127.7 (20.6)	0.802
Mean total cholesterol (SD) mmol/L	4.06 (0.89)	4.61 (0.94)	**0.012**
Mean AIx (SD) %	24.0 (8.9)	21.6 (9.9)	0.314
Mean PWV (SD) m/s	8.76 (1.35)	8.57 (1.82)	0.537

Table 4.7: Comparison of CKD and CF cohorts.

The mean eGFR was 89.9(±12)ml/min/1.73m^2 and 57.4(±10.7)ml/min/1.73m^2 in CF and CKD cohorts respectively (p=0.0001). Mean total cholesterol was higher in the CKD cohort. There was no statistical difference in the prevalence of diabetes mellitus between groups, however CF patients had a lower BMI; 23.9kg/m^2 versus 27.4kg/m^2 (p=0.006).

The prevalence of elevated AIx (>25%) was 51.6% in the CF group (mean 24.0±8.9%) compared to 29% in CKD (mean 21.6±9.9%). After adjustment for low BMI, the OR for increased arterial stiffness in CF patients compared to CKD was 2.95 (95% CI = 1.03-8.45), p=0.045.

The prevalence of elevated CSBP (>140mmHg) was 16.1% in the CF group (mean 130±12mmHg) compared to 32.3% in CKD (mean 128±21mmHg). After adjustment for low BMI, the odds ratio (OR) for increased CSBP in CF patients compared to CKD was 0.43 (0.12-1.47), p=0.178. There was no significant difference in PWV between CKD and CF groups.

4.4.5 QRisk® scoring

One patient in our cohort had a pre-existing diagnosis of cerebrovascular accident (CVA), preceding his CF diagnosis, and as such was excluded from QRisk® analysis. QRisk® data for 84 patients were analysed.

Each patient had both QRisk®2 and QRisk®3 scores calculated simultaneously. Patients with CFRD had separate QRisk® scores calculated for CFRD classified as T1DM and then as T2DM. QRisk®3 scores may be perceived to have greater reliability for CF patients given the inclusion of inflammatory conditions and oral corticosteroid parameters, hence more attention was paid to QRisk®3 during analysis. However, strong correlations were shown between QRisk®2 and QRisk®3, in all diabetic classes, showing consistency of both models in this group; as T1DM r=0.983, T2DM r=0.990, non-CFRD r=0.999, p<0.001 for all. In addition, similar prevalence of raised scores (≥10%) were shown for QRisk®3 and QRisk®2; 31.3 vs 33.7% respectively.

Comparison between QRisk® scores for the total cohort are shown below (table 4.8).

	QRisk®2 (Non-CFRD)	QRisk®3 (Non-CFRD)	QRisk®2 (T1DM)	QRisk®2 (T2DM)	QRisk®3 (T1DM	QRisk®3 (T2DM)
Patients (n)	40	40	44	44	44	44
Mean score (SD)	5.24 (5.97)	4.83 (5.51)	11.1 (6.19)	7.44 (4.56)	10.1 (5.47)	6.80 (4.08)

Table 4.8: Comparison of QRisk® scores in our older CF cohort

Mean QRisk®2 and QRisk®3 scores in the non-CFRD group were 5.24(±5.98)% and 4.83(±5.51)% respectively, p<0.001. For the CFRD group, scores were compared for both T1DM and T2DM. Overall mean scores in non-diabetics were significantly lower than for patients with CFRD; QRisk®3 assuming CFRD equivalent to T1DM: 10.1(±5.47)% and QRisk®3 assuming CFRD is equivalent to T2DM: 6.80(±4.08)% (vs QRisk®3-non-DM; p<0.001). Analysis of table 8 also shows the QRisk®3 scores to be lower than their QRisk®2 equivalent in each diabetic class. This was a consistent finding in all participants.

Within the total cohort, 34.9% of patients had a QRisk® score of ≥10%. Two patients had a QRisk®3 score of >20%, indicating high risk. Both patients were already on primary cardiovascular prevention therapy. There was no significant relationship between ppFEV$_1$, exocrine pancreatic status, BMI and hsCRP and elevated QRisk® score using linear regression analysis.

Analysis of the CFRD group showed a mean HbA1c was 47.9(±9.80) mmol/mol, mean cholesterol of 3.95(±0.85) mmol/L and mean systolic BP of 129(±13.0) mmHg, with no significant difference between age groups (40-49, 50-59 and ≥60 years). The mean(±SD) QRisk®3 scores calculated using T1DM and T2DM as equivalents for CFRD are shown in table 4.9.

Classification of CFRD Mean (SD)	CFRD as T1DM	CFRD as T2DM	P value
QRisk®3	10.1 (5.46)	6.80 (4.08)	**<0.001**
QRisk®2	11.1 (6.19)	7.44 (4.56)	**<0.001**

QRisk®3 ≥10% (%)	19 (43%)	11 (25%)	**0.008**

Table 4.9: Comparison of QRisk® scores between CF diabetics as T1DM and T2DM groups.

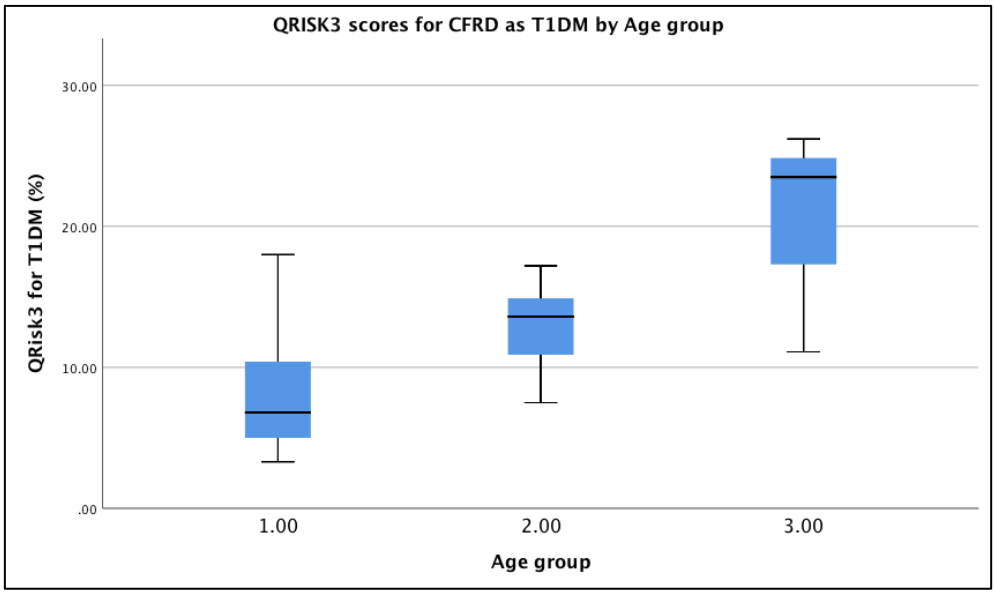


Figure 4.1: Boxplot of QRisk®3 as T1DM between age groups (1=40-49 years, 2=50-59 years, 3=≥60 years).

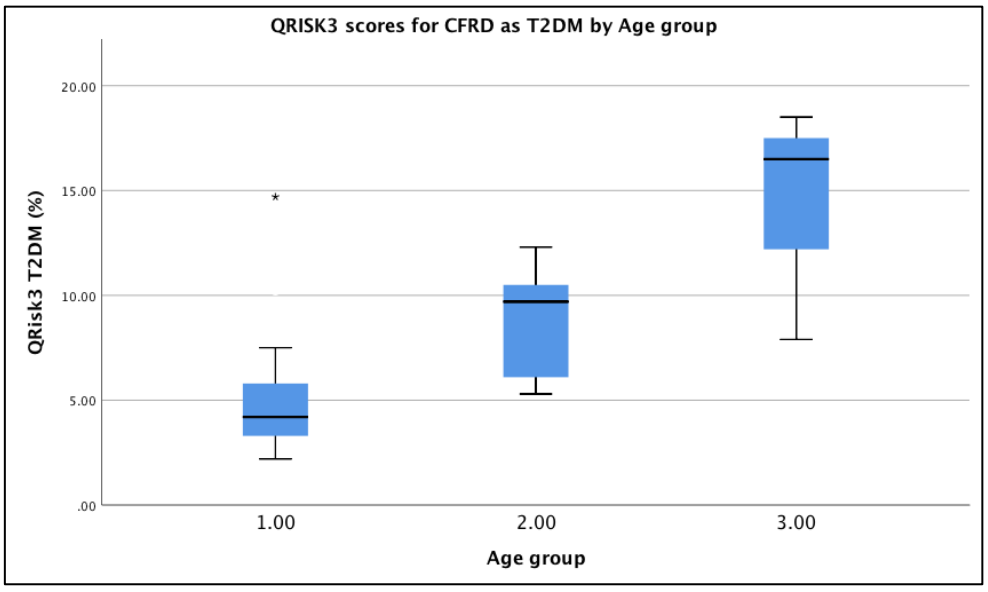


Figure 4.2: Boxplot of QRisk®3 as T2DM between age groups (1=40-49 years, 2=50-59 years, 3=≥60 years).

A significant difference was observed between the two QRisk®3 diabetic groups ($p<0.001$) and all three age groups ($p<0.001$), with significantly higher QRisk® scores shown with advancing age. QRisk®3 scores in patients with and without CFRD also increased with age

when analysed in subsets of 40-49 and ≥50 years; p=0.001 (CFRD as T1DM), 0.003 (CFRD as T2DM) and <0.001 (no CFRD) respectively.

19 patients (43%) scored ≥10% risk with CFRD classified as T1DM group compared with 11 (25%) classified as T2DM (p=0.08) (see table 4.9), highlighting the importance of CFRD classification in this model.

Chi squared testing shows no significant difference between CFTR class for QRisk® scores of ≥10% for any QRisk® diabetic and non-diabetic group (p=0.058).

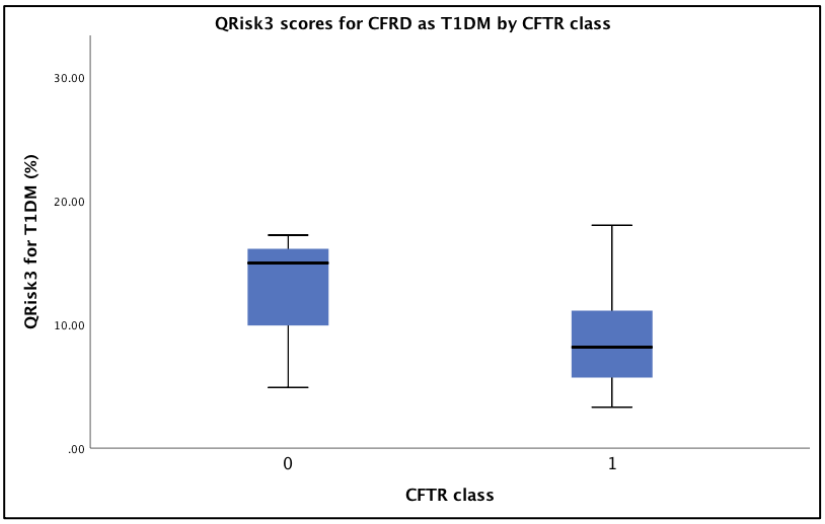


Figure 4.3: Boxplot of QRisk®3 with CFRD assumed to be T1DM and CFTR class (0=CFTR class IV-V, non-severe, 1=CFTR class I-III, severe).

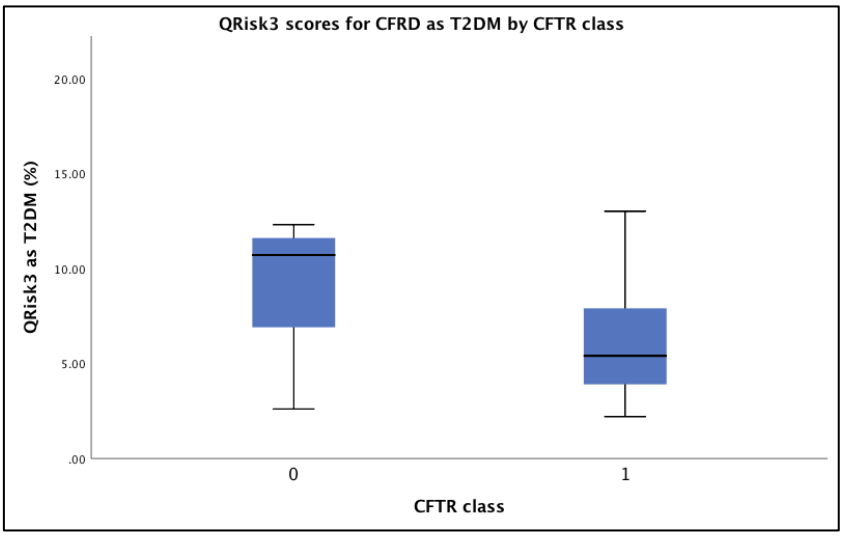


Figure 4.4: Boxplot of QRisk®3 with CFRD assumed to be T2DM and CFTR class (0=CFTR class IV-V, non-severe, 1=CFTR class I-III, severe).

When analysing QRisk® of any diabetic classification, there is a positive correlation between PWV of >11m/s and QRisk® score of ≥10% (r=0.239, p=0.015) with statistical significance on Chi squared testing (p=0.048). This is not reflected in Chi squared analysis of AIx of >25% with QRisk® scores of ≥10%. Linear regression analysis of the relationship between PWV >11 m/s and QRisk® score of ≥10% shows an odds ratio (OR) of 0.118 (95% CI 0.013, 1.11), p=0.062.

4.5 Discussion

4.5.1 General discussion

This is the first study to analyse cardiovascular risk factors in CF patients aged 40 years and above. Results demonstrate a high prevalence of systemic hypertension and hyperlipidaemia in long term CF survivors, increasing with age. A high proportion (34.9%) had QRisk® scores of ≥10%, demonstrating the presence of cardiovascular risk in this ageing CF cohort. The risk of increased arterial stiffness as measured by AIx, was higher in the CF group than a matched CKD cohort, perhaps indicating an inherent cardiovascular risk in an older CF population. A poor correlation between arterial stiffness and hsCRP in the study group may suggest that inflammatory burden is not related to cardiovascular risk in this older CF population, indicating other factors may be more relevant. This older CF cohort has been shown to be heterogeneous, with a spectrum of CFTR genotype and disease phenotype. With this being said, we observed a high prevalence of severe CFTR mutations (72.9%), exocrine pancreatic insufficiency (83.5%) and CFRD (49.4%) in this group.

Cardiovascular disease is proving an area of increasing interest in CF as survival continues to improve. Cardiovascular risk has been shown to be prevalent in patients with chronic respiratory disease, such as bronchiectasis(355), idiopathic pulmonary fibrosis (IPF)(356) and chronic obstructive pulmonary disease (COPD)(357). Alongside traditional risk factors in these predominantly older cohorts, there exists inherent cardiovascular susceptibility from airflow limitation and chronic inflammation. In this way, CF exhibits some similarities to other chronic respiratory conditions. However, could the multisystem impact of CFTR dysfunction contribute to enhanced cardiovascular risk in CF? CFRD and the effects of CFTR modulation are just some of the factors to consider as part of cardiovascular risk assessment in CF.

4.5.2 Cholesterol

Cholesterol has previously been found to be lower in CF than the general population predominantly due to exocrine pancreatic insufficiency and lipid malabsorption. Improved survival in CF, with longevity of high fat diets, exocrine pancreatic sufficient genotypes and the impact of CFTR modulation may alter cholesterol metabolism and subsequent risk of hyperlipidaemia and cardiovascular disease in CF.

In 2008, the Coronary Heart Disease Compendium reported the prevalence of raised cholesterol to be 58% within the UK population across all age groups, increasing to over 70% in those aged 40 years and above(358). Hyperlipidaemia is one of the most potent cardiovascular risk factors in the general population and there exists a wealth of evidence of its relationship to cardiovascular morbidity and mortality(359)(360). A lower prevalence of raised total cholesterol (>5mmol/L) was seen in our older CF patients (15.5%), however there were trends toward higher LDL-C and Apo-B levels with increasing age. A raised LDL-C has also been shown to be related to less severe CFTR mutation classes (p=0.006), exocrine pancreatic sufficiency (p=0.04) and higher BMI (p=0.19). There were also higher levels of TC, TG and a higher TC/HDL ratio seen in patients with preserved exocrine pancreatic function. These results are similar to those observed in a CF cohort with a mean age of 31($\pm$10) years(300). Figueroa and colleagues observed higher TG levels in a younger CF population (mean age 21$\pm$11 years) compared to a control group, but with a lower TC level seen in the CF group(298). There was however a trend towards higher TC with advancing age in CF, the highest age band analysed being 35-44 years. Dyslipidaemia in CF may be predominantly related to non-CFTR factors such as dietary intake and ageing, however there is evidence that chronic inflammation and raised TNF alpha may affect hepatic lipid metabolism. Levy and colleagues observed an association between hypertriglyceridaemia and raised TNF alpha in CF adolescents(361). In addition, CFTR deficient cells have been showed to have altered fatty acid metabolism in vitro which may contribute to dyslipidaemia(362).

Interestingly, Nash and colleagues reported a high prevalence of hyperlipidaemia in CF patients (n=108) following lung transplantation (41.7%), with cholesterol levels increasing post-transplantation when compared with pre-transplant levels(363). However, all but one had a cardiovascular risk score of <10%. The development of at least one cardiovascular risk factor (systemic hypertension, diabetes mellitus and hyperlipidaemia) has been reported in up to 90% of lung transplant recipients with no pre-transplant cardiovascular risk identified(364). With the potential for an increasing age at organ transplantation in long term CF survivors, post-transplant hyperlipidaemia and cardiovascular risk may become increasingly relevant and worth further exploration.

As survival in CF continues to improve, the prevalence of CFRD will increase along with the potential for dyslipidaemia and cardiovascular disease. CFTR modulation may well

contribute to dyslipidaemia due to improvements in gastrointestinal lipid absorption and, given their now widespread use, longitudinal cholesterol data will be useful in measuring modulator impact on cardiovascular risk.

Although the prevalence of raised total cholesterol is lower in this CF cohort compared with the general population, the association of cardiovascular disease with other risk factors in older CF patients must be addressed. Regular, comprehensive lipid review should still be considered in CF patients aged 40 years and above in order to identify and treat hyperlipidaemia early with an aim to prevent cardiovascular morbidity.

4.5.3 Apolipoproteins

Raised serum cholesterol is widely recognised as a risk factor for cardiovascular disease and TC and HDL-C measurements are routinely used in cardiovascular risk prediction. Over recent years, studies have shown the potential for apolipoproteins to be more accurate measures of hyperlipidaemia(107)(365). Since apolipoproteins B and A1 are direct particle counts of LDL and HDL particles respectively, they form a direct atherogenic count and as such may be more reliable markers of cardiovascular risk, particularly for those patients on lipid lowering treatment(366)(367). As such, their measurement has been incorporated into several national cholesterol guidelines(107)(368). To our knowledge, apolipoproteins have not previously been studied in CF cohorts.

In our older CF population, there were strong correlations between apolipoprotein levels and their respective conventional cholesterol equivalents; ApoB to LDL-C; $r=0.559$, $p<0.001$, ApoA1 to HDL-C $r=0.796$, $p<0.001$, indicating these are consistent markers of cholesterol analysis in our study group. 9.5% of patients had a raised ApoB/A1 ratio, compared with 8.3% with a raised TC/HDL-C ratio. Interestingly, one patient had a raised Apo-B level without raised LDL-C, and two with low ApoA1 without corresponding low HDL-C. There was no significant relationship found between abnormal apolipoprotein levels and raised cardiovascular risk scores (QRisk®) in the study population. 11.8% of patients were on statin therapy at the time of sampling, due to a preceding diagnosis of hyperlipidaemia. Four patients in this subgroup remained with raised TC, however none had concurrent apolipoprotein abnormalities. This would seem contradictory to the expectation of greater accuracy in detecting hyperlipidaemia with apolipoprotein measurement, particularly when on statin treatment. However, one patient did have low ApoA1 levels with normal HDL-C,

indicating a degree of cardiovascular risk despite statin therapy. These results portray some inaccuracy in apolipoprotein testing in our older CF patients, particularly in a population with low prevalence of lipid lowering therapy and in whom conventional cholesterol monitoring may thus be sufficient in establishing cardiovascular risk. Albers et al found ApoB and LDL-C to be raised in type two diabetics; 36% and 23% respectively, correlating with poor glycaemic control(369). Bajaj and colleagues observed higher ApoB, lower ApoA1 and higher ApoB/A1 ratio in patients with CKD(370). Since these disease groups are likely to be relevant in CF longevity, apolipoprotein monitoring may still be appropriate in assisting to establish atherosclerotic risk in an ageing CF population. Further research into the accuracy of apolipoprotein measurements in cardiovascular risk assessment in CF would be informative.

4.5.4 Systemic hypertension

Systemic hypertension is one of the most notable risk factors for the development of cardiovascular morbidity and mortality, accounting for over 50% of coronary and cerebrovascular events worldwide(371). An ageing CF population will observe an increased prevalence of CFRD and the potential for increased prevalence of chronic renal disease, further attributed by the use of chronic CF therapies, and the development of atherosclerosis. These factors may contribute to an increasing prevalence of systemic hypertension in an older CF population. Although exocrine pancreatic sufficiency is associated with older age and increased cardiovascular risk in our cohort, survival advantage associated with CFTR modulation (such as Kaftrio®) may see more patients with severe genotypes and exocrine pancreatic insufficiency at risk of comorbid ageing complications. In addition to multiple predicted extra-pulmonary advantages, could CFTR modulation alter the propensity for lower blood pressure in CF in the longer term? If CFTR dysfunction and associated osmotic imbalance has historically contributed to low blood pressure in CF, could restoration of CFTR function in theory contribute to an increase in systemic blood pressure over time? Future study of the longitudinal effects of CFTR modulation will be enlightening and although will likely focus predominantly on pulmonary outcomes, the influence on cardiovascular risk in this context may be important in this ageing CF population.

16 patients (18.8%) had raised CSBP (>140mmHg) using Vicorder® analysis, of whom ten were already on anti-hypertensive treatment and thus indicating suboptimal treatment. The

majority of these patients were on ACE inhibition for anti-hypertensive treatment, with a few on calcium channel blockers. It was noted that a number of these patients may have been started on ACE inhibition due to CFRD and microalbuminuria. The majority of patients with pre-existing systemic hypertension (70%) had concurrent hyperlipidaemia and two had a positive family history of cardiovascular disease. Eight hypertensive patients had exocrine pancreatic insufficiency, nine had CFRD and four had a history of renal dysfunction. A number of new hypertension diagnoses in our older CF cohort highlights the importance of close attention to blood pressure recording during routine clinical review and the early implementation of treatment. One must be mindful of 'white coat hypertension', with perhaps increased utility of ambulatory blood pressure monitoring, alongside the possibility of secondary causes of hypertension, particularly in an older CF population at risk of renovascular disease and diabetic nephropathy.

4.5.5 Arterial stiffness

Central arterial stiffening has been found to be independently related to the development of cardiovascular disease(372). Reduced aortic compliance may be associated with subclinical arterial disease and premature vascular ageing(373). Although a reduction in aortic distensibility is an ageing phenomenon, diabetes mellitus, CKD and chronic inflammatory conditions can accelerate this process, causing premature cardiovascular disease. The systemic inflammatory burden and high levels of pro-inflammatory cytokines, and a high prevalence of CFRD, may influence arterial stiffening in older CF adults.

In our study cohort, a large proportion of patients (44.7%) were found to have a high AIx (>25%), however a smaller proportion (7.1%) had raised PWV (>11m/s). The most pertinent finding was that of an increased risk of raised AIx in our older CF cohort compared to a comparative CKD population (OR 2.95, 95%CI 1.03-8.45, p=0.045), when adjusted for BMI. This is an important result, given that the CKD population has inherent cardiovascular risk and increased arterial stiffness as compared to the general population(147).

Interestingly, there was an inverse relationship between CFRD and arterial stiffness in our cohort, with higher PWV and AIx measurements seen in the non-CFRD cohort. Hull and colleagues studied a younger CF cohort (mean age 28.2±8.2 years) observing patients to have higher AIx than controls, independent of blood pressure, and the risk increasing with the presence of CFRD(227). There was no difference in PWV however, which is a more

reliable marker of arterial stiffness at older ages. In 2011, Buehler and colleagues did not observe increased arterial stiffness in CF children with CFRD(226), perhaps indicating the importance of duration of hyperglycaemia in CF vascular dynamics.

There was no significant relationship between hsCRP and arterial stiffness in our older CF cohort. This is in contrast to Hull et al (2009) who observed higher AIx with increasing log_{10}CRP in a younger CF cohort(227). This difference could reflect the differences in reliability in AIx measurements with advancing age.

Other markers of disease severity, as measured by $ppFEV_1$, BMI and exocrine pancreatic insufficiency similarly did not seem to influence PWV and AIx in the study group. Interestingly, the absence of chronic *Pseudomonas aeruginosa* infection appeared to be related to raised PWV (>11 m/s); p=0.029, and raised AIx (25%); p=0.037. This result could represent the spectrum of disease genotype and phenotype in an older CF cohort and the influence of residual CFTR function on cardiovascular risk. Alternatively, this could simply portray the dominance of non-CFTR related influence on cardiovascular risk in this population. The presence of chronic *Pseudomonas aeruginosa* infection is significantly related to raised hsCRP in our cohort (p=0.001) and the absence of correlation between these factors and the presence of arterial stiffness is unexpected. This indicates that other CFTR or non-CFTR related factors are influencing arterial stiffness and cardiovascular risk in this older CF population.

Measuring arterial stiffness in clinical practice is a non-invasive technique that may add weight to the assessment of cardiovascular risk in an older CF population. Although its widespread use in routine clinical care may not be feasible due to equipment availability and time constraints, its use in select 'high risk' patients may be useful in providing a more comprehensive cardiovascular assessment. It should be noted that the use of applanation tonometry with the Vicorder® (Skidmore Medical) method provides a global measure of arterial stiffness and is subject to a degree of error, particularly in measuring carotid-femoral path length and the importance of this in calculation of PWV. More focal (and hence more accurate) methods may include ultrasound techniques to measure carotid intima-medial thickness (IMT) and cardiac magnetic resonance imaging (CMR) to assess

aortic distensibility(374). These would be interesting areas for further study in long term CF survivors exhibiting cardiovascular risk.

Strategies for reducing arterial wall stiffness and modifying cardiovascular risk have been studied but thus far have not been implemented in clinical practice within the general population. The use of anti-inflammatory medication to modify cardiovascular morbidity in patients with ESRD has been limited due to their negative effects on renal function(375). Research surrounding the benefits of anti-inflammatory medication in CFTR-mediated chronic lung inflammation in CF is promising(376)(377) and this may become more relevant in terms of arterial stiffness and modifying cardiovascular risk in an ageing CF population. The pleiotropic effects of macrolide antimicrobials have been shown to reduce systemic inflammatory response in CF and reduce pulmonary exacerbation rate(78)(378). Could these agents have the additional benefit of modifying arterial stiffness in CF? The use of macrolides and their relationship to systemic inflammation was not specifically studied in this work, however future research in this area would be informative.

46.4% of patients had a raised baseline hsCRP, however no significant relationship was found between $\log_{10}$CRP and AIx or PWV. A study analysing the effect of intravenous antibiotics on large artery haemodynamics in a small CF cohort during pulmonary exacerbation revealed a statistically significant lowering of AIx (but not PWV) and hsCRP at the end of the IV course, most prominent in those with severe pulmonary disease. Although this does not prove causal relationship, there is some evidence that reduction in systemic inflammation may improve arterial stiffness in the short term(225).

14.1% of our cohort were on ACE inhibition as treatment for systemic hypertension and/or microalbuminuria. The use of ACE inhibitors in ESRD cohorts has observed an improvement in aortic distensibility, a reduction in PWV and a decrease in cardiovascular mortality, independent of their antihypertensive effects(379). ACE inhibitors are widely used for the treatment of both systemic hypertension and diabetic nephropathy in the general population and may be very relevant in the treatment of these comorbidities in our older CF population. Renal consequences of ageing in CF will be discussed in detail in subsequent chapters.

Anti-TNF therapy has been shown to reduce both AIx and PWV in RA(343). Could these anti-inflammatory therapies be transferrable to high levels of systemic inflammation in CF patients, particularly in those suffering from inflammatory arthropathies as a consequence of CFTR dysfunction?

Immunomodulatory properties of statins have been observed with reduction in cough and neutrophilic inflammation in non-CF bronchiectasis(380). Van Doornum and colleagues have shown a reduction in arterial stiffness (measured by AIx) with atorvastatin treatment in RA patients in addition to their cholesterol lowering properties(381). These properties could be relevant in the CF population, particularly in those requiring lipid lowering treatment. However, with most patients on chronic macrolide therapy, drug-drug interactions must be carefully considered prior to the implementation of long term statin therapy.

In the short term, inflammatory burden in CF can be modified with the potential to reduce arterial stiffness parameters, however its translation into longer-term alteration of vascular dynamics and cardiovascular risk in CF is unknown. Studies of potential 'de-stiffening' strategies with regards to cardiovascular risk reduction in the general population will continue to inform, however the transferrable advantages of arterial stiffness analysis in the CF population, particularly given the rarity of macrovascular disease as a whole in CF, is debatable. Further studies regarding cardiovascular burden in CF are required.

4.5.6 CKD comparison

Chronic kidney disease and end stage renal disease represent population cohorts with increased arterial stiffness and premature cardiovascular disease. Given the postulation that CF cohorts may have enhanced arterial stiffness due to high inflammatory burden, a CKD cohort was compared to our older CF group for interest.

As expected, mean eGFR was significantly higher in the CF group; 89.9±12 vs 57.4±10.7 ml/min/1.73m^2. A higher prevalence of arterial stiffness, as measured by AIx, was observed in our older CF cohort compared to the CKD cohort, 52% vs 29%; OR 2.95 (95% CI 1.03-8,45, p=0.045). There was no associated significant difference in CSBP or PWV between groups.

Raised AIx in our CF group is a significant finding. This data would support the risk of premature vascular ageing in an older CF population, particularly when compared to a CKD group, arguably a population with inherent cardiovascular risk. Further arterial stiffness data

in larger ageing CF cohorts will be informative. As CF patients age, renal disease will become more prevalent. A study has shown the prevalence of CKD to be around 2% in an adult CF population(382). An ageing kidney in CF will certainly influence the development of CKD, as seen in the general population, however recurrent use of nephrotoxic medication, organ transplantation, CFRD and nephrolithiasis may all increase the prevalence of renal disease in CF as survival continues to improve. In turn, this is likely to contribute to cardiovascular morbidity and mortality, whether related to premature arterial stiffening or to the development other cardiovascular risk factors.

4.5.7 QRisk® scores

QRisk® scoring, as used for cardiovascular risk stratification in the general population, is challenging in its application to CF given the lack of CFRD classification into T1DM and T2DM entities. As more CF patients reach longer term survival in CF, cardiovascular risk scoring should logically become more relevant to their clinical care.

34.9% of patients had a QRisk® score (of any category) of ≥10%. QRisk®3 scores of any diabetic classification increased with advancing age and, given that ageing is a significant contributor to the development of cardiovascular disease, this is in keeping with trends seen in the general population. The only use of cardiovascular risk calculation in CF is by Perrin and colleagues, reporting a modified Framingham cardiovascular risk score of <10% in a CF patient with a diagnosis of non-ST elevation myocardial infarction (NSTEMI)(289). The application of cardiovascular risk scoring in CF is difficult and the use of clinical judgement alongside cardiovascular risk prediction is essential.

Mean QRisk®3 scores were significantly lower than QRisk®2 ($p<0.001$). Lower QRisk®3 scores were seen whether CFRD was classified as T1DM or T2DM, and this was a consistent finding in all participants. QRisk®3, superseding QRisk®2, is thought to be a more accurate predictor of 10-year cardiovascular risk in the general population. However, our study observed similar raised QRisk® prevalence with both scoring systems; QRisk®3 ≥10%=31.3% vs QRisk®2 ≥10%=33.7%, with good correlation between models. It is worth noting that other online cardiovascular risk models exist, such as the Joint British Societies for the Prevention of cardiovascular disease tool (JBS3), developed in 2014 as a derivative of QRisk®2 and found to have greater accuracy than its predecessor(383). This model has less

input parameters than the later developed QRisk®3, however does give a 'heart age.' QRisk®3 is currently recommend for use by NICE for assessment of cardiovascular risk(108).

There was no significant relationship between disease severity (defined by $ppFEV_1$, BMI and exocrine pancreatic status) and QRisk®3 score. Interestingly, higher overall QRisk®3 scores were seen in the non-severe CFTR mutation class. The difference in QRisk®3 scores when classed as T1DM or T2DM were statistically significant, indicating the importance of diabetic classification in QRisk® scoring. A higher mean PWV was observed in patients with QRisk® ≥10% than those <10% (9.39 vs 8.37 m/s, p=0.002). These results show the importance of CFRD classification in QRisk® scoring, the discrepancies between the two scoring systems and the potential issues with using this cardiovascular risk score in CF. Would a CF-specific cardiovascular score therefore be a useful addition to clinical practice for older CF patients? Since QRisk®3 scores are higher with CFRD classed as T1DM and most CF diabetics use insulin therapy, it may be logical to use this diabetic phenotype in CF cardiovascular risk scoring.

There was no significant association between hsCRP and QRisk® scores in our older CF cohort. The predictive risk of raised hsCRP in the context of cardiovascular disease has been extensively studied in the general population. Systemic inflammation plays a major role in atherogenesis and the development of cardiovascular disease(384). A metanalysis by Lagrand and colleagues (1999) concluded hsCRP of >3mg/L to be independently associated with cardiovascular risk(385). The EURIKA trial (2014) observed raised hsCRP in a large proportion of non-diabetics with at least one other cardiovascular risk factor(386). Johns and colleagues (2018) proved hsCRP to be helpful in cardiovascular risk stratification in low risk individuals, i.e. in the absence of diabetes mellitus and hyperlipidaemia, observing the majority of those with risk scores of <10% (as defined by JBS3 and QRisk®2) had elevated hsCRP levels(383). The JUPITER study (2008) assessed the pleiotropic use of rosuvastatin as both a lipid lowering agent and an inflammatory modulator, in non-diabetics without hyperlipidaemia but with raised hsCRP, observing reduced cardiovascular events(387). As a population with a high prevalence of systemic inflammation (46.4% with hsCRP>5mg/L), we may have expected to see the presence of heightened cardiovascular risk in our cohort despite absence of other conventional risk factors. The lack of correlation between raised

QRisk®3 scores and raised baseline hsCRP in our study may confer that risk factors other than raised hsCRP may have a greater influence on cardiovascular risk in long term CF survivors.

Notably, one of the late diagnosis patients in this cohort had a history of CVA preceding his CF diagnosis and, as such, was excluded from QRisk® analysis. 11.8% of our older CF cohort were identified as having a significant family history of cardiovascular disease, a factor of great importance in the development of premature cardiovascular disease. The inclusion of postcode in QRisk® scoring allows the influence of social deprivation and health inequality to be analysed in cardiovascular risk, strong predictors of the development of premature cardiovascular disease in the general population(388). Similar socio-economic inequality and adverse health consequences are evident in the CF population. Schechter and colleagues have shown increased morbidity and mortality in CF children of lower socioeconomic status in the US(389). Socioeconomic divide in CF will inevitably influencing cardiovascular risk with age. Detailed socioeconomic analysis was not within the remit of this study, however the socioeconomic impact on CF survival would be an interesting topic for further research.

Khot and colleagues report up to 20% of a large cohort with established coronary artery disease to have no conventional cardiovascular risk factors(390) and hence we may be missing opportunities for primary prevention using predictive risk scoring. Improving the reliability of cardiovascular risk prediction must be prioritised in order to reduce cardiovascular morbidity and mortality in the general population, disease that accounts for the majority of all-cause mortality worldwide(391). Individualising cardiovascular risk prediction to specific disease cohorts would be extremely challenging, particularly in rarer disease groups such as CF. However, we must recognise emerging cardiovascular risk in some of these groups as a result of improving survival but also from inherent disease-related factors. This may be particularly relevant in chronic respiratory disease. The association between chronic airflow limitation and enhanced cardiovascular risk is well-established, and cardiovascular disease is a leading cause of mortality in chronic obstructive pulmonary disease (COPD)(392). A study by Thillai et al analysed QRisk®2 scoring in a population with idiopathic pulmonary fibrosis, observing that over 60% of patients had a score of >20% and were at high cardiovascular risk, requiring primary preventative treatment(352). Saleh and colleagues observed a heightened cardiovascular risk in a

bronchiectasis population, with potential underestimation of this risk when using QRisk®2(393). These studies highlight the need for cardiovascular awareness in chronic respiratory illness and the observed elevation in cardiovascular risk in chronic respiratory cohorts may call for the consideration of respiratory-specific risk prediction tools to enhance reliability in higher risk groups. Larger studies will be required to assess any meaningful trends in causation of cardiovascular risk in an older CF population.

The management of CF patients with raised QRisk® scores should follow national guidelines(108), starting with lifestyle modifications and then consideration of appropriate primary preventative lipid-lowering therapy. Lifestyle modification in some CF patients may be challenging due to the requirements of higher fat diets in exocrine pancreatic insufficiency, the limitations of exercise with advanced pulmonary disease and the effect of CFTR modulation on BMI. It may still be appropriate to consider a cardioprotective diet in CF patients with a high QRisk® score, CFRD should be optimised and systemic hypertension treated effectively. In current guidance, NICE recommends statin therapy in all type one diabetic patients above 40 years of age, particularly in those with diabetic nephropathy or with other cardiovascular risk factors, all of which may have relevance in older CF patients. Statin treatment may be challenging in CF patients established on long term azithromycin due to drug-drug interactions and adverse effects.

