Bicarbonate and acid-base balance in cystic fibrosis

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Intellectual property and publications

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Chapter 3

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Ricard JD, Roca O, Lemiale V, Corley A, Braunlich J, Jones P, Kang BY, Lellouche F, Nava S, Rittayamai N, **Spoletini G,** Jaber S, Hernandez G. Use of nasal high flowoxygen during acute respiratory failure. *Intensive Care Medicine*, 2020 Dec;46(12):2238-2247 I wrote the section on the use of NHFT in hypercapnic respiratory failure.

Chapter 8

Spoletini G, Fitch G, Gillgrass L, Etherington C, Clifton I, Peckham DG. Metabolic alkalosis and bicarbonate in cystic fibrosis: it's not just Pseudo-Bartter! *Submitted for publication.* I audited collected data, and performed further records review, undertook the statistical. analysis, and wrote the manuscript. GF and DGP designed the protocol. LG completed data collection. All authors critically reviewed the manuscript.

Chapter 10

Spoletini G, Pollard K, Watson R, Darby MJ, Johnstone A, Etherington C, Whitaker P, Clifton IK, Peckham DG. Noninvasive ventilation in cystic fibrosis: clinical indications and outcomes in a large UK adult cystic fibrosis center. Respiratory Care, 2021 Mar; 66

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Chapter 11

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Book Chapters

Chapter 3

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Abstract at international Congresses

Chapter 7

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Spoletini G, Sawant A, Watts S, Townson M, Whitaker P, Clifton I, Sutherland T, Elliott M, Peckham D (2018). A new score to identify patients who need fitness to fly test. European Respiratory Journal, 52: S62: PA 2435. Presented at the ERS 2018 in Paris. I designed the study, collected the data, undertook the statistical analysis, wrote and

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Chapter 10

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Spoletini G, Watson R, Pollard K, Etherington C, Clifton IJ, Whitaker P, Peckham DG (2019). Use of NIV in cystic fibrosis: 10-years experience in a large UK adult CF centre. European respiratory journal Supplements. Presented at the ERS 2019 in Madrid. I designed the study, collected clinical data, undertook the analysis, and wrote and presented the abstract. RW and KP contributed to data collection. All authors critically reviewed the abstract.

Spoletini G, Pollard K, Watson R, Etherington C, Clifton IJ, Whitaker P, Peckham DG (2019) Outcomes at 6 months following NIV in adults with cystic fibrosis: experience of a large UK Centre. European Respiratory Journal Supplement. Presented at the ERS 2019 in Madrid. I designed the study, collected clinical data, undertook the analysis, and wrote and presented the abstract. RW and KP contributed to data collection. All authors critically reviewed the abstract.

Chapter 11

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Chapter 12

Spoletini G, Barth J, Peckham D, Ghosh D, Elliott M (2018). Can serum bicarbonate be used to screen for sleep disordered breathing in obese patients? European Respiratory Journal, 52, S62: PA2565. Presented at the ERS 2018 in Paris. I co-designed the study with MWE, collected the data, undertook the analysis, wrote and presented the abstract. All co-authors critically reviewed the abstract. The abstract was awarded a BLF/King's College Award.

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Contributions

My own contribution in the work in this thesis has been:

- Literature review
- Design of thel study protocols
- Data collection and database construction
- Statistical analysis of data
- Drafting of all manuscript and abstract relating to results of the thesis
- Structuring and writing the whole thesis.

Other members of the group and their contribution has been as follows:

- Critical revision of study protocol design (DGP, PW, MWE)
- Assistance in data collection (RW, KP, AS, WYL, LG, AW)
- Critical review of drafted manuscripts and abstract (DGP, PW, MWE, IJC, CE)

DGP: Professor Daniel Peckham; PW: Dr Paul Whitaker; MWE: Dr Mark Elliott; RW: Ruth Watson; KP: Kim Pollard; AS: Dr Akhil Sawant; WYL: Dr Wang Yng Lim; LG: Lindsey Gillgrass; AW: Anne Wood; IJC: Dr Ian Clifton; CE: Dr Christine Etherington.

Abstract

Background: Metabolic alkalosis is common in people with Cystic Fibrosis (CF), and is often presumed to be the result of Pseudo-Bartter's syndrome (PBS). However, clinical experience suggests that both metabolic alkalosis and hyperbicarbonataemia occur, relatively frequently, in the absence of PBS. The clinical significance and aetiology of these phenomena remain poorly understood.

Aims: The main goals of this thesis were: (1) to characterise the level of serum bicarbonate concentration in clinically stable people with CF and see how commonly it was elevated; (2) to assess the clinically significance of elevated bicarbonate; and (3) to evaluate if acid-base balance was differently altered in CF compared to other respiratory conditions. In addition, this thesis aimed at (4) examining the cause of hyperbicarbonataemia, and (5) to ascertain if respiratory support techniques could normalise bicarbonate levels and gas exchanges in people with CF.

Findings: (1) Serum bicarbonate levels were elevated in stable individuals with CF. Comorbidities, age, and sex were independently associated with hyperbicarbonataemia. (2) In patients with CF, bicarbonate increases significantly in the year prior to death. (3) Metabolic alkalosis occurred frequently in CF, in both the clinically stable and during pulmonary exacerbations. Acute and chronic hypercapnic respiratory failure were the most common acid-base disturbances seen prior to death. (4) Urinary bicarbonate excretion did not increase in patients with CF, despite the hyperbicarbonataemia. (5) Individuals, with isolated raised serum bicarbonate, responded to hypoxic stimulus in a similar way to patients with daytime hypercapnia. (6) Transient hypoventilation during exercise was observed in people with CF and severe lung disease. (7) Nasal high-flow therapy reduced CO_2 during exercise. (8) Non-invasive ventilation stabilised serum bicarbonate levels in individuals with CF.

Conclusion: Raised bicarbonate appears to be a marker of disease severity in CF, and has the potential of being an useful prognostic biomarker, especially in the last few years of life. Metabolic alkalosis and respiratory acidosis are the most common acid-base disturbances seen, with hyperbicarbonataemia being likely the result of a combination of transient hypoventilation, hypercapnia and the kidney's inability to increase urinary excretion due to defective CFTR. Respiratory support techniques are likely to control the ventilatory component of hyperbicarbonataemia by improving gas exchanges, while the new CFTR modulators may normalise renal bicarbonate exchange.

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6MWT	6-minute walking test
AA	Annual assessment
ABC protein	ATP-binding cassette protein
ABG	Arterial blood gas
ACPE	Acute cardiogenic pulmonary oedema
ARDS	Acute respiratory distress syndrome
ASL	Airway surface liquid
BAL	Broncho-alveolar lavage
BCC	*Burkholderia cepacia* complex
BE	Base excess
BMI	Body mass index
BTS	British Thoracic Society
CA	Carbonic anhydrase
CBG	Capillary blood gas
CCD	Cortical collecting duct
CF	Cystic Fibrosis
CFA	Cystic fibrosis associated arthritis
CFRD	Cystic fibrosis related diabetes
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CGM	Continuous glucose monitoring
Cl^-	Chloride
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CPET	Cardiopulmonary exercise test
CPT	Chest physiotherapy
CRP	C-reactive protein

CSA	Central sleep apnoea
CT	Computerised tomography
CXR	Chest X-rays
DIOS	Distal intestinal obstructive syndrome
ELX/TEZ/IVA	Elexacaftor/Tezacaftor/Ivacaftor
ENaC	Amiloride-sensitive epithelial sodium channel
EPR	Electronic patients records
$ETCO_2$	End-tidal carbon dioxide
FEV_1	Forced expiratory volume in 1 second
F_IO_2	Fraction of inspired oxygen
FPE	Faecal pancreatic elastase
FVC	Forced vital capacity
GI	Gastrointestinal
GORD	Gastro-oesophageal reflux disease
H^+	Hydrogenion
HAST	Hypoxic altitude stimulation test
HCO_3^-	Bicarbonate
HRA	Health Research Authority
ICM	Intestinal ion channel measurement
ICU	Intensive care unit
ILD	Interstitial lung disease
IMV	Invasive mechanical ventilation
IQR	interquartile range
IRT	Immunoreactive trypsin
IVA	Ivacaftor
kPa	kilo Pascal
LFT	Liver function test
LTOT	Long-term oxygen therapy
LUM/IVA	Lumacaftor/Ivacaftor

MCID	Minimally clinically important difference
MDT	Multidisciplinary team
MI	Meconium ileus
MRI	Magnetic resonace imaging
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
Na^+	Sodium
NBD	Nucleotide binding domain
NBS	Newborn screening
NHE	Na/H exchanger
NHFT	Nasal high-flow therapy
NIV	Non-invasive ventilation
NMD	Neuromuscular disease
NPD	Nasal potential difference
NTM	Non-tuberculosis mycobacteria
O_2	Oxygen
OGTT	Oral glucose tolerance test
OHS	Obesity hypoventilation syndrome
OSA	Obstructive sleep apnoea
PBS	Pseudo-Bartter syndrome
pCO_2	Partial pressure of carbon dioxide
PEEP	Positive end expiratory pressure
PERT	Pancreatic enzymes replacement therapy
PEx	Pulmonary exacerbation
PI	Exocrine pancreatic insufficiency
pO_2	Partial pressure of oxygen
PS	Pressure support
R&I	Research and Innovation
RCT	Randomized controlled trial

REC	Research Ethics Committee
ROC	Receiver operating characteristic
ROS	Reactive oxygen species
RR	respiratory rate
SDB	Sleep disordered breathing
SO_2 or S_aO_2	Arterial oxygen saturation
TAL	Thick ascending limb
$tcCO_2$	Transcutaneous carbon dioxide
TEZ/IVA	Tezacaftor/Ivacaftor
TMD	Transmembrane domain
TWT	Treadmill walking test
US	Ultrasound
V'A/Q'	Ventilation perfusion ratio
VT	Tidal volume
WD	Walking distance
wt-CFTR	Wild-type CFTR

Part I

Introduction

Chapter 1
Cystic fibrosis: an overview

Cystic fibrosis (CF) is a multi-systemic monogenetic disorder, representing one of the most common life-limiting autosomal recessive genetic disease in the Caucasian population [1]. Approximately 77,000 people are affected by CF worldwide, with just over 10,000 cases in the UK [2], where the carrier prevalence is 1 in 25, and the disease incidence is estimated to be 1 in 2,500 live births.

Although CF has been acknowledged in local folklore and medical transcripts since the sixteenth century, the first pathological description of this disease dates to the late 1930s [3]. Initially, CF was recognised as a single rather than multi-systemic condition, being named, “fibrocystic disease of the pancreas” [3].

In the 1940s and 1950s, CF was characterised in more detail, with the discovery of multiple-organ involvement, and the recognition that the lung involvement was a primary cause of mortality. It also became clear that CF had familial transmission with an autosomal recessive pattern [4,5]. The genetic link for the disease was finally identified in 1985 as the long arm of chromosome 7 [6,7], later established to be the locus of the gene for the *cystic fibrosis transmembrane conductor regulator* (CFTR) [8].

This chapter aims to review the pathophysiology of CF, its main clinical manifestations, and the most important treatments that are available to people with CF.

1.1 Pathophysiology of cystic fibrosis

The CFTR gene, constituted of approximately 180,000 base pairs, codes for the CFTR protein, constituted of a chain of 1,480 amino acids. The CFTR protein is an ion channel protein that conducts chloride ions across epithelial cell membranes. To function correctly, the CFTR protein needs to be correctly synthetized, folded and transitioned to the membrane.

The biogenesis of wild-type CFTR (WT-CFTR) is a complex, but highly inefficient, multi-step process. Along the path from the cell nucleus to the apical membrane, the protein must pass stringent quality-control processes that select only fully functional proteins, and avoid that misfolded or unstable ones can reach the membrane.

Through these processes, up to 80% of CFTR is degraded within the cell due to abnormal folding. On the other hand, the mature protein is highly stable and the ones that are endocytosed (10% of those expressed on the plasmatic membrane) are recycled.

1.1.1 WT-CFTR structure and function

CFTR belongs to the ATP-binding cassette (ABC) protein super-family, a large group of proteins characterised by similar structure. ABC proteins characteristically have two transmembrane domains (TMDs) and two cytoplasmatic nucleotide-binding domains (NBDs). These usually dimerize and form binding sites for ATP, which is subsequently hydrolysed to provide energy for the substrate transport [9,10].

WT-CFTR is a sequence of 1,480 amino acids, which, similarly to other members of the ABC family, is constituted of two TMDs and two NBDs. It relies on ATP hydrolysis to power the transport activity and allow for the conformational shifts required for the pore opening and closing [11].

WT-CFTR is atypical compared to other ABC-proteins, with regards to the presence of a unique regulatory domain (R) [8], the phosphorylation of which induces structural changes in the NBDs, exposing the ATP binding site and activating the transport activity [12,13]. From a structural point of view, CFTR is also characterised by two terminal (N- and C-) extensions, which regulate its interaction with other proteins (Figure **1.1**).

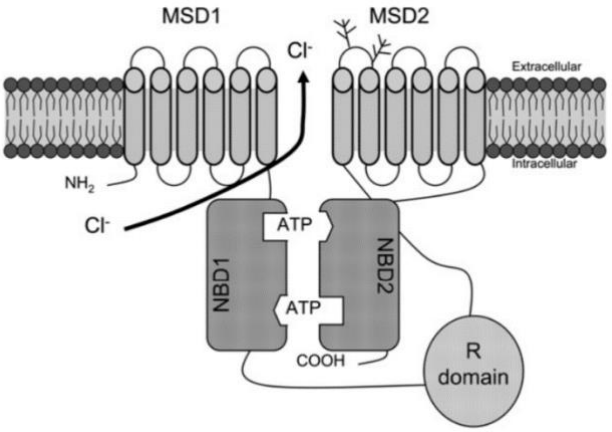

Figure 1.1 Wild-type CFTR structure. CFTR is an ion channel located on the apical membrane of epithelial cells, which belongs to the ABC protein super-family. It is characterised by the presence of two transmembrane domains (MSD), two nucleotide binding domains (NBD), and a regulatory domain (R). Reproduced with permission from [14].

CFTR is, to date, the only known ABC transporter that is an ion channel. Regulated by protein kinase A-dependent phosphorylation of the R domain, CFTR is in fact an active driver of chloride and, to a lesser measure, of bicarbonate [15]. Furthermore, CFTR has been shown to be a conductance regulator for other ion transporters and channel. It functions as a negative modulator of the amiloride-sensitive epithelial sodium channel (ENaC) and interacts with other epithelial channels, including the potassium channel (ROMK) [16,17]. Finally, CFTR interacts with cellular pathways related to inflammation (see also Section 1.4.1.1) [18–20].

1.1.2 Defective CFTR

Any change in the base pairs sequence that meets one of the following criteria is defined as a mutation that causes or is likely to cause CF [21]:

- Change in the amino acid sequence that severely affects CFTR synthesis or function;
- Premature stop signal;
- Variation of the intron splice sites;
- Deletion of one or more exons;
- Introduction of a novel amino acid sequence not occurring in normal variant CFTR from at least 100 CF carriers in the patient's ethnic group;
- Change in a highly conserved residue;
- Creation of a novel splice site.

To date, over 2,000 of these mutations have been identified [22], the vast majority of which are point mutations. These mutations lead to a partial or complete loss of function of the CFTR, resulting in a pleiotropic phenotype, which depends only partially on the genotype [23–25]. While the severity of disease relates to the type of CF-causing mutations, it is also influenced by gene modifiers and environmental exposure [26–28].

Traditionally, six classes of mutations have been recognized, with a seventh class been added more recently [29]. These categories are characterized by similar underlying mechanism leading to defective CFTR (Figure **1.2**).

Class I mutations cause a complete loss of function due to the CFTR protein not being produced [1,30]. These mutations have variable prevalence across European countries (5-35%) and usually result in a severe phenotype [31]. Complete lack of CFTR transcript can be due to the introduction of a premature stop codon in the mRNA (leading to premature termination of protein biosynthesis), or due to splice mutations and chromosomal deletions (leading to a complete absence of CFTR). In 2016, this original class was divided into two separate ones: Class I, characterized by lack of CFTR protein, and Class VII characterized by lack of mRNA transcription [29].

Class II mutations lead to defective intracellular trafficking of CFTR [6,30]. As a result of these mutations, the misfolded protein is retained in the endoplasmic reticulum and is degraded intracellularly, instead of reaching the apical surface membrane. This can lead to a minimal amount of protein to be expressed, and an only partially functioning CFTR [32]. This class of mutations has been widely studied recently, as new targeted therapies have emerged allowing increased expression of CFTR in patients with F508del, the most frequent mutation of this class, by stabilising the faulty protein [33–36].

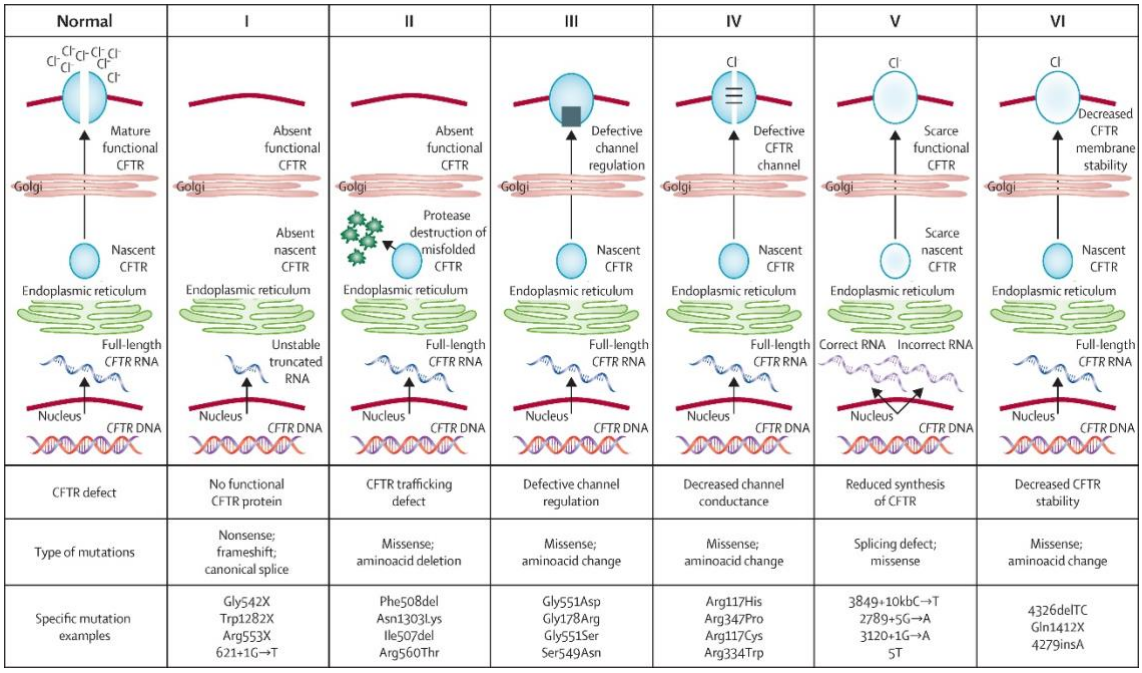

Figure 1.2 Classes of CFTR mutations. Reproduced with permission from [1]

Class III mutations, such as G551D, cause a defective regulation of the CFTR function [1,30]. These so called “gating” mutations are characterised by disruption in the channel regulation with consequent improper ion movement due to prolonged closing and defective opening of the pore. These mutations result in a variable phenotype and affect up to 5% of the individuals with CF worldwide [32]. In the last decade, a drug, Ivacaftor (IVA), capable of rectifying the CFTR defect caused by Class III mutations, has been introduced, allowing for significant clinical improvement [37,38].