Lack of cardiovascular risk stratification in CF may mean failure to identify those at higher risk of cardiovascular disease. QRisk®3 scoring provides an important prediction tool for cardiovascular disease and could provide an opportunity to establish primary cardiovascular prevention in ageing CF population. Since QRisk® scores are higher with CFRD classed as T1DM and most CF diabetics use insulin therapy, it may be logical to use this diabetic phenotype in CF cardiovascular risk scoring. Classification of CFRD in QRisk®3 scoring may have a significant influence on risk stratification for CF patients and warrants further contemplation.

Interestingly, Skolnik and colleagues (2016) performed coronary angiography on 14 CF patients over 40 years of age with advanced pulmonary disease, showing no evidence of established atherosclerosis(222). This may represent a ‘lag’ in the development of quantitative cardiovascular disease in an older CF population with identifiable risk factors

and highlights the importance of ongoing assessment in this cohort as survival continues to improve.

4.6 Limitations

A number of limitations to this study should be recognised. This is an observational, cross sectional study limited predominantly to descriptive data analysis, thus limiting reliability of conclusions made. Longitudinal data of cardiovascular risk factors over time would provide more informative analysis and conclusions.

Although arterial stiffness has been shown to correlate with cardiovascular risk and recommended cut-off values for 'normal' arterial stiffness exist(151)(149), the lack of consistent normative reference ranges for PWV and AIx according to age and systolic blood pressure limits their use in routine clinical practice. The use of applanation tonometry (using the Vicorder®) is a global measure of arterial stiffness and is subject to a degree of error, particularly when measuring carotid-femoral path length. There is also a lack of standardisation of these techniques in the clinical setting(373). More focal (and hence likely more accurate) methods include carotid ultrasound techniques and CMR imaging. The usefulness of these methods in the determination of arterial stiffness in CF is unknown.

The study of CF cohorts in general, but particularly those aged 40 years and above, is associated with the inevitability of small sample sizes. Larger sample sizes are needed to increase the reliability and accuracy of data analysis, and when attributing conclusions to the CF population as a whole. Larger studies may be able to define subsets of CF patients at higher cardiovascular risk, such as those with CFRD, exocrine pancreatic sufficiency and higher BMI.

The classification of CFRD is challenging and there may be some patients included in the non-CFRD group with a degree of impaired fasting glycaemia, affecting between-group analyses. QRisk®3 is yet to be universally adopted by primary care for cardiovascular risk stratification and as such QRisk®2 is more widely used. It would seem logical that QRisk®3 would provide more accurate cardiovascular risk estimates, however the lack of CF-specific cardiovascular risk score limits its application in CF patients.

A proportion of patients were already established on lipid-lowering and anti-hypertensive treatment. Some patients may have had a multi-factorial indication for ACE inhibition, limiting interpretation of results.

A more in depth analysis of cardiovascular risk and appropriate scoring models would be helpful to expand knowledge on risk prediction in CF. The effects of chronic CF therapies, such as macrolide antimicrobials, the use of ACE inhibition and the study of statin therapy in older CF populations would be insightful, to determine their effects on vascular modification, reduction in systemic inflammatory burden and influence on cardiovascular risk reduction. An increasing availability of CFTR modulation may also influence cardiovascular risk in ageing CF patients, and further study in this area would also be informative.

4.7 Conclusion

Our study demonstrates the relevance of cardiovascular risk assessment in CF patients achieving longer term survival. Although the prevalence of hypercholesterolaemia in CF is less than the UK population in general, over one third of our cohort had QRisk® scores of ≥10%. Systemic hypertension and increased arterial stiffness were also seen in our study population. Cardiovascular disease will become more prevalent in an ageing CF population and CFTR modulation may affect cardiovascular risk in CF with disease longevity. An increasing prevalence of CFRD with age will further contribute to cardiovascular and renal disease in this cohort.

The balance of CFTR-related and non-CFTR related factors in CF cardiovascular risk cannot be confidently quantified, but likely both influence the development of cardiovascular disease in CF patients as they age. Areas for future research as survival in CF continues to improve must include assessment of cardiovascular risk, with appropriate and timely implementation of primary preventative therapy, and the study of ageing complications in other organ systems such as the kidney. The development of research in the older CF population is essential in order to optimise management and reduce comorbidity in an already complex and challenging, chronic multisystem disease.

4.8 Recommendations

Based on study findings;

1. Consider cardiovascular risk assessment for all CF patients aged 40 years and above, using QRisk®3 and classifying CFRD as T1DM where appropriate.
2. Those identified as 'at risk' and with QRisk®3 scores of above 10% should receive lifestyle modification advice and/or initiate primary preventative lipid lowering therapy, with concurrent diagnosis and treatment of other cardiovascular risk factors. Patients with scores of >20% should commence primary prevention therapy. Cardiovascular risk management and follow-up should adhere to national guidelines.
3. Subsets of patients with exocrine pancreatic sufficiency and CFRD may require more regular assessment of cardiovascular risk.

The Complexities of Ageing in Cystic Fibrosis

5.0 Chapter 5: The kidney in an older cystic fibrosis cohort

5.1 Abstract

5.1.1 Introduction

Primary renal disease in cystic fibrosis (CF) is rare, however secondary renal dysfunction may become apparent with increasing survival due to the emergence of age-related comorbidities such as CF-related diabetes mellitus (CFRD), systemic hypertension, nephrolithiasis and cardiovascular disease. Inaccuracies in estimations of renal function using creatinine-based glomerular filtration rate (GFR) methods are evident in CF and will have implications for monitoring renal decline, dosing nephrotoxic medication and quantifying cardiovascular risk in an ageing CF population. This study aims to explore renal function and reliability of estimated GFR in an older CF cohort.

5.1.2 Methods

A total of 85 CF patients aged 40 years and above attending MACFC were recruited between August 2018 and August 2020. Prospective sampling for serum creatinine, serum cystatin C, urine albumin-creatinine ratio (ACR) and 24-hour urinary creatinine clearance was performed during periods of clinical stability. Intravenous (IV) aminoglycoside days were calculated for each patient over the preceding ten-year period. The prevalence of CF-related diabetes mellitus (CFRD) and systemic hypertension within the cohort were also determined. Estimated GFR (eGFR) was calculated using MDRD and CKD-EPI equations. A subgroup underwent iohexol clearance 'measured' GFR (mGFR), using a three-point, one compartment protocol. Estimated GFR methods were compared to iohexol GFR to determine their accuracy in this population.

5.1.3 Results

This older CF cohort was phenotypically diverse; mean ppFEV$_1$ 52.9(±23.3)%, the prevalence of severe CFTR mutations was 72.9%, exocrine pancreatic insufficiency 83.5%, CFRD 49.4% and systemic hypertension 18.8%. Mean serum creatinine and cystatin C; 70.7(±16.4) µmol/L and 0.93(±0.17) mg/L respectively (normal range cystatin C; <1.2 mg/L if <50 years, <1.55mg/L if >50 years). 16.9% had raised urine ACR. Estimated GFR range was 40-154

ml/min/1.73m^2 and an eGFR of <90 ml/min/1.73m^2 was seen in up to 47%, depending on the method used. 3.6% had an eGFR of <60 ml/min/1.73m^2 and a diagnosis of CKD stage 3. There was a positive correlation between IV aminoglycoside days and serum cystatin C; r=0.327, p=0.003. Patients with systemic hypertension were more likely to have eGFR <90 ml/min/1.73m^2; p=0.014. The risk of eGFR <90 ml/min/1.73m^2 was not significantly higher in the CFRD group; OR 1.2 (95% CI 0.48-2.7), p=0.756. Mean iohexol GFR of the subgroup was well preserved at 116.1(±20.1) ml/min/1.73m^2. Estimated GFR methods using both serum creatinine and cystatin C underestimated GFR significantly in this population. However, using iohexol mGFR as gold standard, CKD-EPIcreat and CKD-EPIcysC+creat were the most accurate with 77.8% of values within 30% (P30) of measured GFR.

5.1.4 Conclusion

Renal dysfunction is prevalent in this older CF group and increases with age. Intravenous aminoglycoside exposure, systemic hypertension and CF-related diabetes mellitus (CFRD) are related to lower eGFR. A higher burden of IV aminoglycoside days is associated with higher serum cystatin C in this cohort. Estimated GFR using serum creatinine and cystatin C is inaccurate in this population, underestimating measured iohexol GFR. Serum cystatin C may be superior to creatinine in monitoring renal function in older CF patients and its routine use should be considered in an ageing population. Close monitoring of renal function is essential to detect early and progressive renal decline, which will have implications for organ transplantation, nephrotoxic drug dosing and progression of diabetic nephropathy in a population with an emerging cardiovascular risk.

5.2 Introduction

Abnormal CFTR protein in cystic fibrosis (CF) causes disruption of anion movement across epithelial cell membranes, resulting in the accumulation of viscid secretions in multiple CFTR-expressing organ tissues. Although pulmonary disease dominates this pathological process, the effects of abnormal CFTR on other organ systems are varied and reflect the complex interplay between genotype and disease phenotype.

5.2.1 Renal disease in CF

Despite inherent CFTR-related risk factors for renal disease, primary renal pathology in CF remains relatively uncommon. However, improving survival has led to an increasing awareness of the development of ageing comorbidities and the development of secondary renal disease. It is estimated that around 2% of the US CF population are living with moderate to severe renal impairment(220), and this risk increases with age. CFTR protein is present in renal tubular cells and, although its exact influence on the kidney is unknown, several CFTR-related factors may influence the development of renal disease in CF.

5.2.1.1 Nephrolithiasis

Disruption of electrolyte homeostasis, salt depletion and recurrent antimicrobial exposure are attributed to a high prevalence of nephrolithiasis in CF patients. Dysregulation of renal calcium metabolism in CF contributes to hypercalciuria and nephrocalcinosis(394). The absence of oxalate-degrading *Oxalobacter formigenes* bacteria in the gut flora of CF patients, secondary to malabsorption and exacerbated by recurrent antimicrobial use, leads to reduced oxalate absorption, hyperoxaluria and a predisposition for renal stones(395). Nephrolithiasis is more prevalent with age in CF and recurrent renal tract stones may lead to gradual destruction of renal parenchyma and chronic kidney disease (CKD)(69).

5.2.1.2 IgA nephropathy and secondary amyloidosis in CF

As in the general population, IgA nephropathy is the commonest glomerulonephropathy seen in CF patients(396). The prevalence, however, remains rare and only described in case reports(397)(398). High levels of systemic inflammation in CF can cause immunoglobulin deposition in the kidney, which can lead to IgA nephropathy and associated progressive renal decline(397). Other glomerular disease has been reported in CF, including post-infective glomerulonephropathy, focal segmental glomerulosclerosis, minimal change

disease and diabetic nephropathy(398). Chronic inflammation may also contribute to the development of secondary AA amyloidosis in CF and Gaffney et al report two cases of nephrotic syndrome secondary to renal amyloid(399). Systemic amyloidosis and multi-organ amyloid deposits are increasingly being reported in CF autopsy specimens(400)(401)(402). Subclinical, asymptomatic amyloidosis in CF may be more common than first thought and its presence may be related to increasing survival.

5.2.1.3 Pulmonary sepsis and acute kidney injury

Pulmonary sepsis is often accompanied by acute renal impairment and evidence of multi-organ involvement is an important marker for prognosis(403). Recurrent renal insult in relation to systemic infection during CF pulmonary exacerbation, aggravated by intravenous aminoglycoside exposure, may influence longer term renal recovery and contribute to chronic renal impairment over time. At times of clinical instability, CF patients will also be at risk of dehydration from salt depletion and gastrointestinal disturbance, compounding short term renal impairment. Frequent exposure to nephrotoxic medication such as aminoglycosides, non-steroidal anti-inflammatories (NSAIDs), and long term immunosuppressant therapy may impact renal function, causing both acute and chronic renal tubular damage(404).

5.2.1.4 CF-related diabetes mellitus

An increasing prevalence of CFRD with age will also have a significant influence on the development of renal dysfunction. Longevity of hyperglycaemia will contribute to microvascular complications and renal protein loss, and CFRD may also influence the development of hypertension and renovascular disease. The presence of diabetes mellitus also increases the rate of nephron degeneration that occurs as a result of ageing(405).

5.2.1.5 Ageing and renal disease in CF

In the general population, around 20% of renal volume is lost between 50 and 80 years of age. Arteriosclerosis, renal tubular atrophy, glomerular degeneration and nephron loss are all part of the inevitable renal ageing process. Glomerular filtration rate peaks at 30 years of age and subsequently declines with advancing age(406). An ageing kidney in CF may also contribute to renal decline as survival continues to improve. Given that CKD and end stage renal disease (ESRD) are associated with enhanced cardiovascular risk in the general population, an increasing prevalence of renal disease is likely to influence the development

of cardiovascular disease in an older CF population. Accurate monitoring of renal function is essential in CF for early detection of renal disease, adequate management of cardiovascular risk and accurate dosing of nephrotoxic medication. These factors will be of particular importance in the context of lung transplantation, the incidence of which may increase at older ages in longer term CF survivors.

5.2.2 The measurement of GFR

Glomerular filtration rate (GFR) is the most reliable measure of renal function. It can be measured directly by renal clearance of exogenous filtration markers or indirectly via estimations using endogenous serum biomarkers within standardised formulae. Accurate measurements of GFR are important in order to adequately diagnose those with CKD, accurately adjust medications with narrow therapeutic indices and to risk stratify for cardiovascular disease and ESRD.

5.2.2.1 Measured GFR (mGFR)

The gold standard for measuring GFR is through clearance of an 'ideal' filtration marker such as inulin, iohexol or iothalamate(261). These exogenous compounds are exclusively filtered and excreted by the kidneys and, as such, determination of GFR using these methods would result in a direct and accurate measure of renal function, otherwise referred to as 'measured GFR' (mGFR). GFR assessment of renal inulin clearance necessitates continuous intravenous infusion and subsequent multiple timed sampling of urine and serum, making this technique impractical in a routine clinical setting. Although the use of radioisotope mGFR methods, including iothalamate, ^{51}-chromium EDTA and ^{99m}TC-DTPA require only an initial bolus injection and have good method correlation to inulin, the challenges of handling and disposing of radioactive materials in a clinical environment limits their use. In addition, inulin and isotope clearance methods require sampling to be performed over an increasing time interval with declining GFR, such that those with advanced CKD may require samples up to 24 hours after initial infusion to assess the terminal elimination phase and accurate GFR. This obviates clinical implementation.

Iohexol GFR has been shown to correlate highly with both radioisotope and inulin GFR(407)(408). Iohexol may be the most suitable alternative to inulin given its exclusive renal excretion and more practical administration method. Only a single injection of iohexol is required, plasma clearance measurements are preferred over urine and single sample

protocols have been developed(409). Iohexol mGFR has been shown to be a superior measure of renal function and show inaccuracy of creatinine-based estimated GFR calculations in CKD and diabetic populations(410)(411)(412) (see table 5.1, end of chapter 5). However, the logistics of iohexol GFR determination, including the necessity for sampling over a number of hours, currently limits its utilisation outside of research and transplant settings.

5.2.2.2 Estimated GFR (eGFR)

In routine clinical practice, GFR is calculated using endogenous biomarkers, giving rapid estimates of renal function. The most commonly used endogenous biomarker is creatinine. Creatinine is an amino acid and is a product of creatine breakdown in muscle. It is produced at a fairly constant rate, excreted via glomerular filtration, with minimal tubular reabsorption, and can be measured in both serum and urine. Creatinine-based eGFR calculations are the most commonly used and widely available assays, informing the majority of current diagnostic renal guidelines(259). Creatinine-based eGFR is cost-effective and preferred to the 'gold standard,' more time-consuming method of isotope or iohexol clearance mGFR in the standard clinical setting.

5.2.2.3 Inaccuracies in estimated GFR

In most cases, estimated GFR is sufficient to make optimal clinical decisions. However, in certain patient populations eGFR may be inaccurate and measured GFR is thus essential for optimal management. There are a number of significant limitations when using creatinine as an estimate of renal function. Creatinine levels will vary depending on the rate of metabolism within muscles and the degree of proximal renal tubular secretion(413). Factors affecting these variables include muscle mass, age, gender and ethnicity. This results in different 'normal' values between patient groups and must be considered when interpreting results. Patients with chronic disease may have reduced muscle mass and lower serum creatinine, thus creatinine-based calculations may overestimate GFR. Due to the variation in creatinine between patient groups and laboratory reference ranges, there may be a discrepancy between paired eGFR and creatinine levels. A significant decline in eGFR may be seen prior to creatinine reaching abnormal levels and creatine-based eGFR gives no information regarding renal reserve in patients with a normal creatinine(414). Creatinine-

based eGFR may also be inaccurate in non-steady clinical states, such as acute kidney injury, often underestimating the degree of renal dysfunction in this context(415).

5.2.2.4 Cystatin C

The inaccuracies of creatinine-based eGFR in certain patient groups has led to the development of eGFR calculations using other endogenous biomarkers. Cystatin C is a protease inhibitor present in all nucleated cells, filtered by the glomerulus and then reabsorbed by renal tubular epithelial cells(185). Only minimal amounts are excreted in the urine and thus serum levels are the most reliable clinical measure. The concentration of cystatin C within the blood rises in response to declining renal function. A serum Cystatin C level of <1.20mg/L is normal for patients <50 years, and <1.55mg/L normal for patients ≥50 years(416). Serum cystatin C levels in health remain fairly constant and, in contrast to creatinine, vary less with body mass, age and gender(417). Studies have shown serum cystatin C to better predict GFR than serum creatinine(418) and, when compared to iohexol GFR, the addition of cystatin C has shown superiority over creatinine-only calculations in measuring eGFR in patients with lower baseline GFR(419). The use of cystatin C eGFR may be more reliable than creatinine clearance in diabetic populations and in the early detection of renal impairment in the presence of normal serum creatinine(237). Cystatin C estimations of GFR have also been shown to be more accurate in monitoring graft function following renal transplantation(420)(421).

High metabolic demands, malabsorption and chronic inflammation in CF contribute to low muscle mass and the resulting inaccuracies in creatinine-based eGFR may underestimate the degree of renal impairment in CF patients. More robust methods of GFR monitoring in these patients therefore need to be considered. Beringer and colleagues showed cystatin C clearance to be a more accurate measure of GFR in CF patients, compared to both serum creatinine eGFR and urinary creatinine clearance, using iothalamate as the gold standard(189). Halacova and colleagues observed cystatin C to be a more accurate measure of eGFR in CF patients receiving intravenous amikacin(236). In addition, cystatin C may be a more reliable predictor of cardiovascular risk than creatinine in a CKD population(422) which, based on our results from preceding chapters, may have implications for an ageing CF cohort. Accurate GFR measurements in the CF population are important for both optimal medication dosing and early detection of renal disease. This will be particularly relevant for

transplant recipients on long term immunosuppressive agents, in whom progressive renal decline is commonly seen. A deficiency in availability of laboratory assays and higher costs have limited routine clinical use of cystatin C methods thus far in populations where creatinine based eGFR may less inaccurate.

5.2.2.5 Equations to estimate of GFR

The following formulae are most commonly utilised in clinical practice and were chosen as eGFR comparators to iohexol mGFR in this study;

a. The MDRD equation (1999):

GFR = 175 x (Standardized S_{Cr})$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.210 if African American) (ml/min/1.73m^2). {S_{Cr}-serum creatinine}

The most recent version of MDRD is adjusted for body surface area (BSA) along with age, gender and ethnicity, thus is thought to be more accurate than the preceding Cockroft-Gault formula (CG)(180). The MDRD equation was developed using data from patients with chronic renal disease and thus may underestimate GFR in healthy populations. Notably, equations to measure eGFR from serum creatinine seem to be less accurate for eGFR levels of over 60 ml/min/1.73m^2(175). The relationship between GFR and creatinine is disproportionate and a much greater decline in eGFR will be seen for a similar decline in creatinine for patients with better preserved renal function than those without(182).

b. Chronic Kidney Disease Epidemiology Collaboration - CKD-EPI:

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) have been recognised in KDIGO guidelines since 2012(259). They are recently being used in preference to MDRD in calculating eGFR in routine clinical practice. CKD-EPI calculations have three forms to include a creatinine-based equation, a cystatin C-based equation and a combination of the two. All adjust for the same four variables as in MDRD calculations. The CKD-EPI creatinine and the CKD-EPI creatinine-cystatin C calculations have been shown to have improved reliability when compared with MDRD, particularly in the elderly and those in higher GFR ranges(183)(184).

i. CKD-EPI creatinine (2009):

$eGFR = 141 \times \min(SCr/\kappa, 1)^{\alpha} \times \max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] x 1.159 [if Black] *{κ = 0.7 (females) or 0.9 (males), α = -0.329 (females) or -0.411 (males), min = indicates the minimum of SCr/κ or 1, max = indicates the maximum of SCr/κ or 1}.*

ii. CKD-EPI cystatin C (2012):

$eGFR = 133 \times \min(Scys/0.8, 1)^{-0.499} \times \max(Scys/0.8, 1)^{-1.328} \times 0.996^{Age} \times 0.932$ [if female]

{Scys (standardized serum cystatin C) = mg/l, min = indicates the minimum of Scys/0.8 or 1, max = indicates the maximum of Scys/0.8 or 1}.

iii. CKD-EPI cystatin C creatinine (2012):

$eGFR = 135 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-0.601} \times \min(S_{cys}/0.8, 1)^{-0.375} \times \max(S_{cys}/0.8, 1)^{-0.711} \times 0.995^{Age} \times 0.969$ [if female] × 1.08 [if black] *{α = -0.248 (females) or -0.207 (males)}.*

5.2.2.6 Urinary creatinine clearance

The measurement of urinary creatinine excretion over a prolonged period may be a more accurate marker of GFR in populations with nutritional impairment and fluctuating clinical stability in chronic disease. Historically, urinary creatinine clearance has been considered the 'gold standard' for more accurate GFR measurements when compared to serum creatinine eGFR. However, this technique is limited by patient compliance and accurate timing of collection. Since creatinine is secreted by the renal tubules, GFR is often over-estimated, particularly in patients with CKD. For these reasons, the measurement of GFR from urine creatinine clearance has largely been replaced by the above serum creatinine eGFR formulas. Decline in urinary creatinine clearance remains useful in CKD patients as a prediction tool for renal decline and mortality(190).

5.2.3 Proteinuria

Renal protein loss is a sensitive marker of disease progression in CKD. However, patients with proteinuria (microalbuminuria) in the context of normal renal function are also at risk of both progressive renal damage and cardiovascular disease(423). The most frequently observed proteinuric nephropathy in CF is associated with CFRD, although non-diabetic renal protein loss is seen, albeit less commonly, in glomerular disease such as IgA nephropathy. Albumin creatinine ratios (ACR) are routinely used in clinical practice to detect

microscopic proteinuria, particularly in the diabetic population. ACR monitoring in CF diabetics may provide an opportunity to detect early signs of renal damage and to implement timely treatment. Persistent microalbuminuria is also seen in the non-diabetic CF population(192). This, in addition to the risk of CFRD increasing with age, deems regular ACR monitoring relevant in the ageing non-CFRD population to inform both renal and cardiovascular risk.

5.2.4 Aminoglycosides and renal dysfunction

Advances in CF treatment over recent years have seen the introduction of regular intravenous aminoglycoside use for pulmonary exacerbation, particularly effective in those with chronic *Pseudomonas aeruginosa* infection. Aminoglycosides (tobramycin, gentamicin and amikacin), although extremely effective bactericidal antimicrobials, have a narrow therapeutic index and are excreted exclusively by the kidneys. Reduced clearance and accumulation of aminoglycosides in the setting of abnormal renal function leads to damage of the proximal tubular cells, which may lead to acute kidney injury (AKI). Close drug level monitoring and dose adjustments can assist to avoid short term aminoglycoside toxicity, however there is evidence to suggest that recurrent exposure may lead to chronic renal damage(86). Since the majority of CF patients will have frequent exposure to aminoglycosides during their lifetime, accurate renal monitoring is essential to minimise the risk of long term renal complications as survival continues to improve. Touw and colleagues observed a good correlation between tobramycin clearance and creatinine clearance in a CF cohort(238). However, Soulsby and colleagues found tobramycin clearance to correlate poorly with ^{99m}TC-DTPA mGFR in CF patients and showed cystatin C based eGFR formulae to be more reliable(424).

5.2.5 Organ transplantation and renal dysfunction

Renal dysfunction is commonly seen following organ transplantation. Ishani and colleagues reported 91% of lung and heart-lung transplant recipients in their study cohort (8% with CF) to have deterioration in renal function at six months and 7.3% developed end stage renal failure requiring haemodialysis(232). This risk of renal failure following lung transplantation is higher in females, those with lower pre-transplant GFR and in diabetic patients(425). Other significant contributory factors include long term immunosuppressive treatment,

particularly calcineurin inhibitors, and new onset post-transplant systemic hypertension, confounded by corticosteroid use and weight gain(426).

Renal assessment is therefore of paramount importance during lung transplant assessment and ^{51}-chromium EDTA GFR is a technique used to obtain accurate GFR in some centres. Measured isotope GFR is also important in renal pre-transplant assessment, for both graft donor and recipient. Despite the high prevalence of renal dysfunction in transplant recipients, isotope GFR is not routinely measured following lung transplantation(426). Improving survival in CF may lead to older ages at lung transplantation. Pre-existing ageing comorbidities and the potential for organ dysfunction post-transplant will thus become important considerations for both transplant and CF multidisciplinary teams.

5.2.6 Glomerular hyperfiltration

Hyperfiltration is defined as a pathological increase in glomerular filtration rate and can occur in disease states such as CKD, diabetes mellitus and obesity(427). Glomerular hyperfiltration may precede the development of nephropathy in diabetic and hypertensive populations and is associated with more rapid decline in GFR and increased cardiovascular risk in those with CKD(428).

Hyperfiltration has also been observed in CF, postulated to be caused by altered sodium transport within renal tubules secondary to CFTR-related essential fatty acid deficiency(429)(430). Several studies have shown a significantly higher GFR in CF patients compared to healthy controls, particularly when using exogenous methods(429)(424). Hyperfiltration in CF may lead to GFR over-estimation and poor recognition of early renal dysfunction. In addition, hyperfiltration in CF means that larger doses of aminoglycosides are required during pulmonary exacerbations to achieve therapeutic effect(431). Despite normal or raised baseline GFR in CF patients, accurate renal assessment remains essential, particularly as the risk of diabetic nephropathy, systemic hypertension and other cardiovascular risk factors are likely to increase with an ageing CF population.

5.2.7 Iohexol GFR use in the non-CF setting

Iohexol (Omnipaque®) is a non-ionic contrast agent used predominantly during computed tomography (CT) scanning and angiography. Iohexol is exclusively excreted by glomerular filtration, with very minimal extra-renal clearance, and is also present in the extracellular

space, allowing for plasma measurement. Iohexol safety has been exhaustively studied showing good tolerability and a lack of adverse events(261). In addition, only small volumes are required for investigative benefit. As a renal clearance marker, iohexol has many advantages over inulin and radioisotope GFR due to stability in clinical environments and ease of administration. Iohexol concentration can be measured in serum, conferring advantage over multiple urine sampling. Although, iohexol is most commonly measured using high performance liquid chromatography (HPLC)(262), the laboratory at our institution was able to provide iohexol analysis using liquid chromatography-tandem mass spectrometry (LC-MS). LC-MS is a more costly and complex analytical process, however has a greater sensitivity and specificity compared to HPLC(260).

Given the variability in non-renal components of eGFR formulae between disease populations, Delanaye and colleagues report that measured GFR is not only the most accurate determinant of CKD progression, renal graft function and dosing of medications with narrow therapeutic indices, it is also the sole suitable method to assess the relationship between renal dysfunction and cardiovascular risk(261). The lack of accuracy of estimated GFR in patients with normal renal function presents a further challenge of GFR monitoring in disease populations outside of a CKD setting, such as CF, who may still confer risk of renal decline and in whom this decline may impact disease morbidity and organ transplantation. The improved accuracy along with the wide availability, low cost and administration advantages of iohexol present this method as a front-runner in mGFR monitoring, particularly in small disease cohorts such as CF.

Once injected into the venous system, iohexol concentration will diminish in the blood over time, allowing plasma clearance (PC) to be measured. This calculation uses the initial iohexol dose (D) divided by the area under the curve (AUC); $PC=D/AUC$. Iohexol pharmacokinetics involve an initial distribution (fast) phase within extracellular tissues followed by an elimination (slow) phase of renal drug excretion(432).

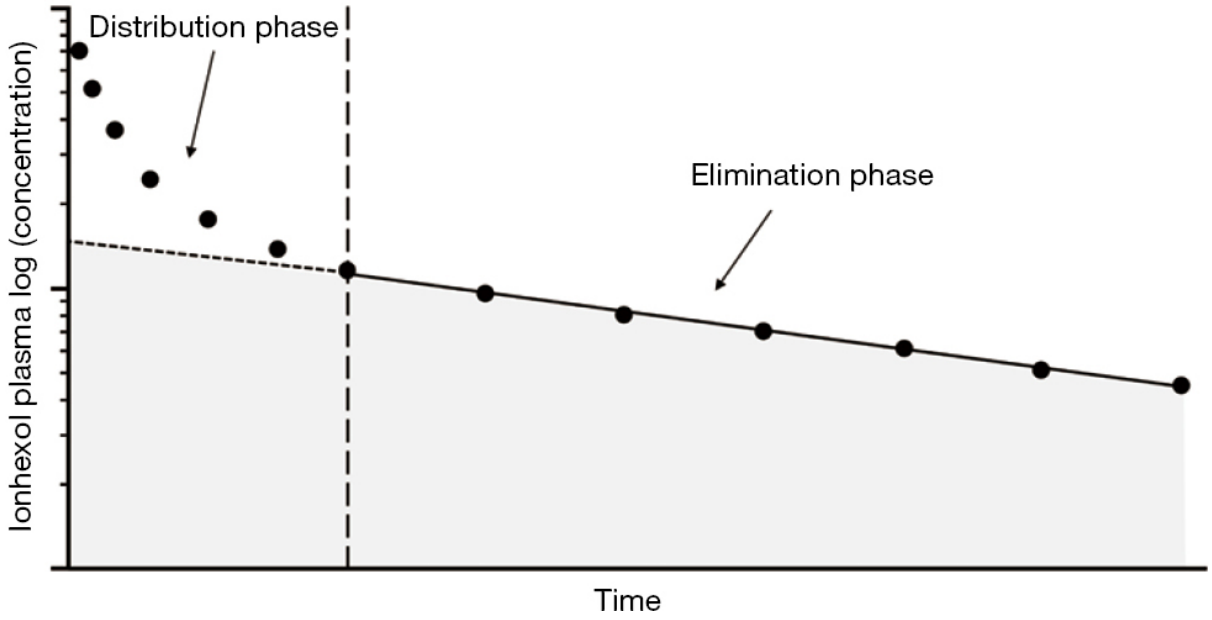


Figure 5.1: The clearance of iohexol in plasma over time, following intravenous injection; correction equation must be applied in one compartment models (i.e. sampling only in elimination phase)(433).

The accuracy of plasma iohexol clearance depends upon the number and timing of samples. The drug distribution phase is usually completed within two hours. Plasma clearance GFR has historically consisted of a two compartment sampling model to account for both drug distribution and elimination phases. However, the development of a one compartment clearance model, with sampling only in the elimination phase and using the Bröchner-Mortensen equation for two-compartment correction(434), has introduced some simplicity but with equitable clinical reliability. Carrara and colleagues (2018) report a final sample at four hours to be a sufficiently reliable determination of mGFR in patients with normal or mildly impaired renal function(433). However, for patients with lower baseline eGFR, a longer duration of sampling is required to produce accurate GFR. Although both single and multi-sample protocols have been used in iohexol studies to date, an increasing number of sample points reduces sampling error and improves accuracy of non-linear regression GFR analysis. Therefore, in practice multi-sample methods are preferred, particularly in patients with lower GFR.

5.2.8 Iohexol clearance GFR calculation

A one-compartment model generates an iohexol clearance GFR using a slope-intercept linear equation(263). This process is shown below;

1. A semi-logarithmic plot of iohexol concentration against time is constructed, allowing calculation of iohexol clearance or "slope-intercept GFR;" *Slope-intercept GFR = k × Iohexol dose (µg)/C_0 (µg/mL)*, where k is the slope of the logarithmic plot and the intercept is the concentration of iohexol at time 0 (C_0).
2. GFR must be corrected for body surface area (BSA) using the DuBois equation; $BSA = 0.007184 \times height\ (metres)^{0.725} \times weight\ (kg)^{0.425}$
3. To account for lack of sampling in initial fast distribution phase, iohexol clearance must finally be corrected using the Bröchner-Mortensen (BM) equation(434); *BSA-adjusted slop-intercept GFR = (0.990778 × GFR) - (0.001218 × GFR^2).* This provides a GFR in ml/min/1.73m^2.

5.2.9 Iohexol evidence in the non-CF setting

Studies analysing the accuracy of iohexol clearance GFR compared to other filtration markers have spanned the last few decades. In 1995, Gaspari et al first showed iohexol to have equivocal accuracy to inulin for GFR measurements in patients with CKD(407). A follow-up precision study proved iohexol to be an accurate determination of GFR in CKD cohorts of varying severity(408). More recent studies have proven eGFR to be unreliable compared to iohexol GFR in diabetic patients, underestimating renal impairment in this group and particularly those with higher baseline GFR(412)(435). However, iohexol GFR has also been compared to eGFR methods in renal transplant recipients, showing GFR overestimation in this cohort(436). Iohexol GFR has also been used to validate the accuracy of cystatin C eGFR methods in CKD populations(437). A summary of recent iohexol studies is shown in table 5.1 (end of chapter 5).

5.2.10 Measured GFR in CF

There are a handful of studies to date analysing the accuracy of estimated GFR in CF renal monitoring, a summary of which is illustrated in table 5.2 (end of chapter 5). Spino and colleagues (1985) studied isotope GFR, concluding it was an accurate measure of renal function in a CF cohort(438). Beringer and colleagues (2009) were the first group to study cystatin C in CF adults, observing cystatin C eGFR to have superior accuracy to creatinine-based eGFR formulae when compared to iothalamate mGFR(189), particularly in those with

lower baseline GFR (cut-off value 90ml/min/1.73m^2). Tidman and colleagues first validated the use of serum cystatin C in eGFR equations in the CKD population in 2007(437), showing improved accuracy when compared to creatinine, using iohexol as gold standard. The improved reliability of cystatin C based eGFR methods may enable mild renal impairment to be detected at an earlier stage and as such its routine utilisation in the CF population may be advantageous. Several studies have also shown inaccuracies in serum creatinine-based eGFR when compared to urinary creatinine clearance(233)(238).

Novel-Caitin and colleagues have been the only group to study iohexol GFR in CF patients, in the context of advanced lung disease and aminoglycoside exposure. They report consistent overestimation of GFR using CKD-EPI eGFR in this population, particularly in those with abnormal baseline eGFR or previous AKI(235). A recent multicentre study by Wallace and colleagues (2020) shows eGFR equations to be unreliable in a paediatric CF cohort when compared to isotope GFR, highlighting the potential inaccuracies of GFR monitoring in CF patients of all ages(263). To date, the methodology of GFR measurement using exogenous filtration markers in CF is diverse and limited by small sample sizes.

5.2.11 Aims

Review of the current literature highlights some important questions regarding renal monitoring in CF;

a. What impact does ageing have on renal disease in CF and what are the potential implications with respect to the need for enhanced renal monitoring?
b. Which eGFR method is the most accurate when compared to mGFR in an older CF population? We aim to compare eGFR values derived from different equations to determine if they show agreement.
c. Presuming there is little agreement, as shown in previous studies, we aim to see which eGFR method is most accurate when compared to mGFR.
a. The advantages of mGFR testing in transplant patients has been established but can these benefits be extended to a large proportion of CF patients and should this change clinical practice as CF survival improves?

Our study will attempt to explore some of these issues and is the first to analyse cystatin C based eGFR and iohexol measured GFR as gold standard in a CF cohort aged 40 years and above.

5.3 Methods

5.3.1 Outline of study methods

A full description of methodology is outlined in chapter two. In brief, renal biomarkers were obtained from 85 CF patients aged 40 years and above attending MACFC (from a total of 92 eligible patients) including baseline serum creatinine, cystatin C and urine albumin-creatinine ratio (ACR). Estimated GFR equations were used to calculate renal function as described above (MDRD, CKD-EPIcreat, CKD-EPIcysC and CKD-EPIcreat+cysC). 24-hour urinary samples for creatinine clearance GFR were also requested from patients.

All measurements were taken during episodes of clinical stability, defined as the absence of pulmonary exacerbation requiring oral or intravenous (IV) antibiotics within the preceding four-week period(279). Total intravenous antibiotic days over the preceding ten-year period were calculated for each patient, each IV course consisting of a dual combination of aminoglycoside (tobramycin) and another agent. Serum high sensitivity C-reactive protein (hsCRP) was also measured at baseline. Serum biomarker reference ranges were used as per local trust guidelines; hsCRP <5mg/L, serum creatinine 59-104 μmol/L, serum cystatin C <1.20mg/L (if aged <50years) and <1.50mg/L (if aged ≥50years).

Reduced urinary creatinine clearance was defined as <80ml/min/1.73m^2(233). Definitions used to classify chronic kidney disease (CKD) and proteinuria (as measured by ACR) were taken from KDIGO guidelines (2012)(259). For study purposes, GFR of >90 ml/min/1.73m^2 and ACR <3 mg/mmol were classed as normal. As per guidelines, a GFR of <60 ml/min/1.73m^2 (G3a, G3b, G4 and G5) was classed as CKD. G1 and G2 were classed as abnormal renal function if there was evidence of proteinuria/albuminuria (and thus renal damage).