Class IV mutations, such as R117H, result in adequate protein expression at the apical surface membrane [30]. However in these cases, CFTR is characterised by a reduced conductance. These mutations are less severe and tend to be associated with milder phenotype. Ivacaftor, or the combination Tezacaftor/Ivacaftor (TEZ/IVA), have been shown to be an effective treatment for R117H and many class IV mutations [39,40].

Class V mutations are associated with reduced synthesis of WT-CFTR [30]. As a results CFTR function is decreased due to reduced protein expressed at apical level, but each protein presents normal function. Ivacaftor, by increasing the conductance activity of WT-CFTR, has been approved for some class V mutation as well [41].

Similarly, *Class VI mutations* results in the expression of functioning CFTR. While the protein is functional at the apical membrane or the cell, it has decreased stability and increased turnover due to the truncation of the C-terminus [30].

Prevalence of the different classes of mutation is characterised by significant geographical variability. In the UK, over 70% of patients have at least one allele carrying the Class II mutation F508del.

Despite over 95% of patient with CF having undergone genetic analysis to identify their genotype, at least one allele remains unknown in 8.9% of individuals [2]. This has become an issue of critical importance recently, in light of the new available CFTR modulators that can target specific genotypes.

1.2 Epidemiology of cystic fibrosis

Cystic Fibrosis affects around 80,000 people worldwide, with a global incidence of 1 in 3,000-4,000 live births, with significant geographical variability. In Europe, the incidence of CF ranges from 1:1300 in Ireland [42] to 1:25,000 in Finland [43], being on average 1:4,500 in Western Europe [44,45] and 1:6,000 in Northern and Central Europe [46,47]. In Australasia and in Canada the incidence is on average 1:3,000 [48,49]. In USA, the incidence is 1:4,000, but large ethnical variations are present [50]. In South America, the incidence of CF is estimated to be between 1:8,000 and 1:10,000 [51]. In Asian populations, the existence of CF has more recently been established, but its incidence remains underestimated in most countries and appears to be higher in the Middle East than in East Asia, possibly as a result of consanguinity [51,52].

In parallel with its major geographic variability in incidence, the distribution of CFTR mutations varies significantly between different ethnic groups, which needs to be taken into account when patients undergo genetic analysis [53].

The majority of CF-causing mutations are European derived, and no alleles have reached the level of incidence of the Class II mutation F508del. The World Health Organization recently produced an overview of the distribution of CFTR mutations across different regions, and especially in non-European-derived populations, where information is more limited [54].

Time trends have shown a declining incidence of CF over the last 20-25 years, which can be attributed to demographic changes, prenatal diagnosis, and family testing among other factors. These trends themselves however show significant geographical variability [52].

While the incidence of CF has reduced in the last few decades, its prognosis has improved significantly. This can be attributed to a major improvement in the management of disease, consisting of standardisation of care, a multidisciplinary approach, better control of infection, aggressive nutritional supplementation, and focus on airway clearance, and, more recently, to the introduction of CFTR modulators.

Data from the European CF Registry estimates an increase in the number of living adults with CF of 75% between 2010 and 2025 [55]. Current UK figures show that 60.6% of patients with CF are over 16 years of age, with an overall median age increased from 19 in 2014 to 21 in 2019 [2].

The expected survival at birth for an individual with CF has been increasing continuously in the last 60 years, reaching the median age of 49.1 years in 2020, a vast improvement compared to an expected death by early childhood in the 1960s (Figure **1.3**).

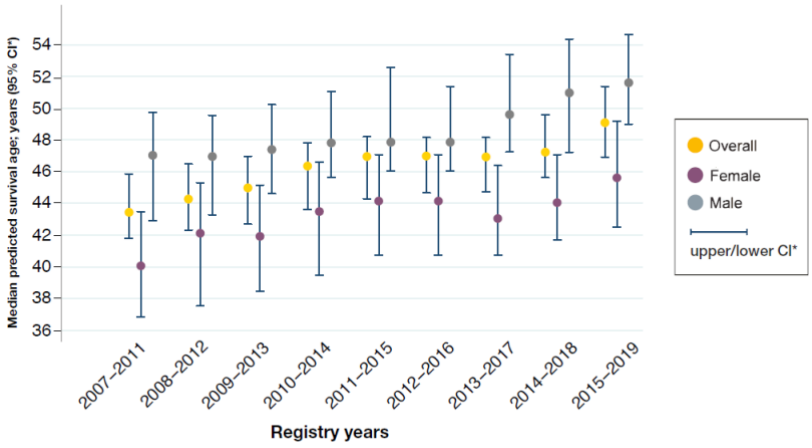

Figure 1.3 Median predicted survival in CF in the UK over the last 12 years. The figure shows the median predicted survival age in years for people with CF in the UK, which has been progressively increasing in the last 12 years. Reproduced with permission from [2].

1.3 Diagnosis of cystic fibrosis

A diagnosis of Cystic Fibrosis is based on the presence of clinical features of the disease, in combination with evidence of CFTR dysfunction or identification of two disease-causing mutations.

Nowadays, most infants who are diagnosed with CF are identified via newborn screening (NBS), based on the immunoreactive trypsin (IRT) assay. Serum IRT levels are, in fact, raised in children with CF in the first few months of life. This test is sensitive, but not specific, for CF, and as such a confirmatory test is required. While repeating the IRT test was previously recommended to confirm an abnormal IRT result, genetic analysis and sweat test have become the gold standard of second-tier testing [53,56–58].

Before the introduction of NBS, Cystic Fibrosis was diagnosed mostly in infancy or childhood, based on clinical features suggestive of the disease, such as meconium ileus at birth, intestinal malabsorption and failure to thrive, and recurrent chest infections.

Presently, children showing symptoms or features suggestive of CF, are recommended to undergo further assessment even if they were negative during NBS [56,57,59]. In upwards of 18% of cases, CF can be diagnosed in adulthood. Some might present with single-organ involvement, having bronchiectasis, or repeated episodes of acute pancreatitis and male infertility [60].

Algorithms to diagnose Cystic Fibrosis depend on its presentation, whether typical or atypical (Figure **1.4** and Figure **1.5**) [57]. Both algorithms are based on sweat test results and genetic analysis.

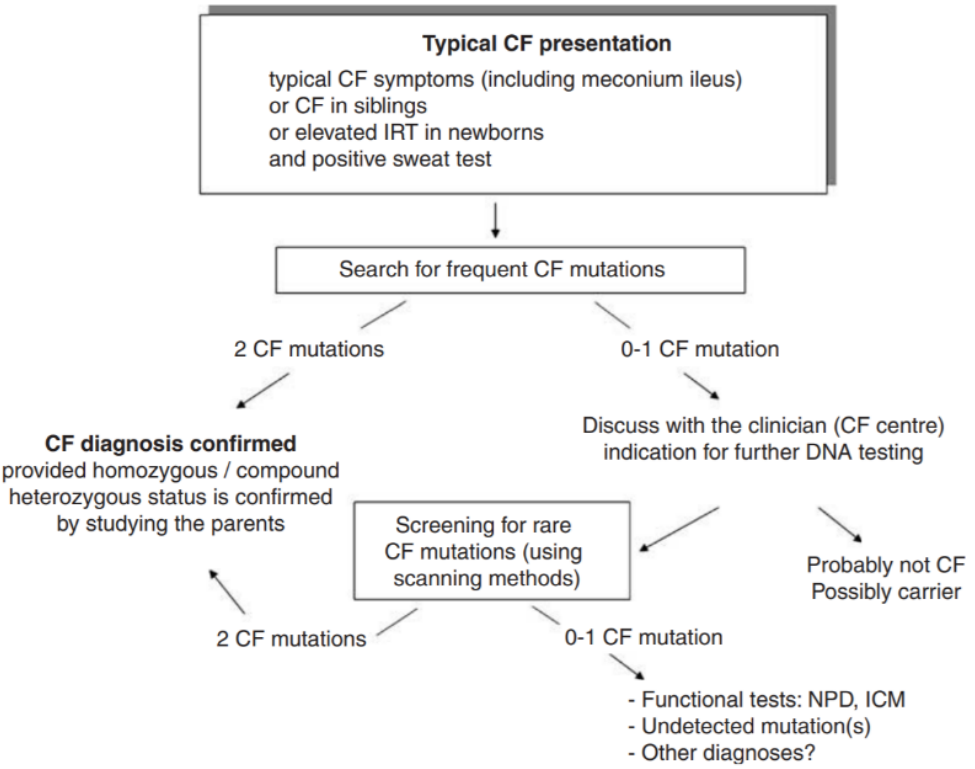

Figure 1.4 Diagnostic testing in typical CF presentation. This algorithm applies irrespective of the age of the CF patient, including newborn screening to late diagnosis in adulthood. Reproduced with permission by [57].

Sweat test has been the gold standard for the diagnosis of CF since the early 60s, when it was first developed. It can be performed in infants who are 2 weeks or older, provided their weight is over 3 Kg, and that they are well hydrated and not acutely unwell. Since CFTR is primarily a chloride channel, measuring the concentration of this anion is the analysis of choice as it relates to CFTR dysfunction.

Pilocarpine iontophoresis is the method of choice for sweat stimulation. Sweat should be collected for 30 minutes, aiming to have a volume of 50-100 ml. A concentration of chloride greater than 60 mmol/L is considered positive for CF, as comparable levels are extremely rare in the healthy population. A sweat chloride concentration lower than 30 mmol/L is considered normal. Concentrations between 30 and 60 mmol/L are considered to be borderline, and tend to be associated with mutations in CFTR leading to a protein with residual function (Class IV and Class V) [53].

Genetic analysis is routinely performed as part of the diagnostic assessment of Cystic Fibrosis and is of particular importance in view of the recent development of mutation-specific treatments. Appropriate testing techniques for CFTR mutations, standardized criteria for CF-causing mutations (as defined in Paragraph 1.1.2) and assessment of the ethnic variability of the CF genotype and phenotype are required to accurately interpret the results of genetic diagnosis.

While the panels and assays used currently can typically identify 90% of mutations, up to 10% of individuals who undergo genetic analysis carry at least an unidentified mutation. This is particularly true for people with CF from ethnic groups other than Caucasian, who have a different distribution and frequency of CF-causing mutations, and therefore more frequently show mutations that are globally rare [53,54,57,58].

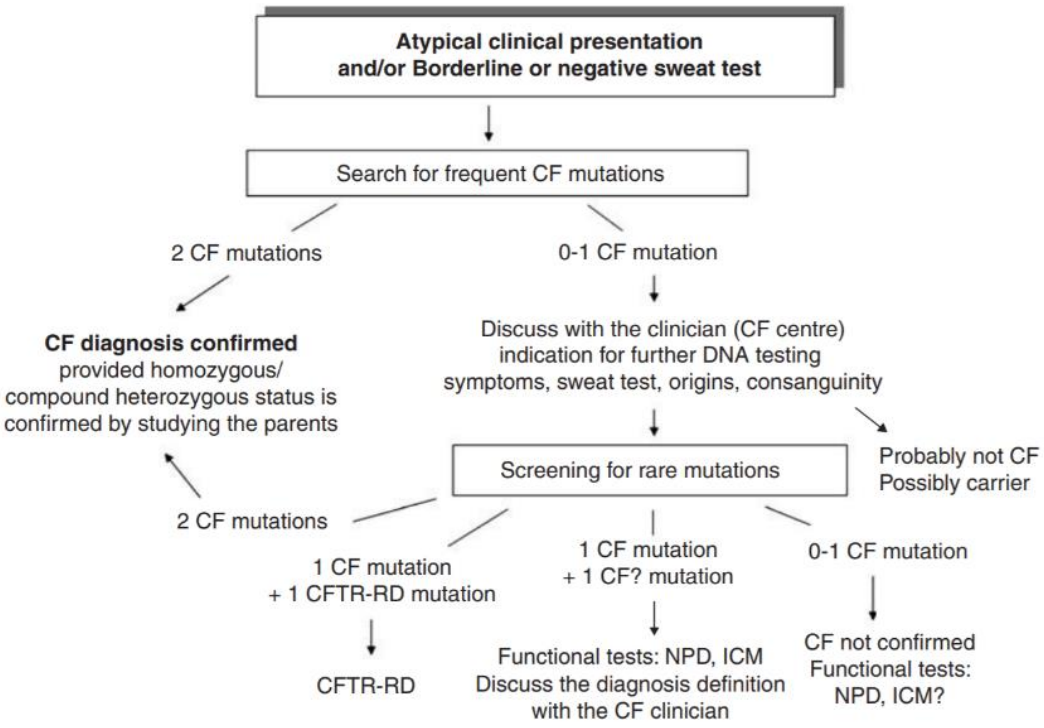

Figure 1.5 Diagnostic testing in atypical clinical presentation. This algorithm present the diagnostic approach for those people who present with atypical clinical features and/or have a normal or borderline sweat chloride concentration. Reproduced with permission from [57]

Additional tests are typically required to establish a diagnosis of Cystic Fibrosis in atypical clinical presentations, inconclusive sweat test or if it is not possible to identify two causing mutations. One such example is nasal potential difference (NPD), which is more negative in people with CF compared to controls. As the technique is easy to learn and reliable, it has been suggested as a complementary test for atypical presentations of CF, borderline or negative sweat tests and/or genetic analysis [57,61,62]. It is however time consuming, repeatability can be variable and is very user dependent.

As a possible alternative or complement, intestinal ion channel measurements (ICM), consisting in measuring CFTR function *ex vivo* from rectal biopsy specimens, has been proposed. While ICM has been shown to be able to differentiate between people with CF and controls, the lack of standardised thresholds and diagnostic criteria do not allow for its routine use. ICM is therefore mostly used for research purposes at the moment [63–65] (Figure **1.5**).

1.4 Cystic fibrosis lung disease

Lung disease accounts for the majority of morbidity and mortality in patients with CF, with respiratory failure being the cause of death in over 85% of individuals [2,66]. As such, promoting early intervention to improve outcome and reduce progression of lung disease is of the utmost importance in this patient population.

1.4.1 Pathogenesis of lung disease

The onset of lung disease appears early in the life of people with CF, with speculation that it might even start *in utero*. CFTR is expressed during early development. Newborn CF piglet models present with airflow obstruction and air trapping in the absence of inflammation or mucus obstruction [67]. Several studies have also shown that asymptomatic infants and children with CF have airflow limitations, ventilation inhomogeneity, and structural parenchymal changes in early life [68].

Multiple factors contribute to the development of lung disease in people with CF (Figure **1.6**). The abnormal CFTR in the airways leads to changes in the volume and acidity of airway surface liquid (ASL), with consequently less effective muco-ciliary transport and clearance and defective defensins. In addition, an increase in the density of the mucus leads to the formation of endobronchial mucus plaques and plugs, which are the main sites of airway infection. Finally, a combination of CFTR dysfunction and endobronchial infection leads to sustaining of inflammation (Section 1.4.1.1) and to a chronic cycle of infection and inflammation resulting in structural damage.

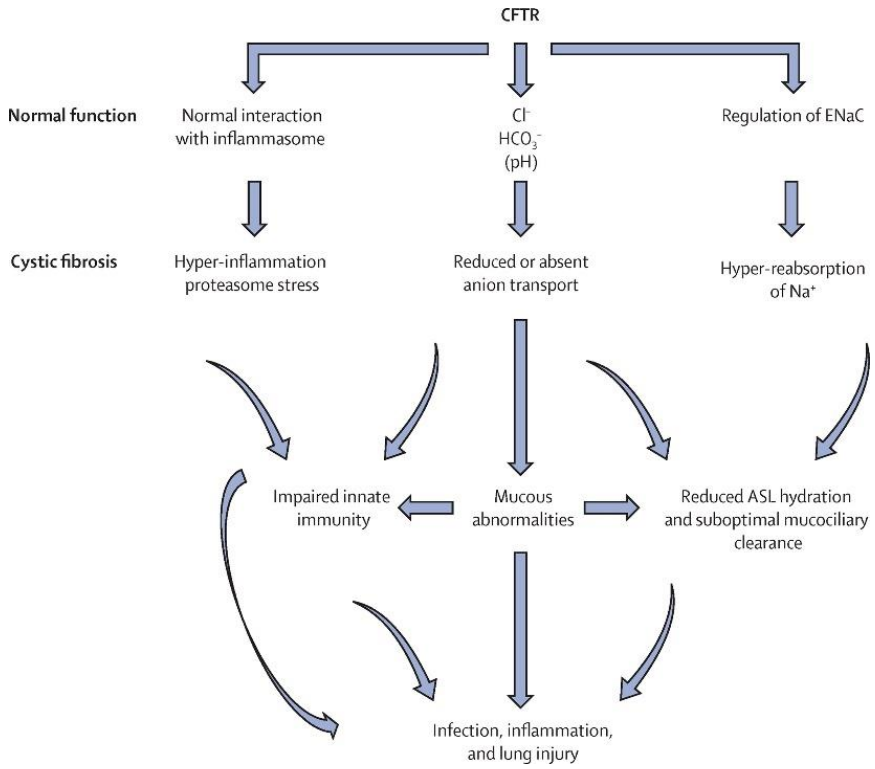

Figure 1.6 Effect of CFTR dysfunction leading to lung damage. CFTR is a key apical membrane channel involved primarily in the exchange of chloride and bicarbonate. However, wt-CFTR is also key-player in inflammation interacting with NLPR3 inflammosome and regulates ENaC. If these key functions of CFTR are defective, as in CF, a sequency of events leading to hyperinflammatory response and mucus abnormalities, is responsible to maintain the vicious cycle of infection, inflammation and lung injury which characterise the disease. Reproduced with permission from [1].