GFR category	**GFR (ml/min/1.73m^2)**	**Terms**	**Previous CKD staging (old system)**(439)
G1	>90	Normal or high*	I (Kidney disease with normal GFR)
G2	60-89	Mildly decreased*	II (Mildly impaired GFR)

G3a	45-59	Mildly to moderately decreased	III (Moderately impaired GFR)
G3b	30-44	Moderately to severely decreased	III (Moderately impaired GFR)
G4	15-29	Severely decreased	IV (Severely impaired GFR)
G5	<15	Kidney failure	V (Kidney failure)

Table 5.3: Categories of CKD according to KDIGO(259)*.*In the absence of evidence of renal damage, G1 and G2 do not constitute CKD.*

Category	**Albumin excretion rate (AER) mg/24hr**	**ACR mg/mmol**	**Terms**
A1	<30	<3	Normal to mildly increased
A2	30-300	3-30	Moderately increased
A3	>300	>30	Severely increased

Table 5.4: Albuminuria categories in CKD according to KDIGO(259)*. (ACR=albumin creatinine ratio).*

Iohexol GFR was measured in order to compare eGFR methods to a 'gold standard' mGFR. Simultaneous measurements of serum creatinine and cystatin C were also recorded for accurate eGFR comparison. Exclusion criteria included a history of iodine or intravenous contrast allergy and eGFR of <30 ml/min/1.73m^2. A recruitment target of 20 patients was set. However, the study was prematurely terminated due to the Covid-19 pandemic and as a result only nine patients have been included in the iohexol study arm thus far.

Iohexol clearance was performed using a standard multi-sample protocol (see appendix 3). Venous blood sampling following iohexol administration was performed (from the contralateral arm) in a four-point sampling protocol at 30, 120, 180 and 240 minutes. Concurrent capillary samples were also taken at each time point using a Mitra device (10

microlitre sample) and a size seven lancet needle at a suitable peripheral site on the index finger.

A 30 minute distribution phase iohexol sample was initially included in the protocol, however these values were not included in final analysis to allow for the use of a one compartment approach.

Plasma iohexol was measured using liquid chromatography-tandem mass spectrometry (LC-MS) and measured GFR was calculated from iohexol plasma clearance over time, using a one compartment model, corrected using the Bröchner-Mortensen factor(434). Iohexol results were compared to a reference method(262)(260), finding only 3.5% variation in results (60 patients compared, MFT assay showed 3.5% positive bias), and hence reducing the chance of laboratory processing error.

The slope-intercept, measured GFR as a derivative of iohexol clearance was calculated from the serial serum iohexol samples using the method below(262)(263);

- The slope-intercept GFR (mL/min) = k × Iohexol dose (µg)/C_0 (µg/mL).

  k = slope of the (neperian) semi-logarithmic plot of plasma iohexol concentration, C_0 is the calculated iohexol concentration at time zero (intercept).

- This value was multiplied by 1.73 and divided by the body surface area (BSA, calculated from the equation: BSA = 0.20247 × height (metres)$^{0.725}$ × weight (kg)$^{0.425}$ (DuBois).
- The BSA-slope intercept GFR value (ml/min/1.73 m^2) was corrected by the Bröchner-Mortensen correction factor = (0.990778 × GFR) - (0.001218 × GFR^2), producing a measured iohexol GFR value (ml/min/1.73 m^2).

Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) Ethics committee (reference 19/EM/0067).

5.3.2 Statistical analysis

Renal data were collated and analysed against the general demographics of this cohort, along with analysis of any emerging trends with increasing age using defined age groups; 40-49 years, 50-59 years and ≥60 years. Independent T-test, one-way ANOVA and Pearson correlations were performed to display normally distributed data. Logarithmic

transformation was performed prior to analysis of data without normal distribution and results displayed as geometric mean/SD or back-transformed mean/CI as applicable. For data without normal distribution on logarithmic transformation, non-parametric testing was used for analysis. Categorical data were analysed using Chi-squared testing. Regression models were used to determine relationships between dependent and independent variables.

Iohexol GFR was compared to eGFR methods using Pearson correlation and Bland Altman plots(440). Precision and accuracy of methods and between-method analysis was expressed as limits of agreement (LOA; within 1.96 SD of the mean and containing 95% of results) and percentage of eGFR values falling within 10% (P10) and 30% (P30) of mGFR. Paired T-testing was used for between-method comparison. Pearson correlations were performed to assess relationships between methods. SPSS® (IBM®, version 25.0) was used for analysis of data and two-tailed p values of <0.05 were deemed statistically significant.

5.4 Results

5.4.1 Demographics

Data from 85 patients were analysed. This study cohort had a mean(±SD) age of 48.4(±7.70) years. Baseline demographics are shown in table 5.5.

Variable	Patients
Age (years) mean (±SD)	48.4 (7.70)
BMI (kg/m^2) mean (±SD)	23.9 (3.13)
$ppFEV_1$ (%) mean (±SD)	52.9% (23.3)
IV AG (days) median (IQR, range)	88.0 (188, 0-562)
PsA % (n)	65.9% (56)
PI % (n)	83.5% (71)
Male % (n)	65.9% (56)
CFTR genotypes I-III (severe) % (n)	72.9% (62)
CFRD % (n)	49.4% (42)
Raised systolic BP % (n)	18.8% (16)
Established on ACE inhibition % (n)	14.1% (12)
History of renal stones	7.1% (6)

Table 5.5: Demographics of study population (AG=aminoglycoside, PsA=chronic Pseudomonas infection, ACE-angiotensin converting enzyme, PI=exocrine pancreatic insufficiency).

18.8% of the total cohort had systemic hypertension, 7.1% had a history of renal stones and 49.4% were diabetic. 72.9% had a severe CFTR genotype (class I-III) and 83.5% have exocrine pancreatic insufficiency. Median IV aminoglycoside days over the preceding ten-year period

was 88.0 (IQR 188, range 0-562) days, in a cohort with relatively severe pulmonary disease and mean ppFEV$_1$ of 52.9(±23.3)%.

5.4.2 Descriptive data analysis

A complete renal data set was obtained for 77 patients. Renal blood biomarkers, excluding cystatin C, were analysed for 84 patients. One patient died during the study period prior to data collection. We were unable to obtain cystatin C samples in two patients and urine ACR samples for seven patients.

Renal parameters are shown in tables 5.6 and 5.7. Mean serum creatinine and cystatin C for the total cohort were 70.7(±16.4)µmol/L and 0.93(±0.17)mg/L respectively. Two patients had a raised baseline creatinine. One patient had a raised cystatin C (1.69mg/L), this patient having undergone renal transplantation in 2015. Fourteen patients (16.6%) had a baseline creatinine below the normal range (<59 µmol/L).

25% (n=21) of patients in our cohort had a baseline eGFR of <90 ml/min/1.73m^2 using CKD-EPIcreat, the recommended eGFR calculation used at our institution. However, only three of these patients had co-existing proteinuria. A diagnosis of CKD stage 3a and above, based on KDIGO classification; eGFR of <60 ml/min/1.73m^2, was seen in 3.6% (n=3) of patients using the CKD-EPIcysC formula (see table 5.8). None of these patients had an eGFR <60 ml/min/1.73m^2 with the remaining three eGFR formulae, however two patients had an eGFR of <90 ml/min/1.73m^2. No patients had a diagnosis of CKD above stage 3b.

Proteinuria (ACR >3mg/mmol) was seen in 16.9% of patients (n=13), with a median ACR of 0.45 (IQR 1.58, range 0-85.3) mg/mmol. Eight of these patients had CFRD and ten had a corresponding eGFR of <90 ml/min/1.73m^2 using GFR analysis from all four estimation equations, ranging from 63 to 87 ml/min/1.73m^2, the lowest results seen with CKD-EPIcysC. Two patients with raised ACR were already on angiotensin converting enzyme (ACE) inhibitors and a further ten with abnormal GFR within the total cohort were also on ACE inhibition

There was poor compliance with 24 hour urinary collection with only 14 patients (16.7%) completing this part of the study. Mean urinary creatinine clearance (CrCl) GFR was 114 (±38.4) ml/min/1.73m^2, with a range from 59 to 175 ml/min/1.73m^2. GFR comparison for this group is shown in table 5.9 (end of chapter 5).

Renal parameter	Total (n)	Minimum	Maximum	Mean	±SD
Creatinine (µmol/L)	84	36	115	70.7	16.4
MDRD eGFR (ml/min/1.73m^2)	84	59	171	98.6	22.7
CKD-EPIcreat eGFR (ml/min/1.73m^2)	84	64	127	100.0	14.2
CKD-EPIcysC eGFR (ml/min/1.73m^2)	82	40	119	90.7	17.8
CKD-EPIcreat+cysC eGFR (ml/min/1.73m^2)	82	61	122	95.4	14.7
Cystatin C (mg/L)	82	0.66	1.69	0.93	0.17
ACR (mg/mmol)	77	0	85.3	0.45*	1.58**
hsCRP mg/L	84	1	42	1.5*	8**

Table 5.6: Renal parameters for total group (=median,**=IQR).*

Mean ±SD	Total n=84	40-49 years n=57	50-59 years n=19	≥60 years n=8	P value
Creatinine (µmol/L)	70.7 (16.4)	70.9 (15.8)	70.2 (18.7)	70.6 (16.4)	0.986
Cystatin C	0.93 (0.17)	0.90 (0.17)	0.99 (0.16)	0.99 (0.14)	0.067

(mg/L)					
eGFR: MDRD (ml/min/1.73m^2)	98.6 (22.7)	100.6 (22.4)	94.7 (25.1)	94.3 (21.2)	0.524
eGFR: CKD-EPIcreat (ml/min/1.73m^2)	100.0 (14.2)	103.1 (13.5)	96.3 (13.9)	87.5 (10.8)	**0.005**
eGFR: CKD-EPIcysC (ml/min/1.73m^2)	90.7 (17.8)	96.1 (17.3)	80.8 (13.8)	76.6 (13.0)	**<0.001**
eGFR: CKD-EPIcreat+cysC (ml/min/1.73m^2)	95.4 (14.7)	100.0 (14.1)	87.4 (10.8)	82.9 (11.9)	**<0.001**
Urine ACR* (mg/mmol)	0.45 (1.58)	0.3 (1.15)	1.4 (5.5)	1.1 (1.35)	0.047
hsCRP* (mg/L)	5.0 (8)	6.0 (9)	5.0 (10)	2.5 (2)	0.471

*Table 5.7: Renal parameters between age groups. (*non-parametric testing used, median/IQR), {n=84 total cohort, n=77 ACR, n=82 cystatin C eGFR}.*

There is significantly lower eGFR with advancing age using the CKD-EPI equations; CKD-EPIcreat (p=0.005), CKD-EPIcysC (p<0.001) and CKD-EPIcreat+cysC (p<0.001) respectively. There was no significant difference in CrCl GFR between age groups.

There was a marginally higher creatinine for patients with CFRD than those without (72.0±17.6 vs 69.4±15.2 µmol/L), but this was not statistically significant (p=0.375). Estimated GFR was not significantly different between diabetic groups using any eGFR formula and although the risk of an eGFR of <90 ml/min/1.73m^2 was slightly higher in the group with CFRD (OR 1.2, 95% CI 0.48-2.7) this was not statistically significant. The prevalence of raised ACR was not significantly different between diabetic and non-diabetic groups using Chi squared testing; p=0.547.

Mean±SD cystatin C was significantly higher in diabetics then non-diabetics; 0.94±0.21 vs 0.91±0.12 mg/L, p=0.007. There was a positive Spearman correlation between cystatin C and IV aminoglycoside days; r=0.274, p=0.015. Cystatin C had no significant relationship to

hsCRP in our cohort. There was no statistical significance in IV aminoglycoside days between those with GFR less than 90 ml/min/1.73m^2 and those above. Patients with systemic hypertension were more likely to have a GFR of less than 90 ml/min/1.73m^2 (Chi squared; p=0.014).

eGFR equation	**G1 % (n)**	**G2 % (n)**	**G3a % (n)**	**G3b % (n)**	**Total abnormal (eGFR<90) % (n)**	**Total CKD G2 (eGFR<90) with proteinuria % (n)**	**Total CKD ≥ 3a (eGFR <60)**
MDRD	62.7% (52)	36.9% (31)	2.4% (2)	0	37.3% (31)	4.8% (4)	2.4% (2)
CKD-EPIcreat	75.3% (64)	24.7% (21)	0	0	25.3% (21)	3.6% (3)	0
CKD-EPIcysC	53% (44)	43.4% (36)	2.4% (2)	1.2% (1)	47.0% (39)	11.0% (9)	3.6% (3)
CKD-EPIcreat+cysC	69.9% (58)	30.1% (25)	0	0	30.1% (25)	7.3% (6)	0

Table 5.8: Proportion of patients with CKD staging G1-3b as per eGFR equation in total cohort.

5.4.3 eGFR method correlations

5.4.3.1 MDRD vs CKD-EPIcreat

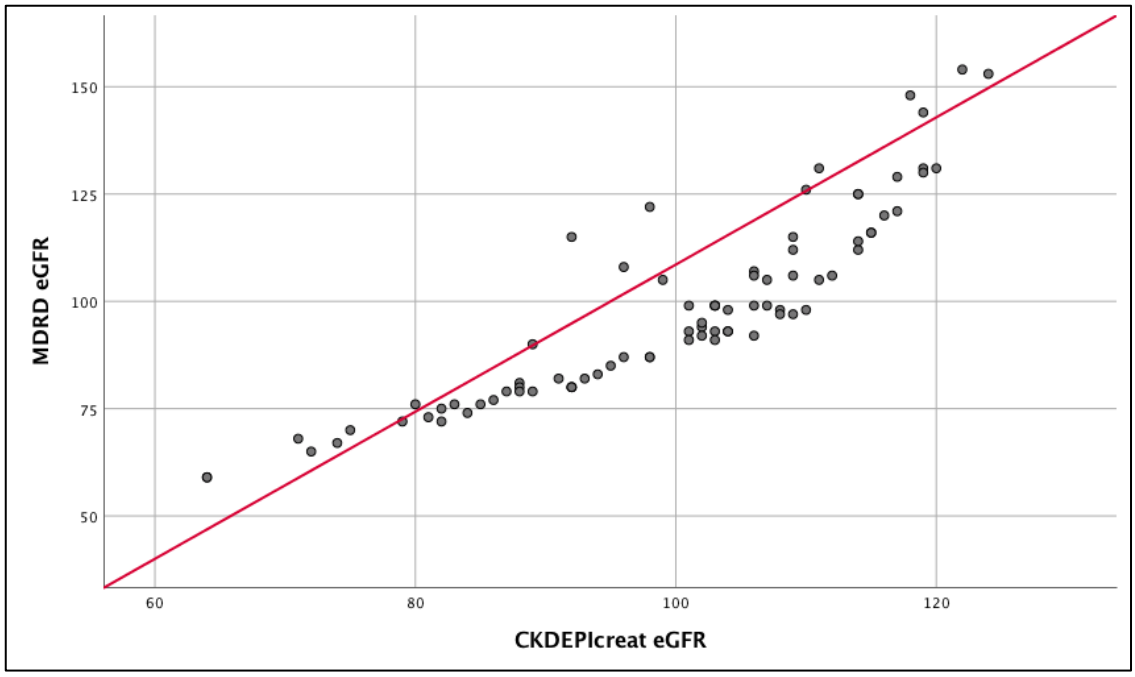


Figure 5.2: Correlation between MDRD and CKD-EPIcreat eGFR, in ml/min/1.73m^2 (Pearson r=0.924, p<0.001).

5.4.3.2 MDRD vs CKD-EPIcys

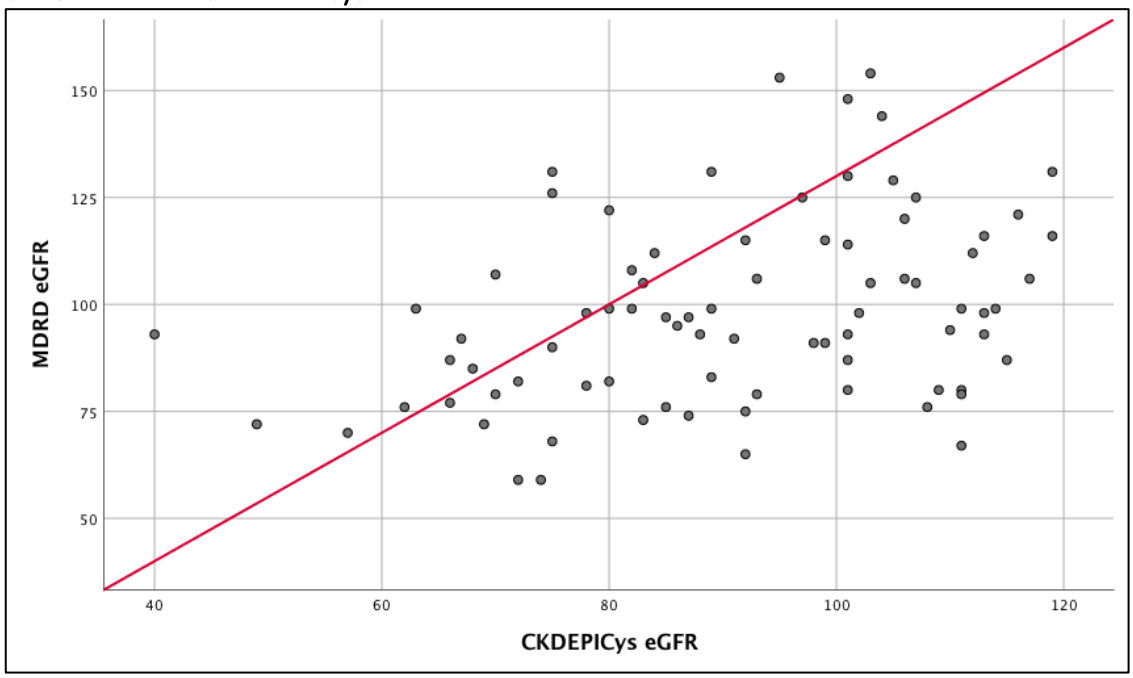


Figure 5.3: Correlation between MDRD and CKD-EPIcysC eGFR, in ml/min/1.73m^2 {Pearson r=0.366, p=0.001).

5.4.3.3 MDRD vs CKD-EPIcreat+cys

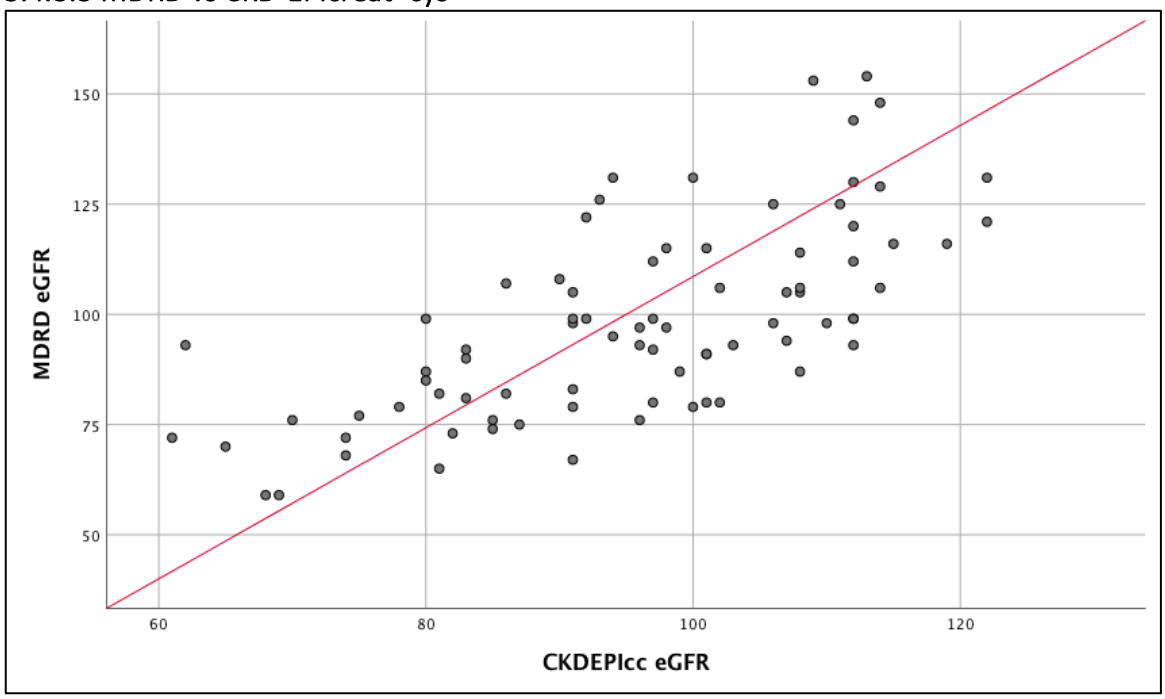


Figure 5.4: Correlation between MDRD and CKD-EPIcreat+cysC eGFR, in ml/min/1.73m^2 {Pearson r=0.703, p<0.001}.

5.4.3.4 CKD-EPIcreat vs CKD-EPIcys

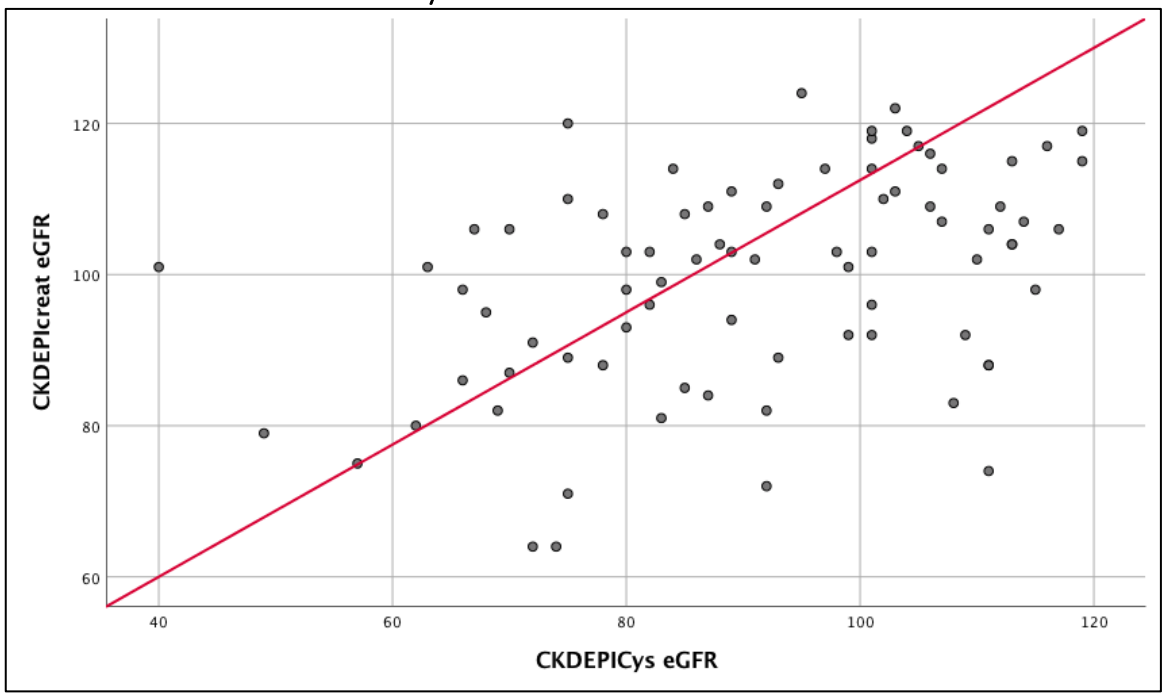


Figure 5.5: Correlation between CKD-EPIcreat and CKD-EPIcysC eGFR, in ml/min/1.73m^2 (Pearson r=0.453, p<0.001).

5.4.3.5 CKD-EPIcreat vs CKD-EPIcreat+cys

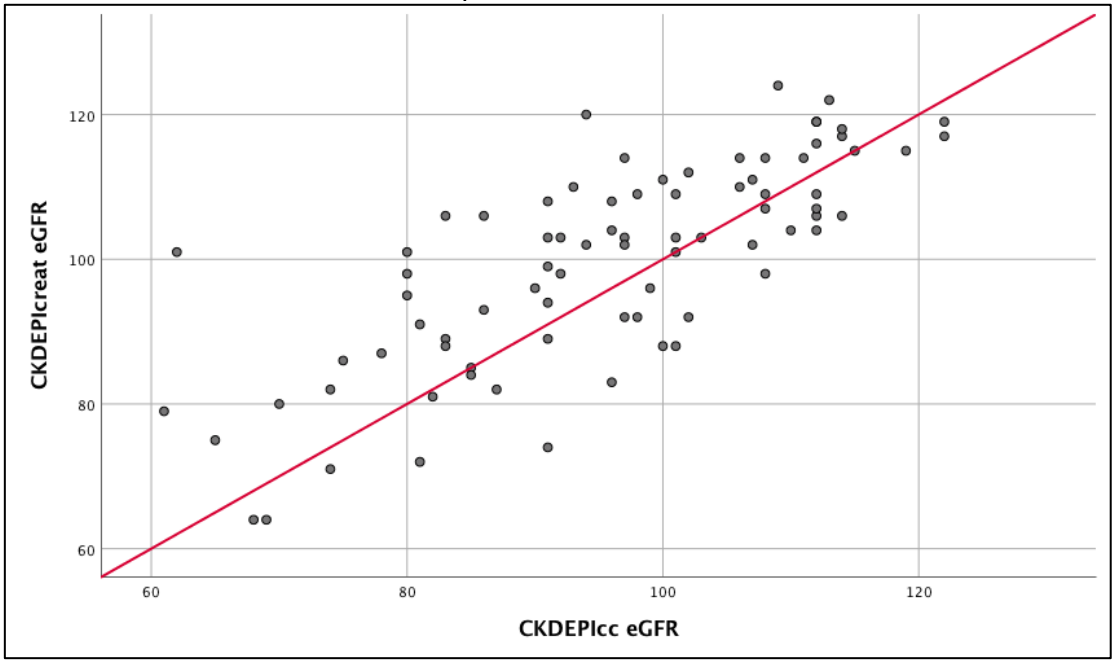


Figure 5.6: Correlation between CKD-EPIcreat and CKD-EPIcreat+cysC eGFR, in ml/min/1.73m^2 (Pearson r=0.769, p<0.001).

5.4.3.6 CKD-EPIcys vs CKD-EPIcreat+cys

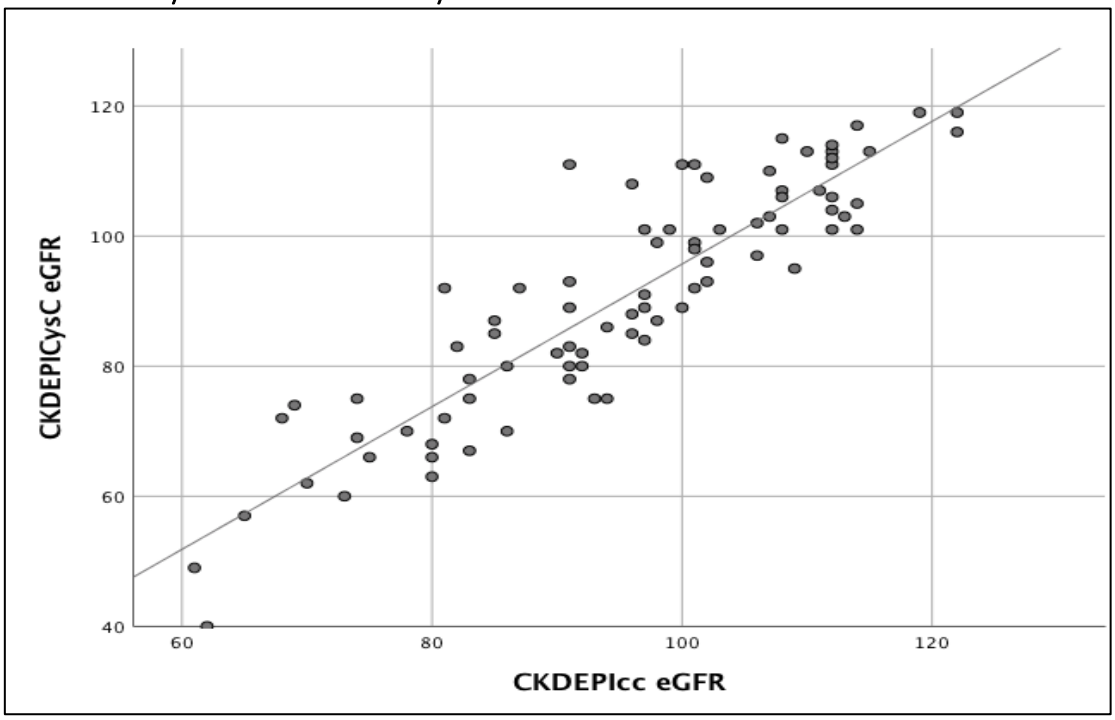


Figure 5.7: Correlation between CKD-EPIcysC and CKD-EPIcreat+cysC (cc) eGFR, in ml/min/1.73m^2 (Pearson r=0.890, p<0.001).

eGFR method comparison	Correlation coefficient (r)	P value	Agreement between methods using T test?	Agreement P value (T-test)	BA plot required?
MDRD vs CKD-EPIcreat	0.891	<0.001	Yes	0.150	Yes
MDRD vs CKD-EPIcysC	0.358	0.001	No	0.006	No
MDRD vs CKD-EPIcreat+cysC	0.684	<0.001	Yes	0.165	Yes
CKD-EPIcreat vs CKD-EPIcysC	0.418	<0.001	No	<0.001	No
CKD-EPIcreat vs CKD-EPIcreat+cysC	0.761	<0.001	No	<0.001	No
CKD-EPIcysC vs CKD-EPIcreat+cysC	0.902	<0.001	No	<0.001	No

Table 5.10: Correlation coefficients and agreement (via one-sample T-test) between eGFR methods (BA=Bland Altman plot).

The strongest correlation was seen between CKD-EPIcysC and CKD-EPIcreat+cysC (r=0.902, P<0.001). Positive correlations were also shown between all other paired eGFR methods, the weakest being between MDRD and CKD-EPIcysC (r=0.358), but all with statistical significance.

Subsequent one sample T-testing only revealed agreement (i.e. a non-significant difference) between MDRD and CKD-EPIcreat, and MDRD and CKD-EPIcreat+cysC (p=0.150 and p=0.165 respectively). Bland Altman plots were then constructed to assess limits of agreement and bias between these methods.

5.4.4 Bland Altman analysis

5.4.4.1 MDRD vs CKD-EPIcreat

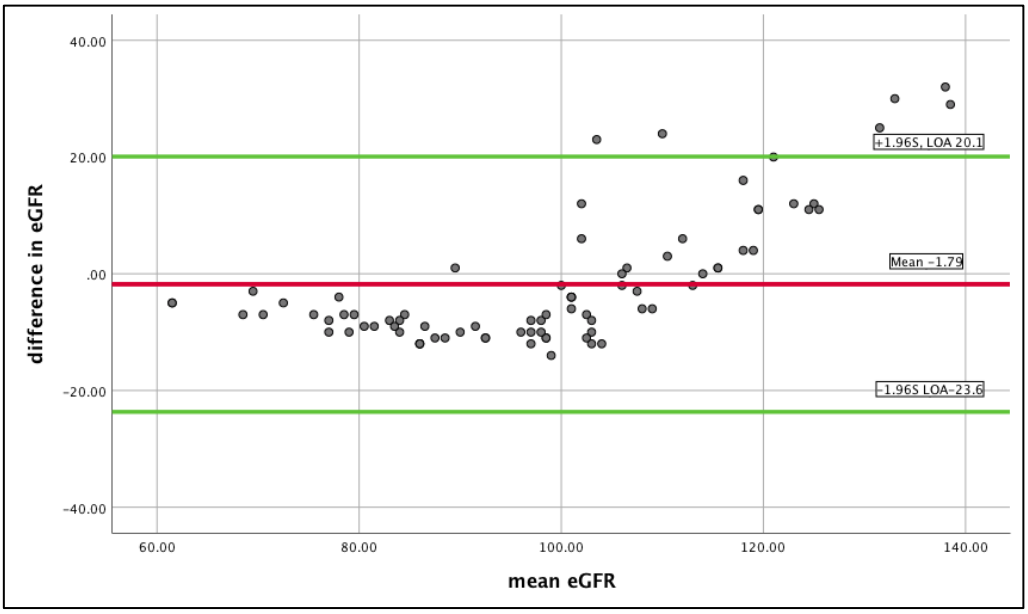


Figure 5.8: Bland Altman plot of the MDRD vs CKD-EPIcreat, expressed in ml/min/1.73m^2 (LOA=limits of agreement).

The majority of values lie within +/-1.96 SD of the mean. Data points show a trend of higher GFR difference with increasing mean GFR, with some results lying outside of the upper limit of agreement (LOA). Logistic regression shows proportional bias between methods; r^2 = 0.502, p<0.001, higher mean eGFR = larger difference in eGFR between methods.

5.4.4.2 MDRD vs CKD-EPIcreat+cys

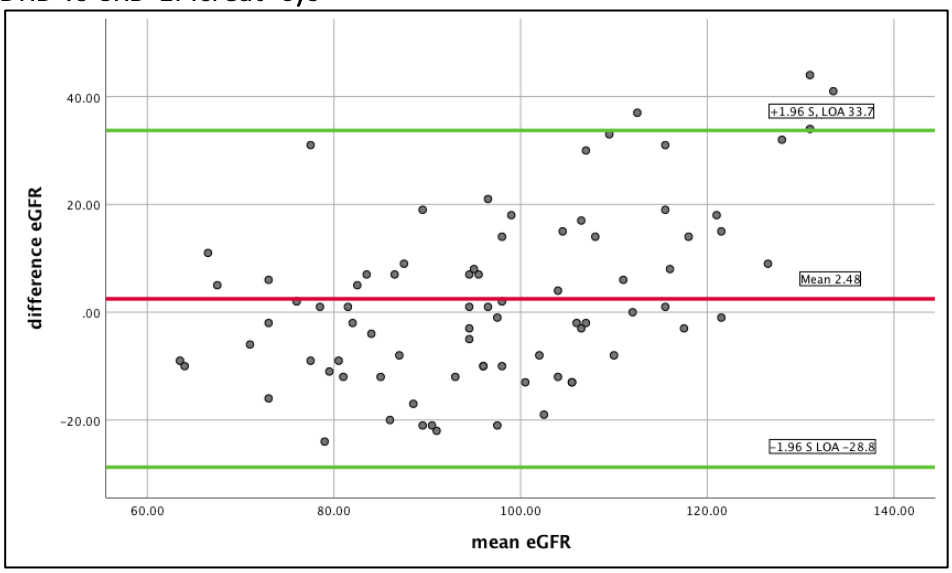


Figure 5.9: Bland Altman plot of the MDRD vs CKD-EPIcreat+cysC, expressed in ml/min/1.73m^2.

The majority of values lie within +/-1.96 SD of the mean. The majority of results lie within the limits of agreement for these methods, however there is again a trend towards higher GFR difference with increasing mean GFR. Logistic regression shows proportional bias between methods; $r^2 = 0.239$, $p<0.001$, with a larger difference in eGFR with increasing mean eGFR

5.4.5 Comparison between iohexol mGFR and eGFR methods

5.4.5.1 General iohexol analysis

Nine patients were recruited for iohexol GFR analysis. The mean age of this smaller cohort was 48.3(±6.4) years. Seven (77.8%) had a diagnosis of CFRD. Mean ppFEV$_1$ was 46.3(±20.2)%, mean BMI was 25.4(±2.0) kg/m^2. Cohort demographics and renal parameters for these patients are shown in table 5.11 and 5.12 (end of chapter 5 results section). Iohexol mGFR and eGFR data is shown in table 5.13 (end of results section). There was a significant difference in GFR results between iohexol mGFR and all eGFR methods using T-testing ($p<0.001$), with underestimation of GFR for all patients using estimated methods.

There was poor correlation between eGFR and iohexol GFR for all estimated methods and mean GFR was significantly different between methods ($p<0.001$). Mean difference between methods (bias) was the highest for CKD-EPIcysC at 30.4(±18.4)ml/min/1.73m^2. Accuracy within 10% (P10) was poor for all eGFR methods, the highest observed for CKD-EPIcreat with 33% of values lying with 10% of iohexol GFR. Accuracy within 30% (P30) was superior for the CKD-EPI equations; CKD-EPIcreat 78%, CKD-EPIcreat+cysC 78%, CKD-EPIcysC 67% (see table 5.13).

Bland Altman plots were constructed for each eGFR method and iohexol GFR for assessment of bias and the extent of inaccuracy between methods.

5.4.5.2 Iohexol vs MDRD

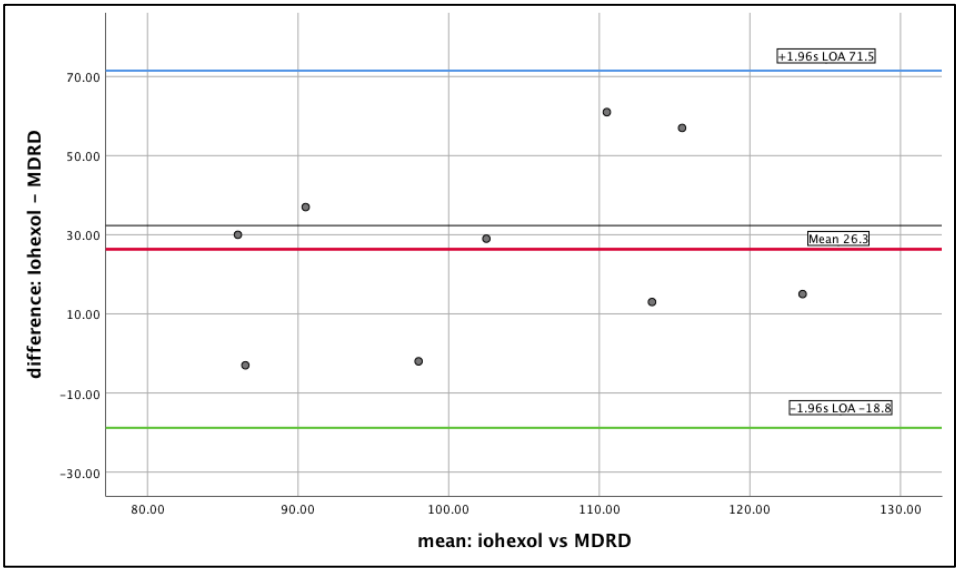


Figure 5.10: Bland Altman plot of iohexol and MDRD GFR method comparison (expressed in ml/min/1.73m^2).

5.4.5.3 Iohexol vs CKD-EPIcreat

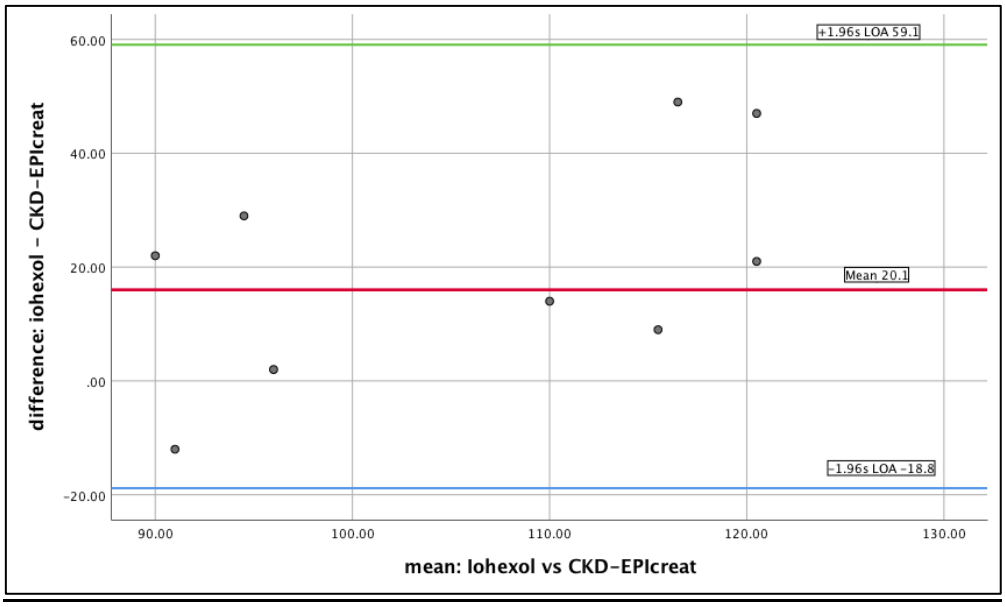


Figure 5.11: Bland Altman plot of iohexol and CKD-EPIcreat GFR method comparison (expressed in ml/min/1.73m^2).

5.4.5.4 Iohexol vs CKD-EPIcys

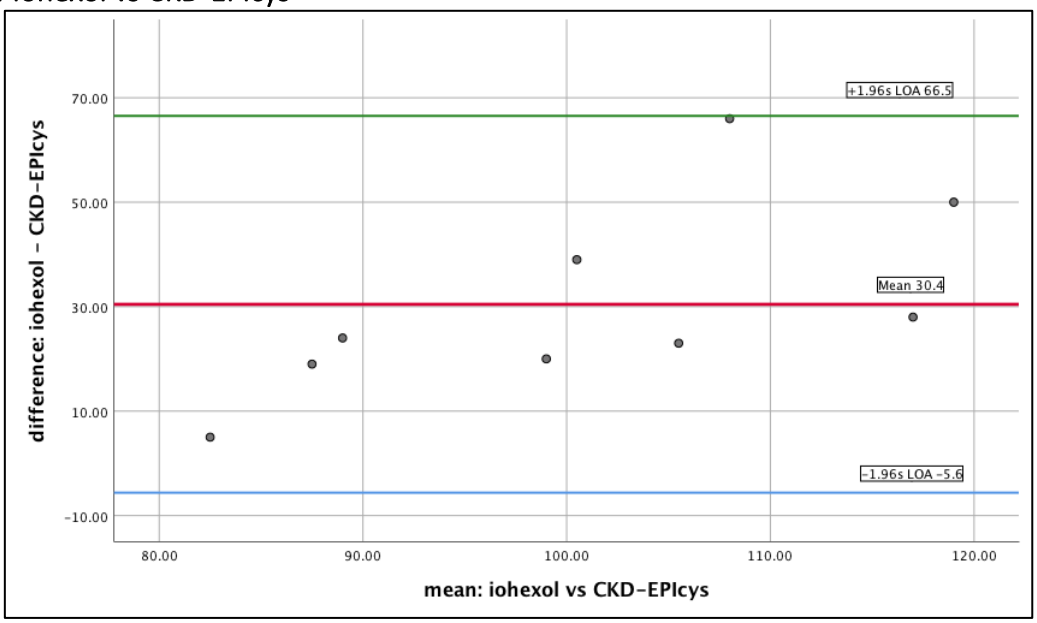


Figure 5.12: Bland Altman plot of iohexol and CKD-EPIcysC GFR method comparison (expressed in ml/min/1.73m^2).

5.4.5.5 Iohexol vs CKD-EPIcreat+cys

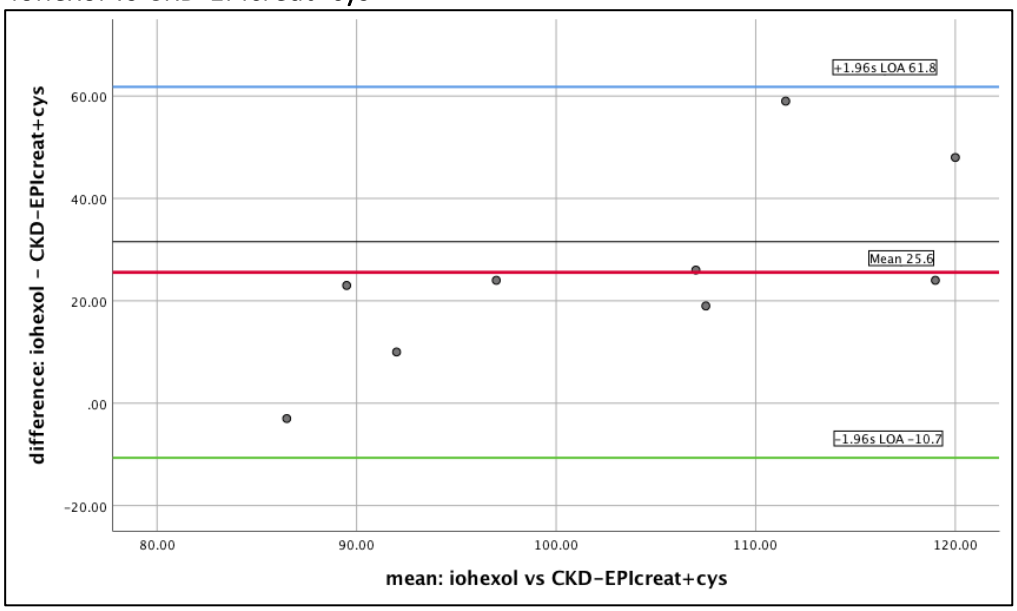


Figure 5.13: Bland Altman plot of iohexol and CKD-EPIcreat+cysC GFR method comparison (expressed in ml/min/1.73m^2).

Although patient numbers are small, we can see that the vertical spread of data points is larger at higher mean GFR values. This indicates that the difference between methods, and hence the bias, becomes more apparent with increasing mean GFR for all eGFR methods compared to iohexol. Small patient numbers also produce large limits of agreement (LOA) for each method comparison, leading to a high systematic difference between methods,

indicating that, in this patient group, each eGFR method underestimates GFR compared to iohexol. We cannot confidently assess the degree of proportional bias with regression analysis due to small patient numbers and lack of significant correlation between iohexol GFR and eGFR methods.

Patient	MDRD eGFR	CKD-EPIcreat eGFR	CKD-EPIcysC eGFR	CKD-EPIcreat+cysC eGFR	CrCl GFR
2	90	89	75	83	109
3	105	111	103	107	117
9	80	92	109	102	117
10	93	104	113	112	94
17	108	96	82	90	63
18	87	96	101	99	145
22	122	98	80	92	127
24	98	108	78	91	77
38	81	88	78	83	90
39	115	92	99	98	189
49	82	91	72	81	59
50	105	107	107	108	175
61	105	99	83	91	135
63	79	87	70	78	100

Table 5.9: Creatinine clearance (CrCl) GFR in comparison to eGFR using MDRD and CKD-EPI (n=14). All data expressed in ml/min/1.73m^2.

Patient	Age (years)	ppFEV1 (%)	BMI (kg/m^2)	BSA (m^2)	Serum creatinine (µmol/L)	Serum cystatin (C mg/L)	ACR (mg/mmol)
1	42	25	26.6	2.17	70	1.02	0.3
2	45	59	21.6	1.54	69	1.02	3.1
3	48	39	24.8	1.61	82	0.90	0
4	42	36	24.4	1.56	64	0.87	1.7
5	46	91	26	1.68	98	1.05	0
6	49	58	27.4	1.90	96	0.93	0
7	63	32	26	1.88	70	0.99	0.9
8	52	42	27.9	1.92	80	1.0	13.3
9	48	35	23.5	1.79	64	0.84	0
Mean(±SD)	48.3 (6.4)	46.3 (20.2)	25.4 (2.0)	1.78 (0.21)	77.0 (12.9)	0.96 (0.07)	2.14 (4.31)

Table 5.11: Demographic data of total renal cohort at time of GFR testing.

Patient	eGFR MDRD (ml/min/1.73m^2)	eGFR CKD-EPIcreat (ml/min/1.73m^2)	eGFR CKD-EPIcysC (ml/min/1.73m^2)	eGFR CKD-EPIcreat+cysC (ml/min/1.73m^2)	24 hour urinary creatinine clearance (CrCl) GFR (ml/min/1.73m^2)	Iohexol GFR (ml/min/1.73m^2)
1	107	111	81	94	77	120
2	80	92	75	82	-	141
3	87	97	94	96	-	144
4	88	103	94	98	-	117
5	71	79	77	78	-	101
6	72	80	89	85	-	109
7	99	95	78	87	-	97
8	88	97	80	88	100	85

9	116	110	103	107	161	131
Mean (±SD)	89.8 (15.2)	96.0 (11.4)	85.7 (9.7)	90.6 (9.0)	112.7 (43.4)	116.1 (20.1)

Table 5.12: Evaluation of GFR methods for each patient undergoing iohexol GFR

eGFR method	**Mean**	**Correlation between eGFR and mGFR; r (95% CI)**	**P value**	**Bias (mean diff) (SD) (ml/min/1.73 m²)**	**95% CI for bias**	**P value (mGFR and bias)**	**Limits of agreement (LOA)**	**Accuracy within 10% (P10)**	**Accuracy within 30% (P10)**
eGFR MDRD ml/min/1.73m²	89.8 (15.2)	0.173 (-0.55, 0.75)	0.65	26.3 (23.0)	8.6, 44.0	<0.001	-18.8, 71.5	22.2%	44.4%
eGFR CKD-EPIcreat ml/min/1.73m²	96.0 (11.4)	0.306 (-0.45, 0.81)	0.42	20.1 (19.9)	4.8, 35.4	<0.001	-18.8, 59.1	33.3%	77.8%
eGFR CKD-EPIcysC ml/min/1.73m²	85.7 (9.7)	0.412 (-0.35, 0.85)	0.27	30.4 (18.4)	16.3, 44.6	<0.001	-5.6, 66.5	22.2%	66.7%
eGFR CKD-EPIcreat+cysC ml/min/1.73m²	90.6 (9.0)	0.400 (-0.36, 0.84)	0.16	25.6 (18.5)	11.3, 39.8	<0.001	-10.7, 61.8	22.2%	77.8%

Table 5.13: Iohexol GFR comparison with eGFR methods; bias and accuracy. (eGFR equations evaluated using NKF/KDIGO guidelines, LOA=limits of agreement – within 1.96 SD of the mean and containing 95% of results. Mean diff/bias = iohexol-eGFR method).

5.5 Discussion

5.5.1 General discussion

This is the first study to analyse GFR using iohexol and cystatin C methods exclusively in a CF population aged 40 years and above. Our findings demonstrate a high frequency of renal dysfunction in our older CF cohort, with up to 46.4% prevalence of eGFR <90ml/min/1.73m^2, a 3.6% prevalence of CKD stage 3 and a 16.9% prevalence of proteinuria. Decreasing eGFR was seen with advancing age, as expected. There was a significant relationship between the presence of systemic hypertension and abnormal GFR (p=0.014) and serum cystatin C showed a positive correlation with IV aminoglycoside days (r=0.274, p=0.015). The risk of GFR <90ml/min/1.73m^2 was slightly higher and the mean serum creatinine slightly higher in the CFRD group compared to the non-CFRD group, although these results were not statistically significant.

An underestimation of GFR using MDRD and CKD-EPI methods was observed in a small subgroup compared to iohexol mGFR, indicating the inaccuracy of estimated GFR in this population. Cystatin-C based eGFR formulae have the highest accuracy on between-method comparison with iohexol mGFR, and hence may be the most useful marker of renal function in ageing CF patients.

Renal dysfunction in the acute and chronic setting has adverse implications for morbidity and mortality in the general population(441). Acute renal failure is being increasingly recognised in CF(442) and the prevalence of CKD (stage 3 and above) in CF is currently estimated to be as high as 11% depending on lung transplant status, age and the presence of CFRD(443). Increasing survival in CF may lead to an increasing prevalence of CKD, influenced by an increasing rate of CFRD, organ transplantation at older ages and cumulative aminoglycoside exposure. The emergence of CKD in an ageing CF population may also have detrimental implications for cardiovascular risk.

Estimated GFR formulae perform optimally in the populations from which they were created. Given that MDRD and CKD-EPI equations were derived from CKD population data, they are the most accurate in those with lower than normal baseline GFR. The reliability of these equations in a CF population, in which the majority of patients are younger and have a normal GFR, may be reduced and the consideration of more accurate GFR methods in CF is

required. The inaccuracies of renal monitoring in CF using creatinine-based estimated GFR has led to the study of novel biomarkers, such as cystatin C, and method comparison using exogenous clearance GFR. However, a lack of universally accepted robust GFR method in CF still exists. The study of GFR in the CF population is paramount in the context of increasing survival, accumulation of ageing comorbidities and an emerging renal risk.

5.5.2 Prevalence of renal dysfunction

In our cohort, there was a spectrum of disease severity, with a ppFEV$_1$ ranging from 18 to 100% (mean 52.9±23.3%). There was a relatively well preserved nutritional status with a mean BMI of 23.9(±3.13)mg/kg/m^2, however the majority (83.5%) were exocrine pancreatic insufficient. 25% of patients in our cohort had a baseline eGFR of <90 ml/min/1.73m^2 using CKD-EPIcreat, the accepted eGFR method at MFT. However, those with a diagnosis of CKD stage G1 and G2, defined by coexistence of proteinuria, were less and ranged from 3 to 11% depending on eGFR formula used. In addition, 3.6% (n=3) had a diagnosis of CKD stage 3 (G3) and eGFR reduction was seen with advancing age. The prevalence of CKD stage 3 in our cohort appears slightly higher than that of the general adult CF population. In a large study by Quon and colleagues (2011), analysing US registry data for CF patients aged ≥18 years, an annual CKD stage 3 prevalence of 2.3% was observed, which increased with age from 0.6% in the 18 to 25 year age to group to 19.2% aged 55 years and above(220). A retrospective study by Wilcox et al reported a 5.1% prevalence of renal disease in an adult CF cohort(444), although additionally reporting episodes of acute kidney injury that were not studied here. The most prevalent pathology found in this study was renal stones at 2%. A higher prevalence of renal stones was found in our older adult cohort (7%), perhaps representing a higher risk if nephrolithiasis with age in CF.

5.5.3 Inaccuracies of estimated GFR

Several studies to date, in younger cohorts, have proven the use of endogenous creatinine-based eGFR calculations inaccurate in the CF population. 16.7% of the patients in our study had a low baseline serum creatinine level and hence creatinine-based eGFR values may over-estimate true GFR. Al-Aloul and colleagues observed overestimation of GFR using MDRD and CG formulae when compared to measured urinary CrCl (by 18.3 and 15.8 ml/min/1.73m^2 respectively), particularly those with a reduced CrCl of <80ml/min(233).

Touw and colleagues similarly observed overestimation of GFR in adult CF patients using predictive GFR equations when compared to measured CrCl(238).

The study of novel endogenous biomarkers has therefore become important in CF renal research. Cystatin C has been studied extensively within the non-CF population as an alternative or in addition to eGFR calculations, in order to improve accuracy. Results are variable but a number of studies have shown its superiority over serum creatinine for eGFR calculation in certain patient groups, particularly in those with type two diabetes mellitus(237). Coll and colleagues observed creatinine-based formulae to overestimate GFR in patients with GFR <90 ml/min/1.73m^2 using iothalamate comparison. This study also reported a rise in cystatin C at higher GFR levels than creatinine (88 vs 75ml/min/1.73m^2), concluding that cystatin C may be a more reliable determination of GFR in those with normal or mildly impaired renal function(445). Cystatin C has also been found to be superior to creatinine in estimating GFR for those with spinal cord injury(446) and liver cirrhosis(447), both of these patient populations exhibiting significant muscle wasting, conferring relevance of cystatin C eGFR study in the CF population. Cystatin C-based eGFR measurement is now recommended by KDIGO as part of CKD assessment if eGFR, using creatinine-based methods, is <60ml/min/1.73m^2(259).

Comparison of estimated GFR formulae in our older cohort showed good correlation between methods, particularly between CKD-EPIcysC and CKD-EPIcreat+cysC (r=0.902, p<0.001). However, significant differences in agreement between eGFR were seen for MDRD vs CKD-EPIcysC and CKD-EPIcreat vs CKD-EPIcreat+cysC. Agreement was shown between MDRD and CKD-EPIcreat, and MDRD and CKD-EPIcreat+cysC using T-testing, however Bland Altman analysis between these methods showed a degree of proportional bias, with higher mean differences with increasing mean GFR. A greater degree of abnormal eGFR (<90ml/min/1.73m^2) was detected using CKD-EPIcysC than with other eGFR formulae in our older CF group; 46.4% compared to 36.9% (MDRD), 25% (CKD-EPIcreat) and 29.8% (CKD-EPIcreat+cysC). It seems possible that the inclusion of both creatinine and cystatin C levels within the CKD-EPIcreat+cysC formula would increase the accuracy of estimated GFR measurement. However, given the unreliability of serum creatinine in the CF population as a whole, CKD-EPIcysC eGFR could be the most useful method, particularly in detecting subtle renal abnormalities in a cohort predominantly with normal renal function.

Several studies to date have investigated the accuracy of cystatin C in CF for GFR determination. Beringer and colleagues studied serum cystatin C in an adult CF cohort, showing greater accuracy of eGFR using serum cystatin C than for creatinine-based methods when compared to iothalamate GFR(189). Halacova and colleagues observed cystatin C to be more reliable in eGFR monitoring in patients receiving intravenous amikacin than comparative creatinine clearance methods(236). Soulsby and colleagues showed cystatin C eGFR to be the most accurate compared to ^{99m}TC-DTPA radioisotope GFR in an adult CF population(424), although this was not reflected in results of a similar method in the paediatric CF population, perhaps reflecting a greater impact of low muscle mass on creatinine levels with longevity of malnutrition(263). In addition, serum cystatin C levels may be affected by a high hsCRP level, corresponding to systemic inflammation, and leading to a risk of GFR underestimation using this method. In our cohort, cystatin C levels had a positive correlation with IV aminoglycoside days ($r=0.327$, $p=0.003$), highlighting a potentially important emerging relationship between aminoglycoside use and renal dysfunction in an older CF population. There was no significant correlation between cystatin C and hsCRP. However, with a raised mean baseline hsCRP (7.8±8.7mg/L) and a high prevalence of severe disease phenotypes, it is possible that systemic inflammation contributed to eGFR underestimation using cystatin-based CKD-EPI equations in this older CF population.

In CF patients, with a low prevalence of overt renal dysfunction, analysis of renal function decline over time may be a more useful management approach, particularly in higher risk groups such as those with CFRD, a high pulmonary exacerbation frequency and following organ transplantation. The ease of cystatin C measurement in serum and its increased reliability over creatinine-based eGFR, may deem this method suitable for more widespread use within the CF population.

5.5.4 Iohexol GFR

From the nine patients undergoing Iohexol GFR, eight had a lower corresponding estimated GFR with all four eGFR formulae compared to measured GFR. Patients two and three had a significantly higher iohexol GFR when compared to the highest eGFR comparator; a difference of 49 and 47 ml/min/1.73m^2 respectively. Patients seven and eight had the closest iohexol GFR when compared to eGFR methods, within 19 ml/min/1.73m^2. However,

MDRD appeared to over-estimate GFR in patient 7, and all eGFR methods except CKD-EPIcysC overestimated GFR in patient 8 (table 5.12). GFR underestimation has been seen in several iohexol GFR studies in type two diabetic populations, including underestimation of GFR decline over time(412)(435).

Although iohexol GFR has been extensively studied in the CKD population, it is less well researched in CF. The majority of CF GFR studies have utilised radioisotope methods as the gold standard exogenous GFR measure and none so far have examined a CF population aged 40 years and above. Studies in a paediatric(263) and adult cohort(189) have used isotope GFR to highlight the inaccuracies in estimated GFR. The only published study to date using iohexol GFR in CF was performed by Novel-Caitin et al in 2016(235), studying the renal function of 25 adult CF patients with advanced pulmonary disease prior to lung transplantation. CKD-EPI eGFR was shown to over-estimate mGFR in this cohort, in one patient by up to 31 ml/min. Although our results are contradictory to this, Prestidge and colleagues reported underestimation of eGFR in CF children using an isotope GFR method, a similar result as observed in our older CF cohort(429). The LC-MS method used for iohexol sample analysis at MFT was compared against a validated method used by a Belgian group(262), showing only a 3.5% bias in our results over a 60-sample comparison process. The difference between iohexol GFR and eGFR in our study could thus not be fully explained by laboratory error. It may be that eGFR underestimation is reflective of an older CF group, however a small sample size undoubtedly limited our analysis. Larger studies would be necessary to develop any firm trends in GFR in this group.

There was poor correlation between eGFR and iohexol GFR for all estimated methods and mean GFR was significantly different between methods ($p<0.001$). Bland Altman analysis, although challenging to interpret with small patient numbers, showed a trend towards higher GFR difference with increasing mean GFR. Although analysis showed no outlying data points, there were wide limits of agreement for each method comparison. Mean difference between methods (bias) was the highest for CKD-EPIcysC at 30.4±18.4 ml/min/1.73m^2. Accuracy within 10% (P10) was poor for all eGFR methods, the highest observed for CKD-EPIcreat with 33% of values lying with 10% of iohexol GFR. Accuracy within 30% (P30) was superior for the CKD-EPI equations; CKD-EPIcreat 78%, CKD-EPIcreat+cysC 78%, CKD-EPIcysC 67%. Although CKD-EPIcysC has the highest detection rate of low GFR in our cohort, CKD-

EPIcreat+cysC appears to give the highest P30 GFR accuracy when compared to iohexol, hence may provide a more accurate GFR estimate in our CF cohort (table 5.13). Tidman and colleagues also observed an improved accuracy within 30% of iohexol GFR with eGFR formulae combining serum creatinine and cystatin C in a population with varying degrees of CKD(437).

The wider use of iohexol GFR in a CF clinical setting is likely not feasible in a multi-sample protocol. A single sample protocol would present more viability, however, still requires a prolonged sampling period after iohexol injection depending on baseline renal function. It may be that this technique could be used to calculate accurate GFR in selective higher risk CF patient groups such as pre and post organ transplantation, those with known renal dysfunction in the context of optimal nephrotoxic medication dosing and in monitoring rate of renal decline.

5.5.5 Urinary creatinine clearance

Only three patients were able to provide a 24-hour urinary sample for calculation of creatinine clearance (CrCl) in the iohexol cohort. The CrCl of patient one showed an underestimation of GFR; iohexol vs CrCl: 120 vs 77ml/min/1.73m^2, whilst patient eight and nine represented over-estimation of GFR using this method; 85 vs 100 and 131 vs 161ml/min/1.73m^2 (see table 5.9). This may reflect the inaccuracy of this method in calculating GFR. In the total cohort, we observed a higher mean eGFR with 24-hour urinary creatinine clearance GFR compared to MDRD and CKD-EPI formulae (114.1±38.4 ml/min/1.73m^2), however this did not significantly increase with advancing age. Further analysis of urinary creatinine clearance was challenging due to low patient numbers. Creatinine clearance has historically been the gold standard measurement of GFR in CF due to an increased reliability over estimated GFR using serum biomarkers. However, a low serum creatinine may overestimate creatinine clearance and GFR, relevant in a CF population with nutritional impairment and low muscle mass(448). The lack of adherence to this part of the study, in both the total and iohexol cohorts, highlights the relative impracticality of this test in CF renal monitoring and emphasises the need for other methods of accurate GFR monitoring in CF to be studied.

5.5.6 Practicalities of iohexol GFR

Iohexol GFR measurement is time consuming and cumbersome, however single sample iohexol protocols may help to alleviate some impracticality and allow for more accurate GFR to be obtained in specific patient groups. Single sample protocols are recommended as an accurate GFR measure only for those with normal or mildly impaired renal function(433). The CF population, as a chronic disease cohort, comprises relatively small numbers within the context of the vast general population, thus may be a good target for further mGFR investigation, particularly in view of emerging renal and cardiovascular risk with increasing survival. In this study, I used a multi-sample iohexol protocol due to its greater weight of evidence of accuracy in the literature(410)(261)(263)(235), however the time-consuming nature of this technique certainly limited patient recruitment.

In 2018, Zhang and colleagues observed accuracy in a single sample iohexol GFR method in participants with eGFR >60ml/min/1.73m^2 (449), a protocol that may have relevance in a CF population the majority of whom will have normal renal function. James and colleagues validated the accuracy of an iohexol GFR method with a single serum sample taken between two and four hours(450), eradicating the need for multiple venepuncture, particularly relevant in CF patients with poor peripheral venous access. A study by Rizk et al in 2018 reported close correlation (r=0.996) between iohexol mGFR and GFR measured by injection of a novel exogenous biomarker, visible fluorescent injectate (VFI), in a study group consisting of patients with both normal and impaired renal function(451). The VFI mGFR method used blood sampling over a shorter time period and required less volume for analysis. Could the development of a rapid and effective bedside mGFR test therefore be feasible in the CF population?

The study of capillary iohexol GFR monitoring, if validated against serum iohexol clearance, could potentially provide an accurate GFR method for patients without the need for venepuncture. Mafham and colleagues published work with capillary dried blood spot analysis for iohexol GFR, observing reliability of one, two and three sample models compared to estimated GFR in those with baseline GFR of <60ml/min/1.73m^2(452). Less accuracy and wider limits of agreement were seen for capillary iohexol and eGFR at higher baseline renal function, indicating the need for further study in these patient groups. We performed capillary iohexol sampling for the nine patients in the iohexol study arm,

however unfortunately we have been unable to process these due to the Covid-19 pandemic. Analysis of these samples against serum iohexol GFR and corresponding GFR will be informative, and may assist to validate this method in patients without CKD.

5.5.7 Renal dysfunction and organ transplantation

One of our study participants was a renal and pancreatic transplant recipient, with a degree of existing renal impairment; estimated GFR ranging from 93 ml/min/1.73m^2 using MDRD to 40 ml/min/1.73m^2 using CKD-EPIcysC. Three patients (3.5%) in our older CF cohort received a double lung transplantation during the study period, indicating not only the severity of CFTR-related disease in this older CF cohort but also the importance of renal monitoring in the pre and post-transplant settings. The risk of chronic renal dysfunction following lung transplantation has been shown to be as high as 90%(232). Quattrucci et al observed CKD to be the most frequent non-infective complication following CF lung transplantation over a six year period(453), and Lefaucheur and colleagues reported a 30% GFR decline in over 30% of their CF transplant group within the first year(454). A recent retrospective study of 29 patients by Florens and colleagues (May 2020), including 16 CF patients, with pre and post-transplant iohexol or inulin mGFR results, observed a 66% prevalence of CKD stage $\geq$3 and 60% of this cohort had >50% decrease in mGFR at one year following lung transplantation(455). Although the preferential use of tacrolimus over ciclosporin has assisted to abate the risk of renal impairment following organ transplantation, isotope GFR and other exogenous methods are not routinely performed in CF patients following lung transplantation in the UK(426). The accumulation of ageing comorbidities with increasing survival in CF may necessitate closer renal monitoring both pre and post-transplantation, and renal disease may affect lung transplant eligibility. Accurate GFR monitoring is essential for optimal immunosuppressant dosing, early detection of renal dysfunction and adequate management of renal complications following organ transplantation.

5.5.8 Intravenous aminoglycoside exposure

The median intravenous aminoglycoside exposure within the previous ten-year period was relatively high for our study group (83.0, IQR 188 days), with a maximum value of 562 days, indicating a potential risk of aminoglycoside-induced renal damage in this cohort. However, there was no significant relationship between IV aminoglycoside days and eGFR <90ml/min/1.73m^2. Intermittent exposure to intravenous aminoglycoside, used for the

treatment of pulmonary exacerbation in CF, will increase in proportion to age and hence accurate dosing is essential to avoid acute and chronic renal tubular damage with cumulative exposure. The risk of acute nephrotoxicity from aminoglycoside exposure is well documented. Pederson and colleagues report a 39% prevalence of eGFR reduction in CF adults following two weeks of intravenous tobramycin therapy(456). Wilcox and colleagues observed over half of their AKI cases to be related to IV gentamicin or tobramycin use(444). However, chronic renal damage from recurrent aminoglycoside use in CF is less well established and challenging to study. Quon and colleagues reported no association between chronic renal dysfunction and pulmonary exacerbation frequency, as a surrogate marker for IV aminoglycoside exposure, in their adult CF cohort(220). Although, Wilcox and colleagues report aminoglycoside toxicity to be the second biggest cause of CKD in their smaller CF cohort(444). The study of cumulative nephrotoxic drug use in an older CF population is essential to establish causation of CKD as survival increases.

Tobramycin is the main intravenous aminoglycoside used in CF, exhibiting less nephrotoxicity then its counter-parts amikacin and gentamicin, however still presenting renal risk despite use of a safer once-daily dosing regimen. Acute renal impairment during pulmonary sepsis will affect renal clearance of aminoglycoside, making regular renal and drug level monitoring essential to individualise safe and optimal dosing. However, it is challenging to detect renal effects of nephrotoxic medication due to the inaccuracy of estimated GFR in CF. Town and colleagues observed a strong correlation between creatinine clearance GFR and tobramycin clearance in CF patients(457), however this was not reflected in a previous study by Levy et al(458). Raised urinary N-acetyl-ß-D-glucosaminidase (NAG) levels, a marker of acute renal tubular damage, has shown promise in identifying aminoglycoside nephrotoxicity(221), however its use for detection of chronic renal impairment has not been validated. Novel-Caitin et al (2016) studied a CF cohort on the lung transplant waiting list with a high cumulative aminoglycoside exposure. In a younger adult cohort, they observed only one patient out of 25 with significant renal impairment, however eGFR using CKD-EPIcreat underestimated GFR compared to iohexol and inulin GFR in this population(235). The unreliability of eGFR equations in CF, particularly at higher baseline GFR levels, continues to make an optimal GFR method in CF elusive.

The efficacy of intravenous aminoglycoside treatment in the setting of chronic *Pseudomonas aeruginosa* infection and pulmonary exacerbation makes this antimicrobial choice essential for a large number of CF patients. In the setting of renal dysfunction, strategies for minimising nephrotoxicity include the use of a nebulised aminoglycoside (with less systemic absorption) or using an alternative antibiotic agent. As always, close monitoring of renal function and drug levels is essential in all CF patients receiving intravenous aminoglycoside treatment, with particular attention to older patients with the propensity for subclinical renal decline.

5.5.9 Proteinuria and CFRD

Almost half of our cohort had CFRD and the incidence of proteinuria (microalbuminuria) was 16.9%, predominantly in diabetics. Within the proteinuric cohort (n=13), ten patients had evidence of reduced GFR using estimated methods. Two patients with raised ACR were already on angiotensin converting enzyme (ACE) inhibitors and a further ten with abnormal GFR within the total cohort were also on ACE inhibition, mostly for systemic hypertension. Although the majority of patients in our study with proteinuria were diabetic, there was no significant difference in eGFR between those with CFRD and those without, which is contrary to what we would expect. Interestingly, Van den Berg and colleagues reported a higher incidence of proteinuria in CFRD than type one diabetes mellitus, postulating the existence of other contributary CF-related factors for renal protein loss(459). In a large US registry study, Quon and colleagues observed an increasing risk of CKD with duration of CFRD in a younger adult CF population(220). The increasing prevalence of CFRD with age and the risk of chronic renal damage with longevity of hyperglycaemia deems accurate GFR monitoring and early detection of renal dysfunction essential in both ageing diabetic and non-diabetic CF patients, particularly in a cohort where renal hyperfiltration may mask the extent of renal damage and GFR decline. A longitudinal study of an ageing CF cohort and analysis of GFR decline with accumulation of age-prevalent comorbidity is highly relevant and necessary as survival in CF improves. Regular, routine monitoring of urine ACR should be performed in diabetic and non-diabetic CF patients as a screening tool for early detection of renal disease in this older CF population.

5.5.10 End stage renal disease

A proportion of CF patients may progress to end stage renal disease (ESRD) with increasing survival. Risk factors include CFRD, IV aminoglycoside exposure, IgA nephropathy and transplantation-related renal dysfunction. Up to 7% of patients progress to ESRD following lung transplantation(232).

Renovascular disease may also become more relevant in CF with ageing. 18.8% of our cohort had systemic hypertension and this was significantly associated with a GFR of <90 ml/min/1.73m^2 (p=0.017). As mentioned previously, one patient was a renal and pancreatic transplant recipient for refractory CFRD and hypertension. Accurate monitoring of graft function following renal transplantation, and in the context of chronic immunosuppression is essential. Radioisotope and iohexol GFR are often utilised in a renal transplant setting.

5.5.11 Glomerular hyperfiltration

Glomerular hyperfiltration has been shown in diabetes mellitus, obesity and hypertension(428). Several studies have reported hyperfiltration to be common in the CF population(429)(460), manifesting as a higher than normal GFR (usually >150ml/min/1.73m^2). As a consequence of hyperfiltration, eGFR equations may overestimate true GFR and renal damage may exist despite a high or normal eGFR. Gaspari et al showed underestimation of GFR decline in hyperfiltering patients with type two diabetes mellitus both at six months and over a four year period(412). Although no patients in our older CF group had renal hyperfiltration based on GFR, the possibility of sub-clinical hyperfiltration in CF means we could be missing subtle changes in GFR and renal decline in CF patients over time. This may be particularly relevant with an increasing prevalence of CFRD with age.

5.5.12 Renal risk and the future in CF

The quantification of renal risk is important in developing our understanding of rarer disease cohorts in an ageing population and to create opportunities for primary prevention in high risk groups. Maple-Brown and colleagues describes the importance of accurate measurement of GFR in indigenous Australians important, given their inherent risk of diabetes mellitus and cardiovascular disease(435). An older CF cohort may be at enhanced cardiovascular risk, as mentioned in previous chapters, and given the close affiliation between renal and cardiovascular disease(461), further research into renal comorbidities

may provide opportunities to reduce risk and implement primary prevention strategies. This study has demonstrated renal disease in an older CF population and the prevalence of renal disease is likely to increase with age. As survival improves and more patient reach older adulthood, further renal studies are essential to reduce additional comorbidity in this complex orphan disease.

5.5.13 Limitations

A number of limitations of this study should be recognised. This is an observational, cross sectional study. Longitudinal data would provide more informative analysis regarding trends, causation and extent of renal disease in this cohort, particularly the influence of accumulative comorbidities such as CFRD, systemic hypertension and nephrotoxic treatment burden. Longitudinal studies with larger sample sizes are required to assess GFR decline over time in the older CF population.

Poor participation in 24-hour urinary CrCl measurements limited comparative analysis of measured CrCl to serum GFR methods. It would have been informative to analyse nebulised aminoglycoside use in the context of renal dysfunction in addition to intravenous use. The small sample size for iohexol GFR limits conclusions relating to accurate GFR methods in this cohort. Patient recruitment was significantly restricted by the Covid-19 pandemic, shortening the recruitment period by four months. Time constraints of iohexol sampling also adversely affected patient participation, and long clinic visits for this part of the study were challenging due to necessities of patient segregation and lack of clinic space.

Capillary iohexol sampling was performed but sample analysis was not possible in the MFT laboratory given the temporary halt in the processing of research samples due to the Covid-19 pandemic. Analysis of these samples in the future may assist to validate a capillary sampling iohexol protocol and, if successful, could provide opportunities for more amenable and accurate GFR testing in a CF cohort with an existing high frequency of healthcare visits.

My hope is that this work will continue in our centre over the next few years to ensure we can provide stronger evidence of accurate GFR testing in an older CF population.

5.6 Conclusion

This study demonstrated a high prevalence of renal dysfunction in long term CF survivors. Risk factors for renal impairment appear to be advancing age, CFRD, IV aminoglycoside exposure and systemic hypertension.