1.4.1.1 CFTR and inflammation

In the healthy lung, the innate immune system plays a critical role against environmental insult and invading pathogens through activation of toll-like receptor expressed on macrophages and dendritic cells. These detect molecular patterns on microbes and trigger the immune response. In addition to the immune system, the pulmonary epithelial cells also play a significant role by orchestrating the inflammatory response through multiple pathways, including the expression of toll-like receptors.

Once the insult is detected, the immune response is initiated and activated via the release of pro-inflammatory cytokines, such as IL-1 and IL-18. These help the recruitment of neutrophils to the sites of infection and promote the continuation of the inflammatory and immune response.

While the normal start of an inflammatory response as a result of exposure to an external damaging stimulus is critical, its resolution is equally important, as an exaggerated pulmonary inflammation can lead to tissue damage as well [19].

It is well established that people with CF have increased local and systemic inflammation. In the broncho-alveolar lavage (BAL) of people with CF, pro-inflammatory cytokines are present at a higher concentration compared to healthy controls. Furthermore, people

with CF have a higher than usual percentage of macrophages that express intracellular cytokines. This hyper-production of pro-inflammatory and decreased release of anti-inflammatory cytokines has been speculated to play a role in the progression of lung disease [69].

It is not fully understood if CFTR is intrinsically proinflammatory or if rather it facilitates inflammation indirectly. CFTR is not expressed exclusively on epithelial cells, but also on neutrophils, monocytes, lymphocytes and alveolar macrophages [70]. A defective CFTR protein on the innate immune cells has been associated with an exaggerated inflammatory response. The combination of excessive response to bacterial infection, and the intrinsic defect in CF, predispose towards a pro-inflammatory phenotype and towards a reduction in the anti-inflammatory one, similarly to what happens in autoinflammatory diseases [19].

Acute and chronic infections are a hallmark of CF disease. In people with CF, infections trigger an enhanced neutrophilic infiltration, as a consequence of a hyper-activation of local environmental cells, such as macrophages and bronchial epithelial cells. This neutrophilic infiltration leads to a pro-inflammatory phenotype. Bacterial infection and colonisation, typical of CF airways, also plays a role in the genesis of inflammation. Common lung pathogens such as *Staphylococcus.aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, as well as many viruses, can trigger the NLRP3 inflammasome and lead to uncontrolled inflammation. A similar pattern of inflammation has been shown in CF animal models growing in a germ-free environment and in the BAL of infants with CF in the absence of infection, suggesting that sterile inflammation is also present [19,71]. Recurring infections act on the background of this pro-inflammatory status and upregulate airway inflammation, driving pulmonary exacerbations and as a central factor in the progression of lung disease [72].

The loss of the inhibitory effect on ENaC as a result of defective CFTR (Section 1.1.1) can lead to further ionic imbalance within the cells and act as a driver to activate the inflammasome [73]. In addition, CFTR is expressed by neutrophils. In this context, the loss of function of CFTR leads to neutrophilic dysregulation with increased migration to the target sites. By potentiating the CFTR function in people with a gating or residual mutation, a change in the neutrophils phenotype was observed suggesting that the CFTR dysfunction itself is a primary driver of inflammation [74]. Similarly, monocytes with CFTR mutations show an exaggerated inflammasome-dependent inflammatory phenotype, which leads to increased production of IL-1 and IL-18 and CFTR modulators have been shown to down regulate the proinflammatory response *in vitro* [75].

Defective CFTR alters the intrinsic characteristics of the ASL leading to a reduction in the luminal pH and an increase in glucose and acid uric levels, factors known to activate the NLRP3 inflammasome. In addition, people with CF have an increased oxidative

stress. The associated production of reactive oxygen species (ROS) activates the innate immune signalling and inflammasome, and leads to an exaggerated response. ROS are usually counteracted by anti-oxidants, which is lacking in CF due to a reduced expression of glutathione, which presence in the epithelial surface fluid relies in fact on functional CFTR.

In summary, people with Cystic Fibrosis are characterised by an imbalance that leads to a pro-inflammatory phenotype, sustained by mechanisms typical of auto-inflammatory conditions. These result from a combined effect of the defective CFTR, and of an exaggerated response to acute and chronic endobronchial infection. The infiltration by innate immune cells at the lungs, combined with a paucity of auto-reactive T cells, an increased production of pro-inflammatory cytokines, and the increased ROS with reduction of anti-oxidants are all hallmarks of autoinflammatory diseases, and are typical in people with CF [18,19,76].

1.4.1.2 CFTR and the airways

In the airways, CFTR is expressed throughout the epithelium and in parts of the submucosal glands. At this level, it is directly responsible for transferring anions (chloride primarily, and in lesser measure bicarbonate) out of the cells in the epithelial surface liquid. In addition, CFTR is indirectly responsible of re-absorption of cations, via the creation of an electrical gradient and regulation of ENaC, and the resulting paracellular flow of water in the same direction (Figure 1.7). As a consequence, CFTR regulates the volume of ASL, the thin layer of fluid sitting above the airway epithelium, which is essential for the innate defense of the lungs and adequate functioning of the muco-ciliary clearance. To ensure an optimal functioning of the ASL, it is crucial that its volume, composition in electrolytes and pH are maintained to their optimal levels.

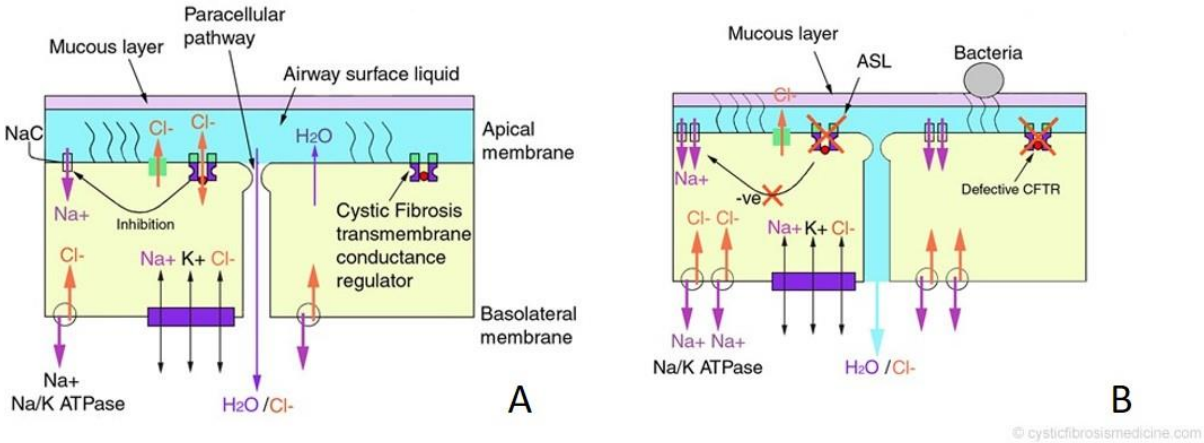

Figure 1.7 Physiology of the airway epithelium in presence of WT (A) and defective (B) CFTR. In the airways of patients with Cystic Fibrosis, defective CFTR leads to dehydration of the airway surface liquid. This is owing to reduced fluid production and increased fluid absorption. The reduced volume of ASL results in viscous mucus and compromised muco-ciliary clearance, which predisposes to bacterial infection and colonisation. Reproduced with permission from www.cfmedicine.com.

Defective CFTR in the airways is responsible of excessive dehydration of the airway surface liquid, causing the mucus to have an abnormally high viscosity (Figure 1.7). This is due to compromised CFTR function leading to the inability to regulate chloride levels, and to its loss of inhibitory effect on ENaC. As a result, a hyper-absorption of sodium and water leads the mucus layer of the ASL to become hyperviscous, which impairs the effectiveness of muco-ciliary clearance, one of the main innate protective mechanisms for the lungs.

The lack of functional CFTR leads to the bicarbonate level in the ASL to be reduced, causing its excessive acidification. Not in its optimal conditions, the ASL leads to reduced antibacterial properties of the mucus with predisposition to bacterial infection and colonization, and impaired mucin expansion with increased mucus viscosity.

The thickened mucus also leads to airflow obstruction, and is a perfect environment for bacterial infection and growth. These infections are one of the triggers for the inflammation cycle that is also intrinsically maintained by defective CFTR itself (Section 1.4.1.1).

1.4.2 Clinical manifestations of lung disease

The mucus lining in healthy lungs is the first line of innate defence against bacterial infections. The defective CFTR in people with Cystic Fibrosis changes the physiological characteristics of their mucus and ASL (Section 1.4.1.1), and plays a central role in facilitating acute and chronic infections, due to the compromised muco-ciliary transport and reduced activity of local antimicrobial peptides facilitating bacterial growth.

This vicious cycle of infection and inflammation leads to the development of structural abnormalities that further contribute to the same damaging cycle. The CF disease has a slow progression with periods of exacerbations and sudden worsening and leads to respiratory failure (Section 2.4).

1.4.2.1 Airway colonisation

While multiple bacteria and fungi have been associated with acute and chronic infections in people with CF, the microbiome in people with CF is far less diverse than in the airways of healthy individuals (Figure **1.8**) [77]. Despite decreasing with age and severity of the disease, this reduced microbial diversity, is not a sole consequence of antibiotic treatment. The introduction of highly virulent bacteria that adapt and become predominant is also thought to play a role. The makeup of the most common strains of bacteria varies with the age of patients with CF, with *S.aureus* and *H.influenzae* being most common in childhood, and *P.aeruginosa* in adults.

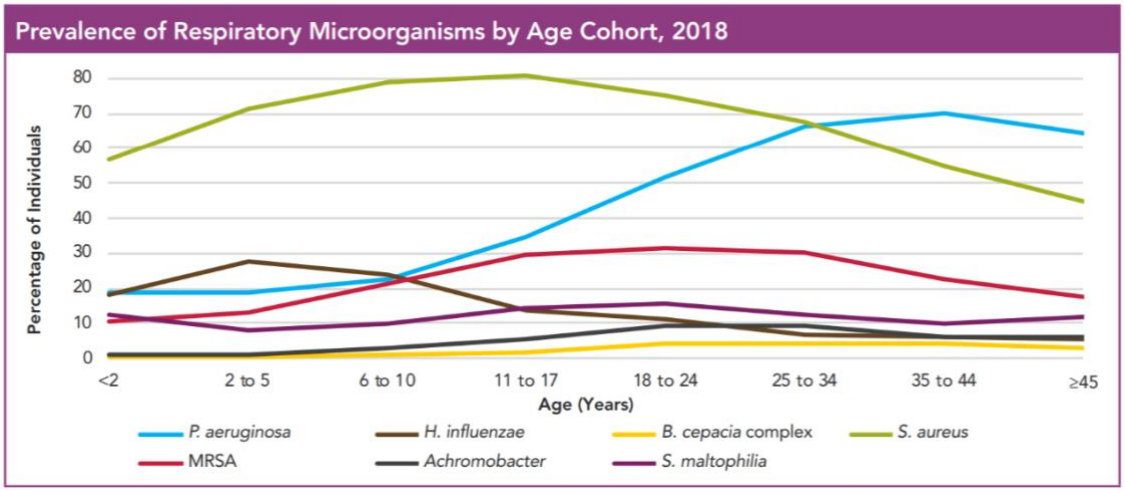

Figure 1.8 Prevalence of bacteria in sputum culture by age cohort. Reproduced with permission from [77].

It is unclear if the presence of methicillin-susceptible *S.aureus* (MSSA) is particularly indicative of a decline in lung function, with some studies suggesting that it can lead to a rapid lung destruction, and others reporting no effect [78,79]. Some authors, conversely, hypothesise that *S.aureus* can prevent colonisation with more pathogenic organisms such as *P.aeruginosa*, with epidemiological studies showing that endobronchial infection with *S.aureus* precedes the one with *P.aeruginosa* being supportive to this hypothesis [79].

While the evidence for a pathogenic role for MSSA remain controversial, colonisation with methicillin-resistant *S.aureus* (MRSA) has clearly been associated with more rapid decline and increased mortality. As such, prompt eradication should be initiated as soon as MRSA is first isolated, although currently there is no internationally established protocol [80] .

Pseudomonas aeruginosa remains the most common bacterial pathogen, being isolated in 55% of adults with Cystic Fibrosis [2,77]. The prevalence of colonisation with *P. aeruginosa* has been progressively declining over the years, possibly as a results of well-established eradication treatments [81].

Since the introduction of strict cross-infection policies in the majority of hospitals treating people with CF, the vast majority of new acquisitions of *P. aeruginosa* nowadays are environmental. As such, new *P. aeruginosa* isolates are usually non-mucoid and susceptible to most antibiotics, leading to successful eradication in over 80% of cases. In contrast, mucoid phenotypes are associated with the production of a biofilm which increases *P.aeruginosa* resistance to antibiotics, and is associated with lung function decline, worsening in quality of life, increase risk of exacerbations and hospitalisations and reduced survival.

Both mucoid and non-mucoid *P. aeruginosa* can lead to chronic colonisation, and feed into the cycle of infection, inflammation and structural damage. Once colonisation with *P.aeruginosa* becomes chronic, the treatment aims to maintaining a stable lung function and to reducing exacerbations.

The *Leeds Criteria* [82] help in the definition of *P. aeruginosa* status, by categorising patients as:

- No history of *P. aeruginosa*;
- *P. aeruginosa* free – *P. aeruginosa* not isolated for 12 or more months;
- Intermittent *P. aeruginosa* – *P. aeruginosa* isolated in less than 50% of the months in the preceding year;
- Chronic *P. aeruginosa* – *P. aeruginosa* isolated in more than 50% of the months in the preceding year.

A CF-specific pathogen that is linked with increased risk of death and worsening pulmonary function is *Burkholderia cenocepacia,* which belongs to the *Burkholderia cepacia* complex (BCC), a group of gram-negative organisms. The prevalence of colonisation with BCC is low at 2-3%, but this group of organisms has been associated with significantly worse pulmonary function and increased risk of death, due to the high virulence, pathogenicity and resistance to antibiotics. Multiple organisms belong to the BCC, and their distribution in the CF population shows a degree of geographic variability [83].

In view of the high risks associated with BCC, and considering that colonisation with *B.cenocepacia* represents a contraindication for lung transplantation, a prompt eradication with three intravenous antibiotics followed by nebulised tobramycin is recommended to be attempted at the first sign of a positive sputum culture [84,85].

In addition to the bacteria previously described, new emerging pathogens have been drawing attention recently, including *Stenotrophomonas maltophilia, Achromobacter xylosoxidans and* non-tubercolosis mycobacteria.

1.4.2.2 Pulmonary exacerbations

In CF, progressive chronic lung disease develops over time due to the presence of mucous plugging, chronic inflammation, lung damage and bacterial infection. Acute on chronic deterioration also occurs as a result of acute pulmonary exacerbations (PExs).

There is no clear consensus on the formal definition of a PEx in CF, and patient symptoms, laboratory data and clinical evaluation are usually assessed holistically to diagnose a PEx. In general, an exacerbation can be defined as a sudden need for additional antibiotic treatment as indicated by at least two of the following: change in sputum volume or colour; increased malaise, fatigue or lethargy; loss of appetite or weight loss; decrease in lung function or radiographic changes; increased dyspnoea [86].

Pulmonary exacerbations aggravate the pulmonary inflammation, and lead to further lung damage. This is reflected by a significant worsening of pulmonary function tests during PExs. As such, although a univocal definition for exacerbation is lacking, consensus has been reached that PExs need to be prevented and aggressively and promptly treated when occurring [86]. This is to attempt to maintaining stability of lung function and prolonging survival and restoring lung function as close as possible to baseline when affected by the exacerbation itself. Despite aggressive and early treatment of pulmonary exacerbations, in fact, up to 25% of patients with CF do not recover to baseline lung function after a PEx, and PExs are responsible of over half of the decline seen in lung function [87–90] (Figure **1.9**).

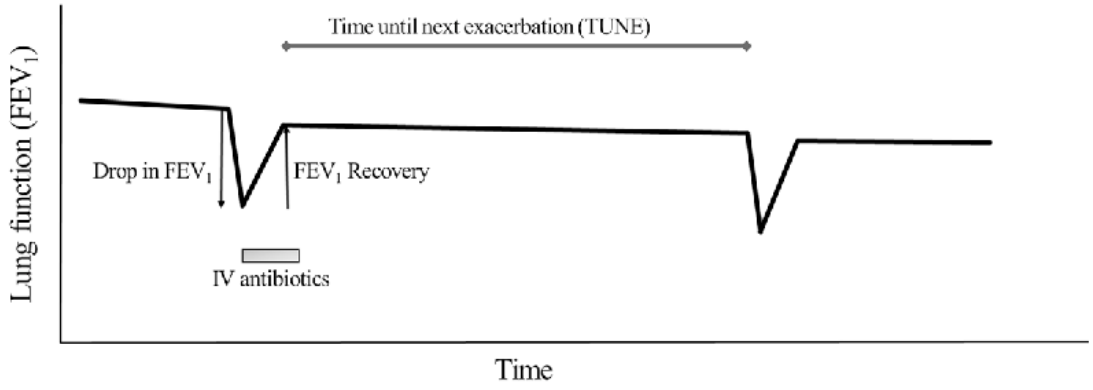

Figure 1.9 Decline in lung function over time and effect of exacerbations. Reproduced under the terms of the Creative Commons license from [90].

The cause of pulmonary exacerbations in patients with CF has not been fully understood. Viruses and bacteria can cause PExs independently, or viruses can be the trigger for bacterial infections. Virus-triggered PExs have been associated with increased severity and worse outcome compared to non-viral exacerbations, possibly as a result of an exaggerated inflammatory response to virus in the CF airway epithelium [86].

1.4.2.3 Complications of lung disease in CF

The two most common complications of lung disease in CF are haemoptysis and pneumothorax.

Haemoptysis consist of coughing blood, ranging from blood stained sputum, to massive haemoptysis, defined as over 240 ml in the 24 hours. Despite massive haemoptysis affecting up to 5% of patients with CF, its pathogenesis remains not fully understood.

Chronic inflammation and infection can lead to the erosion of capillary or arterial walls, with a consequent upsurge of blood into the airways. While more frequent among older individuals with severe lung disease, approximately 25% of episodes of massive haemoptysis occur in paediatric patients and 10% in people with normal lung function. Massive haemoptysis is a life threatening medical emergency due to the risk of death from asphyxiation and hypovolemia. Antibiotic treatment, and support therapies are

required, but selective bronchial artery embolization is recommended to terminate the bleeding [91].

Approximately 4% of individuals with CF experience a pneumothorax in their life. Similarly to haemoptysis, pneumothorax is more common among people with advance lung disease, especially if they present with anatomical risk factors such as cysts, blebs or bullae. The risk of recurrence is estimated to be between 50-90% and it remains high for a year after resolution. This notwithstanding, pleurodesis is not recommended at the first occurrence of pneumothorax, but can be considered in case of recurrent pneumothorax or persistent air leak [92].

1.4.3 Structural lung abnormalities

The most common lung abnormalities in patients with CF are bronchiectasis and air trapping, which is a reflection of small airway disease. Other, less common abnormalities include sacculation, atelectasis, bullae, airway wall thickness and thickening of the septa. A significant heterogeneity of expression in these abnormalities can be observed, and in some patients areas of normal parenchyma can be adjacent to others with severe lung damage [93].

Bronchiectasis is usually the result of chronic inflammation and bacterial infection. Development of bronchiectasis can be detected in up to three quarters of children with CF and is an important prognostic factor. This irreversible widening of the airways represents in fact an ideal reservoir for bacteria, and another trigger point for the vicious cycle of infection and inflammation. The presence of bronchiectasis is an important predictor of future exacerbations, disease progression, and is linked to worse quality of life and mortality in cases of severe lung disease [94,95].

1.4.4 Radiological assessment of lung disease

Chest radiographs (CXR) are commonly performed on a yearly basis in CF clinics, with the purpose of monitoring patients longitudinally, and scoring systems might help in tracking the progression of disease. However, their clinical role is fairly limited since, by providing a bidimensional representation, their sensitivity is low with regards to mild changes and air trapping [96,97].

On the other hand, CT chest scans are more sensitive in defining early abnormalities, and protocols combining inspiratory and expiratory scans allow for assessment of both bronchiectasis and air trapping. Progression of lung disease is also better evaluated with a CT chest than with both conventional radiograms and lung function testing. Up to 50% discordance between lung function testing results and severity of bronchiectasis and other structural changes on CT scan has been reported [98]. This taken together with

the notion that severity of bronchiectasis is an independent risk factor for pulmonary exacerbation and mortality, would support a routine screening with CT chest for individuals with CF. However, concerns for exposure persists and other institutions, including the Leeds Adult CF Centre, prefer to repeat CT chest based on clinical needs rather than at regular intervals.

The longitudinal comparison of CT scans allows for individual evaluation of the typical structural abnormalities of CF (Figure **1.10**), but also to track progression. This should be quantified by using one of the possible scores that have been developed and include all the main airways and parenchymal abnormalities in CF [99].

More recently, MRI has been suggested as a radiation-free diagnostic modality for individuals with CF. While traditionally MRI has been considered a poor method to visualize the lungs, there are now multiple techniques that allow to assess both structure and function. MRI might not be as effective as CT in the early detection of bronchiectasis, but is adequate in reporting mucus plugging. In addition functional and structural scans can be combined and can define regional abnormalities, which would not be possible with traditional pulmonary function tests [100].

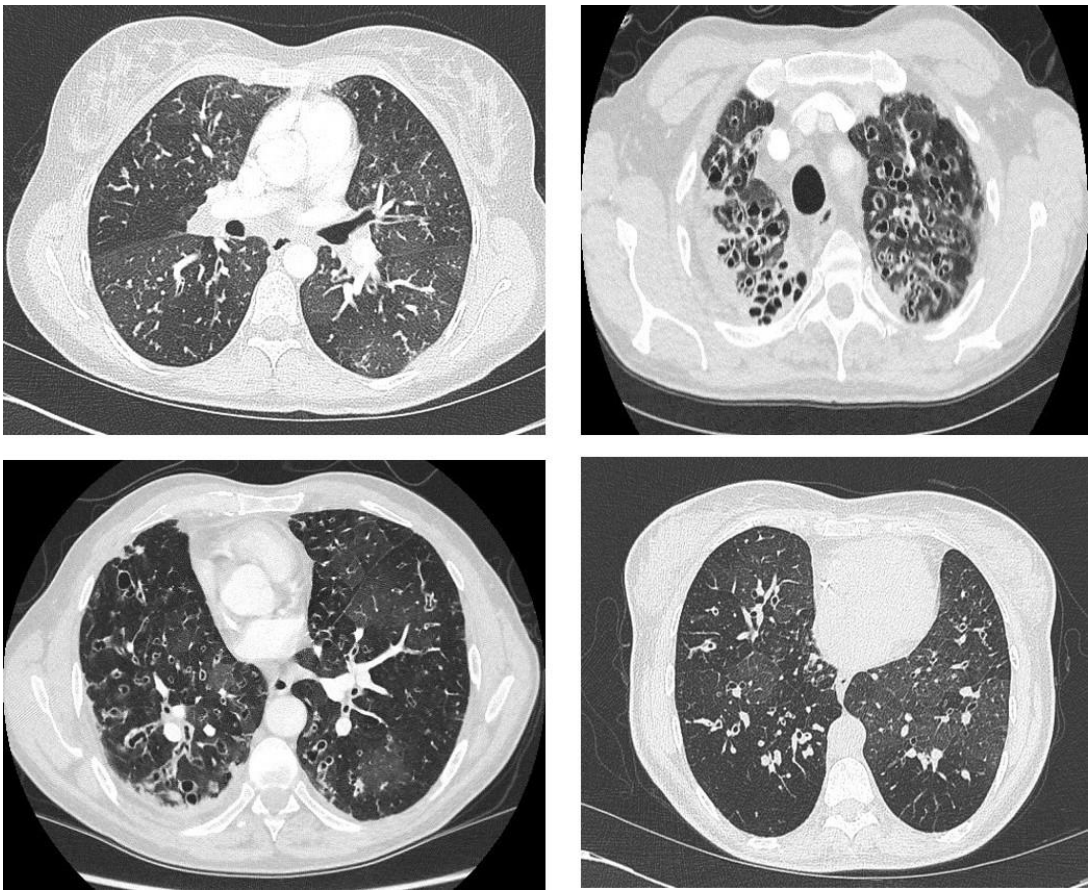

Figure 1.10 CT scan of patients with CF. A combination of several structural abnormalities (bronchiectasis, air trapping, mucus plugging and atelectasis) and areas of relatively spared parenchyma can be observed. Courtesy of the Leeds Adult CF Centre.

1.4.5 Functional assessment of lung disease

Despite the advancement in radiation-free imaging and its low sensitivity in assessing severity of disease, lung function tests remain a key physiological parameter used in the monitoring of people with CF. Pulmonary function tests are non-invasive techniques, inexpensive and easily reproducible, with spirometry being the most commonly performed test in the clinical setting to measure FEV_1 and FVC [101]. Historically, FEV_1 has been considered a better marker compared to FVC of disease severity and prognosis. However, more recently several studies have shown that this parameter lacks sensitivity in determining prognosis and that normal results do not necessarily mean normal lung function and normal lung parenchyma [102,103].

The lung clearance index, measured with the multiple breath washout test, has been used as an endpoint in clinical trials and is now extensively studied to assess its utility in clinical practice. It is a good marker for early lung disease and it is easier to perform in children compared to spirometry, but the variability of measurements increases with disease severity [104].

Finally, as part of the functional assessment, patients with CF need to be evaluate for gas exchange abnormalities at rest, during sleep and exercise. This can be done invasively using blood gas analysis, or non-invasively with measurements of the oxygen saturation (S_pO_2) and transcutaneous carbon dioxide ($tcCO_2$) (Section 2.3). Formal assessment of gas exchanges with arterial blood gases, 6-minute walking test (6MWT) and overnight oximetry, is advised at least yearly for patients who have advanced lung disease, defined as FEV_1 <40% and for those under consideration for lung transplantation [105].

1.5 Other clinical features of cystic fibrosis

Wild-type CFTR is expressed on the apical membrane of epithelial cells of many organs across the body, including lungs, liver, pancreas, gastrointestinal (GI) and reproductive tracts and skin [1]. This accounts for the multi-systemic nature of the disease, in presence of defective CFTR.

1.5.1 Liver disease

CFTR is expressed on the apical membrane of the cholangiocytes and in the gallbladder epithelial cells, where it plays a significant role in bile secretion, but is not expressed on hepatocytes or other liver cells [106]. Here, WT-CFTR contributes to Cl/HCO_3 exchange, hence ensuring adequate hydration of secretions. As a consequence of defective CFTR, bile composition and excretion are impaired. Secretions become indeed more viscous, and this thickened bile leads to plugging of intrahepatic bile ducts. This triggers inflammation and subsequent fibrosis by activating the hepatic stellate cells [107]. As

only a subset of patients with CF develop cirrhosis, gene modifiers play a significant role in the pathogenesis of liver disease [108].

CF-related liver disease can present with a wide variety of clinical manifestations. It is usually diagnosed in childhood and rarely progressing in adults. This notwithstanding, annual screening is recommended through monitoring of liver function tests (LFTs), abdominal examination and ultrasonography (US) in children and adults alike [109]. In the most severe cases, CF-related liver disease can progress towards cirrhosis with portal hypertension and subsequent complications, and represents an indication for liver transplantation [110,111].

Despite CF-related liver disease being the third leading cause of death among patients with CF, accounting for 2.7% of all deaths, and being suggested as a possible independent risk factor for mortality by other cause, there is no consensus on a definition for this condition [112]. The European recommendations [109] suggest a diagnosis of CF-related liver disease when in presence of at least two of the following criteria:

- Abnormal physical examination
- Hepatomegaly, confirmed by US; and/or
- Splenomegaly, confirmed by US.
- Abnormalities of liver function tests (LFTs) in at least 3 consecutive determination over 12 months:
    - Increase of transaminase (ALT and AST) ≥ 3x upper normal limit (UNL),
    - Increase of GGT ≥ 3x UNL.
- Ultrasonographic evidence of liver involvemen (Increased or heterogeneous echogenicity, or irregular margins, or nodularity), or
- Features of portal hypertension (Splenomegaly, large collateral veins, ascites).
- Biliary abnormalities (bile duct dilatation).

While most individuals with CF (up to 70%) present with mild liver steatosis, that does not necessarily progress towards fibrosis [109,110], studies performed *post-mortem* demonstrated the presence of focal biliary cirrhosis in up to 70% of patients studied. In some cases patients will present with hepatomegaly and abnormal elevation of bilirubin and liver enzymes, many patients who are diagnosed with CF-liver disease do not have these features, and diagnosis relies on ultrasonography [113].

1.5.2 Gastrointestinal manifestations

Gastrointestinal manifestations are a frequent occurrence in CF. The gastrointestinal tract as a whole can be interested, with a variety of potential complications.

1.5.2.1 Upper GI tract

The prevalence of gastroesophageal reflux disease (GORD) is 6 to 8 times higher in patients with CF compared to healthy individuals [114]. This might be related to medications, prolonged gastric emptying time, increased abdominal pressure from coughing and increased use of positive pressure as an adjunct to physiotherapy.

GORD can contribute to the worsening of lung function and progressive lung structural changes if associated with micro aspiration, and is a risk factor for bronchiolitis obliterans syndrome and chronic rejection in lung transplant recipients. As such, adequate treatment of GORD is essential, and consists of acid suppression. This, however, is often not effective, and patients might require surgical procedures such as fundoplication or LYNX surgery.

1.5.2.2 Small bowel involvement

Up to 20% of patients present at birth with meconium ileus (MI), consisting in distal small bowel obstruction, most typically at the level of the terminal ileum, owing to the impaction of thick tenacious meconium. While not pathognomonic for CF, MI is highly suggestive for diagnosis when present in full-term infants. It was also found to correlate with severe genotype classes, with patients homozygous F508del having a odds ratio of 13.0. This can be complicated with volvulus, perforation, and peritonitis. As such, while aggressive conservative treatment is the recommended first approach, surgical management is indicated when medical treatment fails [115].

In adulthood, many patients suffer with constipation, and up to 20% of adults can present with distal intestinal obstruction syndrome (DIOS). DIOS is more frequent among those who had MI at birth and in patients who are pancreatic insufficient (PI). It is characterised by partial or complete obstruction secondary to accumulation of viscous muco-faeculant material in the terminal ileum. The mainstay of treatment in this situation is hydration with high dose of laxatives, in the attempt to avoid surgery which can be associated with severe complications [116].

1.5.3 Pancreatic complications

While the genotype has not been shown to directly correlate with the severity of lung disease, there is a strong correlation between genotype and phenotypic expression of pancreatic dysfunction. This can present with defective function of only the exocrine function of the pancreas, or involving the endocrine function as well.

1.5.3.1 Exocrine pancreas and pancreatic insufficiency

Acinar cells in the exocrine pancreas are responsible for the production and secretion of digestive enzymes. CFTR is expressed on the membrane of these cells, and contributes to the high concentration in bicarbonate in the secretions. Defective CFTR results in

abnormal secretions in the pancreaticobiliary ducts. Pancreatic juice in patients with CF is three times more concentrated than in healthy people, due to fluid volume reduction and increase in protein concentration. Thickened secretions lead to pancreatic duct obstruction. The reduction in the bloods flow contributes in ischemic damages leading to atrophy, fibrosis and fatty infiltration of the pancreas . These events happen as early as during foetal life, and malabsorption is often one of the early symptoms of CF [117].

Pancreatic insufficiency is usually diagnosed clinically with the aid of stool collection to measure the faecal concentration of pancreatic elastase (FPE). FPE is resistant to degradation, its reliable and easy to test. Usually a cut-off of <100 μg/g in individuals over age 2 to 3 years is considered indicative of pancreatic insufficiency (PI). Values greater than 200 μg/g are considered to be normal, while between 100 and 200 is indicative of loss of pancreatic function, although not necessarily of sufficient severity to confer PI [118] .

While 90% of patients with CF are pancreatic insufficient since childhood, the remaining 10% might develop this complication at a later age. Individuals who are pancreatic sufficient (PS) can also present with acute and recurrent pancreatitis, characterised by severe epigastric pain with elevated serum amylase. It is hypothesised that recurrent episodes of pancreatitis can also lead to the development of PI [118].

Inadequate exocrine pancreatic function results in malabsorption symptoms including greasy stools, abdominal bloating and poor weight gain. Fat-soluble vitamin deficiency is a main area of concern in patients with CF, even when they are receiving pancreatic enzyme replacement therapy (PERT). Deficiency in vitamin A, D, E and K can lead to anaemia, neuropathy, night blindness, coagulopathy and osteoporosis.

1.5.3.2 Endocrine pancreas and CF-related diabetes

Up to 10-15% of blood flow in the pancreas is received by the islets of Langherans, the endocrine component which represents 1-2% of the organ. The islets of Langherans are responsible of insulin production by the β cells, regulated by CFTR [119].

In individuals with CF, since childhood, the islets of Langherans are reduced in number, and the remaining ones are disorganised and present fatty infiltration. This predisposes to the loss of function, over time, of β cells with resulting reduced production of insulin, leading to progressive increase in prevalence of glucose intolerance and diabetes with age in CF.

Individuals with CF have variable levels of glucose tolerance over time, and several factors including pulmonary exacerbations, gastrointestinal abnormalities and diet can affect it. Patients with CF should regularly be monitored for glucose intolerance and diabetes, by performing the oral glucose tolerance test (OGTT) or continuous glucose monitoring (CGM). The OGTT remains the gold standard to screen for CF diabetes, and

can also help to identify those with impaired glucose tolerance. Patients with CF who have normal or impaired OGTT should be monitored at least annually, as results can vary significantly from year to year. People with CF can fluctuate between hypoglycaemia and diabetic OGTT before developing irreversible diabetes [120]. Furthermore, the diagnosis of diabetes must be confirmed with CGM or flash sensors as in some people with pathological OGTT the glucose profile is normal [121].

Early identification of CF-related diabetes (CFRD) is crucial, since CFRD has a negative impact on morbidity and mortality [122,123]. CFRD is in fact associated with clinical deterioration, worsening lung function and nutritional status. Lung function decline by 20% can be evident up to 4-6 years before the diagnosis of CFRD [102,124]. The negative effects of hyperglycaemia on lung function can be direct or indirect by affecting the parenchyma structurally or predisposing to in lung infection. CFRD can also predispose to a catabolic state, affecting negatively lung function.

1.5.4 Musculoskeletal system

The impact of CF on the musculoskeletal system is quite variable in each individual and includes effects on bone mineral density, muscle function with postural changes, as well as inflammatory arthritis, known as cystic fibrosis associated arthritis (CFA).

1.5.4.1 CF-associated arthritis

People with CF present with rheumatic symptoms, the prevalence of which tends to increase with age and severity of the disease. While specific autoimmune inflammatory arthropathies have been reported among people with CF, this association is rare. However, joint symptoms and arthropathy have been described more frequently as a consequence of inflammation in the context of CF related arthropathy (CFA) [125].

CFA is defined as an arthropathy with clinical features of articular inflammation without sepsis; absence of periostitis on radiograms; absence of another cause of arthritis after evaluation. It can affect between 2.6% and 8.5% of patients with CF, with data from the UK CF registry reporting a prevalence for arthritis at 3% in individuals aged over 16 [2]. The difficulties in the correct estimation of prevalence are related to the variable presentation of CFA itself. Patients can report diverse signs and symptoms, can present with oligo- or poly-arthritis, often remitting-relapsing and flitting. Joints of the hand and the feet are the ones that are most often affected [125] .

Many reports have indicated that relapses of the CFA occur in correspondence with pulmonary exacerbation, with a German observational study showing that pulmonary exacerbations and elevated levels of total serum IgG are associated with CFA. This might suggest that chronic inflammation in patients with CF plays a role in the development of CFA [126].

While hyperuricemia is described frequently in adults with CF, clinical gout is only rarely reported, and CFA is not associated with hyperuricemia and does not meet the typical features of gout [127]. Similarly, rheumatoid factor and auto-antibodies are often positive, but no specific auto-antibodies pattern is linked to this condition. While radiographic imaging of affected joints can be normal, US scan shows inflammatory changes. In lack of clearly defined criteria, diagnosis of CFA remains an exclusion one. Detailed history, joint examination, and serology are therefore essential [125].

CFA is a well-recognised complications of CF, and significantly affects the quality of life and morbidity. There is however at present little data on its management, as a result of lack of clarity on its pathophysiology. However, it appears that the role of CFTR in inflammation and auto-inflammation, as described in Section 1.4.1.1 by activation of the inflammasome, appears to be central in the development of this complication.

1.5.4.2 Bone mineralisation

Multiple factors predispose patients with CF to an increased risk for osteopenia or osteoporosis, including malabsorption of fat-soluble vitamin D and K, intermittent use of steroids to treat some of the respiratory complications (i.e. ABPA), reduced level of physical activity due to exercise-induced desaturation or dyspnoea. Finally, recurrent infection and chronic inflammation might lead to a reabsorption of bone as predispose to a catabolic state.