Chronic renal dysfunction in an ageing CF population will have implications for a variety of aspects in CF care. There may be challenges in the monitoring and dosing of nephrotoxic antimicrobials, and hence achieving optimal treatment of pulmonary exacerbation. However, although an eGFR <90ml/min was common in our study group, CKD to the degree at which drug dose changes may be needed was very rare. This is reassuring. In addition, the small iohexol study showed that eGFR tended to underestimate renal function so the prevalence of CKD may actually be lower based on this finding.

Chronic renal disease will complicate pre and post-transplantation management, in the context of transplant eligibility as well as immunosuppressant efficacy. The presence of CKD will also have an influence on the development of cardiovascular disease in an ageing CF population, making regular assessment of cardiovascular risk, systemic blood pressure and glycaemic control essential as survival continues to improve.

In the absence of measured GFR, the calculation of eGFR using cystatin C-based equations may be more reliable than creatinine. Data demonstrated underestimation of estimated renal capacity in this population, which is dichotomous to previous studies. This may be due to the small sample size or lack of validation of LC-MS method validation in this disease cohort. In the absence of sampling error and size, GFR underestimation may accurately reflect an older CF population with an increasing prevalence of CFRD and larger studies are required assess trends and causation.

5.7 Study recommendations

- Urine ACR should be used in older CF patients both with and without CFRD for regular assessment of renal function and early detection of renal disease.
- Intravenous aminoglycosides should be used with caution in older CF patients with renal dysfunction.

- Although it is likely not be feasible to use iohexol GFR outside of a research setting in CF, measured GFR may be important for high risk patient subgroups; those with chronic renal impairment in the context of nephrotoxic medication dosing, in organ transplant recipients on immunosuppression, those with intra-renal pathology, such as IgA nephropathy, and at risk of renal decline.

Further research is necessary to assess the causes and rate of renal decline in an older CF population, and to determine the most accurate GFR measure in this clinically diverse and challenging group. Renal impairment will undoubtedly affect morbidity and mortality in older adult CF patients, and the preservation of renal function must be a priority in an ageing CF population to reduce the burden of cumulative ageing comorbidity in an already complex multiorgan disease.

Authors	Year	Study type	Patient number	Disease group	eGFR range ml/min/1.73m^2	eGFR formulae used for comparison	Method used for gold standard mGFR	Results
Gaspari et al(407)	1995	Cross-sectional	41	CKD	6-160	NA	Inulin vs iohexol (5ml bolus) Sampling 5 to 600 mins (13 samples)	Significant correlation between methods for a range of GFRs.
Gaspari et al(408)	1998	Longitudinal	24	CKD	14-104	NA	Iohexol (5ml bolus) – precision study. 2 groups: 1. eGFR ≥40 Sampling at 120 to 240 mins 2. eGFR ≤40 Sampling at 120 to 600 mins	Iohexol shows good precision for GFR in those with normal and abnormal renal function.
Gaspari et al(410)	2004	Longitudinal	81	Renal transplant recipients	21.8-86.1	CG, MDRD	Iohexol (5ml) Sampling at: 120 to 600mins	eGFR equations over-estimate rate of GFR decline in renal

								transplant recipients.
Tidman et al(419)	2008	Cross sectional	644	CKD	12-125	Cystatin C validation in eGFR CG, MDRD	Iohexol (5ml) Single sample method Sampling 3.5 to 24 hours (timing based on eGFR from serum creatinine).	Addition of Cystatin c in eGFR equations increases accuracy
Ruggenenti et al(411)	2012	Longitudinal	111	Autosomal dominant polycystic kidney disease (ADPKD)	Mean 78.6	MDRD, CKD-EPI (creatinine)	Iohexol (5ml) 2 groups: 1. eGFR≤40: sampling at 120 to 480 mins 2. eGFR>40: sampling at 120 to 240 mins	eGFR equations under-estimate GFR change over time.
Evans et al(530)	2013	Cross-sectional	2,198	CKD IV/V	8-27	MDRD, CKD-EPI (creatinine)	Iohexol (2-10ml bolus) 2 groups: 1. eGFR 15-30 – sampling at 360-420mins 2. eGFR≤15 – sampling extended to 48hours.	eGFR equations may be inaccurate in elderly patients and those with diabetic nephropathy.

Gaspari et al(412)	2013	Longitudinal	600	Type 2 diabetes mellitus	71-108	MDRD, CG, CKD-EPI (creatinine)	Iohexol (5ml) No sampling times stated 8 year study period, 6-monthly iohexol data	GFR underestimated at 6 months. GFR decline missed in hyperfiltering patients and underestimated over 4 years.
Maple-Brown et al(435)	2014	Longitudinal	224 (Indigenous Australian)	Type 2 diabetes mellitus	68-119	MDRD, CG, CKD-EPI (creatinine)	Iohexol (5ml) Sampling up to 240mins	CKD-EPI is the most accurate eGFR equation in this population, however underestimates GFR in diabetic patients with normal renal function
Luis-Lima et al(436)	2015	Cross-sectional	193	Renal transplant recipients	Mean 52.2	MDRD, CG, CKD-EPI (creatinine and cystatin C)	Iohexol (5ml) Sampling up to 480mins.	Poor correlation of eGFR equations with iohexol, over-estimation of GFR in this population.
Florens et al(455)	2020	Cross-sectional, retrospective	91	Lung transplant recipients	Mean 106 (pre-transplant)	NA	Pre and one year post-transplant mGFR (iohexol or inulin)	All had >25% reduction in mGFR at one year, 60% had

				(32 CF patients)	Mean 58 (one year post-transplant)			>50% mGFR reduction

Table 5.1: Iohexol studies in the non-CF population.

Authors	**Year**	**Study type**	**Patient number**	**Mean age (years)**	**Disease group**	**Mean eGFR ml/min/1.73m^2**	**eGFR formulae used for comparison**	**Method used for gold standard mGFR**	**Results**
Spino et al(438)	1985	Cross-sectional	8	17	Cystic fibrosis	>90	NA	^{99m}Tc-DTPA vs 125-OIH Blood sampling up to 180mins (13 samples), urine up to 24hours	Renal clearance similar in CF and control group
Touw et al(238)	1996	Cross-sectional	18	27	Cystic fibrosis	148	CG, Jeliffe Comparison of tobramycin clearance	24 hour urinary creatinine clearance mCrCl = urine Cr x urine vol/Serum Cr (ml/min)	Good correlation between tobramycin clearance and creatinine clearance in CF.

Al-Aloul et al(233)	2007	Cross-sectional	83	24.3	Cystic fibrosis	92.6	CG, MDRD	24 hour urinary creatinine clearance mCrCl = urine Cr x urine vol/Serum Cr (ml/min)	Over-estimation of renal function in patients with eGFR <80.
Beringer et al(189)	2009	Cross-sectional	19	29.3	Cystic fibrosis	104	CG, MDRD, Cystatin C clearance (Tidman)	Iothalamate (456mg) Blood sampling at 15 to 180mins. Urine sampling at 30 to 180mins	Cystatin C estimates of GFR are superior to creatinine based formulae, especially for lower GFR.
Prestidge et al(429)	2010	Cross-sectional	67	9.6	Cystic fibrosis – paediatric	140	Nil comparison	^{99m}Tc-DTPA Sampling at 2 and 3 hours.	6% had GFR<90, 40% had evidence of hyperfiltration
Soulsby et al(424)	2010	Cross-sectional	53	18	Cystic fibrosis		CG, MDRD, cystatin C,	^{99m}Tc-DTPA	Cystatin C eGFR correlates most closely

							tobramycin clearance	Sampling at 2, 3 and 4 hours	with mGFR method
Jain et al(531)	2012	Cross-sectional	50	-	Cystic fibrosis	115	MDRD	Chromium^{51} EDTA	Renal hyperfiltration in CF patients
Novel-Caitin et al(235)	2016	Cross-sectional	25	32	Cystic fibrosis – on lung transplant waiting list	106	CKD-EPI	15 patients – iohexol (6ml); 120 to 240 mins 6 patients – inulin (+urine)	CKD-EPI over-estimated GFR in CF patients with advanced lung disease.
Wallace et al(263)	2020	Cross-sectional	17	11.5	Cystic fibrosis – paediatric cohort	136	CG, MDRD, CKD-EPI, + other paediatric-specific Cystatin C equations	^{99m}Tc DTPA 120 to 360 mins	Within 10% correlation eGFR to mGFR of 5-47%. Poor accuracy of eGFR formulae in paediatric CF population.

Table 5.2: GFR comparison studies in CF

6.0 Chapter 6: Cardiac magnetic resonance imaging in an older cystic fibrosis population

6.1 Abstract

6.1.1 Introduction

Ageing in cystic fibrosis (CF) may be associated with an increased cardiovascular risk. Previous chapters have provided an insight into cardiovascular disease in an older CF population, however the prevalence of intrinsic cardiac pathology in this group is unknown. The use of echocardiography to determine cardiac function may be inaccurate in CF, particularly in the presence of advanced pulmonary disease. Cardiac magnetic resonance imaging (CMR) may provide a more reliable alternative method to assess the prevalence and extent of myocardial disease in an older CF population.

6.1.2 Methods

CF patients aged 40 years and above, attending MACFC, were recruited for CMR participation. CMR was performed at MFT using a 1.5T MR scanner (Avanto, Siemens). The CMR protocol was developed and CMR analysis performed in conjunction with the cardiac imaging research department at MFT. Investigation parameters included biventricular function and volumetrics, T1 and T2 time, pulse wave velocity (PWV), the presence of late gadolinium enhancement (LGE) and calculation of extracellular volume fraction (ECV), to determine the presence of diffuse myocardial disease. Images were analysed using CVI Circle Imaging^{TM} software. Data were inputted into a pre-constructed excel spreadsheet and corrected for body surface area (BSA) and haematocrit. Data were analysed as a total cohort using predominantly descriptive methods, then categorised into severe ($ppFEV_1$ <40%) and non-severe ($ppFEV_1$ ≥40%) lung disease groups for comparison. Statistical analysis was performed using SPSS® (version 25.0, IBM®) with significance reported at the 0.05 level.

6.1.3 Results

Nine patients underwent CMR, with good tolerance to scan duration, positioning and breath-holding. Mean(SD) age was 46.7(±3.39) years and 66% were male. Mean $ppFEV_1$; 50.1(±23.5)%, exocrine pancreatic insufficiency; 88.9%, chronic *Pseudomonas aeruginosa*

infection; 77.8% and CF-related diabetes mellitus (CFRD); 66.7%. One participant had systemic hypertension, two had hyperlipidaemia and two had a positive family history of cardiovascular disease. On CMR, all patients had normal left ventricular (LV) and right ventricular (RV) systolic and diastolic function. No significant correlations were observed between biventricular function and severity of airflow limitation, as measured by $ppFEV_1$. 44.4% had localized LGE due to right ventricular insertion point fibrosis (RVIP). Five patients had raised ECV; mean(SD) 26.5 (±2.30)%. ECV values were higher in women than men; mean 29.1 (±1.27) vs 25.2 (±1.4)%, p=0.005. All patients had normal PWV on CMR. There were no statistically significant differences in demographic or CMR data seen between severe and non-severe lung disease groups.

6.1.4 Conclusion

This is the first study of CMR in the older adult CF population. Results show a high prevalence of RVIP fibrosis as identified by LGE on CMR, the significance of which is uncertain. The significance of raised ECV in the context of CF warrants further investigation. CMR is well tolerated and could be a useful imaging tool in assessment of the CF heart, particularly in those at higher risk of cardiovascular disease.

6.2 Introduction

The relationship between cystic fibrosis (CF) lung disease, pulmonary hypertension and right ventricular (RV) failure is well documented(462). Alterations in right ventricular morphology and function arise as a result of progressive lung disease and chronic hypoxaemia, leading to altered pulmonary vascular resistance and vessel remodelling. This ultimately causes raised systolic pulmonary artery pressure (sPAP), pulmonary hypertension, RV strain and hypertrophy and cor pulmonale(463).

A correlation has been shown, using 2D/M-mode echocardiography, between the extent of RV dysfunction and severity of underlying CF lung disease (table 6.1, end of chapter 6). The relationship between left ventricular (LV) function and CF, however, is less well reported. There are some important questions to consider in this context;

i. Is there intrinsic cardiac pathology in CF as a result of CFTR dysfunction?
ii. Is the presence of cardiac dysfunction related to or independent of lung disease severity in CF?
iii. Is cardiac dysfunction in CF secondary to ageing and associated increased cardiovascular risk?

Understanding the prevalence and causation of cardiac disease in CF will be of particular importance in an ageing CF population, a group with cardiovascular risk and emerging age-related multi-organ comorbidity. In this era of CFTR modulator therapy, there is potential for further survival advantage and the possibility of a paradigm shift in CF epidemiology, from mortality at a relatively young age to the reality of a chronic, comorbid, older adult disease.

6.2.1 Cardiac disease in CF

The myocardium is the muscle layer of the heart and is responsible for cardiac contraction. It is comprised of layers of myofilaments within sarcomeres, the integral components of cardiac muscle. Sarcomere function within the myocardium is predominantly regulated by calcium and potassium movement. In a complex process of ion dependent excitation-contraction coupling, the release of calcium from the sarcoplasmic reticulum leads to myocardial contraction(464).

6.2.2 CFTR and the heart

CFTR protein is present in myocardial cells, both atrial and ventricular myocytes. Primary CFTR dysfunction causes defective ion movement in myocardial cells, affecting myocyte contraction, intracellular calcium signalling, arrhythmogenesis and ventricular remodelling(465). Sellers at al observed abnormal ventricular cardiomyocyte function in CF mice models, postulating the risk of myocardial dysfunction from loss of normal CFTR(466). Could CF patients therefore be at risk of primary CF-related cardiomyopathy independent of pulmonary disease severity?

6.2.3 Secondary cardiac pathology in CF

6.2.3.1 Hypoxaemia

In patients with chronic lung disease, severe hypoxaemia may result from exercise, sleep or may occur at rest depending on pulmonary disease severity. Hypoxaemia affects myocyte function and can cause ventricular dysfunction, reduction in coronary blood flow and arrhythmogenesis. Several studies have shown ventricular arrythmias secondary to hypoxia in CF, at rest and during exercise(467)(468). Wolf et al report exercise-induced left ventricular perfusion defects in CF adults(469). Fraser et al observed a relationship between hypoxaemia and pulmonary hypertension in severe CF pulmonary disease in the context of normal LV and RV function(470). Several other studies have also shown the role of hypoxaemia in the development of RV dysfunction in CF(471)(472). Chronic hypoxaemia in CF leads to the development of pulmonary hypertension and cor pulmonale. The resulting RV enlargement may cause abnormalities in interventricular septal motion, causing ventricular discordance, reduction in LV stroke volume and left ventricular impairment(473).

6.2.3.2 Chronic inflammation

Longstanding systemic inflammatory burden can alter homeostatic vascular dynamics and integrity, increasing arterial wall stiffness. Chronic systemic inflammation has been shown to be related to atherosclerosis and cardiovascular disease in the non-CF setting(474). Studies in rheumatoid arthritis (RA) cohorts have shown endothelial dysfunction and increased cardiovascular risk secondary to inflammatory burden.

An increased level of high sensitivity C-reactive protein (hsCRP), a serum maker of systemic inflammation, has been shown to correlate with pulmonary artery pressure and RV

dysfunction in CF(462). Ionescu et al observed reduced RV systolic dysfunction with raised hsCRP levels in a CF cohort with severe pulmonary disease(475).

Large artery stiffness has been well established as a risk factor for cardiovascular disease in the general population and is also associated with LV dysfunction. Its presence may be related to systemic inflammation and represent a non-atherosclerotic vasculopathy. Large artery stiffness has been shown to correlate with hsCRP and, as measured by augmentation index (AIx), has been shown to be higher in CF when compared to healthy controls(227). This may reflect a similar pathological process to COPD, in which arterial stiffness is prevalent and their cardiovascular risk enhanced when compared to the general population(476).

Elevated inflammatory markers such as TNF alpha have shown a positive correlation with hypertriglyceridaemia in CF(298). Although interestingly, analysis of systemic inflammation using hsCRP in the same study did not concur. The infusion of TNF alpha, an inflammatory mediator, in dog models has been shown to contribute to LV dysfunction(477).

Maki-Petaja and colleagues have shown modification of cardiovascular risk and reduction in arterial stiffness in the RA population using statin and anti-TNF alpha therapy(340), strategies that may be relevant in CF with similar premature arterial stiffness and cardiovascular risk (see chapter 4).

A number of anti-inflammatory agents have been studied in CF, mainly targeted at pulmonary inflammation and infection. The study of statin therapy in non-CF bronchiectasis has shown pulmonary symptom reduction(478). The anti-inflammatory role of statin therapy in CF has been evaluated by Jouneau et al(479), analysing the role of Fluvastatin and IL-8 production in whole CF red blood cells in the context of chronic *Pseudomonas aeruginosa* infection. Could there be a role for statins, independent of their cholesterol lowering effects, in lowering arterial stiffness in the context of chronic inflammation contributing and cardiovascular risk in an older CF population? Further studies will be informative.

6.2.3.3 Endothelial dysfunction

Endothelial dysfunction is a major step in the development of atherosclerotic disease and has been described in CF cohorts(474). Poore and colleagues report endothelial dysfunction,

as measured by ultrasound brachial artery flow mediated dilation (FMD), in a young adult CF cohort with preserved LV function(480). Endothelial dysfunction has been shown to be related to the persistent systemic inflammatory burden in CF(481), and as such both factors may contribute to the development of premature atherosclerosis and cardiovascular disease.

Interestingly, Sellers and colleagues report LV dysfunction and aortic stiffness in Phe508del mouse models, independent of lung disease(465), perhaps reflecting additional inherent CFTR associated cardiac influence, independent of conventional cardiovascular risk factors. Could there be a CF-related cardiomyopathy as a result of CFTR dysfunction? If this is the case, inherent cardiac dysfunction in CF is likely to be exacerbated by the ageing process and the development of age-prevalent cardiovascular risk.

6.2.4 Ischaemic heart disease in CF

Ischaemic heart disease (IHD) in CF has only been documented in a handful of case reports to date and is rare outside of the organ transplantation setting. One of the first case reports of coronary artery disease in CF was reported by Onady and colleagues in 2006, in a 52-year-old patient, with a late diagnosis of CF, presenting with dyspnoea and vague chest discomfort. A left anterior descending coronary artery occlusion was found on angiography(224). In 2010, Perrin and colleagues reported a non-ST elevation myocardial infarction (NSTEMI) in a 48-year-old CF patient, with an early diagnosis, CF-related diabetes mellitus (CFRD) and a significant family history of premature IHD(289). In both cases, electrocardiography showed no ST segment changes and perfusion defects were diagnosed on thallium stress testing. These cases highlight the potential for atypical presentations of ischaemic cardiac disease in CF and the importance of cardiovascular risk prediction and primary prevention for higher risk patients. It is thus essential to identify cardiovascular risk factors relevant to the CF patient with ageing, such as CFRD and systemic hypertension. Eaden and colleagues (2013) reported a case of accelerated atherosclerosis of the coronary arteries in a 19-year-old CF patient who had received cardiac and bilateral lung transplantations (at four years of age)(482). Although this is a rare case, could post-transplant vasculopathy become more relevant in an ageing CF population in which the prevalence of solid organ transplantation may increase with advancing disease severity? Cardiac ischaemia has also been reported in younger CF patients, with Aronsson et al

describing a transmural myocardial infarction in a two-year-old patient with undiagnosed CF and in the context of bilateral pneumonia, acute cardiac failure and raised serum cardiac enzymes(483).

6.2.5 Chronic respiratory disease and cardiovascular risk

The risk of cardiovascular disease in other chronic respiratory disease is well established. Chronic respiratory tract inflammation has been linked to cardiovascular disease, evidenced in chronic obstructive pulmonary disease(484) and bronchiectasis(355), with an association between low lung function and cardiovascular risk. A study by Sabit and colleagues observed subclinical LV dysfunction in COPD patients secondary to chronic to airflow obstruction(485). Baum and colleagues observed the presence of LV dysfunction in 15 COPD patients, independent of right heart abnormality(486). Although predominantly older cohorts, undoubtedly with secondary cardiovascular risk, could these findings be relevant for chronic airflow obstruction associated with CF?

The presence of chronic respiratory tract and systemic inflammation in CF may render these patients at similar risk of cardiovascular events as the aforementioned population groups, particularly as survival increases. Reduction in ppFEV$_1$ with age, and the emergence of age-related comorbidities with imporving survival, could accentuate this risk in older CF patients.

6.2.6 Other contributors to LV dysfunction in CF

As CF survival increases, atherosclerosis of ageing and the increasing prevalence of systemic hypertension, hyperlipidaemia and CFRD will all become relevant in the development of cardiovascular disease(289). Hyperlipidaemia is an important risk factor for the development of atherosclerosis in the general population. Figueroa and colleagues showed excessive plasma triglycerides in CF patients(298), independent of diabetic status and liver disease, common causes of hyperlipidaemia in the general population. Hypertriglyceridaemia may be compounded by the risk of hyperglycaemia and impaired glucose tolerance as CF patients age. Diabetic cardiomyopathy should also be considered in the context of ageing CF adults(487). The increasing prevalence of systemic hypertension may have significant effects on LV structure and function in long term CF survivors, as seen in the general hypertensive population, and would also be an interesting area for further review.

Exocrine pancreatic function and malabsorption may also be paramount in cardiovascular risk in CF. McGiven (1962) postulates that pathology of the left ventricle in CF, specifically the development of myocardial fibrosis, may be caused by deficiencies of thiamine and tocopherol(488). Furthermore, the impact of CFTR modulation on exocrine pancreatic function, improved fat absorption and weight gain cannot be understated in CF and has been reported in ivacaftor monotherapy studies. Longitudinal data examining the newer CFTR modulator compounds in this area of efficacy will be informative.

As shown in previous chapters, patients with less severe CFTR genotypes and preservation of exocrine pancreatic function may be at a higher risk of hyperlipidaemia with age(300). The risk of macrovascular disease in CF may therefore have some relationship to CFTR genotype. The relationship between genotype and phenotype in CF is complex and notoriously difficult to study due to significant phenotypic diversity within CFTR mutation classes. However, the phenotypic differentiation within CF cohorts may indeed be important for the determination of cardiovascular risk and targeting higher risk groups for primary prevention therapy.

6.2.7 Cardiovascular risk in CF

Defining cardiovascular risk in CF is challenging. The historically poor prognosis of CF has meant that cardiovascular disease is a relatively novel concept in what is now an ageing CF population. In the general population, cardiovascular risk scoring is performed in the primary care setting and includes those aged 40 years or above. A high frequency of appointments with specialist CF services usually means a lack of input from primary care and hence cardiovascular risk identification and opportunities to implement primary prevention may be overlooked in CF care. Reliable cardiovascular risk scoring using QRisk®3(162) requires a definition of CFRD as being either type one or type two diabetes mellitus. Although most patient require insulin treatment, CFRD is characterised by both insulin deficiency and insulin resistance, hence equitable classification is difficult. This presents limitations and lowers reliability of cardiovascular risk scoring in this cohort.

There may be a lack of correlation between abnormal LV ejection fraction (LVEF) and cardiac failure. In the non-CF population, Owan et al showed a proportion of patients with preserved ejection fraction (EF) (i.e. diastolic heart failure) progressing to congestive cardiac

failure(489). If the majority of CF patients have normal LV function, could alternative pathology be contributing to cardiac disease?

6.2.8 Myocardial fibrosis

The myocardium consists of both cellular components (cardiac muscle and structural entities) and extracellular components (fluid, collagen, glycoproteins)(490). Myocardial fibrosis is caused by excessive extracellular matrix deposition within the myocardium, the cause of which can be cardiac or extra-cardiac. Expansion of the myocardial interstitium/extracellular space is an important pathological process in ventricular remodelling, a common process in end stage cardiac disease and a predictor of major adverse cardiovascular events(490). There are two types of myocardial fibrosis; *replacement* and *interstitial*. The causes of each are shown in table 6.2.

Replacement myocardial fibrosis	Myocardial infarction (commonest), Hypertrophic obstructive cardiomyopathy (HOCM), myositis, sarcoidosis, drug induced/toxic cardiomyopathy, chronic renal insufficiency
Diffuse interstitial myocardial fibrosis	**Reactive:** ageing, systemic hypertension, diabetes mellitus, non-ischaemic dilated cardiomyopathy **Infiltrative**: amyloid, Anderson-Fabry disease

Table 6.2: The causes of myocardial fibrosis(491)

6.2.9 Myocardial disease in CF

Chronic inflammation, hyperglycaemia, hypoxaemia and activation of the renin-angiotensin aldosterone system (RAAS) are all likely to play a role in the dysregulation of myocyte function and fibroblastic cell proliferation in CF-related myocardial fibrosis(492). Myocardial fibrosis can progress to biventricular dysfunction. Some potential contributors to a myocardial fibrotic process in CF are shown in figure 6.1.

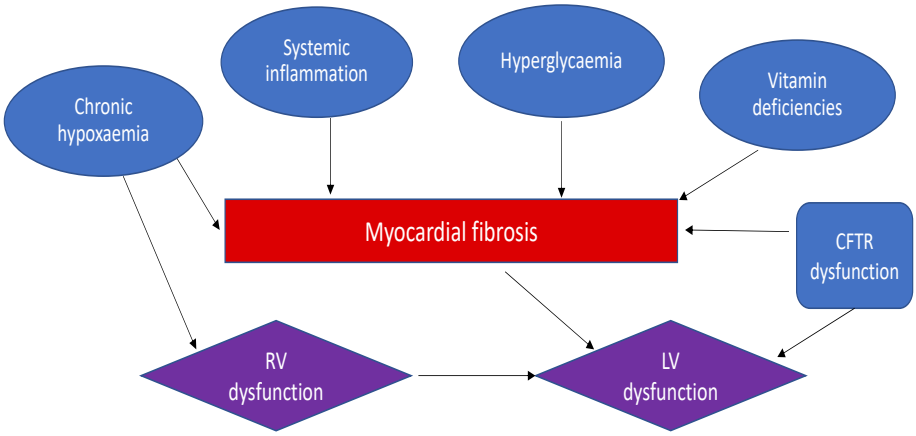


Figure 6.1: Potential causes of myocardial fibrosis in CF.

To date, myocardial fibrosis has only been described in paediatric CF patients. The first case series were reported by McGiven et al (1962)(488) and Barnes et al (1970)(493), observing posthumous findings of myocardial fibrosis possibly related to exocrine pancreatic insufficiency, vitamin deficiency and subsequent myocardial destabilisation in CF infants. However, with the use of pancreatic enzyme replacement therapy (PERT) and vitamin supplementation now commonplace in CF management, there may be an assumption that myocardial disease related to malabsorption will likely have decreased as their use has expanded.

A number of other studies followed, also observing multifocal myocardial fibrosis on necropsy specimens of CF children(494)(495)(496). Myocardial fibrosis has been reported as a cause of sudden, unexpected death from cardiac insufficiency in CF infants, independent of pulmonary disease(497), observing its possible relationship with Phe508del homozygosity. Ambrosi and colleagues described myocardial fibrosis as a cause of thallium perfusion defects and sub-normal LV ejection fractions in young CF children (mean age 16.7 months)(498), a potential complication of advancing disease in CF.

Given the causal link between ageing, systemic hypertension and diabetes mellitus in the development of myocardial fibrosis in the general population, the study of myocardial disease and LV function in older adult CF patients seems logical in a quest to better

understand the cardiac complications associated with ageing in CF. The inaccuracies associated with echocardiographic analysis in CF calls for a superior cardiac imaging technique, such as CMR, to be studied.

6.2.10 Cardiac imaging in CF

Echocardiography is reported as being around 80% sensitive at detecting cardiac failure(499). Echocardiographic studies in CF are extensive and the majority have been performed in relation to right heart structure and function in the context of progressive CF pulmonary disease. However, studies analysing LV morphology and function as a separate entity have shown LV abnormalities independent of pulmonary status and right heart disease (table 6.1). Chipps and colleagues (1979) observed abnormal LVEF, used as a measure of LV function, in a proportion of CF patients both at rest and during exercise(468). Johnson and colleagues (1991) reported diastolic LV filling defects in CF patients with severe pulmonary disease using doppler echocardiography(471). Strain echocardiography, a more recently developed imaging technique, has allowed the detection of subclinical LV diastolic and systolic impairment in CF adults with severe pulmonary disease(492)(500). LV abnormalities in CF warrant further investigation, particularly as survival increases and both CFTR-related and non-CFTR related factors may contribute to cardiac pathology.

The use of radionuclide angiography in the detection of cardiac dysfunction allows additional analysis of myocardial function. Matthay and colleagues (1980) used radionuclide angiography in 22 young adult CF patients, showing normal LV function but RV dysfunction related to severity of pulmonary disease(501). Benson and colleagues (1984) observed abnormal LVEF with exercise in 29% (n=31) during radionuclide assessment of CF patients (mean age of 17 years) with a range of pulmonary severity(502).

Both imaging modalities have their limitations in the CF population. Respiratory motion artefact, abnormal breathing patterns, pulmonary hyperinflation and inadequate positioning may limit image quality in some CF patients. Strain echocardiography has been shown to detect LV abnormalities and is a useful additional echocardiographic technique, however a standardized protocol in CF is yet to be developed(503). Tissue doppler echocardiography, first described in CF by Ionescu and colleagues (2002)(475) may identify heart failure in presence of normal LV ejection fraction, and hence may be a useful tool in CF to identify early signs of cardiac dysfunction, particularly in higher risk patients.

Radionuclide angiography may assist to alleviate inaccuracies associated with echocardiographic assessment in CF, however it is an invasive technique. Cardiac MR imaging may be a suitable alternative technique in CF cardiac assessment.

6.2.11 Cardiac MR

6.2.11.1 Clinical use of cardiac MR

Cardiac magnetic resonance imaging (CMR) is an important diagnostic tool in a range of cardiovascular pathologies, specifically in the diagnosis of ischaemic and non-ischaemic cardiomyopathies. CMR has superior sensitivity and specificity to echocardiography and enables acquisition of three and four-dimensional imaging sequences for reliable evaluation of cardiac structure and function(504). CMR imaging can provide circumferential analysis of the myocardium, not provided by echocardiography alone, enabling accurate myocardial tissue characterisation(276). CMR also has superior accuracy and reproducibility, thus requiring smaller sample sizes to produce meaningful conclusions(505). Invasive biopsy has historically been the only tool to accurately identify myocardial disease, however CMR is increasingly being used as a non-invasive alternative in evaluating the myocardium using T1 mapping and extracellular volume analysis(506).

Magnetic resonance imaging is also increasingly being used to define the extent of pulmonary disease and pulmonary perfusion abnormalities in CF(507). Although echocardiographic research is vast, no published studies to date have utilised CMR in the analysis of cardiac function in CF. Several factors deem CMR a useful next step in CF cardiac exploration. Cardiac MR imaging, in addition to its superior image quality, is safe and uses no ionizing radiation, particularly favourable in a CF population with a high burden of previous lung imaging. Although breath hold techniques can be used in CMR protocols to reduce image artefact, these are not essential for good quality imaging and respiratory correction methods may be utilised. However, the necessity to lie flat for a prolonged period of time may limit MRI tolerability for patients with more severe pulmonary disease and orthopnoea.

6.2.11.2 Applications of CMR in an ageing CF population

LV mass, as measured by CMR, has been shown to correlate with cardiovascular risk in hypertensive patients(508)(509) and biventricular assessment may be useful in an ageing CF

population with an emerging cardiovascular risk. CMR in CF will also achieve a detailed assessment of the myocardium, abnormalities of which may be relevant in the setting of CFRD, systemic hypertension and chronic systemic inflammation.

6.2.12 CMR imaging techniques

CMR protocols vary between centres, however all exhibit high reproducibility in study contexts(491). The majority use gadolinium-enhanced imaging techniques, T1 and T2 mapping and calculations of extracellular volume.

The use of late gadolinium enhancement (LGE) and T1/T2 mapping in CMR allows detailed analysis of myocardial tissue. The addition of extracellular myocardial analysis allows detection of both focal and diffuse myocardial disease, used in the quantification of, and differentiation between, ischaemic and non-ischaemic cardiomyopathy. Two specific myocardial pathologies can be detected using CMR; myocardial oedema; seen in acute myocardial infarction and inflammatory cardiac pathology, and myocardial fibrosis (localised and diffuse); seen in chronic ischaemic scar and non-ischaemic cardiomyopathies.

6.2.12.1 Late gadolinium enhancement (LGE)

LGE techniques are used to diagnose localised myocardial fibrosis and oedema. Gadolinium contrast distribution time equates to the degree of myocardial disease. Typical gadolinium washout from the myocardium is between 5 and 20 minutes. In localised myocardial fibrosis, the gadolinium chelates and expands into the extracellular space thus delaying its washout time. The extent of myocardial fibrosis using LGE techniques are identified qualitatively using visual interpretation, and hence may subject to inter-operator variation(276). LGE alone cannot diagnose diffuse myocardial fibrosis.

6.2.12.2 T1 mapping

T1 time in CMR analysis measures the longitudinal (spin lattice) relaxation time of the myocardium. Native T1 time is an important indicator of myocardial disease and may be increased in the presence of myocardial oedema, fibrosis and amyloid deposition, and shortened in excess myocardial lipid or iron content. The Modified Look-Locker inversion recovery (MOLLI) method calculates T1 time over a single breath hold (17 successive heart beats)(490) and is the most widely used CMR protocol. Native T1 values (i.e those in the absence of gadolinium contrast) are influenced by the MR field strength used (higher on 3T than 1.5T) and will also vary depending on the local CMR protocol. Native T1 may be higher

in men and with advancing age. The mean reference value for native T1 time on a 1.5T MR scanner has been quoted by Dabir and colleagues as 931(±21) milliseconds (ms) in healthy subjects(510).

Despite method variability, native T1 times display high reproducibility between studies. Poor breath holding may influence image quality and respiratory motion compensatory mechanisms may need to be used. This will be of relevance in CF patients, particularly those with advanced pulmonary disease. T1 mapping alone may be used in myocardial tissue determination in patients with renal failure, in whom the use of gadolinium contrast may be contraindicated.

6.2.12.3 T2 time

T2 time is a measure of myocardial transverse relaxation time and a marker of myocardial water content. T2 time is increased in the presence of myocardial oedema, relevant in acute myocardial infarction and myositis. Normal T2 time has been quoted by Giri and colleagues as 52.18(±3.4)ms in healthy subjects on a 1.5T MRI scanner(511).

6.2.12.4 Contrast-enhanced T1 mapping for quantification of extracellular volume fraction (ECV)

Analysis of both native and post-contrast (LGE) T1 time can be used to detect the presence of diffuse myocardial fibrosis and scar. In the presence of LGE, T1 mapping can be used to calculate the extracellular volume fraction (ECV), an important marker of myocardial tissue remodelling(275). T1 time and ECV have been shown to correlate well with LV function, and abnormalities in ECV may be present in early stage cardiomyopathy(490). A dilated extracellular space in the presence of diffuse myocardial fibrosis causes contrast accumulation and higher ECV values. Low ECV is seen in thrombus formation or fat accumulation(276). A normal ECV, using a 1.5T scanner, is between 20 and 26%(512), and values may be higher in women.

ECV fraction estimates are calculated using pre and post-contrast T1 values and the patient haematocrit value. In preparation for ECV calculation, a serum sample for haematocrit is taken from the patient just prior to scanning. ECV is calculated using the following formula;

$$ECV = (1 - hct)\frac{1}{post\ contrast\ T1\ myo} - \frac{1}{native\ T1\ myo} \div \frac{1}{post\ contrast\ T1\ blood} - \frac{1}{native\ T1\ blood}$$

The relationship between abnormal T1 time and ECV measurements in cardiac pathology are shown in figure 6.2.

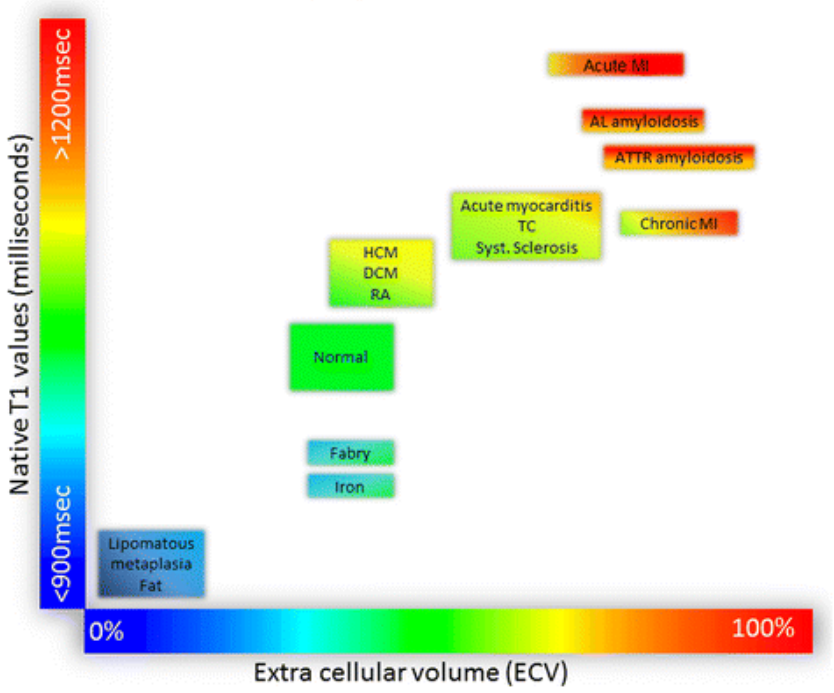


Figure 6.2: T1 mapping and extracellular volume fraction in myocardial tissue characterisation(276).