1.6 Management of cystic fibrosis

Over the last six decades, the survival of individuals with CF has improved dramatically, as a result of the introduction of a multitude of therapies directed to the downstream effects of CFTR dysfunction (Section 1.2). Therapies include strategies for airway clearance, replacement of pancreatic function, antibiotic treatment, vitamin supplementation. While associated with a significant improvement in quality of life and survival, they represent a significant burden of treatment for individuals with CF (Figure **1.11**).

Following the discovery of the CFTR gene, and consequent better understanding of the pathophysiological mechanisms of the disease, the therapeutic approach to CF has shifted towards the treatment of the underlying cause of CF, in the form of CFTR modulators.

Figure 1.11 Treatment for cystic fibrosis. The panel on the left represents the evolution of treatment in CF since 1940. With the improvement in treatment available for people with CF, a significant increase in survival has been observed with the downside of patients needing to take multiple medication on a daily basis, reproduced with permission from [1]. The two panels on the right represent the dosset boxes of adult patients attending the CF Unit (Courtesy of patients attending the Leeds Adult Regional CF Centre).

1.6.1 Symptomatic treatment for CF

Impaired mucociliary clearance and hyperviscous mucus is one of the hallmark of CF lung disease. As such, it is not surprising that airway clearance techniques to aid expectoration with the help of nebulised treatment are a mainstay in the management of this condition. Dornase alfa (known by its commercial name in the UK of *Pulmozyme*) cleaves DNA and reduces the viscosity of the mucus in people with CF allowing for better clearance. It was one of the first treatment to show results in terms of improvement in lung function and reduction in the exacerbation rate [128,129]. In some cases, hypertonic saline is used in adjunct to DNAase to target the mucus dehydration via an osmotic effect and by triggering coughing spells [130]. These nebulised therapies are often ineffective if undertaken without chest physiotherapy. Different airway clearance techniques can be used, varying from hands-on treatment to the use of positive pressure devices, such as PEP mask or noninvasive ventilation.

Inhaled antibiotics are often needed to eradicate new bacterial growths, control chronic endobronchial infection and reduce the frequency of pulmonary exacerbations (i.e. for *P. aeruginosa* or BCC). Oral, intravenous and nebulised antibiotics play an important role in the early and aggressive treatment of pulmonary exacerbations. In this context, antibiotic choice should be guided based on the bacteria usually isolated in the sputum cultures, taking into consideration response to treatment in previous courses and allergies. Susceptibility testing can be important in the management of multi resistant pathogens but does not seem to predict clinical response in patients with CF and acute exacerbations [131].

Progression of disease despite maximal treatment, including maintenance antibiotics, nebulisers, CPT, is inevitable. This might lead to respiratory failure requiring respiratory or ventilatory support in the form of oxygen therapy or noninvasive ventilation (Section 3.3), and consideration for lung transplantation.

Dietetic review to tailor patients' diet, address specific nutritional needs, including the requirement for nutritional supplementation and review the dosing of supplemental pancreatic enzymes is of extreme importance. This can not only aid the weight management of patients with CF, but also to prevent GI complications such as constipation and obstruction.

Fat-soluble vitamins should be replaced in people with CF, particularly in those who are PI. An assessment of calcium intake should be performed in all patients in view of the risks of low bone mineral density in CF.

Patients who suffer with CF related diabetes should be adequately reviewed so that insulin might be started as required. Optimal glucose control is linked with better outcomes among people with CF and diabetes.

1.6.2 CFTR modulators

Over the past decade there has been a paradigm shift in the treatment of CF following the introduction of CFTR modulators, a new class of drugs that target the underlying mechanisms of CF rather than the disease complication.

After many years of basic and clinical research, the first CFTR modulator, Ivacaftor, was approved in 2012 for clinical use. Ivacaftor is a potentiator that has extreme effectiveness in improving CFTR function for people who have a gating mutation (i.e. G551D) or Class IV (i.e. R117H) and Class V (3789+10kb C>T) mutations [37,38,132].

Ivacaftor binds to CFTR and increases the open time of the pore, allowing an improved conductance when WT-CFTR is expressed, as is the case in Class V mutations. In clinical trials, Ivacaftor was shown to improve lung function by 15%, to normalize sweat chloride, reduce exacerbation rate and improve quality of life [37,38]. Almost ten years since its introduction in clinical practice, real-life data have shown that while the improvement in lung function observed in the general population was in line to that of the clinical trials, decline in lung function is unfortunately continuing among people with gating mutation on treatment, albeit more slowly than controls [133].

People with mutations other than those responsive to Ivacaftor, however, do not express CFTR at all, due to misfolding or intracellular trafficking blockage (Section 1.1.2). In recent years, the development of a corrector to stabilise the CFTR protein and allow it to reach the membrane has led to the development of two drugs, Lumacaftor (LUM) and Tezacaftor (TEZ). Both these correctors have been studied in combination to the potentiator Ivacaftor and were shown to provide similar results with an increase in lung function of approximately 4% among individual who are homozygous for the F508del mutation (Class II). Lumacaftor, however, appeared to be less tolerated especially among people with more advanced lung disease, who presented with severe pulmonary side effects [33,34]. Both drug combinations (LUM/IVA and TEZ/IVA) were approved in

the UK in 2019 for routine clinical practice for individuals with two copies of the F508del mutation, covering approximately 40% of people with CF.

A recent analysis of people treated with the LUM/IVA combination in real life showed that while 40% of patients who had a baseline FEV_1 between 40-90% (as per the clinical trial inclusion criteria) improved their lung function more than 10%, those who had a lower or higher baseline FEV_1 did not have any benefit with this combination [134].

The highly effective combination of Elexacftor/Tezacaftor/Ivacaftor (ELX/TEZ/IVA) was introduced into clinical practice in 2020 and has since been approved for people who are homozygous for F508del and for those who are heterozygous for F508del with a minimal function mutation. In these patients, triple therapy was shown to improve lung function between 10 and 14%, reduce exacerbation rate and sweat chloride [36,135].

1.6.3 Future directions

CFTR modulators have certainly changed the outlook for the management of people with Cystic Fibrosis. However, the lack of long-term data warrants caution in the extrapolation of any long-term persistence of the positive impact introduced by these drugs and the impact that these new drugs can have on the progression of lung disease towards respiratory and ventilatory failure remains still uncertain.

In addition, triple therapy is not available to 100% of patients with Cystic Fibrosis, due to some mutations still being excluded from eligibility. As such, further research will be needed to provide an answer to the need of this excluded population. Long-term registry data will provide crucial longitudinal evaluation to monitor and assess long-term outcomes of these new treatments.

Chapter 2

Respiratory failure and acid-base disturbances

Respiratory failure is a state characterised by the inability of the respiratory system to maintain normal gas exchanges, leading to dysfunction in other organs, and potentially threatening life.

This Chapter reviews the principles of gas exchange physiology and acid-base metabolism, describes the main features of respiratory failure and acid base disturbance in relation to cystic fibrosis, and summarises how to diagnose these impairments.

2.1 Basic concepts of gas exchange physiology

The pulmonary system, in conjunction with the cardiovascular system and the body tissues, is at the core of the oxygen/carbon dioxide pathway (Figure **2.1**).

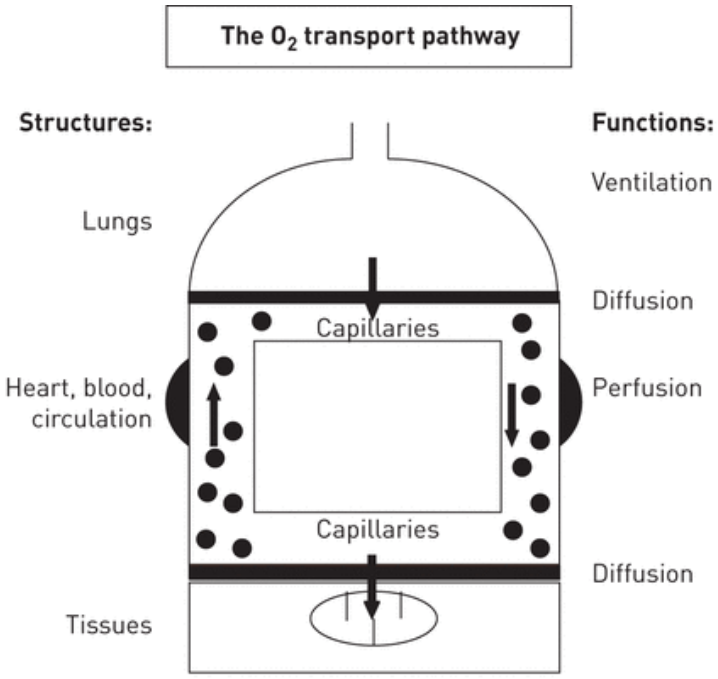

Figure 2.1 Schematics of the oxygen transport. The structures involved in the transport of oxygen from the lungs to the body tissues are part of a series of processes that include ventilation, diffusion and perfusion. The CO_2 pathway mirrors that of the oxygen, but in reverse. Reproduced with permission from [136].

The lung anatomy consists of approximately 300 million alveoli with an extensive capillary network which is supported by an interstitial matrix and allows efficient gas exchange by passive diffusion.

In healthy adults, inspired air enters the trachea and is delivered to the alveoli which have a total surface area of approximately 140 m^2. The pulmonary vasculature includes a network of capillaries that cover up to 95% of alveolar surface. Only a thin membrane separates the alveolar gas and blood compartments [137]. The pulmonary gas exchange

is a continuous process involving ventilation, diffusion and perfusion, with these processes are regulated by the law of conservation of mass [136].

Ventilation is the first step of this complex multi-step gas exchange process that leads to tissue oxygenation. Pulmonary ventilation through breathing results in alveolar ventilation. Not all the air inspired during each breathing cycle reaches the alveoli and is actively involved in gas exchanges. Some of the inspired air simply fills some of the respiratory dead spaces where gas exchange does not occur, such as the upper airways and part of the bronchial tree. These spaces constitute the anatomical dead space and during expiration, the air in the dead space is the first to be expired, making the presence of dead space disadvantageous for gas exchange.

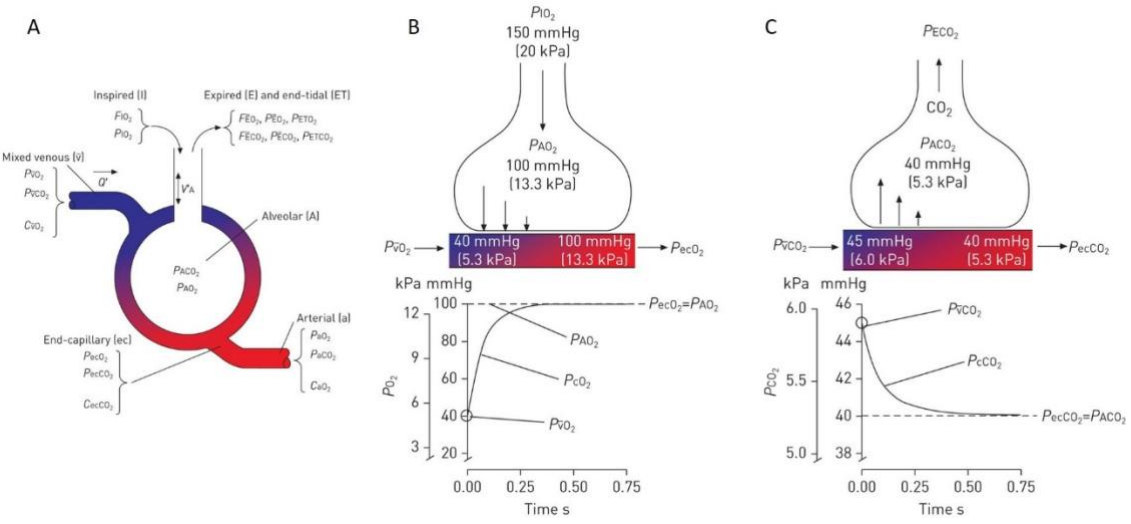

Figure 2.2 Gas exchange in a single lung unit model. A single lung unit schematic is represented in Panel A with the notations used for partial pressures, fractions of gas and O_2 content for different compartments in the Unit. Diffusion of O_2 (B) and CO_2 (C) in a single lung unit to allow PeO_2 and $PeCO_2$ to equal PAO_2 and $PACO_2$ at the end of the process. The time scale in the panels is the transit time of red blood cells through the capillaries. Reproduced with permission and modified from [138].

After the alveoli are filled with fresh air thanks to the ventilation process, the next step of gas exchange is the passive *diffusion* of oxygen into the capillaries (Figure **2.2**) where the uptake rate depends on the partial pressure of oxygen in alveolar air, the partial pressure in capillary blood and on the pulmonary diffusing capacity [137].

CO_2, the product of the body's metabolism, is cleared by the lungs via passive diffusion across the alveolar-capillary membrane in a direction opposite to that of O_2, from blood to alveolar gas, taking advantage of the lower resistance of CO_2 diffusion compared to O_2 (Figure **2.2**, Panel C). Through the process of passive diffusion, alveolar ventilation can eliminate 15 mol of carbon dioxide per day, and maintain arterial CO_2 at normal levels, between 4.5 and 6 kPa. The ventilatory process is controlled by chemoreceptors in the medulla oblongata and in the carotid body. These respond to variations in pH,

controlling an increase or decrease in alveolar ventilation to maintain a constant level of pCO_2 [137–140].

While oxygen and carbon dioxide in the alveoli and are dependent on the rate of alveolar ventilation, and rate of gas transfer across the respiratory membrane, other factors should be considered beyond those influencing the diffusion capacity across the membrane itself. Lungs comprise thousands of respiratory units, with variable degrees of perfusion and ventilation. In particular, the *ventilation perfusion ratio* (defined as V'A/Q'), is highly heterogeneous across the lungs. It can range within two extremes:

- V'A/Q' = 0, corresponding to a shunt. This corresponds to no ventilation, even with adequate perfusion. In this case the air in the alveoli comes to equilibrium with the blood oxygen and carbon dioxide as these can continue to diffuse passively.
- V'A/Q' = ∞, corresponding to dead space. This corresponds to an absence of perfusion, even with adequate ventilation. In this case the air in the alveoli comes to equilibrium with the inspired air.

In reality, areas of the lung with normal V'A/Q' coexist with others with a V'A/Q' below normal (a physiological shunt), and others with a V'A/Q above normal (a physiological dead-space). In healthy individuals, there is a higher degree of physiological dead-space in the upper zones of the lungs and of physiological shunt in the lower parts [138,141].

2.1.1 Respiratory failure

Effective gas exchange relies on multiple processes working correctly including normal chest wall expansion, respiratory muscles function, and central nervous system stimuli. All these factors are key in ensuring normal tissue oxygenation, and the appropriate elimination of carbon dioxide. If any of these elements doesn't function properly, gas exchange can be impaired, leading to respiratory failure.

Respiratory failure is often classified into two categories, depending on levels of both oxygen and carbon dioxide, into type I respiratory failure (hypoxaemic), and type II respiratory failure (hypercapnic). Based on its onset, respiratory failure can also be classified into acute, chronic, or acute on chronic respiratory failure [142,143].

Type I respiratory failure is characterised by arterial hypoxaemia caused by intrinsic lung disease with V'A/Q' mismatch, changes in diffusion capacity, shunt or alveolar hypoventilation.

A ventilation/perfusion mismatch develops in presence of decreased ventilation in regions of the lung that are normally perfused, or when there is a disproportionate reduction in ventilation compared to perfusion. Multiple situations can lead to V'A/Q' mismatch including atelectasis, airway obstruction, pneumonia, or bronchospasm. In

these conditions, increasing the fraction of inspired oxygen (F_IO_2) through the use of oxygen therapy can improve the hypoxaemia.

Shunts, where venous blood bypasses ventilated alveoli without being oxygenated, occur in conditions of sepsis, liver failure, or as a result of anatomical shunts. Any disease affecting the alveolar-capillary membrane can result in impaired diffusion capacity, especially for gases with reduced solubility (i.e. this defect will be more relevant for O_2 than for CO_2).

Type II respiratory failure is characterised by the presence of hypoxaemia and hypercapnia. Similarly to any form of respiratory failure, type II can occur acutely, chronically, or it can comprise an acute event developed on top of chronic hypercapnia. To develop type II respiratory failure, alveolar ventilation needs to be insufficient to compensate for CO_2 production, and is generally associated with airflow obstruction, decreased respiratory muscle strength or decreased respiratory drive. In addition, lung disease can cause type I respiratory failure which can evolve into type II as the underlying condition worsens.

2.2 Basic concepts of acid-base physiology

Acid-base homeostasis is a critical process for the regulation of blood pH. In normal physiology, arterial pH is maintained between values of 7.35 and 7.45. Any deviation from this range leads to changes in the intracellular pH, and in the pH of other bodily fluids, with consequent pathological effects.

In physiological conditions, a condition of steady state is maintained, with the amount of produced acid equalling the amount of acid being excreted. Multiple sources of acid and base are present in the body, and act as buffer systems. Of these, the most important to maintain the correct acid-base homeostasis is the HCO_3^-/CO_2 buffer system (also known as the bicarbonate buffer system), regulated by the lungs and kidneys. The bicarbonate buffer system has sufficient capacity to buffer both acids and alkali independently [140].

The relationship of this buffer system to pH is described by the Henderson-Hasselbalch equation:

$$pH = 6.1 + log\frac{HCO_3^-}{0.03 \times PCO_2}$$

While the HCO_3^-/CO_2 buffer is the most widely studied, other buffers also play a role in the response to acid-base disturbances.

In the event of acute changes of pCO_2, variations of serum bicarbonate are minimal, because of the interval time required for the kidneys to adapt the reabsorption and production of bicarbonate, and because of the role of other buffers in compensating for the change.

2.2.1 Bicarbonate in the kidneys and the role of pendrin

The kidneys play a crucial role in the acid-base balance, being responsible for the reabsorption of bicarbonate, excretion of acid (proton and ammonium), and production of new bicarbonate.

Bicarbonate is freely filtered at the glomerulus. However, urine is virtually bicarbonate free as a result of the reabsorption taking play in the tubular system. The majority of bicarbonate reabsorption takes place in the proximal tubule (85-90%) [140], with the remainder being attributable to the downstream segment. About 15% of bicarbonate is reabsorbed by the loop of Henle via the transcellular pathway [140].

The apical membrane is impermeable to bicarbonate. As such, in the proximal tubule and in the intercalated cells, carbonic anhydrase (CA) plays a crucial role, by facilitating a reaction combining HCO_3^- with H^+, secreted by the Na^+/H^+ exchanger (NHE), into water and carbon dioxide [144].

CO_2 can easily cross the cell membrane, and, within the cell, another CA leads to the production of H^+ and HCO_3^- from carbon dioxide and water. The newly generated bicarbonate can cross the basolateral membrane via a Na/HCO_3^- symporter (NBCe1-a, or SLC4A4), which transfers three HCO_3^- ions for every Na [140,145]. The combined action of the symporter and the sodium pump lead to the reabsorption of one molecule of bicarbonate and one molecule of sodium from the lumen for each molecule of secreted H^+ (Figure **2.3**).

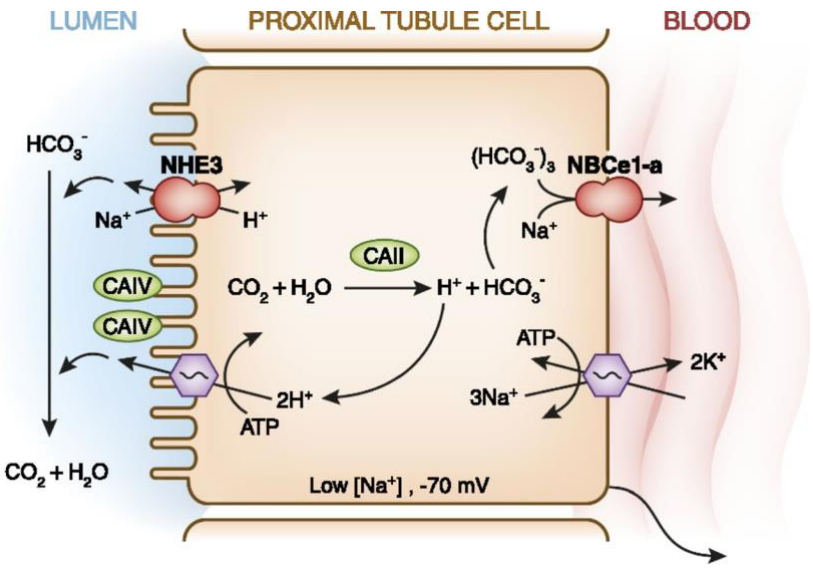

Figure 2.3 Schematics of bicarbonate reabsorption in the proximal tubule cells. The apical membrane in the proximal tubule cells is impermeable to bicarbonate. Carbonic anhydrase (CA) facilitate the formation of carbon dioxide from bicarbonate and protons, secreted via the Na^+/H^+ exchanger. CO_2 crosses the cell membrane and another CA generates bicarbonate to be reabsorbed via a symporter in the basolateral membrane. Reproduced with permission from [140]

While the proximal tubule and the thick ascending limb (TAL) contribute to acid-base homeostasis by reabsorbing bicarbonate, the cortical collecting duct (CCD) can also secrete acid and bicarbonate via the intercalated cells [140,146].

In type A intercalated cells, the interaction of the proton pump and the anion exchangers contributes to the reabsorption of bicarbonate. In type B intercalated cells, the coordination of pendrin and proton pumps contributes to bicarbonate secretion [140,147,148].

Pendrin is a Na^+-independent Cl^-/HCO_3^- exchanger localised on the luminal membrane of type B intercalated cells in the CCD, where it interacts with Na^+-dependent exchangers. While the latter mediates the absorption of Na^+ and HCO_3^-, pendrin mediates the secretion of HCO_3^- and the absorption of Cl^-, recycling both Cl^- and HCO_3^- across the apical membrane. Across the basolateral membrane, Cl^- exits the cell through the ClK channel, and Na^+ via the Na^+/HCO_3^- cotransporter. Type A intercalated cells secrete H^+ through the apical membrane H^+-ATPase, resulting in a net HCO_3^- efflux across the basolateral membrane via the anion exchanger. Finally, in the principal cells, ENaC is responsible for the reabsorption of Na^+, which then exits the cell across the Na/K ATPase. While pendrin is not expressed significantly in the principal cells, it contributes to the regulation of ENaC (Figure **2.4**) [140,147–150].

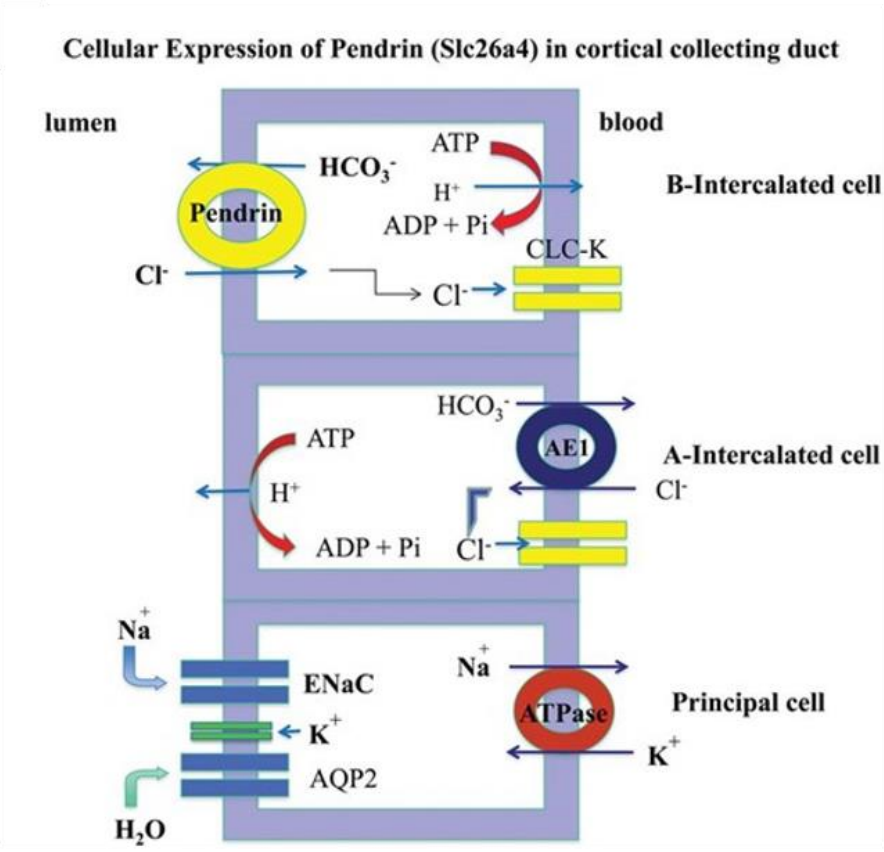

Figure 2.4 Schematics of electrolyte exchange in the collecting cortical duct. Reproduced with permission from [149].

Pendrin has an adaptive role in managing bicarbonate shifts in the CCD. In baseline conditions, the expression and activity of pendrin are minimal, as it is heavily regulated by shifts in the acid-base balance and interaction with the aldosterone-angiotensin II system and, in lesser measure by CFTR. Pendrin activity is in fact upregulated in response to metabolic alkalosis and following angiotensin II stimulation, whereas it is downregulated in the event of acid loading [140,149].

2.2.2 Response to acid-base disturbances

The physiological response to acid-base disturbances is alteration in ventilation and absorption and generation of bicarbonate in the nephrons.

Arterial CO_2 is regulated by alveolar ventilation as described in Section 2.1. Variations in levels of pCO_2 lead to changes in pH that are reflected in the cerebral interstitium. This determines a rapid change in ventilation in order to maintain acid-base homeostasis. The response to a change in serum HCO_3^- is slower, as these chemoreceptors are relatively insulated [140,151–153].

In the kidneys, the rate of reabsorption of bicarbonate is modulated by the proton pump and NHE antiporter directly following changes in pH, but mostly via hormonal stimulation. Adrenergic agonists, angiotensin II and parathyroid hormone (PTH) all stimulate the reabsorption of HCO_3^-. Volume regulators also play a role, as a reduction in the extracellular volume leads to an increased reabsorption of both sodium and bicarbonate through increased activity of the NHE [140,146,154].

In the presence of chronic changes in pCO_2, the kidneys alter the level of serum bicarbonate to maintain pH within range. These changes are slow, taking up to several days to complete, and do not necessarily restore the pH within normal limits [140].

In response to metabolic acid or base loads, a combined compensatory mechanism is triggered. This involves both the respiratory system (with changes in alveolar ventilation), as well as the kidneys, and normally leads to the pH being restored within the normal range. Metabolic acid loads result in increased ventilation to eliminate volatile acid (CO_2). Metabolic alkali loads are usually compensated by hypoventilation and a reduction in proximal tubule reabsorption [140].

Another compensatory mechanism for metabolic loads is the excretion of bicarbonate in the cortical tubules. The renal response to acid-base disturbances is linked to changes in the expression and activity of pendrin, with its up-regulation in presence of alkalosis and down-regulation in the event of acidosis [149].

Although acid-base compensations involve multiple intervening systems, changes in concentrations of either component of the CO_2/HCO_3^-buffer system are monitored in routine practice to identify and classify the main acid-base disturbances in metabolic or respiratory disorders. Figure 2.5 shows the classical approach to the classification of

acid-base imbalances, based on the concentration of pH, HCO_3^-, and pCO_2, into four imbalance categories, and their respective physiological compensations.

Acid-base disturbances are observed often among hospitalised patients, happening in response to numerous conditions. In the general population, metabolic alkalosis is the most commonly detected imbalance (51%), followed by respiratory alkalosis (29%), respiratory acidosis (27%) and metabolic acidosis (12%) [155].

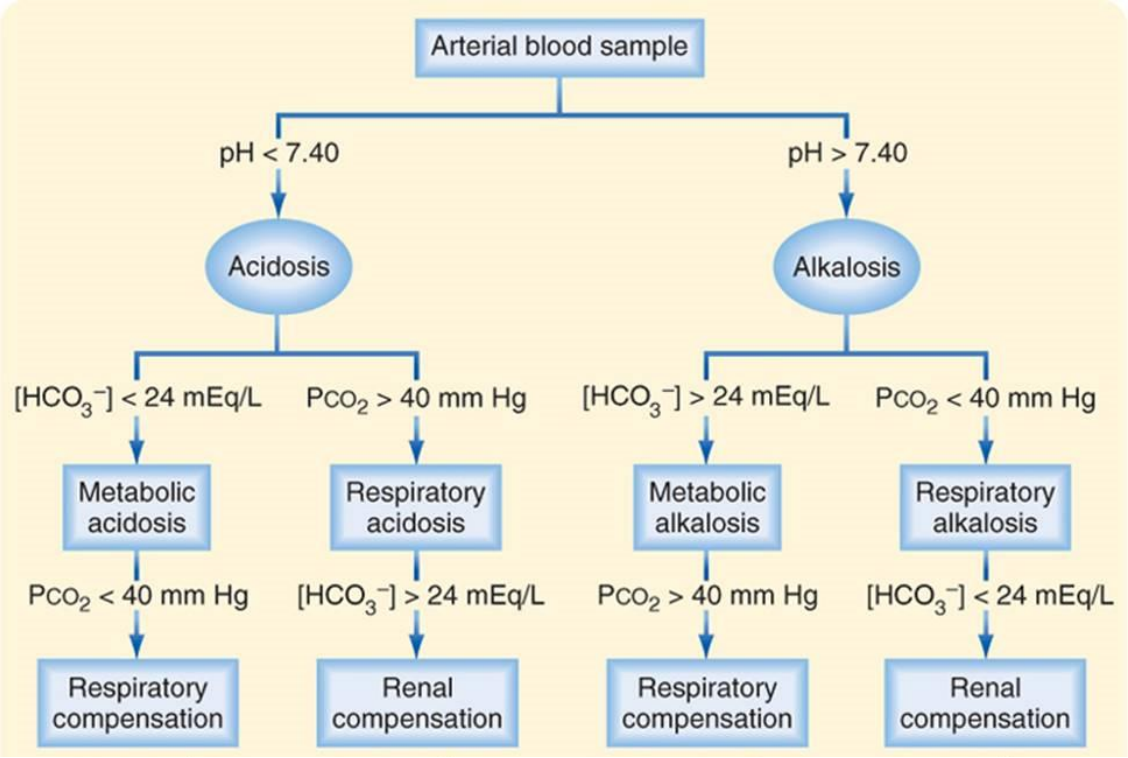

Figure 2.5 Simplified classification of acid-base disturbances.

2.3 Clinical assessment of gas exchange and acid base

Gas exchanges and acid-base balance can be assessed clinically:

- Through blood gas analysis
- By evaluating the gas exchange directly, or by using an indirect measure of the CO_2/HCO_3^- buffer.

2.3.1 Blood gas analysis

Blood gas analysis is a widely used, reliable technique to assess oxygenation, ventilation and acid-base status as a diagnostic and monitoring tool, both in the acute and chronic setting.

A blood gas analyser can measure pO_2, pCO_2 and pH directly, and derive HCO_3^- and base excess (BE) indirectly (Table 2.1)

Arterial blood gas (ABG) is the gold standard method of blood gas analysis. Samples are traditionally drawn from an artery, ideally a radial artery, with alternative sites being the

brachial or femoral arteries. ABG can be painful for patients, and can lead to rare but serious complications, such as vascular injuries (hematoma, ischemia, fistula, aneurism) and infections [156].

Table 2.1 Common parameters reported in a blood gas analysis

Parameter	Normal range	Significance
pH	7.35-7.45	Arterial content of hydrogen ion (measured)
pCO_2	4.7-6 kPa	Partial pressure of arterial carbon dioxide (measured)
HCO_3^-	22-26 mmol/L	Amount of base buffer in arterial blood (derived)
BE	-2 to +2 mmol/L	Amount of acid required to restore 1L of blood to a pH of 7.4 (derived)
pO_2	10.7-13.3 kPa	Partial pressure of arterial oxygen (measured)

Alternative approaches have, therefore, been suggested to reduce the number of ABGs performed in clinical practice, including venous blood gas (VBG) and arterialised capillary blood gas (CBG) [157,158]. To date, neither one of these techniques have been shown capable to provide the same data with accuracy and precision comparable to ABG.

In particular, arterio-venous agreement between these tests and ABG is good for pH and HCO_3^-, such that these values on ABG and VBG are almost interchangeable [159–162]. However, venous sampling cannot reliably test for pO_2 and pCO_2. Similarly, CBG reflects arterial pH and pCO_2 adequately, but underestimates pO_2 [163,164].

2.3.1.1 Interpretation of blood gas analysis

Blood gas analysis can be interpreted to assess the level of oxygenation, ventilation and acid-base status. While oxygenation can be assessed in a relatively simple way by looking at the at pO_2 and SO_2 values, the assessment of ventilation and acid-base status requires a systematic approach, involving the diagnosis of primary disorders and compensatory mechanisms, looking holistically at pH, pCO_2, HCO_3^-, and base excess (BE).

Three approaches have been proposed and are currently in use to assess acid-base problems; the Boston or physiological approach, the Copenhagen or base-excess method, and the Stewart's strong ion difference or physicochemical method.

The Boston approach, also known as "the traditional approach", is based on the Henderson-Hasselbach equations and it focuses on the centrality of the bicarbonate buffer system in the acid-base homeostasis [165]. This approach assumes that the component of the HCO_3^-/CO_2 buffer are in equilibrium with non-bicarbonate and non-volatile buffers such as albumin, phosphate and haemoglobin. In this method, the pH value is a measure of the degree of acidity or alkalinity, pCO_2 is a marker of the respiratory component and bicarbonate of the metabolic one. The Boston method suffers from a few limitations: it is mostly qualitative in nature, it does not allow to reliably assess the non-respiratory component of the acid-base disorders, and it does not provide

visibility on the nature of acids other than carbonic, as it focuses on HCO_3^- [165–167]. The Davenport normogram, shown in Figure 2.6, is a useful guide for the interpretation of acid-base disturbances according to the Boston approach.

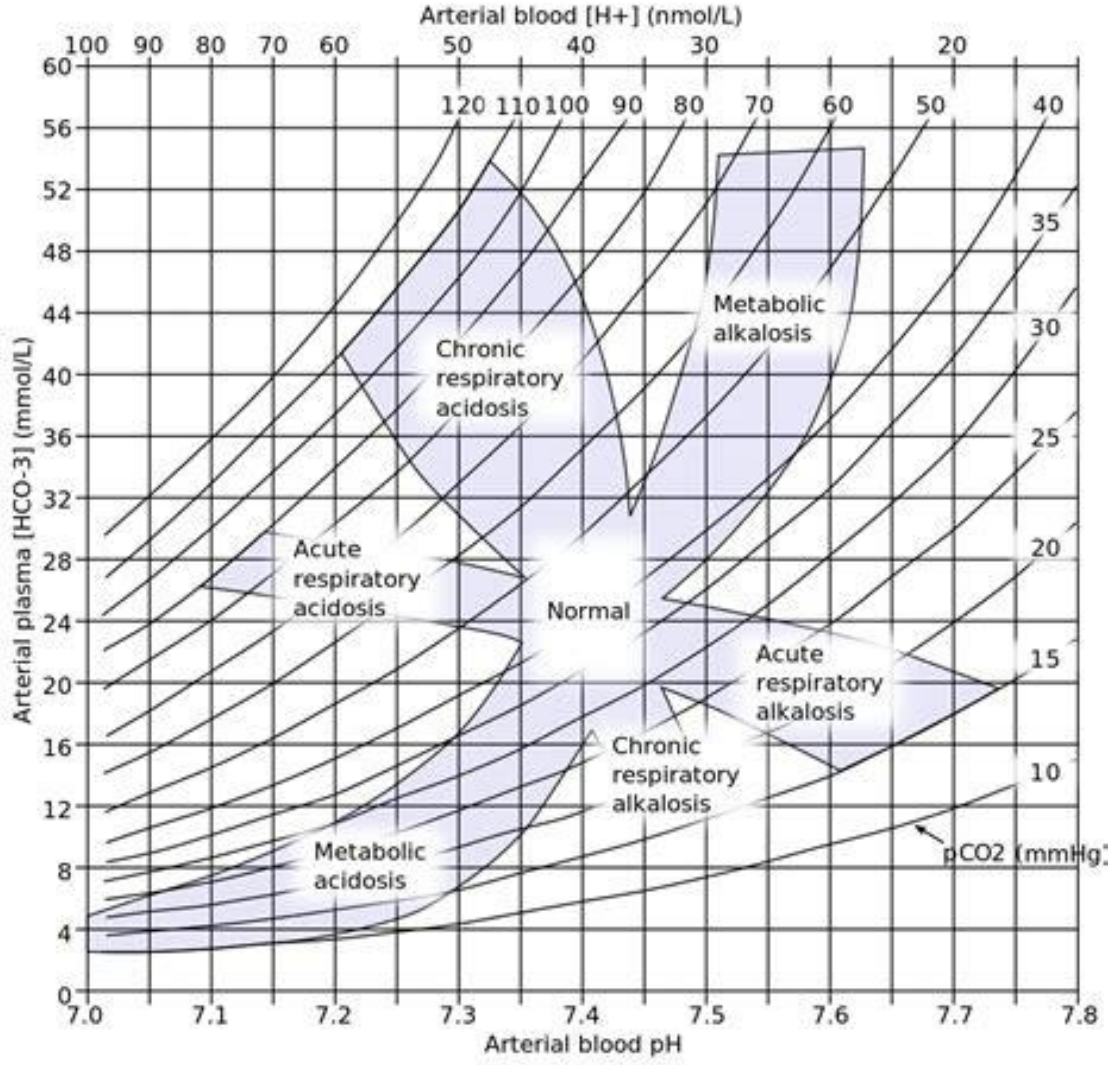

Figure 2.6 Davenoport acid-base normogram.