6.2.12.5 Aortic distensibility

Aortic distensibility (stiffness) is an important marker of premature, subclinical arterial disease(374). Central aortic stiffness (i.e. reduced aortic compliance) is an independent risk factor for cardiovascular disease and mortality(338). Aortic stiffness can be measured using pulse wave velocity (PWV) or by using aortic distensibility calculations on CMR imaging. PWV measurements on CMR may be more accurate than non-invasive applanation tonometry methods of carotid-femoral (aortic) PWV.

CMR measurements of aortic distensibility uses contouring software to calculate the maximal ascending aortic diameter (A_{max}) in systole and minimal aortic diameter (A_{min}) in diastole. The change in aortic diameter is then used in a calculation with pulse pressure to give a value for ascending aortic distensibility (AAD) in mmHg(374); $\Delta A=A_{max}-A_{min}$ and then, $AAD=\Delta A/(A_{min}-PP)$.

CMR measurements of PWV use 2D image slices through the aortic arch to provide two flow waveforms from the ascending and descending thoracic aorta. Normal PWV varies

depending on age and degree of systemic hypertension. However, a cut off value of <11 m/s has been defined as normal for non-invasive methods in recent literature. Normal values of between 6 and 10 m/s for those over 30 years of age, has been identified in a large multi-centre CMR study of data from healthy individuals(373). Normal aortic distensibility in healthy individuals has been observed as 6.1(±2.5)mmHg in men and 8.6(±2.7)mmHg in women(513).

6.2.13 Use of CMR in clinical practice

CMR is increasingly being utilised in the diagnosis of cardiomyopathy and myocardial disease without the need for invasive endocardial biopsy, particularly in the context of cardiac amyloidosis, in which early identification and treatment is key. Myocardial fibrosis is an important mechanism in the development of congestive cardiac failure from a variety of disease mechanisms (see table 6.2). Su and colleagues reported an increased ECV in heart failure with preserved ejection fraction compared to healthy controls(514). CMR has also been used in the detection of subclinical LV impairment in patients with rheumatoid arthritis, due to myositis, showing increase in native T1 time compared to controls(515). Could CMR thus be useful in the detection of subclinical cardiac impairment in older CF patients?

6.3 Methods

6.3.1 Study overview

CF patients aged 40 years or above were recruited into the study. Eligibility included cardiovascular disease naivety and the participant group encompassed a range of pulmonary disease severity (as determined by $ppFEV_1$).

Ethical approval was obtained from the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) Ethics committee (reference 19/EM/0067).

The cardiac MR scanning was performed on a Tesla 1.5T MR scanner (Avanto, Siemens; Alliance Medical) according to a protocol developed in collaboration with cardiac imaging colleagues at MFT. The CMR protocol used is shown in appendix 5. Written consent was obtained in CF clinic prior to the scan date. On the day of scanning, each patient completed a standard MRI safety questionnaire, had a peripheral venous cannula inserted and a single serum sample taken for haematocrit. The scan time was approximately 45 minutes and was performed by specialist Alliance CMR radiographers, with supervision from Dr Christopher Orsborne (Cardiac imaging research fellow) and myself.

Standard long and short axis cine CMR imaging sequences were performed, following localisation of the LV within the thorax. LGE imaging began at six minutes following delivery of an intravenous gadolinium bolus (0.20mmol/kg). T1 mapping (by Modified Look-Looker Inversion Recover {MOLLI}) was obtained at the basal and mid left ventricular short axis level before and 15 minutes following the final bolus of gadolinium. LGE and both native and post-contrast T1 mapping were performed to assess for localised myocardial fibrosis. ECV fractions, based on native and post-contrast T1 values of blood and myocardium, were calculated to evaluate the presence of diffuse myocardial fibrosis.

6.3.2 CMR image analysis

CVI Circle Imaging™ software was used for CMR image analysis. CMR measurements were calculated using the stages described below. All calculations were corrected for body surface area (BSA), haematocrit and gender where applicable.

6.3.2.1 Biplanar long axis (LAX)

For biventricular and bi-atrial analysis, the biplanar long axis (LAX) method was used. Left atrial (LA) analysis was performed by defining the maximal LA area during the cardiac cycle.

Biplanar and monoplanar LA analysis were performed to give two and four chamber functional measurements.

The tricuspid annular plane systolic excursion (TAPSE) was measured as a marker of global right ventricular function. This is the distance between the tricuspid annulus and RV apex. This give values for LA and right atrial (RA) volumes, indexed to BSA. The cardiac valves were then assessed to look for any significant abnormalities.

6.3.2.2 Short axis 3D

Short axis cine imaging was used to analyse ventricular structure from base to apex. This technique was used to assess end diastolic (ED) and end systolic (ES) volumes in the RV and LV.

LV mass is calculated from contouring of the ventricular perimeter and calculating maximal wall thickness. This volumetric data was also indexed to BSA. The short axis method enhances the accuracy of ventricular analysis when used in combination with long axis methods.

6.3.2.3 Tissue characterisation

Tissue characterization was performed used LGE imaging. Gadolinium should be distributed in and out of myocardium uniformly. As an extracellular contrast agent, if there is an area of extracellular expansion, LGE will be present (in different patterns and distribution). T2 is a marker of myocardial oedema and is abnormal if elevated. This is demonstrated by the region of interest drawn in the LV septum to quantify T2 time. Septal analysis is the most reliable in the absence of an area of focal oedema. T2 is calculated at the basal and mid slice of the LV image, then an average of the two measurements is taken.

6.3.2.4 Tissue tracking

Strain analysis feature tracking was used in analysis. Endocardial and epicardial contours were drawn using available software. This gives a peak global longitudinal strain measurement, which is reduced in subclinical LV function and a method used to detect early dysfunction.

6.3.2.5 T1 Time

The MOLLI method was used to measure native T1 time. Post-contrast T1 was also measured with native T1 to calculate extracellular volume fraction (ECV). Epicardial

contours were drawn, focusing on the middle third of the myocardium. RV insertion point was selected, giving six segments for overall T1 time, pre and post contrast, for basal and mid LV areas. Blood pool and myocardial T1 then provides final data sets.

6.3.2.6 Aortic distensibility

Aortic distensibility and blood pressure were measured using the largest and smallest ascending aortic diameter during the cardiac cycle. The PWV was then measured using flow waveforms at a transection point through the aortic arch.

6.3.2.7 Flow

The ascending and descending aorta structure and waveforms were traced by endothelial contouring and used to calculate aortic blood flow.

All above data parameters were inputted into a pre-formed spreadsheet and values adjusted for BSA and haematocrit (appendix 9), and compared to normal reference ranges as provided by Petersen et al (appendix 10)(512). SPSS® (IBM®, version 25.0) was used for statistical analysis, with two-tailed p values of <0.05 deemed statistically significant.

6.4 Results

6.4.1 Demographics of the study population

The demographics of the study population are shown in table 6.3 and 6.4. Ten patients were recruited, of whom nine underwent CMR scanning. Due to lack of confirmation of CMR-safety of one participant's port-a-cath, this patient was deemed unfit for scan at the discretion of the CMR imaging team.

All patients tolerated the scan well and completed the required duration of imaging, including relevant breath-hold sections. There were no complications during or following scanning. All participants had normal baseline renal function (eGFR>90ml/min/1.73m^2). Subsequent renal function at the next routine clinic appointment (tested within 4 weeks) was normal for all participants.

Mean age of the cohort was 46.7(±3.39) years. There was a range of pulmonary disease severity (ppFEV$_1$ 25-91%) with a mean of 50.1(±23.5)%. For participants, 88.9% were exocrine pancreatic insufficient, 66.7% had CFRD and 77.8% had chronic *Pseudomonas aeruginosa* infection.

All patients were non-smokers. Patient 1 and 4 had known hyperlipidaemia. Patient 2 had systemic hypertension and patients 2 and 4 had a positive family history of premature cardiovascular disease (see table 6.3).

6.4.2 CMR analysis

Full CMR data is shown in tables 6.5 to 6.9 (end of chapter 6 results section) Normal CMR reference values used(512) are shown in appendix 10.

All CMR parameters were corrected for body surface area (BSA), age and gender using the CMR calculator (version 4.0.1, European Society of Cardiology).

- Mean RVEF was 56.0(±6.40)% (normal range 45-68%) and mean LVEF was 61.7(±6.22)% (normal range 48-70%).

- Two patients had slightly low LV end systolic volume (ESV) and one had low LV end diastolic volume (EDV), based on normal reference parameters.

- All patients had normal RVEF, despite five patients having severe lung disease ($ppFEV_1$ <40%).
- All patients had normal T1 and T2 times; mean 1033.3(±26.1) and 47.7(±2.75) milliseconds (ms) respectively.
- The presence of LGE was found in four out of nine patients. This was specifically at the right ventricular insertion point (RVIP), suggestive of RVIP fibrosis. Although, three of the patients with LGE had $ppFEV_1$ <40%, there was no significant difference in RVEF between the LGE and no LGE group overall; 55.5(±2.3)% vs 56.4(±8.7)%, p=0.850.
- PWV was normal in all nine patients; mean 4.6(±2.9) m/s. Aortic distensibility was also normal for all participants; mean 4.5(±2.1) mmHg {normal values; (mean±SD) 6.1±2.5mmHg in men and 8.6±2.7mmHg in women}.
- The mean ECV for the total group was 26.5(±2.30)% (normal range 20-26%). ECV values ranged from 21.2 to 30.2%, observing higher values in females; patient 1, 2 and 8 (females) had ECV values of 27.7, 29.2, 30.2% respectively, giving a mean of 29.1(±1.27)% compared to 25.2(±1.4)% in male participants; p=0.005.
- There was no significant difference in ECV values between lung function groups (p=0.282). Higher ECV values were seen in diabetics compared with non-diabetics; 27.2(±2.1)% vs 25.1(±2.3)%, although this was not statistically significant using T-testing (p=0.216).
- Patients were divided into severe and non-severe lung disease groups according to baseline lung function; severe=$ppFEV_1$ <40%, non-severe=$ppFEV_1$ >40%. Comparison between these groups showed no statistical difference in demographic or cardiac MRI parameters (table 6.4 and 6.5).
- There were no statistically significant correlations between $ppFEV_1$ and biventricular functional parameters; $ppFEV_1$ vs LVEF: r=0.319, p=0.413, $ppFEV_1$ vs RVEF: r=0.559, p=0.117.
- There was no statistically significant difference in RVEF, LVEF, aortic distensibility, PWV or ECV with advancing age, as categorised into 40-49 and ≥50 years (table 6.10).

CMR parameter mean±SD	40-49 years	≥50 years	P value
RVEF (%)	56.3 (7.3)	55.0 (1.4)	0.520
LVEF (%)	62.5 (7.0)	59.9 (0)	0.821
Aortic distensibility (mmHg)	4.7 (2.4)	3.7 (0.1)	0.588
PWV (m/s)	5.4 (1.6)	2.1 (5.4)	0.162
ECV (%)	26.6 (2.5)	26.1 (2.3)	0.776

Table 6.10: Comparison of CMR parameters between age groups. (CMR=cardiac MRI, PWV=pulse wave velocity, ECV=extracellular volume fraction).

Patient	Age (years)	Gender	Genotype	ppFEV$_1$	BMI	Predominant organism	Pancreatic status	CFRD status	Presence of CV risk	Baseline hsCRP (mg/L)
1	50	F	Phe508del/Phe508del	34	22.2	PsA	PI	N	⬆ Chol	1
2	45	F	Phe508del/Arg553X	58	24.9	PsA	PS	N	+ve FH	2
3	41	M	Phe508del/Phe508del	25	26.6	PsA	PI	N	⬆ BP	12
4	45	M	Phe508del/1078delT	54	24.4	*B.multivorans*	PI	Y	⬆ Chol, +ve FH	19
5	53	M	Phe508del/Asp579Tyr	32	21.5	PsA	PI	Y	Nil	4
6	47	M	Phe508del/Phe508del	39	24.8	PsA	PI	Y	Nil	6
7	45	M	Phe508del/Phe508del	91	26	PsA	PI	Y	Nil	2
8	47	F	Phe508del/Phe508del	83	22.2	*B.cenocepacia*	PI	Y	Nil	13
9	47	M	Phe508del/Phe508del	35	21.8	*B.cenocepacia*	PI	Y	Nil	7

Table 6.3: Demographic data for CMR participants (B=*Burkholderia*, PsA=*Pseudomonas aeruginosa, PI=exocrine pancreatic insufficient, PS=exocrine pancreatic sufficient, FH=family history of premature CV disease, BP=blood pressure, Chol=cholesterol*

Parameter	Total CF group n=9	$ppFEV_1$ <40% n=5	$ppFEV_1$ >40% n=4	P value
Age (years) mean±SD	46.7 (3.39)	47.6 (4.5)	45.5 (1.0)	0.391
% male	66.7	80%	50%	0.524*
BMI mean±SD	23.8 (1.95)	23.4 (2.2)	24.4 (1.6)	0.478
BSA (m^2) mean±SD	1.74 (0.18)	1.8 (0.2)	1.7 (0.1)	0.379
SBP (mmHg) mean±SD	113.9 (15.2)	112.6 (8.9)	115.5 (22.4)	0.797
DBP (mmHg) mean±SD	71.6 (6.97)	73.0 (4.1)	69.8 (10.0)	0.524
$ppFEV_1$ (%) mean±SD	50.1 (23.5)	33 (5.14)	71.5 (18.3)	**0.003**
Pancreatic status - %PI	88.9%	100%	75%	1.000*
CFRD (%)	66.7%	60%	75%	0.167*
Chronic PsA (%)	77.8%	80%	50%	0.524*
CRP (mg/L) median (IQR, range)	6.0 (10.5, 1-19)	6.0 (7, 1-12)	7.5 (15.5, 2-19)	0.730**

*Table 6.4: Mean demographic parameters of total CMR cohort, then comparison between severe and non-severe groups (severe=$ppFEV_1$ <40%, non-severe=$ppFEV_1$ ≥40%); BSA=body surface area, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, ppFEV1=percent predicted forced expiratory volume in one second, CFRD=CF-related diabetes mellitus, PsA=Pseudomonas aeruginosa. {*Fishers exact test, **Mann Whitney U test).*

Cardiac parameter	CF group (mean±SD) n=9	$ppFEV_1$ <40% n=5	$ppFEV_1$ >40% n=4	P value
LV EDV (ml)	116.5 (21.3)	110.4 (22.6)	124.0 (19.8)	0.375
LV EDV indexed (ml/min^2)	67.0 (11.0)	61.5 (9.0)	73.9 (10.2)	0.096
LV ESV (ml)	44.4 (10.0)	43.6 (4.5)	45.4 (14.4)	0.809
LV ESV indexed (ml/min^2)	25.7 (6.0)	24.6 (4.4)	27.0 (8.2)	0.590
LV EF (%)	61.7 (6.22)	59.9 (5.9)	64.0 (6.7)	0.360
LV mass (g)	88.8 (17.8)	89.2 (18.2)	88.3 (19.9)	0.902
LV mass indexed (g/m^2)	50.8 (7.2)	49.6 (5.6)	52.4 (9.6)	0.606
LV max wall thickness (cm)	10.2 (1.24)	9.9 (1.1)	10.6 (1.4)	0.436
RV EDV (ml)	128.1 (19.2)	123.8 (23.3)	133.5 (13.9)	0.491
RV EDV indexed (ml/min^2)	73.7 (8.7)	69.1 (7.3)	79.6 (6.9)	0.062
RV ESV (ml)	55.9 (9.41)	56.9 (5.2)	54.7 (14.0)	0.749
RV ESV indexed (ml/min^2)	32.2 (5.2)	32.0 (1.8)	32.6 (8.1)	0.872
RV EF (%)	56.0 (6.40)	53.4 (4.9)	59.3 (7.2)	0.189
LA volume (ml)	61.2 (16.3)	58.5 (15.4)	73.5 (15.0)	0.185

RA volume (ml)	60.1 (19.2)	54.6 (23.3)	67.0 (11.9)	0.298
TAPSE (cm)	3.2 (3.9)	4.0 (5.3)	2.1 (0.7)	0.506
Aortic parameters				
Aortic distensibility (mmHg)	4.5 (2.1)	4.1 (1.7)	5.0 (2.9)	0.587
Pulse wave velocity (PWV) (m/s)	4.6 (2.9)	4.7 (3.8)	4.5 (0.9)	0.943
Myocardial tissue characterisation				
T1 (ms)	1033.3 (26.1)	1041 (31.4)	1024 (17.5)	0.389
T2 (ms)	47.7 (2.75)	46.8 (2.6)	48.9 (2.8)	0.285
ECV fraction (%)	26.5 (2.30)	25.7 (1.9)	27.5 (2.7)	0.282
Absolute myocardial ECM volume (ml)	22.2 (3.26)	21.7 (3.4)	22.8 (3.4)	0.635
LGE present (%) (n) **Size (g)** **Size (%)**	44.4 (n=4) 2.11 (1.11) 1.98 (1.09)	60% (n=3) 2.7 (0.3) 2.5 (0.4)	25% (n=1) 0.48 0.4	-
Infarct present (n)	n=0	n=0	n=0	-

Table 6.5: Cardiac MR measurements for total cohort and comparison between severe and non-severe groups (severe=ppFEV$_1$<40%, non-severe= ppFEV$_1$ ≥40%); ECV=extracellular volume fraction, LGE=late gadolinium enhancement, TAPSE=The tricuspid annular plane systolic excursion, PWV=pulse wave velocity, EDV=end diastolic volume, ESV=end systolic volume, EF=ejection fraction, LV=left ventricle, RV=right ventricle.

Patient	LV EDV (ml)	LV EDV index (ml/m^2)	LV ESV (ml)	LV ESV index (ml/m^2)	LV mass (g)	LV mass index (g/m^2)	LV EF (%)	LV max wall thickness (cm)
1	113	69.2	47	28.8	67	41.1	59	8.7
2	109	66.0	36	21.8	76	46.0	67	10.0
3	138	63.7	42	19.4	112	51.7	70	9.2
4	150	85.0	61	34.6	117	66.3	59	12.0
5	115	67.4	47	27.6	85	49.9	59	9.5
6	75	46.5	33	20.5	79	49.0	56	10.6
7	108	64.4	31	18.2	86	51.3	72	11.5
8	129	80.0	54	33.5	74	45.9	58	8.9
9	111	60.8	49	26.9	103	56.4	56	11.5

Table 6.6: Left ventricular CMR data (adjusted for age and body surface area (BSA)).

Patient	RV EDV (ml)	RV EDV index (ml/m^2)	RV ESV (ml)	RV ESV index (ml/m^2)	RV EF (%)	TAPSE (cm)
1	121	74.3	55	34.0	54	1.3
2	133	80.5	57	34.5	57	2.0
3	161	74.3	66	30.5	59	1.5
4	151	85.6	67	38.0	55	2.3

5	127	74.5	56	32.8	56	1.8
6	99	61.4	53	32.9	46	13.5
7	117	69.8	35	20.6	70	2.9
8	133	82.5	60	37.2	55	1.3
9	111	60.8	54	29.6	52	2.0

Table 6.7: Right ventricular CMR data (adjusted for age and BSA), TAPSE=The tricuspid annular plane systolic excursion.

Patient	LA vol (ml)	LA vol index (ml/m^2)	RA vol ml	RA vol index (ml/m^2)
1	71	43.5	66	43.5
2	80	48.4	63	38.1
3	60	27.7	46	21.2
4	66	37.4	78	44.2
5	55	32.5	49	28.6
6	34	21.1	25	15.5
7	57	34.0	52	31.0
8	91	56.4	75	46.5

9	72	39.5	87	47.7

Table 6.8: Atrial CMR data (adjusted for age and body surface area (BSA))

Patient	Aortic distensibility (mmHg)	PWV (m/s)	ECV (%)	Absolute myocardial ECM vol (ml)	Absolute myocardial cell vol (ml)	Native T1 (ms)	Native T2 (ms)	Is LGE present	Is infarct present	LGE size (g)	LGE size (%)
1	3.6	1.8	27.7	17.7	46.1	1078	49	No	No		
2	5.2	4.8	29.3	21.2	51.1	1032	51	No	No		
3	3.6	6.1	23.2	24.8	81.9	996	45	Yes	No	2.3	2.1
4	NA	NA	25.0	27.9	83.5	1023	47	Yes	No	0.5	0.4
5	3.8	5.9	24.4	19.7	61.2	1029	48.5	Yes	No	2.7	2.6
6	7.1	8.2	27.1	20.4	54.9	1061	43	No	No		
7	7.8	3.5	25.4	20.8	61.1	1001	46	No	No		
8	2.1	5.3	30.2	21.3	49.2	1042	51.45	No	No		
9	2.5	5.1	26.2	25.7	72.3	1040	48.35	Yes	No	2.9	2.8

Table 6.9: Aortic and myocardial CMR data (adjusted for age and body surface area (BSA))

6.5 Discussion

6.5.1 General discussion

This is the first cardiac MRI study in older CF adults to date. Participants had no prior history of cardiovascular disease. Four out of nine patients had pre-imaging cardiovascular risk factors identified, in the form of systemic hypertension, hyperlipidaemia and/or a positive family history of cardiovascular disease. Over 60% of participants had CFRD. A high prevalence of localized LGE (44%) and raised ECV (56%) was found in this study group. Higher ECV was found in women. Biventricular function was within the normal range for all participants and was not affected by pulmonary disease severity. No patient had increased central arterial (aortic) stiffness based on CMR analysis.

Previous echocardiographic studies have eluded to left ventricular dysfunction in CF, however results have been variable and the conclusive detection of CF-related cardiomyopathy or overt myocardial disease in CF is yet to be achieved. The limitations of echocardiography in CF myocardial assessment may call for the utilisation of a more sensitive technique, with the ability to detect subtle or subclinical cardiac pathology, allowing timely management and reduction of cardiac comorbidity.

Although the right heart has been extensively studied in CF, in the context of cor pulmonale and advanced pulmonary disease, we must not discount other causes of cardiac abnormality in CF, especially in an ageing population with emerging cardiovascular risk. Study results of left ventricular function in CF to date, although not consistent, have shown abnormal LV function both at rest and with exercise(468)(502). The variation in results may indicate that the presence of LV dysfunction is associated with particular CF groups. This highlights a number of important questions;

1. Does CFTR genotype affect the susceptibility of cardiac disease in CF?
2. What are the role of modifier genes and the environment on cardiovascular risk in CF?

The optimisation of cardiac health in the ageing CF patient is of great importance as survival continues to increase and the cardiovascular challenges of ageing begin to emerge(503). Morbidity and mortality from cardiac disease in CF may become a reality in the ageing CF population, and the presence of cardiac disease in CF will have implications for quality of

life, organ transplantation and prognosis. Cardiac MR imaging has the ability to diagnose clinical and subclinical myocardial disease and, since myocardial fibrosis is related to adverse outcomes in a spectrum of cardiovascular pathology, may be an invaluable future imaging technique in CF.

6.5.2 Biventricular function

Normal RV function in this cohort is interesting given that five patients had advanced pulmonary disease. Importantly, none of the study participants were using long term oxygen therapy or non-invasive ventilation at the time of data collection. Right heart disease is classically associated with advanced lung pathology(516)(501)(470) and the presence of right heart failure has been shown to correlate with the degree of pulmonary severity in CF(470). However, results are variable and in fact some studies have shown normal RV function in those with severe lung disease(500)(517).

The minor abnormalities in left ventricular ESV and EDV in the context of normal ejection fraction and LV mass in two patients is likely a result of subtle analytical method differences in the contouring process and precision variation in CMR software used. Hence, we are not taking these results to be pathological. Given there are no other CMR studies in CF for comparison, it is difficult to interpret the clinical ramifications of our ventricular data. No significant correlation existed between ppFEV_1 and biventricular function in our older CF cohort, as we might have expected. A study by Sellers et al most closely reflects our findings, with essentially normal RV and LV echocardiographic parameters in CF adults with varying pulmonary severity, and a higher mean age than preceding studies (mean of 35 years)(500). It would be interesting to assess in a comparative younger disease cohort if longevity in CF might be influenced by preservation of right ventricular function even in the context of severe pulmonary disease. Using strain echocardiography, Labombarda and colleagues observed subclinical LV dysfunction with severe CF pulmonary disease and lower LVEF in CF patients (mean age 24 years) compared to healthy controls(492). Ozcelik et al observed no correlation between reduced RV and LV function in a paediatric CF cohort(518). Comparison of LV function with a healthy control group would be informative in an older CF population.

6.5.3 Aortic parameters

Aortic distensibility and PWV were within the normal range for all participants. Non-invasive arterial stiffness measurement (using the Vicorder®, Skidmore Medical) were not compared to CMR results for the study participants due to small sample sizes and lack of validation of either method in the CF population. However, we note that CMR measurements of aortic stiffness may have superior accuracy(373). There was no significant difference in aortic stiffness with advancing age in our study group, a trend we may have expected given our Vicorder® results (see chapter 4) and that of arterial stiffness studies in the general population(337)(519). It is likely that this study group was not large enough to provide a conclusive arterial stiffness trend.

6.5.4 Late gadolinium enhancement (LGE)

LGE was present in four out of nine patients. This appears to be localised to the RV insertion point, known as RV insertion point fibrosis (RVIP). This is usually a benign process and requires no further investigation. However, right ventricular insertion fibrosis has been seen in association with certain disease entities and the accumulation of gadolinium at ventricular insertion points can be due to the presence of hypertrophied myocardium(520). Several CMR studies observing localized LGE due to RVIP fibrosis have been reported. Yi and colleagues describe RVIP fibrosis and LGE in the context of non-ischaemic dilated cardiomyopathy(521). This finding, however, had no bearing on future risk of adverse cardiac events in this cohort. The presence of LGE and RVIP fibrosis has been found in patients with pulmonary hypertension(522) and may be associated with more severe RV disease. Sato and colleagues identified RVIP fibrosis to be associated with abnormal interventricular septal movement in pulmonary hypertension(523). Freed and colleagues observed a relationship between RVIP fibrosis and severe pulmonary hypertension, associated with poorer prognosis. Freed et al also showed lower RVEF in patients with RVIP-induced LGE(524). However, this was not the case for our CF cohort, all with normal RVEF and no significant difference in RVEF between those with or without LGE ($p=0.850$). Interestingly, three out of the four patients with evidence of RVIP fibrosis were in the severe lung disease group ($ppFEV_1 <40\%$), however there was no significant difference in RVEF between lung function groups ($p=0.189$).

Although not within the remit of our study, it would be prudent to combine the detection of RVIP fibrosis (as evidenced by LGE on CMR) with pulmonary arterial pressure studies, to assess the relationship between pulmonary hypertension and RVIP fibrosis in CF. However, in the absence of significant RV dysfunction in our cohort, interpretation of these findings and their associated clinical impact would be challenging. Blyth and colleagues report the use of NTpro-BNP levels as a measure of RV systolic dysfunction, showing good correlation to CMR analysis of RV function(525). Could this be applied in a CF context to detect subclinical RV impairment using NTpro-BNP as an alternative to invasive imaging techniques?

The presence of RV insertion fibrosis in a large number of study participants may elude to the possibility of a high prevalence of this in CF patients, and is consistent with pilot data from another study at our institution, assessing for myocardial abnormalities during CF pulmonary exacerbation. However, larger studies and those with comparative control groups will be required to assess a causal relationship.

6.5.5 ECV

The mean ECV for the total group was 26.5 (±2.30)%. Sado and colleagues report a normal CMR ECV range of 20 to 26%(526). ECV indices for myocardial infarction may be over 50%, however more subtle elevations in ECV of up to 28-29% may be seen with non-ischaemic cardiomyopathies(527). Miller et al reports ECV values of up to 30% in healthy controls(506). Therefore, a mean ECV of just over 26% in our CF group may not necessarily be an indicator of myocardial disease. However, if we were to take an ECV of 26% as the cut off for normal, 55.6% of our study group would have raised ECV and potential evidence of diffuse myocardial abnormality.

ECV values were significantly higher in female than male participants; mean of 29.1(±1.27) vs 25.2(±1.4)% (p=0.005). Higher ECV in women, as assessed by CMR, may be a normal physiological variant. Miller et al showed similar results in a group of healthy volunteers, with mean ECV 29.6±3.0% in women and 25.4±3.0% in men on the same MR scanner as used in our study(506). Sado and colleagues also reported higher ECV values in healthy females compared to males, also on a Siemens 1.5T MR scanner; x0.04% (SD difference 1.4)(526). Possible explanations for this include gender variation in cardiac disease expression, cardiac anatomy and haematocrit levels (required for ECV calculation). Given

that ECV measurement by CMR is a relatively new concept, this may warrant further investigation.

No significant difference was seen in biventricular function and ECV with age in our study. ECV has not been found to increase with advancing age in normal subjects(527). Based on normal reference data from Peterson et al(appendix 10)(512), there is no significant reduction in left and right ventricular function with age, particularly within the age range covered in our study. Arterial stiffness measurements using the Vicorder® were seen to increase with age in our previous study (see chapter 4), however this relationship was not seen with CMR. This may reflect the higher reliability of CMR at detecting abnormal aortic distensibility or, since we know that aortic distensibility does decrease with age(374), it may simply reflect inaccuracies of a smaller sample size used here.

There was a higher mean ECV seen in participants with CFRD than those without; 27.2(±2.1)% vs 25.1(±2.3)%, although this was not statistically significant (p=0.216). Wong and colleagues observed higher ECV values in patients with type two diabetes mellitus than non-diabetic comparators, with an association between raised ECV, a higher rate of hospital admission rates with cardiac failure and increased mortality(528). Interestingly, lower ECV was seen in patients already established on ACE inhibitors. CMR analysis of ECV may therefore represent a method with which to detect early myocardial change in diabetic patients.

Myocardial extracellular matrix enlargement indicates left ventricular remodelling and abnormal myocardium. CMR is an accurate and non-invasive method of assessing the myocardium and in calculating ECV(506). In addition to infiltrative cardiomyopathies such as amyloidosis, raised ECV is found in a number of other cardiac pathological processes to include myocardial infarction, hypertensive heart disease, diabetes mellitus and systemic inflammatory disorders, such as rheumatoid arthritis. All of the above, as previously discussed in the context of cardiovascular risk, may be relevant in CF, particularly in an ageing population with emerging multiorgan comorbidity. Analysis of ECV may thus be a useful marker of subclinical cardiac pathology and detecting future adverse cardiovascular events in CF.

Pilot data from a cardiac MR study performed at our institution has shown raised ECV in a younger adult CF cohort (median age 28 years) during pulmonary exacerbation, possibly related to transient alterations in myocardial capillary permeability during acute infection. In addition, a strong negative correlation between ECV and severity of airflow obstruction was observed. Could recurrent myocardial injury during pulmonary exacerbation contribute to the development of myocardial fibrosis in CF? It would be interesting to assess the influence of pulmonary exacerbation on both biventricular function and myocardial composition in an older adult CF group.

6.5.6 Limitations of study

The high sensitivity and specificity of cardiac MR imaging confers accuracy in studies with small sample sizes. However, a CMR study in CF is likely to require a larger number of participants in order to provide conclusive results. Variability in cardiac image acquisition, assessment and cardiac contouring in CMR analysis is always a limitation of CMR studies and is also relevant here. CMR imaging in CF may also be limited by a lack of MRI safety data related to older port-a-cath devices, as was the case for one study participant. In severe pulmonary disease, CMR image quality may be affected by poor breath holding capacity and lack of ability to lie flat for the scan duration, limiting the viability of CMR in older CF patients with advanced pulmonary disease. Although not relevant in our cohort, intravenous contrast allergy and reduced renal function may also be limitations of CMR in older CF patients.

CMR data correction for BSA and gender allows for greater accuracy, however the lack of definite myocardial parameter reference ranges and uncertainty of causation of gender variation in ECV means that interpretation of myocardial findings in CF must be done cautiously. Further studies of CMR in CF are required.

6.6 Conclusion

Cardiac MR imaging is well tolerated in CF patients and provides high quality assessment of biventricular function, cardiac morphology and myocardial structure. An absence of right ventricular abnormalities was found in this older CF cohort, even in the presence of advanced pulmonary disease. Left ventricular function was also within the normal range for all participants. The prevalence of LGE due to RVIP fibrosis in this older CF cohort was high (44%), the significance of which is uncertain. RVIP, as shown by localised LGE on CMR, has been shown to be associated with pulmonary hypertension and worse prognosis in previous non-CF cardiac studies. Similar research in CF would be informative.

Higher ECV values were found in female CF patients, corroborating results from previous CMR studies, the significance of which is again uncertain. However, with the majority of ECV values higher than the recommended reference range in our study group, it is possible that this indicates a signal of raised ECV in older CF patients and the possibility of underlying myocardial abnormality. Larger studies with a focus on specific comorbidities would be useful in the assessment of causation and clinical impact of raised ECV in CF.

The presence of raised ECV in 44% of patients may highlight the need for closer attention to be paid to cardiac involvement in longer term CF survivors. The existence of myocardial disease is relevant in CF adults, not just a rarity in CF children, and the prevalence of cardiac abnormality in CF may be greater than originally thought.

Expense and availability of CMR is likely to limit its widespread use within the CF population in the determination of cardiac pathology. The use of NT-pro BNP measurements may be a useful adjunct to CF cardiovascular assessment as shown by Blyth et al(525). Cardiovascular pathology undoubtedly exists in older adult CF patients and its presence is likely to increase with improving survival. CFTR and non-CFTR related cardiac influences will be challenging to differentiate, however an increased awareness and exploration of cardiac disease in CF will allow the CF multidisciplinary team to be better equipped at early detection and treatment of additional comorbidity in this exigent chronic disease.

6.7 Study recommendations

This pilot study of cardiac MRI in an older CF cohort was well tolerated and provided suitable images for cardiac analysis.

1. CMR may be a useful adjunctive tool in CF cardiac assessment, although greater numbers are required to determine its feasibility on a larger scale.
2. Cardiovascular assessments should be performed regularly in long term CF survivors to determine cardiovascular risk and requirements for primary preventative treatment.
3. CMR may be a useful adjunct in older CF patients with high cardiovascular risk and/or cardiac symptoms.
4. It may be interesting to perform CMR in older CF patients during and after pulmonary exacerbation to assess the relationship between pulmonary and cardiac function in the acute setting.
5. CMR imaging pre and post CFTR modulator initiation may also be useful to assess the cardiac benefits of CFTR function restoration, and similarly CMR analysis may be informative before and after lung transplantation in CF.
6. Further research into the benefits of cardiac MR imaging in CF is required and CMR may provide an option for non-invasive diagnosis of myocardial pathology, allowing early management and treatment of cardiovascular comorbidity.

Study	Date	Number of participants (n) and age range (years)	Patient group	Cardiac imaging modality	Results	LV studied?
Chipps et al(468)	1979	21 4 to 27 years	Range of CF severity – ppFEV$_1$ 13 to 83%	M-mode echocardiography, radionuclide angiography	Decreased RV EF in 72%	LV EF abnormal at rest in 19%, on exercise in 14%
Allen et al(516)	1979	34 5 months to 35 years	Range of symptom severity	M-mode, 2D echocardiography	RV hypertrophy	Increased thickness of LV posterior wall, correlated with pulmonary severity
Matthay et al(501)	1980	22 14 to 27 years	Range of CF symptom severity	Radionuclide angiography	Abnormal RV in 41%, RV impairment correlate with CF severity	LV function normal in all patients
Panidis et al(517)	1985	17 CF, 10 controls 6-38 years	Range of severity: ppFEV$_1$ 19-87%	M-mode, 2D and Doppler echocardiography	Well maintained RV in moderately severe CF lung disease	Preserved LV in presence of RV dysfunction
Johnson et al(471)	1991	25	Range of CF severity: ppFEV$_1$ 13-99%	Doppler echocardiography	NA	Abnormal LV filling with severe pulmonary disease

		4 to 29 years				
Fraser et al(470)	1999	n=33 >18 years	Severe group (18): $ppFEV_1$ <40% and moderate group (15): $ppFEV_1$ 40-65%	M-mode, 2D and Doppler echocardiography	RV dysfunction correlates with severe pulmonary disease	LV dysfunction in one patient (aged 40, with CFRD)
Florea et al(472)	2000	103 15 to 57 years	Awaiting lung transplantation	Doppler echocardiography	RV dysfunction in severe CF pulmonary disease	No abnormalities in LV detected
Labombarda et al(492)	2011	42 CF 42 controls Mean age 24±7.5 years		Strain echocardiography		Subclinical LV abnormalities, correlating with severity of pulmonary disease. Lower LV EF in CF group.
Sellers et al(500)	2015	8 Mean age 35±13 years	Range of pulmonary severity: $ppFEV_1$ 39-106%	2D and strain echocardiography	Normal RV EF	Normal LV EF but subclinical LV systolic and diastolic abnormalities in CF patients

Giacchi(529)	2015	55 (25 adults, 30 children)	Range of pulmonary severity: ppFEV$_1$ 22-130%	Doppler echocardiography	RV EF and TAPSE impaired in adult CF patients compared to controls.	Lower LV EF also seen (NS).

Table 6.1: Echocardiographic studies commenting on left ventricular function in CF (NS=not significant, LV=left ventricle, RV=right ventricle, EF=ejection fraction, EF=ejection fraction, TAPSE=tricuspid annular plane systolic excursion).

The Complexities of Ageing in Cystic Fibrosis

7.0 Chapter 7: Summary and further work

7.1 Chapter three – The demographics of an older CF cohort

Cystic fibrosis as a disease entity has experienced a paradigm shift since its discovery in 1938. Treatment advances have rapidly evolved such that survival has improved exponentially, from less than one year to survival into the fifth decade and beyond. The most notable observation in long term CF survivors at MACFC was that they comprise a significant proportion of the total CF cohort at just over 20%. The number of older adult CF patients will undoubtedly continue to rise over the coming years with the recent increased availability of CFTR modulator therapy and further research into this fascinating group is therefore essential.