To overcome the limitations of the Boston method, the Copenhagen approach introduces the concept of base excess (BE), which allows a better quantification of the metabolic component of the imbalance. Base excess is defined as the amount of acid or base that needs to be added to a sample of blood to restore the pH to 7.40 if pCO_2 is kept at 5.33 kPa. In clinical practice, standard BE (SBE) should be used, to take into account the role of haemoglobin as a buffer. SBE, however, can be variable and be unreliable only in presence of significant hypoalbuminemia or low phosphate [165–168]. The Siggaard-Andersen diagram, shown in Figure 2.7, simplifies the interpretation of acid-base balance based on the Copenhagen approach.

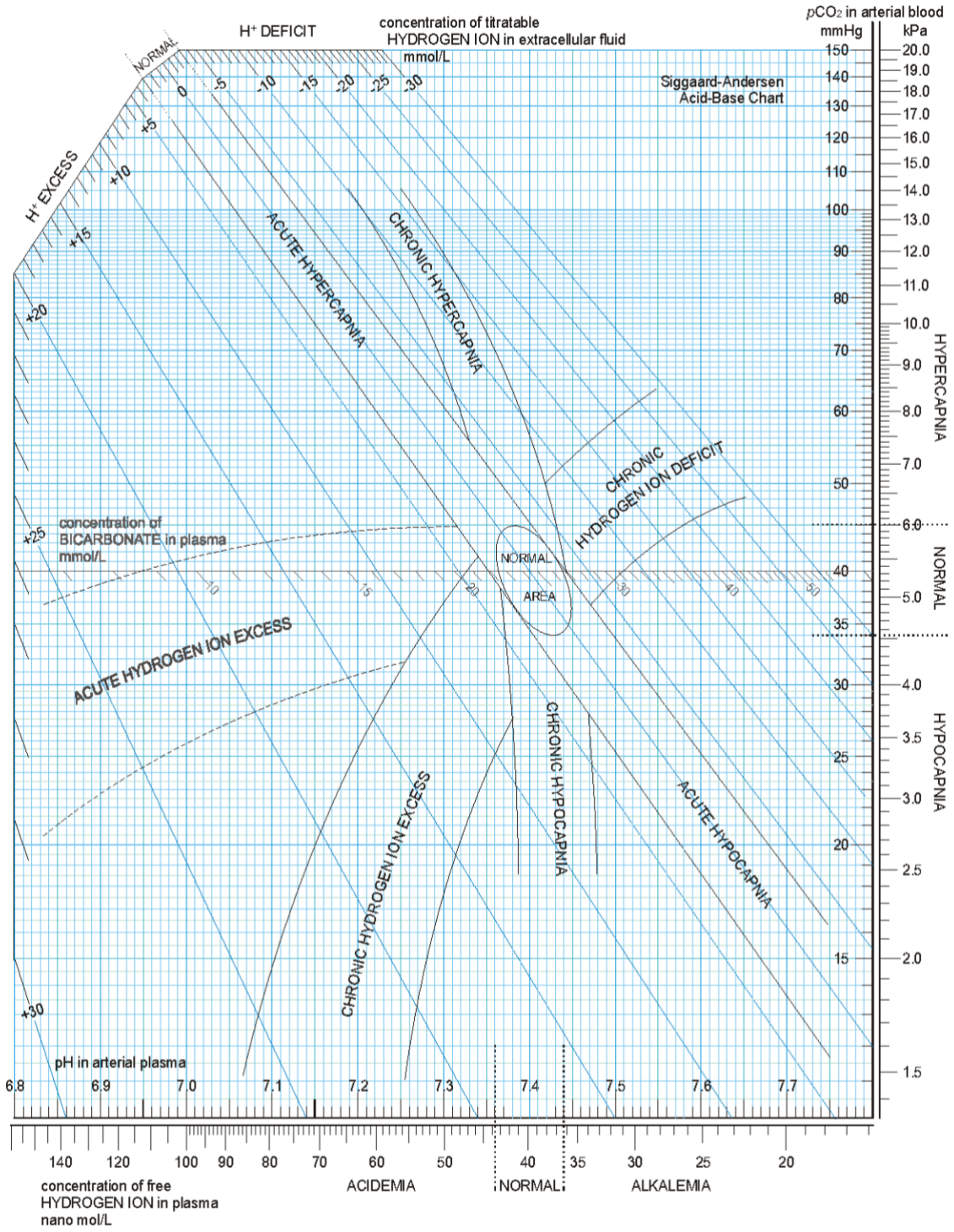

Figure 2.7 Siggaard-Andersen Diagram.

Finally, the Stewart's approach, based on the principles of electroneutrality, conservation of mass and on the law of mass action, reduces bicarbonate to a minor role. According to Stewart's method, pH and HCO_3^- are seen as dependent variables, answering exclusively to changes of three independent variables: strong ion difference (SID, difference between the sums of all the strong cations and all the strong anions), total concentration of non-volatile weak acid (A_{TOT}), and pCO_2. The Stewart method allows for a quantification of the magnitude of each acid-base disorder and of the effect of each component. Thanks to this, it can offer explanations for many acid-base phenomena

seen in clinical practice which are otherwise difficult to characterise with traditional interpretation approaches such as metabolic alkalosis associated with decreased albumin concentration [169].

2.3.2 Non-invasive assessment of gas exchange

While acid-base balance cannot be assessed without blood sampling, information about gas exchange can also be obtained with non-invasive measurements such as pulse-oximetry and measurement of carbon dioxide in the expired air (capnometry, $ETCO_2$), or using skin sensors (transcutaneous CO_2).

2.3.2.1 Oxygenation

Pulse oximetry is a non-invasive method to measure oxygenation, but not ventilation, using spectrophotometric technology. The device uses LEDs emitting red and infrared wavelengths, which are absorbed differently by oxygenated and deoxygenated haemoglobin, to estimate the saturation of oxygen in arterial blood (S_pO_2).

This non-invasive measure has been shown to be accurate in comparison to arterial oxygen saturation assessed invasively. In healthy volunteers, oximeters have in fact an average difference <2% compared to arterial saturation when this is >90%. Accuracy declines however in case of poor perfusion or when in presence of hypoxia [170]. Bias ± precision has been shown to be 1.7 ± 1.2% for S_aO_2 values > 90%, increasing to 5.1 ± 2.7% when S_aO_2 is ≤ 90% [170–172].

Pulse-oximetry is a simple, cheap, and reliable device, and its use is now widespread. This includes the outpatient setting, where it is used for spot measurements of oxygen saturation, and general, respiratory wards and ITUs, where it is used to titrate oxygen requirements, including for patients who are ventilated.

Continuous measurements of oxygen saturation are often used as a screening tool for assessment of sleep disordered breathing (SDB) or nocturnal hypoxaemia, and to monitor oxygenation during exercise, either during incremental cardio-pulmonary exercise test (CPET) or field tests such as the 6 minute-walking test.

2.3.2.2 Oxygenation and ventilation

Non-invasive approaches are available to assess ventilation, but don't provide good measures of pH, or estimates of bicarbonate. In particular, CO_2 can be measured non-invasively in the expired air, or by using skin electrodes.

Capnography is the continuous, breath-by-breath measurement and graphic representation of the partial pressure of CO_2 from expired air (end-tidal CO_2, $ETCO_2$). A gradient represents the difference between $ETCO_2$ and pCO_2, which is a result of alveolar dead space, and is on average 2-5 mmHg (0.27-0.67 kPa[173].

Changes in pulmonary perfusion and ventilation can affect the gradient, and thus this measure. Despite this, $ETCO_2$ provides reliable and useful information on ventilation, and, indirectly, on perfusion and metabolism. It is used routinely during anaesthesia and sedation, in emergency situations, such as to monitor the spontaneous return of circulation after cardiac arrest, and for monitoring ventilated patients. Its role outside the emergency department, operating rooms, and ITUs is however more limited [173,174].

In the assessment of chronic patients, and whenever $ETCO_2$ is not used, transcutaneous CO_2 ($tcCO_2$) monitoring is usually preferred. Transcutaneous CO_2 monitoring utilises skin electrodes to quantify the amount of CO_2 diffusing via the tissue to the surface of the skin. This is possible because the sensor is heated at a temperature between 40 and 44 C, to achieve arterialisation of the capillary bed. This technique is mainly limited by the stabilisation and reaction time, the need for membrane care, restoration and replacement, and baseline calibration. However, it provides reliable, accurate non-invasive measures, and allows for continuous monitoring for up to 8 hours, although technical drift might occur and should be acknowledged and corrected for [174,175].

2.3.3 Pre-flight assessment and hypoxic altitude simulation test

A specific assessment of gas exchange is often advised when people with CF, and other chronic lung diseases, are planning air travel.

During air travel, passengers are in fact exposed to hypobaric hypoxia because airplane cabins on commercial flights are pressurised at an equivalent altitude of 8000 feet, corresponding to a fraction of inspired oxygen of 0.15 at sea level. This can induce changes in intrathoracic pressure and accentuate V/Q mismatch. In most cases these changes cause only a small reduction in oxygenation which is well tolerated [176–178]. It is, however, conceivable that those with chronic respiratory conditions might experience hypoxaemia and hypoxia-related symptoms [178]. Evidence correlating in-flight hypoxaemia with symptoms during or after air travel are conflicting [179].

This notwithstanding, among the 44,000 in-flight medical emergencies occurring worldwide each year, those of respiratory nature are the second most commonly reported [180]. In addition, an increase in access to healthcare resources after flying has been observed among passengers with chronic lung disease who have not used supplemental oxygen in flight, despite an anticipated drop in oxygenation [177,181].

British Thoracic Society recommendations [182], as well as other International guidelines [183–185], favour routine pre-flight assessment for individuals with chronic respiratory diseases.

This assessment might include baseline measurement of SpO2 and partial pressure of oxygen, the use of predictive equations for hypoxia at altitude, and a hypoxic challenge test which could be performed in hypobaric or normobaric conditions. Measurements of baseline SpO2 and oxygenation at sea-level have been recognised as poorly reliable methods of predicting in-flight

hypoxaemia [178]. Predictive equations have been developed in small numbers of patients with COPD, and do not correlate with hypoxic challenge test in identify the need for supplemental oxygen among patients with interstitial lung disease or cystic fibrosis, as tend to overestimate the requirement for in-flight oxygen [186]. In view of these limitations, a formal hypoxic challenge test is often preferred. Hypobaric hypoxemic challenge test is the most accurate way of mimicking the in-flight condition, however it is expensive and not routinely available. Normobaric hypoxic altitude simulation test correlate well with the hypobaric challenge.

A standard hypoxic altitude simulation test requires the patient to breathe a blend of 15% of oxygen in nitrogen for 20 minutes (Venti-mask method) to simulate the oxygen tension at 8000 feet. This methods correlates well with the more accurate, but not widely available hypobaric hypoxic challenge test. Blood gas measurements are taken before and after the test, and a fall in the pO_2 below 6.7 kPa is considered indicative for use of in-flight oxygen. Expert consensus recommends supplemental oxygen at 2 L/min, or 2 L/min over usual flow-rate for patients on oxygen therapy, in individuals deemed at risk for in-flight hypoxemia[182].

2.4 Respiratory failure in CF

As discussed in Section 1.4, cystic fibrosis is a slowly progressive disease that leads to increasing lung damage and results in respiratory failure, through a vicious cycle of infection and inflammation. Cystic fibrosis is characterised by multiple structural lung changes including bronchiectasis and atelectasis, and is associated with airflow obstruction.

It is therefore not surprising that respiratory failure in CF is caused by a combination of contributing factors: a ventilation/perfusion mismatch, an increased dead space ventilation, a state of inflammation, and the presence of exudate in the air space with consequent shunt (Figure **2.8**). All these factors lead to the development of both hypoxaemia and hypercapnia, further complicating the picture of the acid-base status in people with CF (Section 2.5) [66,187,188].

Gas exchange abnormalities in people with CF can present as acute respiratory failure in the context of a pulmonary exacerbation, or develop gradually over time, especially among patients with advanced lung disease. Studies have shown that patients with CF can experience nocturnal hypoxaemia with hypoventilation during REM sleep, irrespective of age, weight and lung function [189,190]. With the progression of the disease, hypoxemia becomes evident also during daytime, and can be associated with hypercapnia due to increased work of breathing [66].

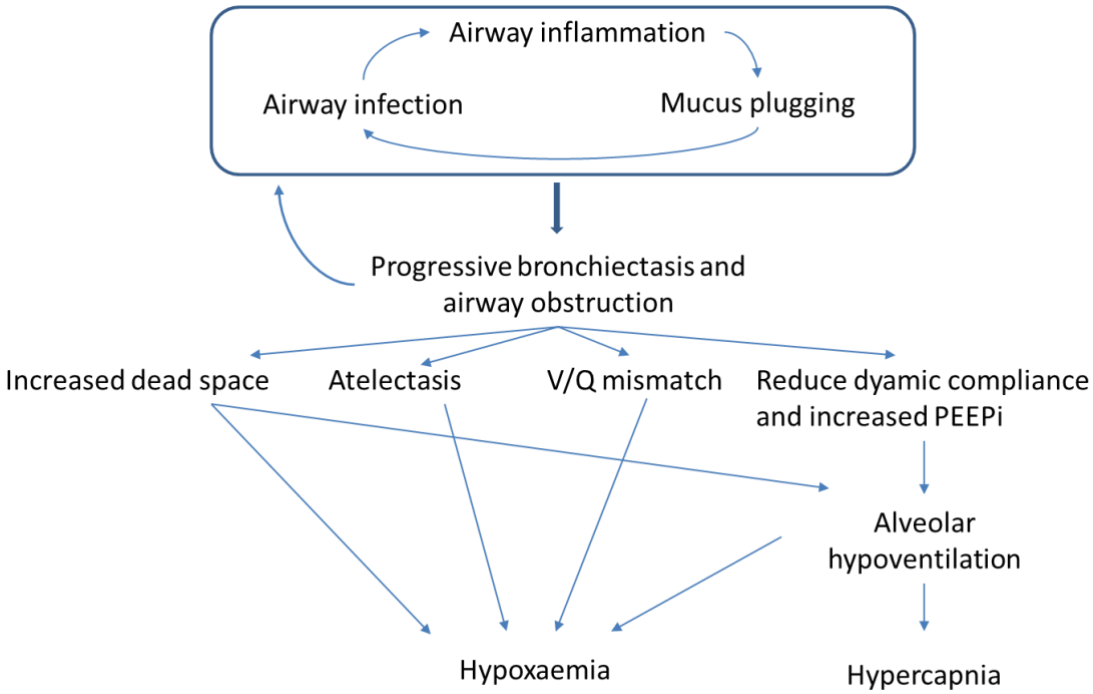

Figure 2.8 Multifactorial origin of respiratory failure in cystic fibrosis. A vicious cycle consisting of airway infection, inflammation and mucus plugging leads to progressive lung disease with bronchiectasis and airway obstruction. These feedback into the afore-mentioned vicious cycle but also responsible for increased dead space, atelectasis, V/Q mismation and reduction of dynamic compliance, resulting therefore in hypoxaemia and hypercapnia.

2.4.1.1 Nocturnal hypoxaemia in CF

During sleep, healthy individuals experience a fall in ventilation by 10-15% due to a reduction in tidal volume and an increase in the resistance of the upper airways [191,192].

This response is exaggerated in people with CF, who also present reduced tidal volume with no compensatory changes in respiratory rate during sleep compared to wakefulness. In combination with alveolar hypoventilation and V'A/Q' mismatch, this results in nocturnal oxygen desaturation and hypercapnia, which is exacerbated during REM sleep [193–195]. Nocturnal desaturation can present in people with CF even in the absence of significant hypoventilation, but is rarely associated with obstructive or central apnoea [196].

While these events are more frequent in patients with advanced lung disease, no independent predictor of nocturnal hypoxaemia or hypercapnia has yet been identified [197], and night-to-night variability has been observed [196]. It is recognised that oxygen desaturation during sleep is more prevalent among people with FEV_1 <65%, and hypercapnia in those with FEV_1<29% [187,197–200].

Nocturnal desaturations have been proposed as a trigger for pulmonary hypertension [201]. In this context, the known sleep fragmentation in people with CF has been

suggested as a mechanism to restore normal breathing and to correct gas exchange abnormalities [197]. It has been suggested that, over time, chemoreceptors may adapt and that the vicious cycle linking depressed respiratory drive, longer periods of abnormal breathing and sleep fragmentation, can lead to daytime respiratory failure [197]. Further, nocturnal desaturation and sleep disruption have been associated with increased difficulties in performing treatment for people with CF, as well as with an impairment in physical and neurocognitive functions.

In the majority of cases, nocturnal desaturation is, at least initially, asymptomatic and as such can go undetected for a long time. There is no recommendation or guideline on the frequency of monitoring for sleep disordered breathing or nocturnal desaturation in patients with cystic fibrosis.

2.4.1.2 Exercise-induced desaturation

Changes in respiratory mechanics do not appear to be the cause of nocturnal desaturation. They are however strictly connected with the development of exercise-induced desaturation, a particularly evident effect in people with CF with severe lung disease.

Patients with advanced lung disease have increased airway resistance, and combined static and dynamic hyperinflation, which significantly limits their peak minute ventilation. This is due to a reduction of the tidal volume during exercise, as a consequence of the relatively higher dead space [202–206].

Static hyperinflation contributes to the flattening of the diaphragm, and to the recruitment of accessory muscles of breathing. A flattened diaphragm is highly inefficient and consumes more oxygen to generate a given V_T compared to a normal diaphragm. Dynamic hyperinflation worsens as respiratory rate (RR) increases, such as during exercise. This exacerbates air trapping and intrinsic positive end-expiratory pressure (PEEP), posing an inspiratory threshold load that consumes energy to lower intra-alveolar pressure enough to initiate air flow for the next breath. Dynamic hyperinflation contributes to inefficient breathing and diminishes exercise capacity [207]. This is on top of the metabolic cost of ventilation being up to 40% higher in patients with advanced lung disease compared to healthy individuals. In addition, exercise worsens the V'A/Q' mismatch, leading to desaturation [208].