Chapter three addresses the first study aim: *To characterise the demographics of an older adult cystic fibrosis population, specifically those aged 40 years of age and above.*

This is one of only a handful of studies analysing the demographics of CF patients aged 40 years and above. This population is heterogeneous and diverse. I observed a high prevalence of severe CFTR mutations, a wide range of pulmonary disease severity and the majority had exocrine pancreatic insufficiency. Almost half had CF-related diabetes mellitus, a phenomenon expected with increasing age in CF. However, there existed a distinct group of late diagnosis, long term CF survivors with less severe phenotypic disease in our study cohort. The relationship between CFTR genotype and clinical phenotype is complex, however it is clear that less severe CFTR genotypes may confer survival advantage and may often present to clinical services in adulthood. With this said, the disease trajectory of these milder disease phenotypes may be unpredictable and we must not underestimate the importance of accurate diagnosis and adequate follow up of these patients within the CF multidisciplinary team.

A high proportion of our older cohort were in part or full time employment (55.3%) and a significant number had biological children (34.1%). This in itself is fascinating, given the poor prognosis of the majority of this group at the time of their CF diagnosis. The rapid

development of treatment over the latter part of the 20th century and beyond has led to an unrivalled survival increase in this orphan disease. Survival influences are multifactorial and unpicking their individual clinical influence is challenging.

Notably, almost 20% of our CF study group aged 40 years and above had systolic hypertension and just over 15% had hyperlipidaemia, evidencing some age-related cardiovascular risk in this population. This is an important area for further study in order to reduce further comorbidity in an already complex multi-system disease.

7.2 Chapter four – Cardiovascular risk in an older CF cohort

Cardiovascular disease accounts for 30% of deaths worldwide, observing no geographical or socioeconomic boundaries. Cardiovascular risk assessment and management is therefore essential and relevant to all disease populations. Historically, the early mortality in CF has meant that cardiovascular disease is extremely rare. However, similar to other inflammatory chronic diseases, CFTR dysfunction may present a degree of cardiovascular risk and the prevalence of cardiovascular disease is likely to increase in an ageing CF population. Potential cardiovascular risk factors also include fat malabsorption, salt intake and high fat diets. Therefore, cardiovascular risk modification must not be overlooked in long term CF survivors and a high prevalence of systemic hypertension and hyperlipidaemia in this cohort emphasises this point. The usefulness of apolipoprotein measurement as an adjunct to conventional lipid monitoring is unclear in CF, given the good correlation between equivalent cholesterol parameters and the relatively low prevalence of statin use in CF.

Chapter four addresses the study aims two and three:

- *To assess the cardiovascular risk and contributory factors in this cohort, including the prevalence of systemic hypertension, hypercholesterolaemia and CF-related diabetes mellitus (CFRD).*
- *To assess arterial stiffness parameters in the older adult CF patient, whether this is augmented in CF when compared with the general population, and a chronic kidney disease (CKD) cohort, and thus if CF patients are at risk of premature vascular ageing as part of their disease process.*

In this first study of cardiovascular risk prediction in CF, over 30% of participants had a cardiovascular risk score of over 10% using the QRisk® calculators. This is an important

finding and may present an opportunity to diagnose and implement primary prevention prior to cardiovascular morbidity in CF. Inherent CFTR-related cardiovascular risk exists, including chronic systemic inflammation, arterial stiffening and endothelial dysfunction, independent of age-related atherosclerotic disease. Our older CF cohort had a high prevalence of increased aortic stiffness and arteriosclerosis, as measured by non-invasive methods, and showed a higher risk of increased AIx than a matched CKD group. This is significant and may represent premature vascular ageing in CF. The high prevalence of CF-related diabetes mellitus in an older CF group presents cardiovascular challenges, as does organ transplantation at older ages.

Total cholesterol levels appear to increase with age in our study cohort, and just over 15% had hyperlipidaemia. Although the prevalence of hyperlipidaemia in our CF cohort was lower than see in the general population, lipid monitoring in CF should form an integral part of cardiovascular risk assessment, along with adequate management of CFRD, systemic hypertension, renal disease and obesity.

The measurement of arterial stiffness, although non-invasive, is time consuming and likely not feasible in CF outside of a research setting. However, it would be recommended for CF patients aged 40 years and above to undergo regular comprehensive cardiovascular assessment including serum lipid profiles, blood pressure monitoring and cardiovascular risk scoring using QRisk®3. Classification of CFRD as type one or type two diabetes mellitus (T1DM, T2DM) has implications for QRisk® scoring, showing lower results with QRisk®3, a more sensitive predictive tool than its predecessor and currently recommended by NICE. Since QRisk® scores are higher with CFRD classed as T1DM and most CF diabetics use insulin therapy, it may be logical to use this diabetic phenotype in CF cardiovascular risk scoring. Further work into cardiovascular risk scoring in CF is certainly needed to assess benefit in this setting.

Cardiovascular disease often co-exists with renal dysfunction, as evidenced by the enhanced cardiovascular risk seen in the CKD population. Therefore, it is a logical step in older CF patients to assess cardiovascular and renal risk simultaneously. There are many factors that may contribute to renal decline in CF and this study begins to explore some of these in the context of an older CF cohort.

7.3 Chapter five – The kidney in an older CF cohort

Chapter five addresses the study aims four and five.

- *To assess the prevalence and causative factors of renal disease in an older adult CF population.*
- *To better define the most accurate method of calculating renal function (using glomerular filtration rate, GFR) in order to diagnose early renal disease in higher risk groups, such as those with CFRD.*

This is the first study of renal disease in an older adult CF cohort, observing a high prevalence of renal dysfunction. Abnormal GFR and the presence of proteinuria is high, although the prevalence of CKD stage 3 is relatively low and in line with previous studies in adult CF. Renal pathology in CF has many influencing factors. The renal impact of chronic treatment with cumulative intravenous aminoglycoside exposure, immunosuppressant use following organ transplantation and an increasing prevalence of CFRD with age, to name the most significant. Glomerulonephropathies are rare in CF, however early detection of proteinuria is essential in order to diagnose and manage intra-renal pathology effectively. Nephrolithiasis, recurrent pulmonary sepsis and the impact of associated acute renal impairment have also been shown to contribute to chronic renal disease over time. The development of atherosclerotic renovascular disease, systemic hypertension and an ageing kidney are all also likely to contribute to renal decline in older CF patients.

Accurate determination of renal function in CF, as measured by GFR, is crucial for renal monitoring in the context of nephrotoxic medication use, post-transplantation and in early detection of renal decline. Estimated GFR using creatinine based methods are inaccurate in this population, however cystatin C based eGFR equations seem to be the most accurate in our study population, and cystatin C has been shown to rise with increasing aminoglycoside burden in this cohort. However, endogenous renal biomarkers still represent limitations and underestimate GFR when compared to gold standard iohexol GFR. Underestimation of GFR in this population was unexpected, however similar GFR underestimation has been seen in some diabetic populations. This is the first study of iohexol GFR in an ageing CF cohort and was limited by small numbers. Further iohexol research would be extremely informative in order to understand accurate GFR determination in CF and to monitor renal function effectively over time in this population with emerging renal disease.

Recommendations for renal monitoring in CF include urine ACR measurements in both diabetics and non-diabetics and an increasing frequency of testing with age, allowing early identification and treatment of renal disease. Our results showed a relationship between greater intravenous aminoglycoside exposure and higher cystatin C levels, perhaps an indicator of aminoglycoside-related renal damage. The measurement of serum cystatin C may be a useful guide in determining safety and suitability of intravenous aminoglycoside in older CF patients with renal dysfunction. Although it is likely not feasible to use iohexol GFR outside of a research setting in CF, measured GFR may be important for higher risk patient subgroups; those with chronic renal impairment in the context of nephrotoxic medication dosing, organ transplant recipients on immunosuppression and those with intra-renal pathology, such as IgA nephropathy, and at risk of renal decline. A single sample iohexol protocol would provide more clinical practicality than multi-sample, and may therefore be feasible in these higher risk CF patient groups to determine accurate GFR.

7.4 Chapter 6 – Cardiac MRI in an older CF cohort

Although the right heart in the context of CF pulmonary disease has been extensively studied using echocardiographic methods, there is sparse data reported for left ventricular function and myocardial pathology in CF. Emerging cardiovascular risk in this older CF population has been observed and may influence the prevalence of ischaemic heart disease and cardiomyopathy with ageing. Abnormal CFTR protein has been shown to affect myocardial function and this inherent CFTR-associated cardiac risk may contribute to primary cardiomyopathy, compounded by the emergence of secondary cardiovascular risk in this ageing CF population.

Chapter six addresses study aim number six: *To investigate cardiac structure, function and the presence of myocardial fibrosis in older adult CF patients.*

Cardiac MR imaging (CMR) represents a highly sensitive and specific technique in the assessment of biventricular structure and function, particularly in the detection of myocardial pathology. Advantages over echocardiography include superior image acquisition and less influence of pulmonary hyperinflation and breathing artefact on image quality. This is the first study of CMR in CF. Interestingly, although normal left and right atrial and ventricular function was seen in all patients, evidence of raised ECV was seen in over 50%, possibly indicative of abnormal myocardial composition. Delayed gadolinium

contrast washout (LGE) was seen in four patients, related to RVIP fibrosis, the relevance of which is uncertain but warrants further investigation given its link with pulmonary hypertension and poorer prognosis in the non-CF population. Subclinical myocardial pathology may be present in older adult CF patients, perhaps representing the influence of CF longevity on cardiovascular disease. Abnormal myocardial studies in older adult CF patients is a new and additional finding to the childhood CF myocardial fibrosis phenomenon reported in multiple previous post-mortem studies.

The role of CMR in CF outside of a research setting is contentious due to cost, timing and requirement of intravenous contrast. However, its high sensitivity in detailing cardiac function presents a definite advantage in further assessment in patients at high risk of or with suspected cardiovascular disease. Further research into cardiac structure and function in CF adults is essential in order to understand the effect of emerging cardiovascular risk in this cohort and the impact on disease morbidity and mortality as survival continues to increase.

7.5 Recommendations

- CF patients aged 40 years and above should receive a comprehensive cardiovascular risk assessment including lipid profiles, blood pressure monitoring and QRisk®3 scoring. As in the general population, the timely implementation of primary prevention therapy is paramount to avoid cardiovascular morbidity and mortality.
- Urine ACR should be used in older CF patients both with and without CFRD for regular assessment of renal function and early detection of renal disease.
- Intravenous aminoglycosides may need to be avoided in older CF patients with renal dysfunction. Serum Cystatin C measurement may assist to identify older CF patients at higher risk of aminoglycoside-induced nephrotoxicity.
- The use of CMR in older adult CF patients is currently no more than a research entity. However, given its feasibility in this cohort, the implementation of CMR on a wider scale could be considered in long term CF survivors with emerging cardiovascular risk to provide detailed analysis of cardiac structure and function.

7.6 Study strengths

This is a large study of the CF population aged 40 years and above, and one of only a handful of its kind. This study area is very relevant in this era of CFTR modulation and increasing survival. This is the first study to examine the use of arterial stiffness measurements, QRisk® scoring, iohexol GFR and CMR in an older adult CF cohort, with good patient recruitment and study feasibility. Significant findings in some of these novel areas may warrant their further investigation in larger studies of comorbid ageing CF patients. Vast amounts of data have been collated for this patient group at MACFC, which will act as a valuable database for future studies in this older CF population.

7.7 Study weaknesses

A number of weaknesses of this study should be recognised. This is a cross sectional, observational analysis of an older CF cohort and therefore assumptions regarding factors contributing to disease longevity must be interpreted with caution. Longitudinal data would provide more informative analysis and conclusions.

A lack of standardisation of Vicorder® arterial stiffness measurements and QRisk® scoring in CF makes these techniques less transferrable to CF care and may limit their future use.

The study of CF cohorts in general, but particularly those aged 40 years and above, is associated with the inevitability of small sample sizes. Larger sample sizes are needed to increase the reliability and accuracy of data analysis, and when attributing conclusions to the CF population as a whole. Larger, multicentre epidemiological studies are required to analyse and interpret survival influences in the older CF community.

Longitudinal studies with larger sample sizes are required to assess GFR decline over time in the older CF population. Iohexol sampling may be impractical in CF settings and there is thus necessity for the development of simpler mGFR techniques and further research into the reliability of cystatin C GFR monitoring in CF.

CMR study in CF is likely to require a larger number of participants in order to provide conclusive results. Variability in cardiac image acquisition, assessment and cardiac contouring in CMR analysis is always a limitation of CMR studies and varies greatly between

imaging centres. Interpretation of myocardial findings in CF must be done cautiously and further studies of CMR in CF are required.

7.8 Further work

Surviving longer with CF will give rise to ageing comorbidities in this already complex and challenging disease. Multidisciplinary CF teams must strategically adapt to account for ageing complications of CF disease in order to optimise management, costing and structure of services as patient numbers and comorbid disease burden increases.

Further research into the CFTR-related and non-CFTR-related influences of ageing in CF is essential to increase our understanding of these long term CF survivors and their disease characteristics. It seems increasingly possible for CF patients to live long, full lives without the burden of multiple chronic therapies, particularly as we see the roll-out of CFTR modulators on a larger scale. CF patients have changing perspectives of their disease, allowing thoughts of a reality of living with chronic disease rather than suffering its all-encompassing consequences. As CF healthcare professionals, we perhaps also need to change historical perspectives of CF as a poor prognostic disease entity and shift management towards an ageing chronic disease. However, there will remain a proportion of CF patients with early mortality and genetic ineligibility for CFTR modulation, hence the importance of traditional therapies and maintaining quality of life remains a priority. The high burden of cardiovascular and renal disease within an ageing general population deems the further study of these areas in older CF cohorts essential. This is of particular importance given the relatively novel concepts that cardiovascular and renal disease burden represent in long term CF survivors, with consequent lack of available data, and during a time of significant CF treatment change with the widespread use of CFTR modulation.

Based on this study, I would recommend the following further research;

1. The use of QRisk® scores on a wider scale in CF and the efficacy of statin treatment and macrolide antimicrobials on reduction of inflammatory burden and cardiovascular risk in long term CF survivors.
2. The study of carotid intima-medial thickness in CF may further inform cardiovascular risk in this group.

3. Further study into the practicality of cystatin C in older CF patients and its accuracy in determination of GFR in this population.
4. The use of iohexol mGFR as capillary and one-sample protocols to determine the most accurate method of renal function monitoring, the feasibility of this test and to assess renal decline over time, in higher risk ageing CF patient groups.
5. Larger studies assessing the use of cardiac magnetic resonance imaging in older CF patients.

8.0 Appendix 1: The QRisk®3 score for cardiovascular risk prediction

Age 25-84	
Gender	Male Female
Ethnicity	White or not stated, Indian, Pakistani, Bangladeshi, other Asian, Black Caribbean, Black African, Chinese, Other ethnic origin
UK Postcode (a measure of deprivation)	
Smoking status	Non-smoker Ex-smoker Light smoker (less than 10) Moderate smoker (10 to 19) Heavy smoker (20 or over)
Diabetes status	None Type 1 Type 2
Angina or heart attack in a 1st degree relative < 60?	Yes/No
Chronic kidney disease stage 3*, 4 or 5?	Yes/No
Atrial fibrillation?	Yes/No
Are you on blood pressure treatment?	Yes/No
Do you have rheumatoid arthritis?	Yes/No
Do you have SLE?*	Yes/No
Do you have migraines?*	Yes/No
Do you have severe mental illness?* **(this includes schizophrenia, bipolar disorder, moderate/severe depression)**	Yes/No

Are you on anti-psychotic medication?*	Yes/No (Amisulpride, aripiprazole, clozapine, lurasidone, olanzapine, paliperidone, quetiapine, risperidone, sertindole, or zotepine)
Are you on regular steroid tablets?*	Yes/No
Do you have erectile dysfunction or are on treatment for this?*	Yes/No
Cholesterol/HDL ratio	
Systolic blood pressure (mmHg)	
Standard deviation of at least two most recent systolic blood pressure readings (mmHg)*	
Weight (kg)	
Height (cm)	
Calculate SCORE (risk)	

Figure 1.7: Example of QRisk®3 score parameters(162).

9.0 Appendix 2: Patient study questionnaire

Complexities of Ageing in Cystic Fibrosis

Patient Questionnaire

Version 2.0: 21st December 2018 **IRAS No: 257209**

Patient number ……..

Age ……

Genotype (if known) ……

Age at diagnosis …….

Clinic group ……

Are you currently on any modulator therapy? (e.g. Ivacaftor, symdeko, orkambi)

Y ☐ **N** ☐

Do you take creon? Y ☐ **N** ☐

Do you have diabetes Y ☐ **N** ☐

Are you on insulin? Y ☐ **N** ☐

Have you ever had allergic bronchopulmonary aspergillosis, or ABPA? Y ☐ **N** ☐

Are you on steroids currently? Y ☐ **N** ☐

Are you on itraconazole? Y ☐ **N** ☐

Do you have liver problems? Y ☐ **N** ☐

Do you have low bone mineral density? Y ☐ **N** ☐

Have you ever had a pneumothorax? (lung collapse) Y ☐ **N** ☐

If yes, have you every required a chest drain? Y ☐ **N** ☐

Have you ever had problems with haemoptysis? (blood in the sputum) Y ☐ **N** ☐

If Yes, have you ever had a procedure to stop this bleeding called a bronchial artery embolization?

Y ☐ **N** ☐

Have you ever had kidney problems or renal stones? Y ☐ **N** ☐

Details ……..

Do you have high blood pressure? Y ☐ **N** ☐

If yes, what medication are you on? ……

Do you have high cholesterol? Y ☐ **N** ☐

If yes, are you on medication and if so what? ……

Have you ever had any problems with your hearing? Y ☐ **N** ☐

Have you ever had a hearing test (audiogram)? Y ☐ **N** ☐

<u>Social</u>

Who do you live with? ……..

Are you married? Y ☐ **N** ☐

Please choose one of the following that is most applicable;

- **In full time study** **Details……..**
- **In full time employment** **Details ……..**

- **In part time study** **Details ……..**
- **In part time employment** **Details ……..**
- **Unemployed currently** **……**

Do you have any children? Y ☐ **N** ☐

If so, how many? ……

Have you ever smoked? Y ☐ **N** ☐

If yes, How many cigarettes per day? ……..

How many years did you smoke for? …….

Do you have a family history of any lung disease? Y ☐ N ☐

If yes, what lung disease and who in the family? ……..

Do you have a family history of heart disease? Y ☐ N ☐

If Yes, what heart disease? …….

Which member of your family and how old were they when they first event?

…….……

Do you have a family history of high cholesterol? Y ☐ N ☐

Have you ever suffered from anxiety? Y ☐ N ☐

If yes, are you on medication for this? ……

Have you every suffered with depression? Y ☐ N ☐

If yes, are you on medication for this? ……..

Do you have problems with erectile dysfunction? Y ☐ N ☐ NA ☐

Have you ever had a diagnosis of rheumatoid arthritis? Y ☐ N ☐

Have you ever had a diagnosis of systemic vasculitis (SLE)? Y ☐ N ☐

Have you ever had a HIV test? Y ☐ N ☐

Thank you for taking the time to complete this questionnaire.

Dr Sarah Paterson

Manchester Adult Cystic Fibrosis Centre, Wythenshawe Hospital

10.0 Appendix 3: Iohexol sampling protocol

1. Verbal consent will be taken from each patient.
2. A cannula is inserted into a peripheral arm vein and baseline blood samples taken to include creatinine, eGFR and cystatin C.
3. An injection of Omnipaque®/iohexol (300mg IU, 5mls) is administered via the cannula over a 20 second to two minute period, with the time precisely recorded.
4. The cannula is then flushed with 10mls of 0.9% normal saline.
5. The patient will be observed for 30 minutes to ensure, in the rare event, there is no allergic reaction.
6. The second blood sample is taken at 30 minutes, from the contralateral arm to the iohexol injection.
7. Further blood samples are then taken from contralateral arm at 120, 180 and 240 minutes after iohexol injection.
8. Capillary samples will be taken at 120, 180 and 240 minutes along with serum samples, using a Mitra device (10 microlitre sample) and a size seven lancet needle at a suitable peripheral site on the index finger.
9. Samples are transported to the laboratory at Wythenshawe Hospital. Patient height, weight, baseline observations and precise date and time of each sample will be recorded on the laboratory request form.
10. Venous blood samples are centrifuged to separate plasma and serum, labelled and then stored in aliquots at -80 degrees centigrade within six hours of venepuncture (performed by researcher).
11. Mitra capillary samples will be processed and stored next to serum samples, labelled accordingly (done by researcher).
12. Following completion of sample collection for all patients, these will be processed in one group so as to avoid any limitations of inter-batch processing variation.
13. Samples will be processed using liquid chromatography-tandem mass spectrometry (LC-MS)(262) by David Marshall, senior laboratory biochemist, MFT.

11.0 Appendix 4: The Wescor macroduct sweat test procedure

- The area preferred for stimulation of sweat is the inside of the forearm. It is important that the selected site is free of breaks, fissures, inflammation and any observable abnormality.
- The selected area of skin is cleaned thoroughly with deionised water to remove dirt, fatty material and dead cells to minimise electrical impedance. The area is then left wet or a drop of water added to the skin to minimise the risk of burns.
- The pilogel discs are placed on the underside of both the positive and negative electrode.
- The discs are gently rotated, applying light continuous pressure, until contact between disc and electrode is uniform and air free.
- The positive electrode (red) is placed onto the forearm followed by the negative electrode (black), a few centimetres proximal to the positive.
- A velcro strap is used to ensure firm and secure attachment of each electrode, such that the pilogel disc portion of the electrode is pressed flat against the skin. There should be moderate pressure to minimise discomfort, but not so much as to damage the gel discs.
- The electrodes are connected to the small electrical device which will provide iontophoresis. The iontophoresis process is then activated by holding the control switch until a beep is heard and a green light appears.
- Iontophoresis then proceeds automatically for 5-6 minutes.
- The macroduct sweat collector is prepared during the iontophoresis process. The macroduct sweat collector is removed from its packaging and a macroduct strap of suitable size is prepared to hold this in position on the skin for the collection phase of the process.
- At completion of iontophoresis, an audible tone will sound briefly, and the instrument will switch off.
- The negative (black) electrode is removed first and the skin cleaned with water. Then positive (red) electrode is then removed.

- The stimulated skin surface underlying the positive electrode is cleaned thoroughly with water and dried thoroughly with tissue in order to emphasise the erythema in this area.
- The macroduct sweat collector is attached firmly to the limb by applying the concave surface of the macroduct collector precisely over the area of skin contacted by the pilogel disc of the red electrode. The Velcro strap is wrapped around the limb and secured, ensuring a complete seal around the skin. This must not be overtightened as this will impede capillary attraction and result in inadequate sweat volume.
- Sweat should be collected for between 20 and 30 minutes, unless the macroduct tube is full earlier, according to national guidelines. This should give 20-90 milligrams (mg) of sweat, with a minimum of 20mg being required.
- The sweat sample is then removed and stored. This must be carried out while the macroduct is intact to the limb by removing the cover using a blunt needle, peeling open the micro tubing and attaching securely to a blunt needle and syringe. The spiral tubing is fully extended by lifting and the tube is then cut as closely as possible to the collector surface.
- The tubing containing the sweat can then be transferred to a sealable 10ml container for storage prior to analysis.
- The collector is then removed from the patient and discarded. Electrodes are cleaned with deionised water and wiped dry.

12.0 Appendix 5: Cardiac MRI study protocol

1.5T CMR scanner (Avanto, Siemens).

1. Orthogonal localisers
2. Bright blood - TrueFISP (Axial Stack)
3. CH2, CH4, SA localisers
4. CH4 cine
5. CH2 cine
6. CH3 cine
7. LVOT cine
8. Aortic valve cine
9. RVOT
10. MOLLI – CH4, basal and mid short axis. Ensure quality.
11. T2 – basal and mid short axis
12. Give contrast (gadolinium)
13. SA cine stack. 8mm slices. 2mm gap.
14. TI Scout (6 mins after contrast)
15. CH4, CH2, CH3 PSIR DE TruFISP
16. SA PSIR DE TruFISP
17. MOLLI (448B) – basal and mid short axis. Ensure quality / 15mins after contrast
18. Aortic candystick cine
19. Cine perpendicular to the ascending and descending aorta at pulmonary bifurcation level (40 phases). Measure BP during acquisition.
20. Phase encoded velocity mapping (VENC 1.5 m/s) of the aorta perpendicular to the ascending and descending aorta at pulmonary bifurcation level i.e. copy image position from Step 20. (40 phases).

13.0 Appendix 6: Demographic data for total study population

Patient	Age	Gender	BMI	Allele 1	Allele 2	CFTR modulator	Age at diagnosis (years)	FEV_1 (L)	ppFEV1 (%)	FVC (L)
1	46	M	25	Phe508del	Phe508del	1	1	0.71	20	1.82
2	68	M	26.6	Phe508del	Asp1152His	0	60	2.28	88	3
3	40	M	22.5	Phe508del	Phe508del	1	1	1.37	34	2.61
4	48	M	26.6	Phe508del	Gly551Asp	1	3	1.83	49	3.46
5	47	F	22.9	Phe508del	Phe508del	0	1.5	1.44	52	3.14
6	47	M	24.8	Phe508del	Phe508del	1	0.25	1.22	39	3.15
7	45	M	24.4	Phe508del	1078delT	0	-	1.85	52	2.73
8	47	M	28.8	Gly551Asp	Gly551Asp	1	1.5	2.35	65	3.25
9	45	F	24.9	Phe508del	Arg553X	0	3	1.66	58	2.93
10	47	F	22.2	Phe508del	Phe508del	0	1.2	2.11	75	2.87
11	45	M	23	Phe508del	1461insAGAT	0	2.5	1.48	43	3.43
12	63	M	26	Phe508del	3272-26A-G	1	0	1.05	32	2.74
13	46	M	24.1	Phe508del	Arg117His-7T	0	-	4.02	94	5.88
14	51	M	26.4	Phe508del	Ser945Leu	0	12	2.19	61	3.74
15	47	M	23.1	1507del	2711delT	0	0	2.8	75	3.77

16	50	M	21.4	1507del	2711delT	0	4	2.03	55	3.33
17	67	M	20.2	Phe508del	C.1538A>G	0	47	0.67	19	1.79
18	49	M	28	Phe508del	Phe508del	0	4	1.91	51	4.18
19	41	M	24.9	Phe508del	Phe508del	0	-	2.42	59	6.17
20	58	F	21.8	Phe508del	Phe508del	1	43	0.86	39	1.54
21	50	M	23.4	Gly551Asp	Gly551Asp	1	0	0.95	25	2.37
22	70	F	17.5	Phe508del	Arg117His-5T	1	38	1.19	56	1.96
23	47	F	19.3	Phe508del	Pro67Leu	1	19	2.24	75	4.01
24	41	M	26.6	Phe508del	Phe508del	1	0.25	1.12	25	3.64
25	48	F	22.3	Phe508del	Val520Phe	0	0	0.86	33	2.33
26	66	M	23.4	Phe508del	TG12-5T	0	58	1.83	56	3.84
27	42	F	21.3	Phe508del	Arg117His-5T	1	26	0.77	26	1.81
28	66	F	30.5	Phe508del	TG12-5T	0	64	1.96	87	2.5
29	44	M	29.5	Phe508del	Asp1152His	0	-	3.53	92	4.69
30	50	M	25.2	Phe508del	Phe508del	0	5	3.11	75	5.32
31	52	F	24.7	Phe508del	Gly551Asp	1	1	0.7	31	1.53
32	49	M	26	Phe508del	711+3A>G	0	34	1.44	41	2.73

33	55	M	23.4	Phe508del	Phe508del	1	0.5	1.61	39	3.62
34	49	M	25.7	Phe508del	Arg117His-7T	0	21	3.64	86	4.91
35	53	M	23.2	Phe508del	Ser945Leu	0	25	0.7	23	2.95
36	40	F	20.2	Phe508del	Phe508del	1	0	0.56	19	1.82
37	48	F	22.4	Phe508del	Phe508del	0	0	1.82	62	2.72
38	58	M	28.3	Arg117His-7T	Arg117His-7T	0	55	4.3	110	4.2
39	79	M	24.1	Phe508del	TG12-5T	0	72	2.64	96	3.82
40	41	F	24.4	Phe508del	Phe508del	1	6	0.95	36	2.13
41	53	F	18.4	Phe508del	Phe508del	0	0.25	0.75	33	1.5
42	45	M	26	Phe508del	Phe508del	0	0.75	2.91	91	4.22
43	48	M	22.2	Phe508del	Phe508del	1	0	1.39	40	3.47
44	43	M	27.4	Phe508del	Arg560Thr	0	0.5	2.97	63	5.17
45	48	M	22.7	Phe508del	1154insTC	0	1.5	1.72	44	4.63
46	52	F	21	Phe508del	c.3874-4522A>G	0	16	0.65	25	1.85
47	53	M	21.5	Phe508del	Asp579Tyr	0	10	1.15	32	3.46
48	47	F	30.3	Phe508del	Phe508del	0	3	1.35	48	2.21
49	56	F	23	Arg117His-7T	Arg117H-7T	0	54	2.62	94	3.42

50	47	M	21.8	Phe508del	Phe508del	0	0.3	1.39	35	3.39
51	48	F	18.7	Phe508del	Phe508del	0	-	0.63	21	1.55
52	45	M	24.2	Phe508del	3600G>4	0	19	1.73	47	3.3
53	40	M	23.2	Phe508del	Phe508del	0	3	2.28	55	5.08
54	48	M	27.4	Phe508del	Gly551Asp	0	0.5	2.1	58	4.68
55	42	M	26.1	3659DelC	Leu633Pro	0	0	2.9	72	4.5
56	41	M	25	Phe508del	621+1G>T	0	3	0.75	18	1.41
57	47	F	27	Phe508del	711+3A>G	0	40	3.06	100	3.56
58	50	F	22.2	Phe508del	Phe508del	0	-	0.95	34	2.3
59	54	M	21.5	Phe508del	Arg117His-7T	0	27	2.47	73	4.3
60	42	F	19.4	Phe508del	Phe508del	0	5	0.88	33	1.77
61	61	M	25.3	Phe508del	711+1G>T	0	0	1.04	30	2.72
62	47	M	23.5	Phe508del	621+1G>T	0	0.5	1.25	35	3.2
63	51	M	27.9	Phe508del	Gly542X	0	0	1.42	42	3.05
64	50	F	21	Arg553X	Gln493X	0	1.5	1.62	54	2.62
65	40	M	23.3	Phe508del	Phe508del	0	0	2.05	52	4.15
66	44	F	21.6	Phe508del	Arg792X	0	0.25	1.66	59	2.29

67	40	M	19.9	Phe508del	Phe508del	1	0	1.68	45	4.08
68	51	F	22.5	Phe508del	Gly551Asp	1	0.75	1.26	46	2.63
69	40	F	25	Phe508del	Phe508del	1	0	0.97	31	2.77
70	49	M	30	Phe508del	c.3874-4522A>G	0	35	3.1	85	3.73
71	41	F	19.4	Phe508del	Trp1310X	0	1	0.99	30	1.76
72	49	M	24	Phe508del	Arg352Gln	0	0	1.68	48	3.02
73	52	F	23	Phe508del	Phe508del	0	-	0.9	34	1.68
74	40	M	22	Phe508del	Phe508del	0	1.5	2.15	57	3.56
75	40	F	23.5	Phe508del	Gly551Asp	1	2	1.33	46	2.19
76	51	F	23.6	Phe508del	Phe508del	0	1.5	2.26	80	3.43
77	44	M	26.5	Phe508del	Phe508del	0	3	3.33	81	5.08
78	40	M	23.4	Phe508del	621+1G>T	0	1	2.73	73	4.11
79	44	M	22.8	Phe508del	Phe508del	0	-	0.92	24	2.49
80	47	M	23	Gly551Asp	pAsn13O3Lys	0	9	2.2	61	3.32
81	40	M	24	1154insTC	2118delAACT	0	0.25	2.27	65	3.34
82	46	M	25.2	Phe508del	Arg117His-5T	0	39	4.94	103	6.98
83	47	M	16.9	Phe508del	1078delT	0	7	0.81	19	2.09

84	43	M	25.8	Phe508del	Phe508del	0	0.5	2.03	47	3.58
85	41	M	35.8	Phe508del	Arg117His-7T	0	30	3.41	75	4.12

Table 3.1a: General demographics of total study cohort (for analysis purpose, 0=no/not present, 1=yes/present), ppFEV$_1$-percent predicted forced expiratory volume in one second, FVC=forced vital capacity.

Patient	Age	Gender	Allele 1	Allele 2	PsA	BCC	MRSA	NTM	IV days	Oral Corticosteroids	Neb Abx	Neb mucolytic
1	46	M	Phe508del	Phe508del	1	1	0	0	276	0	1	1
2	68	M	Phe508del	Asp1152His	0	0	0	0	0	0	0	0
3	40	M	Phe508del	Phe508del	1	0	0	0	138	0	1	1
4	48	M	Phe508del	Gly551Asp	1	0	0	0	83	0	1	0
5	47	F	Phe508del	Phe508del	1	0	0	0		0	1	0
6	47	M	Phe508del	Phe508del	1	0	0	0	273	0	1	1
7	45	M	Phe508del	1078delT	0	1	0	0	100	1	1	0
8	47	M	Gly551Asp	Gly551Asp	1	0	0	0	0	0	1	0
9	45	F	Phe508del	Arg553X	1	0	0	0	0	0	0	0
10	47	F	Phe508del	Phe508del	1	1	0	0	65	0	1	1
11	45	M	Phe508del	1461insAGAT	1	0	0	0	6	0	1	0

12	63	M	Phe508del	3272-26A-G	0	0	0	0	42	0	0	1
13	46	M	Phe508del	Arg117His-7T	0	0	0	1	0	0	0	0
14	51	M	Phe508del	Ser945Leu	0	0	0	0	150	0	1	0
15	47	M	1507del	2711delT	1	0	0	0	0	0	1	1
16	50	M	1507del	2711delT	1	0	1	0	48	0	1	0
17	67	M	Phe508del	C.1538A>G	0	0	0	0	90	1	0	0
18	49	M	Phe508del	Phe508del	1	0	0	0	0	0	1	0
19	41	M	Phe508del	Phe508del	1	1	0	0	188	0	1	1
20	58	F	Phe508del	Phe508del	0	1	0	0		0	1	0
21	50	M	Gly551Asp	Gly551Asp	0	1	0	0	170	0	0	0
22	70	F	Phe508del	Arg117His-5T	1	0	0	0	110	0	1	1
23	47	F	Phe508del	Pro67Leu	1	0	0	0	62	1	1	1
24	41	M	Phe508del	Phe508del	1	0	0	0	398	0	1	1
25	48	F	Phe508del	Val520Phe	1	0	0	1	28	0	1	0
26	66	M	Phe508del	TG12-5T	0	0	1	0	14	0	1	0
27	42	F	Phe508del	Arg117His-5T	0	1	1	0	150	0	1	1
28	66	F	Phe508del	TG12-5T	0	0	0	0	6	0	0	0

29	44	M	Phe508del	Asp1152His	0	0	0	0	0	0	0	0
30	50	M	Phe508del	Phe508del	1	0	0	0	63	0	1	1
31	52	F	Phe508del	Gly551Asp	1	0	0	0	126	0	1	1
32	49	M	Phe508del	711+3A>G	1	0	0	0	423	1	1	1
33	55	M	Phe508del	Phe508del	1	0	0	0		0	1	1
34	49	M	Phe508del	Arg117His-7T	0	0	0	0	0	0	0	0
35	53	M	Phe508del	Ser945Leu	1	0	0	0	440	1	1	1
36	40	F	Phe508del	Phe508del	1	0	0	0		1	1	1
37	48	F	Phe508del	Phe508del	1	1	0	0	203	0	1	0
38	58	M	Arg117His-7T	Arg117His-7T	0	0	0	0	0	0	0	0
39	79	M	Phe508del	TG12-5T	0	0	0	0	0	0	0	0
40	41	F	Phe508del	Phe508del	1	0	0	0	504	0	1	1
41	53	F	Phe508del	Phe508del	1	0	0	0	216	0	1	1
42	45	M	Phe508del	Phe508del	1	0	0	0	56	0	1	0
43	48	M	Phe508del	Phe508del	0	1	0	0	0	0	1	1
44	43	M	Phe508del	Arg560Thr	0	1	0	0	136	0	1	1
45	48	M	Phe508del	1154insTC	1	0	0	0	121	0	1	1

46	52	F	Phe508del	c.3874-4522A>G	1	0	0	0	170	0	1	1
47	53	M	Phe508del	Asp579Tyr	1	0	0	0	130	0	0	0
48	47	F	Phe508del	Phe508del	0	0	0	0	0	0	1	1
49	56	F	Arg117His-7T	Arg117H-7T	0	0	0	0	0	0	0	0
50	47	M	Phe508del	Phe508del	0	1	0	0	46	0	1	1
51	48	F	Phe508del	Phe508del	1	0	0	0	314	1	1	1
52	45	M	Phe508del	3600G>4	0	0	0	0	0	0	0	0
53	40	M	Phe508del	Phe508del	1	0	0	0	0	0	1	1
54	48	M	Phe508del	Gly551Asp	1	0	0	0	86	0	0	0
55	42	M	3659DelC	Leu633Pro	0	1	0	0	0	0	0	1
56	41	M	Phe508del	621+1G>T	1	0	0	0		0	1	0
57	47	F	Phe508del	711+3A>G	1	0	0	0	10	0	0	0
58	50	F	Phe508del	Phe508del	1	0	0	0	39	0	1	1
59	54	M	Phe508del	Arg117His-7T	0	0	0	0	9	0	0	0
60	42	F	Phe508del	Phe508del	1	0	1	0	295	0	1	1
61	61	M	Phe508del	711+1G>T	1	0	0	0	117	0	1	0
62	47	M	Phe508del	621+1G>T	1	0	0	0	193	0	1	0

63	51	M	Phe508del	Gly542X	1	0	0	0	562	1	1	1
64	50	F	Arg553X	Gln493X	1	0	0	0	105	0	1	0
65	40	M	Phe508del	Phe508del	1	0	0	0	94	0	1	1
66	44	F	Phe508del	Arg792X	1	0	0	0	477	0	1	0
67	40	M	Phe508del	Phe508del	1	0	0	0	42	0	1	1
68	51	F	Phe508del	Gly551Asp	1	0	0	0	230	0	1	1
69	40	F	Phe508del	Phe508del	0	1	0	0	376	0	1	0
70	49	M	Phe508del	c.3874-4522A>G	0	0	1	0	0	0	0	1
71	41	F	Phe508del	Trp1310X	1	0	1	0		0	1	1
72	49	M	Phe508del	Arg352Gln	0	0	0	0	234	0	0	1
73	52	F	Phe508del	Phe508del	1	0	0	0	545	0	1	0
74	40	M	Phe508del	Phe508del	1	0	0	0	53	0	1	1
75	40	F	Phe508del	Gly551Asp	1	0	0	0	86	0	1	0
76	51	F	Phe508del	Phe508del	0	1	0	1	0	0	0	1
77	44	M	Phe508del	Phe508del	1	0	0	0	0	0	1	0
78	40	M	Phe508del	621+1G>T	1	0	0	0	10	0	1	1
79	44	M	Phe508del	Phe508del	1	0	0	0	264	0	1	1

80	47	M	Gly551Asp	pAsn13O3Lys	1	0	0	0	91	0	1	1
81	40	M	1154insTC	2118delAACT	1	0	0	0	255	0	1	1
82	46	M	Phe508del	Arg117His-5T	0	0	0	0	0	0	0	0
83	47	M	Phe508del	1078delT	1	0	0	0	90	1	1	1
84	43	M	Phe508del	Phe508del	1	0	0	0	54	0	1	0
85	41	M	Phe508del	Arg117His-7T	1	0	0	0	0	0	0	0

Table 3.1b: Respiratory demographics for study cohort (0=no/not present, 1=yes/present), PsA=Pseudomonas aeruginosa, MRSA=methicillin resistant staphylococcus aureus, BCC=Burkholderia cepacian complex, NTM=non-tuberculous myocbacteria, IV=intravenous, neb=nebulised, abx=antibiotic.

Patient	Age	Gender	Allele 1	Allele 2	CFRD	PI	GORD	Osteoporosis	CFA	ABPA	Ptx	Haemoptysis
1	46	M	Phe508del	Phe508del	1	1	1	0	0	0	0	1
2	68	M	Phe508del	Asp1152His	0	1	0	1	0	0	0	0
3	40	M	Phe508del	Phe508del	1	1	0	0	0	0	0	0
4	48	M	Phe508del	Gly551Asp	0	1	1	1	1	0	0	0
5	47	F	Phe508del	Phe508del	1	1	0	0	0	0	0	1
6	47	M	Phe508del	Phe508del	1	1	1	1	0	0	0	1
7	45	M	Phe508del	1078delT	1	1	1	1	1	1	0	0

8	47	M	Gly551Asp	Gly551Asp	0	1	1	0	1	0	0	0
9	45	F	Phe508del	Arg553X	0	0	0	0	0	0	0	0
10	47	F	Phe508del	Phe508del	1	1	1	0	0	0	0	1
11	45	M	Phe508del	1461insAGAT	1	1	1	1	0	0	0	0
12	63	M	Phe508del	3272-26A-G	1	1	1	1	0	0	0	0
13	46	M	Phe508del	Arg117His-7T	0	1	0	0	0	0	0	0
14	51	M	Phe508del	Ser945Leu	1	1	1	0	0	0	0	0
15	47	M	1507del	2711delT	1	1	0	1	0	0	0	0
16	50	M	1507del	2711delT	1	1	0	1	0	0	0	0
17	67	M	Phe508del	C.1538A>G	0	0	1	1	0	0	0	0
18	49	M	Phe508del	Phe508del	1	1	0	0	0	0	0	0
19	41	M	Phe508del	Phe508del	0	1	1	0	0	0	0	1
20	58	F	Phe508del	Phe508del	1	1	1	1	0	0	0	0
21	50	M	Gly551Asp	Gly551Asp	1	1	1	0	0	0	0	0
22	70	F	Phe508del	Arg117His-5T	0	0	1	1	0	1	0	0
23	47	F	Phe508del	Pro67Leu	0	0	0	0	0	1	0	0
24	41	M	Phe508del	Phe508del	0	1	1	1	0	0	0	1

25	48	F	Phe508del	Val520Phe	0	1	0	0	0	0	0	0
26	66	M	Phe508del	TG12-5T	0	0	1	0	0	1	0	0
27	42	F	Phe508del	Arg117His-5T	1	1	1	0	0	0	0	1
28	66	F	Phe508del	TG12-5T	0	1	1	1	0	0	0	0
29	44	M	Phe508del	Asp1152His	0	0	0	0	0	0	0	0
30	50	M	Phe508del	Phe508del	1	1	1	0	0	0	0	1
31	52	F	Phe508del	Gly551Asp	0	1	1	1	0	0	0	0
32	49	M	Phe508del	711+3A>G	1	1	1	0	0	1	0	0
33	55	M	Phe508del	Phe508del	1	1	1	0	0	0	1	0
34	49	M	Phe508del	Arg117His-7T	1	0	0	0	0	0	0	0
35	53	M	Phe508del	Ser945Leu	0	1	1	0	0	0	0	1
36	40	F	Phe508del	Phe508del	1	1	0	0	0	0	0	1
37	48	F	Phe508del	Phe508del	0	1	1	0	0	0	0	0
38	58	M	Arg117His-7T	Arg117His-7T	0	0	0	0	0	0	0	0
39	79	M	Phe508del	TG12-5T	0	0	0	1	0	1	0	0
40	41	F	Phe508del	Phe508del	0	1	0	1	0	0	0	1
41	53	F	Phe508del	Phe508del	1	1	1	0	0	0	0	1

42	45	M	Phe508del	Phe508del	1	1	0	1	0	0	0	0
43	48	M	Phe508del	Phe508del	1	1	1	0	0	0	0	0
44	43	M	Phe508del	Arg560Thr	0	1	0	0	0	0	0	0
45	48	M	Phe508del	1154insTC	0	1	1	0	1	0	0	1
46	52	F	Phe508del	c.3874-4522A>G	0	1	1	0	0	1	0	0
47	53	M	Phe508del	Asp579Tyr	1	1	1	0	1	0	0	0
48	47	F	Phe508del	Phe508del	0	1	0	0	0	0	0	0
49	56	F	Arg117His-7T	Arg117H-7T	0	0	0	0	0	0	0	0
50	47	M	Phe508del	Phe508del	1	1	1	0	0	0	0	0
51	48	F	Phe508del	Phe508del	1	1	1	0	1	0	0	0
52	45	M	Phe508del	3600G>4	0	0	0	0	0	0	0	1
53	40	M	Phe508del	Phe508del	0	1	1	0	0	0	0	0
54	48	M	Phe508del	Gly551Asp	0	1	1	0	0	0	0	0
55	42	M	3659DelC	Leu633Pro	0	1	1	0	0	0	0	0
56	41	M	Phe508del	621+1G>T	1	1	1	0	0	0	0	1
57	47	F	Phe508del	711+3A>G	0	0	0	0	0	0	0	0
58	50	F	Phe508del	Phe508del	0	1	1	1	1	0	0	0

59	54	M	Phe508del	Arg117His-7T	1	1	1	0	0	0	0	0
60	42	F	Phe508del	Phe508del	1	1	1	0	0	0	0	0
61	61	M	Phe508del	711+1G>T	0	1	0	0	0	0	0	0
62	47	M	Phe508del	621+1G>T	1	1	1	0	0	0	0	1
63	51	M	Phe508del	Gly542X	1	1	1	1	0	0	0	1
64	50	F	Arg553X	Gln493X	0	1	0	0	0	0	0	0
65	40	M	Phe508del	Phe508del	0	1	0	0	0	0	0	0
66	44	F	Phe508del	Arg792X	1	1	1	1	1	0	0	1
67	40	M	Phe508del	Phe508del	1	1	0	1	0	0	0	0
68	51	F	Phe508del	Gly551Asp	0	1	1	0	0	0	0	0
69	40	F	Phe508del	Phe508del	0	1	0	0	0	0	0	1
70	49	M	Phe508del	c.3874-4522A>G	1	1	1	0	0	0	0	0
71	41	F	Phe508del	Trp1310X	1	1	1	1	0	0	0	1
72	49	M	Phe508del	Arg352Gln	0	1	1	0	0	0	0	0
73	52	F	Phe508del	Phe508del	0	1	0	0	0	0	0	0
74	40	M	Phe508del	Phe508del	0	1	0	0	0	0	0	0
75	40	F	Phe508del	Gly551Asp	0	1	1	0	0	0	0	0

76	51	F	Phe508del	Phe508del	1	1	1	0	0	0	0	0
77	44	M	Phe508del	Phe508del	1	1	0	0	0	0	0	0
78	40	M	Phe508del	621+1G>T	1	1	1	1	0	0	0	1
79	44	M	Phe508del	Phe508del	1	1	0	0	0	0	0	1
80	47	M	Gly551Asp	pAsn13O3Lys	0	1	1	0	0	0	0	0
81	40	M	1154insTC	2118delAACT	1	1	0	0	0	0	0	0
82	46	M	Phe508del	Arg117His-5T	0	0	1	0	0	0	0	0
83	47	M	Phe508del	1078delT	1	1	1	0	0	0	0	1
84	43	M	Phe508del	Phe508del	1	1	0	0	0	0	1	0
85	41	M	Phe508del	Arg117His-7T	0	0	1	0	0	0	0	0

Table 3.1c: Other comorbidities of study cohort (0=no/not present, 1=yes/present) CFRD-CF-related diabetes mellitus, GORD-gastro-oesophageal reflux disease, PI-exocrine pancreatic insufficiency, ABPA=allergic bronchopulmonary aspergillosis, CFA=CF-related arthropathy, Ptx=pneumothorax.

Patient	Age	Gender	Allele 1	Allele 2	Hypertension	Hyperlipidaemia	FH +ve for CVD	Mental health issues	Working	Children
1	46	M	Phe508del	Phe508del	0	0	1	1	0	0
2	68	M	Phe508del	Asp1152His	1	1	0	0	0	0
3	40	M	Phe508del	Phe508del	0	0	1	0	1	1

4	48	M	Phe508del	Gly551Asp	0	0	0	0	1	0
5	47	F	Phe508del	Phe508del	0	0	1	1	0	1
6	47	M	Phe508del	Phe508del	0	0	0	1	0	0
7	45	M	Phe508del	1078delT	0	1	1	0	1	1
8	47	M	Gly551Asp	Gly551Asp	0	0	0	0	1	1
9	45	F	Phe508del	Arg553X	0	0	1	0	1	0
10	47	F	Phe508del	Phe508del	0	0	0	1	1	0
11	45	M	Phe508del	1461insAGAT	0	0	0	0	0	0
12	63	M	Phe508del	3272-26A-G	1	0	0	0	1	0
13	46	M	Phe508del	Arg117His-7T	0	1	1	0	1	1
14	51	M	Phe508del	Ser945Leu	1	0	0	0	1	1
15	47	M	1507del	2711delT	1	1	1	0	1	0
16	50	M	1507del	2711delT	0	1	1	0	1	0
17	67	M	Phe508del	C.1538A>G	1	0	0	1	0	0
18	49	M	Phe508del	Phe508del	0	0	0	1	1	0
19	41	M	Phe508del	Phe508del	0	0	0	0	1	0
20	58	F	Phe508del	Phe508del	1	0	0	0	0	0

21	50	M	Gly551Asp	Gly551Asp	1	0	0	0	0	0
22	70	F	Phe508del	Arg117His-5T	1	1	0	0	0	0
23	47	F	Phe508del	Pro67Leu	0	0	0	1	1	1
24	41	M	Phe508del	Phe508del	1	0	0	1	0	0
25	48	F	Phe508del	Val520Phe	0	0	0	0	1	0
26	66	M	Phe508del	TG12-5T	0	0	0	0	0	0
27	42	F	Phe508del	Arg117His-5T	0	0	0	1	0	0
28	66	F	Phe508del	TG12-5T	0	1	0	0	0	1
29	44	M	Phe508del	Asp1152His	0	0	0	0	1	0
30	50	M	Phe508del	Phe508del	0	0	1	1	1	0
31	52	F	Phe508del	Gly551Asp	0	0	0	0	0	0
32	49	M	Phe508del	711+3A>G	1	1	1	1	0	0
33	55	M	Phe508del	Phe508del	0	0	0	0	0	0
34	49	M	Phe508del	Arg117His-7T	0	0	1	0	1	0
35	53	M	Phe508del	Ser945Leu	0	0	0	0	0	0
36	40	F	Phe508del	Phe508del	0	0	0	0	0	1
37	48	F	Phe508del	Phe508del	0	0	0	0	1	0

38	58	M	Arg117His-7T	Arg117His-7T	1	1	0	0	1	0
39	79	M	Phe508del	TG12-5T	0	0	0	0	0	0
40	41	F	Phe508del	Phe508del	0	0	0	1	0	0
41	53	F	Phe508del	Phe508del	0	0	0	0	0	0
42	45	M	Phe508del	Phe508del	0	0	0	0	1	1
43	48	M	Phe508del	Phe508del	0	0	0	0	1	0
44	43	M	Phe508del	Arg560Thr	0	0	0	0	1	1
45	48	M	Phe508del	1154insTC	0	0	0	0	1	0
46	52	F	Phe508del	Nil	0	0	0	0	0	1
47	53	M	Phe508del	Asp579Tyr	0	0	0	0	0	0
48	47	F	Phe508del	Phe508del	0	0	0	0	1	1
49	56	F	Arg117His-7T	Arg117H-7T	0	0	0	0	1	0
50	47	M	Phe508del	Phe508del	0	0	0	0	1	1
51	48	F	Phe508del	Phe508del	0	0	0	0	1	0
52	45	M	Phe508del	3600G>4	0	0	0	0	1	0
53	40	M	Phe508del	Phe508del	0	0	0	0	1	1
54	48	M	Phe508del	Gly551Asp	1	1	0	0	1	0

55	42	M	3659DelC	Leu633Pro	0	0	0	0	1	0
56	41	M	Phe508del	621+1G>T	0	0	1	0	0	0
57	47	F	Phe508del	711+3A>G	0	0	0	0	1	1
58	50	F	Phe508del	Phe508del	0	0	0	0	0	1
59	54	M	Phe508del	Arg117His-7T	1	1	0	1	1	0
60	42	F	Phe508del	Phe508del	0	0	0	0	0	0
61	61	M	Phe508del	711+1G>T	0	0	0	0	1	0
62	47	M	Phe508del	621+1G>T	0	0	0	0	1	0
63	51	M	Phe508del	Gly542X	1	0	0	1	0	0
64	50	F	Arg553X	Gln493X	0	0	0	0	1	1
65	40	M	Phe508del	Phe508del	0	0	0	0	1	0
66	44	F	Phe508del	Arg792X	0	0	0	0	0	1
67	40	M	Phe508del	Phe508del	0	0	0	0	0	1
68	51	F	Phe508del	Gly551Asp	0	0	0	0	1	1
69	40	F	Phe508del	Phe508del	0	0	0	0	1	0
70	49	M	Phe508del	c.3874-4522A>G	0	0	0	0	1	0
71	41	F	Phe508del	Trp1310X	0	0	0	1	0	0

72	49	M	Phe508del	Arg352Gln	0	0	0	0	0	0
73	52	F	Phe508del	Phe508del	0	0	0	0	0	0
74	40	M	Phe508del	Phe508del	0	0	0	0	1	0
75	40	F	Phe508del	Gly551Asp	0	0	0	0	0	1
76	51	F	Phe508del	Phe508del	0	0	0	0	1	0
77	44	M	Phe508del	Phe508del	0	0	0	0	1	0
78	40	M	Phe508del	621+1G>T	0	0	0	0	1	0
79	44	M	Phe508del	Phe508del	0	0	0	0	0	0
80	47	M	Gly551Asp	pAsn13O3Lys	0	0	0	0	0	0
81	40	M	1154insTC	2118delAACT	0	0	0	0	1	1
82	46	M	Phe508del	Arg117His-5T	0	0	0	0	1	1
83	47	M	Phe508del	1078delT	0	0	0	0	1	0
84	43	M	Phe508del	Phe508del	1	0	0	0	1	0
85	41	M	Phe508del	Arg117His-7T	0	0	0	0	1	1

Table 3.1d: Other comorbidities of study cohort – cardiovascular, social (0=no/not present, 1=yes/present); +ve FH=family history of cardiovascular aged 60 years or below in first degree relative, CVD=cardiovascular disease.

14.0 Appendix 7: Sweat chloride analysis for the study group

Patient	Age	Allele 1	Allele 2	Age at diagnosis (years)	ppFEV1 %	Sweat chloride (mmol/L)	Sweat conductivity (mmol/L)	Sweat weight (mg)
1	46	Phe508del	Phe508del	1	20	97	121	63
2	68	Phe508del	Asp1152His	60	88	26	53	44
3	40	Phe508del	Phe508del	1	34	79	105	25
4	48	Phe508del	Gly551Asp	3	49	112	121	72
5	47	Phe508del	Phe508del	1.5	52	98	118	47
6	47	Phe508del	Phe508del	0.25	39	89	111	51
7	45	Phe508del	1078delT	-	52			
8	47	Gly551Asp	Gly551Asp	1.5	65	105	126	52
9	45	Phe508del	Arg553X	3	58	109	129	54
10	47	Phe508del	Phe508del	1.2	75	91	111	52
11	45	Phe508del	1461insAG AT	2.5	43			
12	63	Phe508del	3272-26A-G	0	32	103	124	30
13	46	Phe508del	Arg117His-7T	-	94	62	90	45

14	51	Phe508del	Ser945Leu	12	61	36	58	41
15	47	1507del	2711delT	0	75	109	127	72
16	50	1507del	2711delT	4	55	105	128	40
17	67	Phe508del	C.1538A>G	47	19	61	83	46
18	49	Phe508del	Phe508del	4	51	106	120	49
19	41	Phe508del	Phe508del		59	93		
20	58	Phe508del	Phe508del	43	39			
21	50	Gly551Asp	Gly551Asp	0	25	114	126	42
22	70	Phe508del	Arg117His-5T	38	56	92	107	34
23	47	Phe508del	Pro67Leu	19	75	30	57	32
24	41	Phe508del	Phe508del	0.25	25	56	51	25
25	48	Phe508del	Val520Phe	0	33	123	136	40
26	66	Phe508del	TG12-5T	58	56			
27	42	Phe508del	Arg117His-5T	26	26	73	97	21
28	66	Phe508del	TG12-5T	64	87	59	88	45
29	44	Phe508del	Asp1152His		92			

30	50	Phe508del	Phe508del	5	75	101	120	83
31	52	Phe508del	Gly551Asp	1	31	47	55	42
32	49	Phe508del	711+3A>G	34	41			
33	55	Phe508del	Phe508del	0.5	39			
34	49	Phe508del	Arg117His-7T	21	86			
35	53	Phe508del	Ser945Leu	25	23	23	47	28
36	40	Phe508del	Phe508del	0	19			
37	48	Phe508del	Phe508del	0	62	94	113	35
38	58	Arg117His-7T	Arg117His-7T	55	110	38	64	41
39	79	Phe508del	TG12-5T	72	96	67	81	48
40	41	Phe508del	Phe508del	6	36	87	104	37
41	53	Phe508del	Phe508del	0.25	33	92	115	21
42	45	Phe508del	Phe508del	0.75	91	113	136	44
43	48	Phe508del	Phe508del	0	40			
44	43	Phe508del	Arg560Thr	0.5	63	108	133	52
45	48	Phe508del	1154insTC	1.5	44	100	122	54

46	52	Phe508del	c.3874-4522A>G	16	25	68	92	23
47	53	Phe508del	Asp579Tyr	10	32	102	122	47
48	47	Phe508del	Phe508del	3	48			
49	56	Arg117His-7T	Arg117H-7T	54	94	38	66	69
50	47	Phe508del	Phe508del	0.3	35	98	118	42
51	48	Phe508del	Phe508del		21			
52	45	Phe508del	3600G>4	19	47			
53	40	Phe508del	Phe508del	3	55	104	124	79
54	48	Phe508del	Gly551Asp	0.5	58	135	147	29
55	42	3659DelC	Leu633Pro	0	72	100	121	59
56	41	Phe508del	621+1G>T	3	18			
57	47	Phe508del	711+3A>G	40	100	34	56	30
58	50	Phe508del	Phe508del		34	95	116	50
59	54	Phe508del	Arg117His-7T	27	73	60	81	56
60	42	Phe508del	Phe508del	5	33	107	128	52

61	61	Phe508del	711+1G>T	0	30	103	124	62
62	47	Phe508del	621+1G>T	0.5	35	104	126	62
63	51	Phe508del	Gly542X	0	42	97	115	41
64	50	Arg553X	Gln493X	1.5	54	104	124	43
65	40	Phe508del	Phe508del	0	52	74	96	45
66	44	Phe508del	Arg792X	0.25	59	113	132	41
67	40	Phe508del	Phe508del	0	45			
68	51	Phe508del	Gly551Asp	0.75	46	117	128	66
69	40	Phe508del	Phe508del	0	31	90	113	30
70	49	Phe508del	c.3874-4522A>G	35	85	86	108	30
71	41	Phe508del	Trp1310X	1	30			
72	49	Phe508del	Arg352Gln	0	48	101	124	67
73	52	Phe508del	Phe508del		34			
74	40	Phe508del	Phe508del	1.5	57	82	98	94
75	40	Phe508del	Gly551Asp	2	46	116	132	30
76	51	Phe508del	Phe508del	1.5	80	101	125	32

77	44	Phe508del	Phe508del	3	81	103	128	60
78	40	Phe508del	621+1G>T	1	73			
79	44	Phe508del	Phe508del		24			
80	47	Gly551Asp	pAsn13O3Lys	9	61	120	120	46
81	40	1154insTC	2118delAACT	0.25	65	112	129	79
82	46	Phe508del	Arg117His-5T	39	103			
83	47	Phe508del	1078delT	7	19	85	107	26
84	43	Phe508del	Phe508del	0.5	47			
85	41	Phe508del	Arg117His-7T	30	75	62	89	64

Table 3.5: Sweat chloride results for the study cohort.(Patients highlighted in red had no pre-modulator sweat chloride result and were not included in analysis).

15.0 Appendix 8: The characteristics of the late diagnosis group

Gender	Age in study (years)	Age of Diagnosis (years)	Genotype	Sweat chloride (mmol/L)	FEV_1 (% predicted)	BMI (kg/m^2)	Predominant lung pathogen	Pancreatic status
M	70	60	Phe508del/Asp1152His-7T	26	88	26.6	PsA	PS
M	67	47	Phe508del/C.1538A>G	61	19	20.2	Nil	PS
F	59	43	Phe508del/Phe508del	-	39	21.8	BCC	PI
F	71	38	Phe508del/Arg117His-5T	92	56	17.5	PsA, MRSA	PS
F	48	19	Phe508del/Pro67Leu	-	75	19.3	PsA	PS
M	67	58	Phe508del/TG12-5T	-	56	23.4	MRSA	PS
F	43	26	Phe508del/Arg117His-5T	73	26	21.3	MRSA	PI
F	67	64	Phe508del/TG12-5T	59	87	30.5	Nil	PS
M	49	34	Phe508del/711+3A>G	-	41	26	PsA	PI
M	49	21	Phe508del/Arg117His-7T	-	86	25.7	Nil	PS
M	43	25	Phe508del/Ser945Leu	-	23	23.2	MRSA	PI

M	58	55	Arg117His/Arg117His-7T	38	110	28.3	Nil	PS
M	79	72	Phe508del/TG12T5	67	96	24.1	Nil	PS
F	52	16	Phe508del/c.3874-4522A>G	68	25	21	PsA	PI
F	57	54	Arg117His/Arg117His-7T	38	94	23	Nil	PS
M	45	19	Phe508del/3600G>A	-	47	24.2	*Achromobacter dolens*	PS
F	47	40	Phe508del/c.579+3A>G	34	100	27	PsA	PS
M	54	27	Phe508del/Arg117His-7T	60	73	21.5	Nil	PI
M	49	35	Phe508del/c.3874-4522A>G	86	85	30	MRSA	PI
M	45	39	Phe508del/Arg117His-7T	-	103	26	*Staph aureus*	PS

Table 3.8: Clinical characteristics of late diagnosis group. (PS=exocrine pancreatic sufficient, PI=exocrine pancreatic insufficient, PsA=Pseudomonas aeruginosa, MRSA=methicillin resistant staphylococcus aureus, BCC= Burkholderia cepacia complex).

16.0 Appendix 9: CMR data workbook

CF COA CMR WORKBOOK		**PATIENT MEASUREMENTS**		
LV / RV VOLUME AND FUNCTION - Short Axis Cine		**BSA**	**T1/T2/ECV**	
LV end diastolic volume	0dm		MOLLI Basal Pre T1	0dm
LV end systolic volume	0dm		MOLLI Mid Pre T1	0dm
LV ejection fraction	0dm	**Haematocrit**	MOLLI Average Native Pre T1 **(MYO PRE)**	0dm
LV mass	0dm		MOLLI Basal Blood Pre T1	0dm
LV end diastolic volume (index)	1dm		MOLLI Mid Blood Pre T1	0dm
LV end systolic volume (index)	1dm	**Systolic BP**	MOLLI Average Blood Pre T1 **(BLOOD PRE)**	0dm
LV mass (index)	0dm			
RV end diastolic volume	0dm		Basal T2	0dm
RV end systolic volume	0dm	**Diastolic BP**	Mid T2	0dm
RV ejection fraction	0dm		Average Native T2	0dm
RV end diastolic volume (index)	1dm			
RV end systolic volume (index)	1dm		MOLLI Basal Post T1	0dm
LV Maximal Wall thickness	(cm) 1dm		MOLLI Mid Post T1	0dm
			MOLLI Average Post T1 **(MYO POST)**	0dm

LA / RA VOLUME - Short Axis Cine	
LA Volume	0dm
LA Volume (index)	1dm
RA Volume	0dm
RA Volume (index)	1dm
LV / RV Measurements - A4Ch	
TAPSE	(cm) 1dm
PULSE WAVE VELOCITY	
Aortic distance	mm (1dm)
Pulse Wave Velocity	1dm
AORTIC DISTENSIBILITY	
Maximum aortic area	1dm
Minimum aortic area	1dm
Aortic distensibility	1dm

MOLLI Basal Blood Post T1	0dm
MOLLI Mid Blood Post T1	0dm
MOLLI Average Blood Post T1 **(BLOOD POST)**	0dm
ECV	1dm
Peak Global GLS	1dm

17.0 Appendix 10: Normal CMR values for Caucasians in UK population(498)

	Abnormal low		Normal zone		Abnormal high
Left ventricle					
LVEDV (ml)	<93	Borderline zone*	109 - 218	Borderline zone*	>232
LVESV (ml)	<34		39 - 97		>103
LVSV (ml)	<49		59 - 132		>140
LV mass (g)	<56		64 - 141		>148
indexed LVEDV (ml/m^2)	<52		60 - 110		>117
indexed LVESV (ml/m^2)	<19		21 - 49		>52
indexed LVSV (ml/m^2)	<28		32 - 67		>70
indexed LV mass (g/m^2)	<33		35 - 70		>72
LVEF (%)	<47		48 - 69		>70
LV mass to volume ratio (g/ml)	<0.40		0.42 - 0.84		>0.87
Right ventricle					
RVEDV (ml)	<99		124 - 248		>260
RVESV (ml)	<34		47 - 123		>135
RVSV (ml)	<54		62 - 131		>140
indexed RVEDV (ml/m^2)	<55		68 - 125		>128
indexed RVESV (ml/m^2)	<19		25 - 63		>67
indexed RVSV (ml/m^2)	<30		34 - 67		>69
RVEF (%)	<40		45 - 65		>68

LV and RV ranges - men.

	Abnormal low		Normal zone		Abnormal high
Left ventricle					
LVEDV (ml)	<80	Borderline zone*	88 - 161	Borderline zone*	>175
LVESV (ml)	<25		31 - 68		>73
LVSV (ml)	<47		49 - 100		>110
LV mass (g)	<44		46 - 93		>96
indexed LVEDV (ml/m^2)	<50		54 - 94		>101
indexed LVESV (ml/m^2)	<16		19 - 40		>43
indexed LVSV (ml/m^2)	<29		30 - 59		>63
indexed LV mass (g/m^2)	<28		29 - 55		>55
LVEF (%)	<50		51 - 70		>72
LV mass to volume ratio (g/ml)	<0.35		0.39 - 0.71		>0.81
Right ventricle					
RVEDV (ml)	<83		85 - 168		>192
RVESV (ml)	<26		27 - 77		>95
RVSV (ml)	<47		48 - 99		>107
indexed RVEDV (ml/m^2)	<51		53 - 99		>110
indexed RVESV (ml/m^2)	<16		17 - 46		>55
indexed RVSV (ml/m^2)	<29		30 - 59		>61
RVEF (%)	<45		47 - 68		>70

LV and RV ranges - women

	Abnormal low		Normal zone		Abnormal high
Left atrium					
Max. LA volume (2Ch) (ml)	<22		30 - 104		>112
Max. LA volume (4Ch) (ml)	<23		36 - 124		>125
Max. LA volume (Biplane) (ml)	<26		37 - 108		>112
LA SV (Biplane) (ml)	<16		23 - 62		>66
indexed Max. LA volume (2Ch) (ml/m^2)	<12		16 - 53		>56
indexed Max. LA volume (4Ch) (ml/m^2)	<14	Borderline zone*	19 - 62	Borderline zone*	>63
indexed Max. LA volume (Biplane) (ml/m^2)	<15		19 - 55		>56
indexed LA SV (Biplane) (ml/m^2)	<9		12 - 32		>33
LA EF (Biplane) (%)	<44		47 - 73		>75
Right atrium					
Max. RA volume (4Ch) (ml)	<36		43 - 143		>150
RA SV (4Ch) (ml)	<9		10 - 66		>66
indexed Max. RA volume (4Ch) (ml/m^2)	<19		22 - 74		>79
indexed RA SV (4Ch) (ml/m^2)	<5		5 - 33		>35
RA EF (4Ch) (%)	<21		23 - 58		>60

LA and RA ranges - men

	Abnormal low		Normal zone		Abnormal high
Left atrium					
Max. LA volume (2Ch) (ml)	<19		24 - 90		>97
Max. LA volume (4Ch) (ml)	<23		36 - 108		>114
Max. LA volume (Biplane) (ml)	<26		33 - 93		>100
LA SV (Biplane) (ml)	<17		21 - 53		>60
indexed Max. LA volume (2Ch) (ml/m^2)	<12		15 - 53		>56
indexed Max. LA volume (4Ch) (ml/m^2)	<15	Borderline zone*	23 - 63	Borderline zone*	>67
indexed Max. LA volume (Biplane) (ml/m^2)	<16		21 - 55		>57
indexed LA SV (Biplane) (ml/m^2)	<10		13 - 32		>34
LA EF (Biplane) (%)	<44		49 - 74		>77
Right atrium					
Max. RA volume (4Ch) (ml)	<34		38 - 101		>107
RA SV (4Ch) (ml)	<10		14 - 52		>54
indexed Max. RA volume (4Ch) (ml/m^2)	<20		23 - 59		>63
indexed RA SV (4Ch) (ml/m^2)	<6		8 - 31		>32
RA EF (4Ch) (%)	<26		31 - 63		>66

LA and RA ranges - women

	Age groups (years)								
	45-54			55-64			65-74		
	lower	mean	upper	lower	mean	upper	lower	mean	upper
LVEDV (ml)	109	170	232	108	169	230	93	156	218
LVESV (ml)	39	71	103	39	71	102	34	66	97
LVSV (ml)	58	99	140	59	98	137	49	90	132
LV mass (g)	64	106	148	64	104	143	56	99	141
indexed LVEDV (ml/m^2)	60	86	112	55	86	117	52	81	110
indexed LVESV (ml/m^2)	21	36	51	20	36	52	19	34	49
indexed LVSV (ml/m^2)	32	50	68	30	50	70	28	47	67
indexed LV mass (g/m^2)	35	54	72	34	53	72	33	51	70
LVEF (%)	47	58	70	48	58	69	47	58	69
LV mass to volume ratio (g/ml)	0.42	0.63	0.84	0.40	0.62	0.85	0.41	0.64	0.87
RVEDV (ml)	124	192	260	109	181	252	99	173	248
RVESV (ml)	47	91	135	42	82	123	34	81	129
RVSV (ml)	62	101	140	60	98	136	54	92	131
indexed RVEDV (ml/m^2)	68	97	126	56	92	128	55	90	125
indexed RVESV (ml/m^2)	25	46	67	21	42	63	19	42	66
indexed RVSV (ml/m^2)	34	51	68	31	50	69	30	48	67
RVEF (%)	40	53	65	45	55	65	40	54	68

Age specific ventricular ranges in men

	Age groups (years)								
	45-54			55-64			65-74		
	lower	mean	upper	lower	mean	upper	lower	mean	upper
LVEDV (ml)	88	131	175	80	121	161	81	122	163
LVESV (ml)	31	52	73	26	47	68	25	48	70
LVSV (ml)	49	79	110	47	74	100	47	74	100
LV mass (g)	46	71	96	45	69	93	44	69	94
indexed LVEDV (ml/m^2)	54	78	101	50	72	94	50	73	96
indexed LVESV (ml/m^2)	19	31	43	16	28	40	16	29	42
indexed LVSV (ml/m^2)	30	47	63	29	44	59	29	45	60
indexed LV mass (g/m^2)	29	42	55	28	41	55	28	42	55
LVEF (%)	50	60	70	51	61	72	50	61	72
LV mass to volume ratio (g/ml)	0.39	0.55	0.71	0.36	0.58	0.8	0.35	0.58	0.81
RVEDV (ml)	85	138	192	83	125	168	84	128	171
RVESV (ml)	27	61	95	27	52	77	26	54	82
RVSV (ml)	48	78	107	47	73	100	48	74	99
indexed RVEDV (ml/m^2)	53	81	110	51	75	99	53	77	101
indexed RVESV (ml/m^2)	17	36	55	16	31	46	17	32	48
indexed RVSV (ml/m^2)	30	46	61	29	44	59	30	44	59
RVEF (%)	45	56	68	47	59	70	46	58	70

Age specific ventricular ranges for women

	Age groups (years)								
	45-54			55-64			65-74		
	lower	mean	upper	lower	mean	upper	lower	mean	upper
Maximal LA volume (2Ch) (ml)	25	68	112	30	68	105	22	63	104
Maximal LA volume (4Ch) (ml)	33	79	124	36	80	125	23	74	124
Maximal LA volume (Biplane) (ml)	33	72	112	37	73	110	26	67	108
LA SV (Biplane) (ml)	20	43	66	23	44	65	16	39	62
indexed Maximal LA volume (2Ch) (ml/m^2)	13	35	56	16	34	53	12	33	53
indexed Maximal LA volume (4Ch) (ml/m^2)	18	40	62	19	41	63	14	38	63
indexed Maximal LA volume (Biplane) (ml/m^2)	18	37	56	19	37	55	15	35	55
indexed LA SV (Biplane) (ml/m^2)	11	22	33	12	22	33	9	21	32
LA EF (Biplane) (%)	45	59	73	47	61	75	44	59	74
Maximal RA volume (4Ch) (ml)	38	93	148	43	93	143	36	93	150
RA SV (4Ch) (ml)	10	38	66	10	38	66	9	38	66
indexed Maximal RA volume (4Ch) (ml/m^2)	20	47	75	22	48	74	19	49	79
indexed RA SV (4Ch) (ml/m^2)	5	19	33	5	20	34	5	20	35
RA EF (4Ch) (%)	23	40	58	21	41	60	22	41	60

Age specific atrial ranges for men

	Age groups (years)								
	45-54			55-64			65-74		
	lower	mean	upper	lower	mean	upper	lower	mean	upper
Maximal LA volume (2Ch) (ml)	24	60	97	19	56	92	21	56	90
Maximal LA volume (4Ch) (ml)	36	75	114	27	68	108	23	68	113
Maximal LA volume (Biplane) (ml)	33	66	100	26	60	95	28	61	93
LA SV (Biplane) (ml)	21	41	60	17	36	55	18	35	53
indexed Maximal LA volume (2Ch) (ml/m^2)	15	35	56	12	33	54	14	34	53
indexed Maximal LA volume (4Ch) (ml/m^2)	23	44	65	17	40	63	15	41	67
indexed Maximal LA volume (Biplane) (ml/m^2)	21	39	57	16	36	56	18	36	55
indexed LA SV (Biplane) (ml/m^2)	13	24	34	10	22	33	11	21	32
LA EF (Biplane) (%)	49	62	75	44	61	77	45	59	74
Maximal RA volume (4Ch) (ml)	38	70	101	34	67	101	36	71	107
RA SV (4Ch) (ml)	14	33	53	10	31	52	11	33	54
indexed Maximal RA volume (4Ch) (ml/m^2)	23	41	59	20	40	60	23	43	63
indexed RA SV (4Ch) (ml/m^2)	8	20	31	6	19	31	7	20	32
RA EF (4Ch) (%)	31	48	65	26	46	66	28	45	63

Age specific atrial ranges for women

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