As a consequence of these mechanisms, 15% to 30% of patients with CF experience hypoxaemia during exercise, defined as a drop >4% in oxygen SpO_2 and/or a decrease in SpO_2 below 90% [209,210]. Conclusive data are lacking on the effects of exercise induced hypoxaemia, but a number of studies have linked it with cardiac arrythmias and suggest it should be avoided [210,211].

2.4.1.3 Acute respiratory failure

Patients with CF can present with acute hypoxaemic respiratory failure and/or acute hypercapnic respiratory failure. This can be resulting from pulmonary exacerbations, or from pulmonary complications such as pneumothorax, atelectasis or the rare occurrence of pulmonary embolism [212].

Patients with severe lung disease have a higher risk of developing respiratory failure and complications in the context of a pulmonary embolism. This notwithstanding, patients with relatively preserved lung function are also at risk, and might present with acute respiratory failure requiring aggressive treatment [188,212,213].

2.4.1.4 Chronic respiratory failure

Chronic respiratory failure is the ultimate cause of death for the majority of patients with CF, and is one of the main indications for lung transplantation. Many patients with severe lung disease who present with decompensated respiratory failure, might actually have acute on chronic ventilatory failure on the background of a previously undiagnosed chronic hypercapnia [66].

The CF Foundation recommendations advise to monitor asymptomatic patients with advanced lung disease with yearly blood gas analyses, in order to increase the likelihood of an early diagnosis of chronic ventilatory failure, and to start long-term NIV treatment in response to it (Section 3.3) [105].

2.5 Acid-base disturbances in CF

Cystic fibrosis is a multi-systemic condition in which morbidity and mortality are mostly due to lung disease. Its origin however lies in a defective anion channel responsible of the transport of chloride and bicarbonate, expressed ubiquitously on all epithelia (Section 1.1). It is therefore conceivable that patients with CF might have unique acid-base balance characteristics, and a unique electrolyte homeostasis profile compared to that of the general population.

The main acid-base disturbances described in the CF population are metabolic alkalosis and chronic respiratory acidosis. *Metabolic alkalosis* can be observed in children and adults with CF at a prevalence up to 86% higher than expected in the general population in condition of clinical stability, and during exacerbations [214–218]. Metabolic alkalosis can be an initial manifestation of CF, both in adults and children, or a possible complication of pulmonary exacerbations, associated with electrolytes abnormalities and dehydration in the context of Pseudo-Bartter's syndrome [219–222].

When the lung involvement becomes more severe, and patients, especially adults, develop hypercapnia, metabolic alkalosis can be a contributing factor to CO_2 retention and hypercapnic respiratory failure during exacerbation [223]. CFTR has a central role

in the transport of bicarbonate [224,225], and interacts with a multitude of ion channels at the epithelial level, including in the kidneys [226]. As such, metabolic alkalosis can be a primary disorder leading to compensatory hypoventilation and hypercapnia.

Adults with CF and severe lung disease have episodes of transient hypercapnia during sleep, which can contribute to *chronic respiratory acidosis*, or can present with acute respiratory acidosis during pulmonary exacerbations [227]. In these cases, the concentration of serum bicarbonate increases in response to the acidosis, as a compensatory mechanism.

Respiratory alkalosis and metabolic acidosis are less frequently observed compared to other acid-base disturbances, but are seen occasionally in clinically stable patients at annual review [218]. Metabolic acidosis is seen mainly in post-transplant patients as a consequence of renal tubular dysfunction and bicarbonate loss, secondary to immunosuppresants. In this scenario oral treatment with oral bicarbonate replacement is usually highly effective.

Since acid-base abnormalities and any electrolytes disturbances are associated with poor prognosis, an appropriate recognition of these abnormalities and a good management thereof are required. Both chronic respiratory acidosis and acute ventilatory failure are often treated with non-invasive ventilation (Section 3.3) [228,229], whereas no specific treatment, except for fluids and electrolyte repletion, is available for metabolic alkalosis and electrolyte disturbances in CF. This is partly due to the underlying mechanisms of electrolyte imbalance not being fully understood in this population.

2.5.1 Pseudo-Bartter Syndrome and Cystic Fibrosis

As described in Section 2.2.1, the proximal tubules and the TAL of the Henle's loop play a central role in acid-base homeostasis. In addition, the tubular system is responsible for the regulation of electrolytes, with the TAL reabsorbing approximately 25% of the filtered sodium, being central in the regulation of the extracellular volume, with the involvement of several channels, partially regulated by CFTR.

Bartter syndrome is a group of rare tubulopathies, secondary to several autosomal recessive genetic defects resulting in the dysfunction of one or more of the multiple channels and transporters involved in the reabsorption of salt in the TAL. Despite multiple variants of Bartter syndrome having been described, patients present very similarly either *in utero*, at birth or in early childhood. Clinical presentation is characterised by polyuria, dehydration, hypokalaemic metabolic alkalosis, with hypermagnesiura and hypercalciuria leading to nephrocalcinosis [230,231].

Nephrotoxic drugs, such as polimixins or aminoglycosides, have been associated with a Bartter-like, or acquired Bartter, syndrome presenting with hypokalaemic metabolic alkalosis as a result of a iatrogenic tubulopathy [232–236].

Pseudo-Bartter syndrome (PBS) is a condition characterised by hyponatraemic, hypochloraemic metabolic alkalosis without a tubulopathy, but with a low urinary excretion fraction of sodium. This can be observed in patients with extrarenal loss of sodium chloride as a consequence of excessive loss via the gastrointestinal tract, or the skin, such as in CF [221,237].

Hypokalaemia, hypochloraemia and hyperbicarbonataemia in association with metabolic alkalosis are commonly found in patients with CF. In these patients, the prevalence of PBS has been estimated by multiple reports to be between 12 and 16.5% [221,237]. A systematic review on a total of 262 patients with cystic fibrosis, dyselectrolytaemia and PBS found that over 75% of cases were children, and that in 60% of cases electrolyte abnormalities led to the diagnosis of CF [238].

In CF, two distinct clinical presentations of PBS have been described: acute PBS, generally associated with hot weather and heat exhaustion or diarrhoea, and chronic PBS, aggravated by intercurrent exacerbations [237]. A correct identification and treatment is essential to avoid complications associated with electrolyte unbalance and metabolic alkalosis.

The mechanisms underlying PBS and dyselectrolytaemia in patients with CF are not fully understood. Speculative explanations have been provided, particularly for infants, in whom these conditions have been more often described in association with, clinically inapparent, fluid volume depletion (Figure **2.9**) [238].

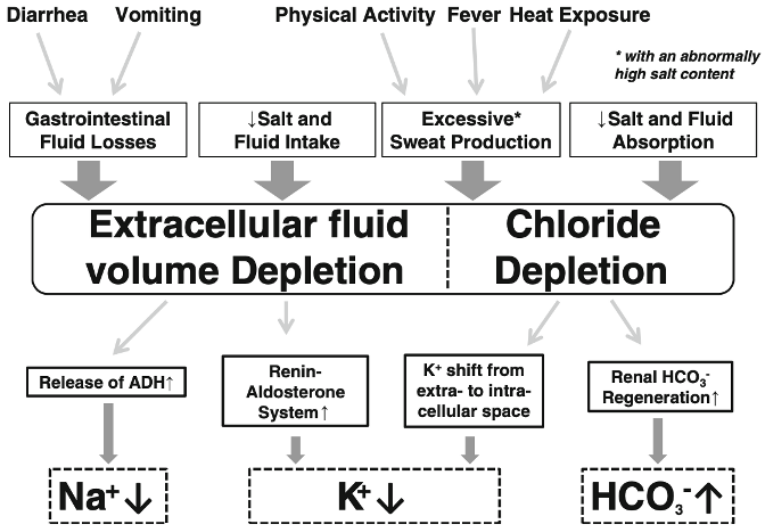

Figure 2.9 Mechanisms leading to dyselectrolytaemia in CF. The increase in bicarbonate as result of chloride depletion leads to metabolic alkalosis in PBS. Reproduced with permission from [238].

2.5.2 Role of pendrin in CF

Acid-base balance abnormalities in CF might also be a consequence of defective CFTR acting directly or indirectly in kidneys and in other organs. CFTR is in fact crucial in the regulation of bicarbonate in the duodenum and pancreatic duct, as it activates the pendrin SLC26A3, forming a bicarbonate-secreting complex [239].

A similar interaction has been observed in the kidney, were a colocalization of CFTR and pendrin has been confirmed in the CCD [239,240]. Wild-type CFTR and pendrin interact and lead to an increase secretion of bicarbonate in the urine [240]. While CFTR is not the only regulator of pendrin activity and expression, it is plausible that these processes are impaired in people with CF due to defective CFTR [149,240]. While data on urinary excretion of bicarbonate in people with CF are limited [241,242], murine models of CF confirmed that pendrin remains inactive leading to reduced bicarbonate excretion from the CCD even in response to a base load [149,240,243].

2.6 Assessment of gas exchange and acid-base in CF

As discussed in this Chapter 2 (Sections 2.4 and 2.5), patients with CF can develop respiratory failure and acid-base abnormalities. As such, a clinical assessment of their gas exchange and acid-base status is indicated. However, no accepted guidelines or recommendations on when to perform these assessments exists.

The European and the UK CF Trust Standards of Care recommend a non-invasive assessment of oxygen saturation using a pulse-oximeter, at every outpatient review and during inpatient stays, and to perform arterial or arterialised capillary blood gas only when clinically indicated [244,245].

As discussed in Section 2.4.1.1, people with CF might present with nocturnal desaturation, while having a relatively preserved lung function and/or a normal daytime S_pO_2. Nocturnal abnormalities can often go undetected, due to the lack of symptoms, especially in their initial stages.

Furthermore, no recommendation is available on the timing and methodology to be used to screen for oxygen desaturation during sleep. The gold standard to diagnose SDB, such as central (CSA) or obstructive sleep apnoea (OSA), is polysomnography, or respiratory polygraphy. These tests can in fact detect and differentiate respiratory events such as central or obstructive apnoeas and flow-limitations, and, as such, help characterising SDB. However, respiratory events similar to those of sleep apnoea are rarely the cause of nocturnal desaturation or hypoventilation in people with CF. Therefore simple continuous monitoring of oxygen saturation and/or transcutaneous monitoring of CO_2 provide valuable information in clinical practice, and more complex studies are rarely required.

Exercise is an integral part of the treatment of people with CF, as aerobic fitness is associated with improved survival, better quality of life and reduced lung function decline [246–252]. The ability to exercise, however, is often limited in these patients by dyspnoea, desaturation and deconditioning. Exercise testing is recommended in people with CF as it offers an integrated and objective assessment of exercise performance, that cannot be obtained from other methods. Exercise evaluations are recommended at least annually, and as part of the assessment for changes in the overall management by the UK CF Trust and the European CF Society [245].

Cardio-pulmonary exercise testing (CPET) is the gold standard for the combined assessment of the cardiovascular, respiratory and muscular-skeletal systems [253]. CPET should be regularly performed according to UK CF Standard of Care, however, due to their complexity are rarely performed and field tests are often preferred [245,254]. Field tests include incremental shuttle test, walking tests and step test. Although these test can be incremental and maximal, not all patients can elicit a peak response.

The 6-minute walking test is simple and requires minimal equipment, although being often performed on a treadmill to minimize risks of infection in patients with CF. Irrespective of the type of testing, monitoring should be continuous and recorded if possible, to allow for a more thorough assessment of the collected data. In addition to measurements of variables such as heart rate and oxygen saturation, an assessment of symptoms, particularly dyspnoea and fatigue, should be completed throughout exercise [255].

In the event of oxygen desaturation during exercise, an assessment for ambulatory oxygen therapy to allow for use of supplemental oxygen during exercise or other daily life activity should be considered.

2.7 Conclusion

Gas exchange and acid-base balance are complex processes that are strictly inter-connected. CFTR is widely distributed in the organism, and its defective function can lead to lung disease with subsequent oxygenation and ventilation disturbances, but can also have an effect in the kidneys leading to acid-base disturbances. Gold standard for diagnosis remains arterial blood gas. Oximetry and transcutaneous monitoring provide valuable alternatives for gas exchange, but no information on acid-base homeostasis. Serum bicarbonate might help in further defining ventilation and compensatory mechanisms, but its role needs to be explored further in view of the multiple possible confounding factors.

Chapter 3
Respiratory and ventilatory support

In clinical practice, hypoxaemic and hypercapnic respiratory failure often leads to the need of providing adequate support for oxygenation and/or ventilation. This assistance can be provided invasively, via endotracheal intubation or tracheostomy, or, where appropriate, non-invasively, via external interfaces.

Over the last century, a wide range of devices has been developed to deliver effective respiratory support, ranging from simple nasal cannulae, face and nasal masks, to more sophisticated equipment for ventilatory support [256].

The first non-invasive mechanical ventilator to be developed was the “iron lung”, the use of which was widespread during the poliomyelitis epidemics. However, these devices were cumbersome and the size and lack of portability of these negative pressure ventilators led to the development of positive pressure devices, which deliver pressurised gas via a tight fitting mask [257].

Recent advancements in the technology of flow-generators, humidification systems and materials for nasal cannulae, led to the development of nasal high-flow therapy (NHFT). NHFT is a non-invasive means to deliver high flows up to 60 L/min of actively humidified gas mixtures, with controllable F_IO_2, through loose-fitting nasal cannulae. Despite NHFT remains a relatively new therapy, its use has increased significantly in the management of respiratory failure, under certain scenarios [258].

This Chapter focusses on the physiological mechanisms of actions of both NIV and NHFT, and summarises the role of these adjunct therapies in the treatment of patients with CF.

3.1 Non-invasive ventilation

Non-invasive ventilation (NIV) is a means to provide respiratory assistance while avoiding the invasion of the airways. It is widely used in patients with a range of conditions leading to acute and chronic respiratory failure.

The most common application of NIV is non-invasive positive pressure ventilation (NPPV[1]). In NPPV, pressurized gas flows from a positive pressure ventilator to the patient via an external interface that is fitted tightly over their nose and/or mouth [259].

[1] Throughout the dissertation NIV will be used to describe NPPV

Non-invasive ventilation can eliminate the typical complications of intubation and invasive mechanical ventilation (IMV), particularly ventilator-associated pneumonias. NIV also avoids the trauma associated with intubation, and reduces the need for analgesia and sedation.

NIV is suitable for intermittent use, allowing breaks for eating and communicating. It has also been shown to potentially reduce morbidity and mortality, shorten intensive care unit (ICU) stays, and avoid the need for ICU altogether, when compared to IMV [259,260]. Despite its significant advantages, the success of NIV treatment is often limited by it being poorly tolerated, due to either discomfort in wearing the interface, or because of patient-ventilator asynchrony [261]. Patient selection, timing of initiation and breaks, device settings, as well as clinical expertise remain essential in improving outcome.

3.1.1 Clinical indications of NIV

NIV combines positive end expiratory pressure (PEEP or EPAP) to counterbalance auto-PEEP, with pressure support (PS) to assist inspiratory muscles, thereby increasing tidal volume, ensuring adequate alveolar ventilation and reducing the work of breathing. This leads to improvement in dyspnoea score, reduced respiratory fatigue, CO_2 levels and heart rate [262]. Based on its mechanism of action, NIV is widely used in the acute setting in emergency departments, respiratory and general wards, and in intensive care units. It is also applied extensively in the chronic setting.

Non-invasive ventilation is recommended as the first-line ventilatory modality in acute hypercapnic respiratory failure secondary to COPD exacerbation, acute cardiogenic pulmonary oedema (ACPE) and hypoxemic respiratory failure in immunocompromised patients [259,263].

In other clinical scenarios, the evidence for using NIV in the acute setting is weaker and more controversial. However, it can be considered a viable method of ventilatory support, as long as there is strict monitoring by intensivists, for acute on chronic respiratory acidosis in morbidly obese patients [262,264,265], and selected cases of pneumonia and acute respiratory distress syndrome (ARDS) [266,267].

Many patients with chronic respiratory conditions remain unsuitable for intubation and in this scenario, NIV can provide the necessary support and improve outcome during acute exacerbation [268,269].

Finally, NIV can be used for symptom relief as part of palliative care. Stopping NIV can sometimes be difficult in end of life care of people with chronic respiratory disease and

each case needs to be sensitively managed as part of a wider clinical and palliative care team [268,269].

In the chronic setting, NIV is primarily used in patients with COPD, chest wall deformities and/or neuromuscular conditions [270–277]. In COPD, NIV is used mainly in patients with severe lung disease who remain hypercapnic after severe exacerbations. NIV has been shown to improve survival, sleep, quality of life, reduce frequency of hospitalisation and appears to contribute to stabilisation of FEV_1 [272,273].

In neuromuscular and chest wall disorders, NIV is used primarily to reduce the symptoms of nocturnal hypoventilation, and improve quality of life [275,278]. In these conditions, the main pathophysiological feature is a restrictive ventilatory defect, with decreased compliance of the chest wall in the presence of a normal respiratory drive. This leads to preserved ventilation/perfusion matching with hypercapnia being caused by an unbalance between the forces generated by the respiratory muscle and the additional load secondary to the reduced compliance. In these settings, the use of NIV is recommended for patients with daytime hypercapnia, or symptomatic for nocturnal hypoventilation [279].

3.1.2 Practical aspects of NIV

In the acute setting, all patients deemed suitable for a trial of NIV should start treatment in a timely manner. As such, an assessment of potential contraindications to NIV, such as respiratory arrest, septic shock with multi-organ failure, or inability to fit the mask, should be completed [259]. In acute care, NIV can be trialled in cases at higher risk of failure under the supervision of intensivists and, whenever appropriate, intubation should be undertaken without delay if no improvement is visible [280].

The choice of an appropriate interface (Figure **3.1**) is key to a favourable NIV outcome. Discomfort in wearing the interface is a common problem with NIV, and can reduce patients' tolerance and adherence to the treatment, introduce air leaks, and affect patient-ventilator synchrony [281,282]. In the acute setting, oro-nasal masks (full-face) are generally preferred, since they are associated with less air leaking through the mouth. On the other hand, nasal masks can facilitate speech and expectoration and may be better tolerated by claustrophobic patients.

Helmets are alternative interfaces consisting of a soft clear plastic cylinder that seals over the neck and shoulders. They have proven effective in delivering CPAP, but require high air flows to minimize rebreathing, and are quite noisy. When used to deliver pressure support and PEEP, patient-ventilator asynchrony can be a problem with the helmet, but a number of recent studies have shown significant advantages in delivering NIV in critically ill patients [281–284]. In chronic settings, and especially in patients with

neuromuscular conditions, nasal masks are usually preferred.

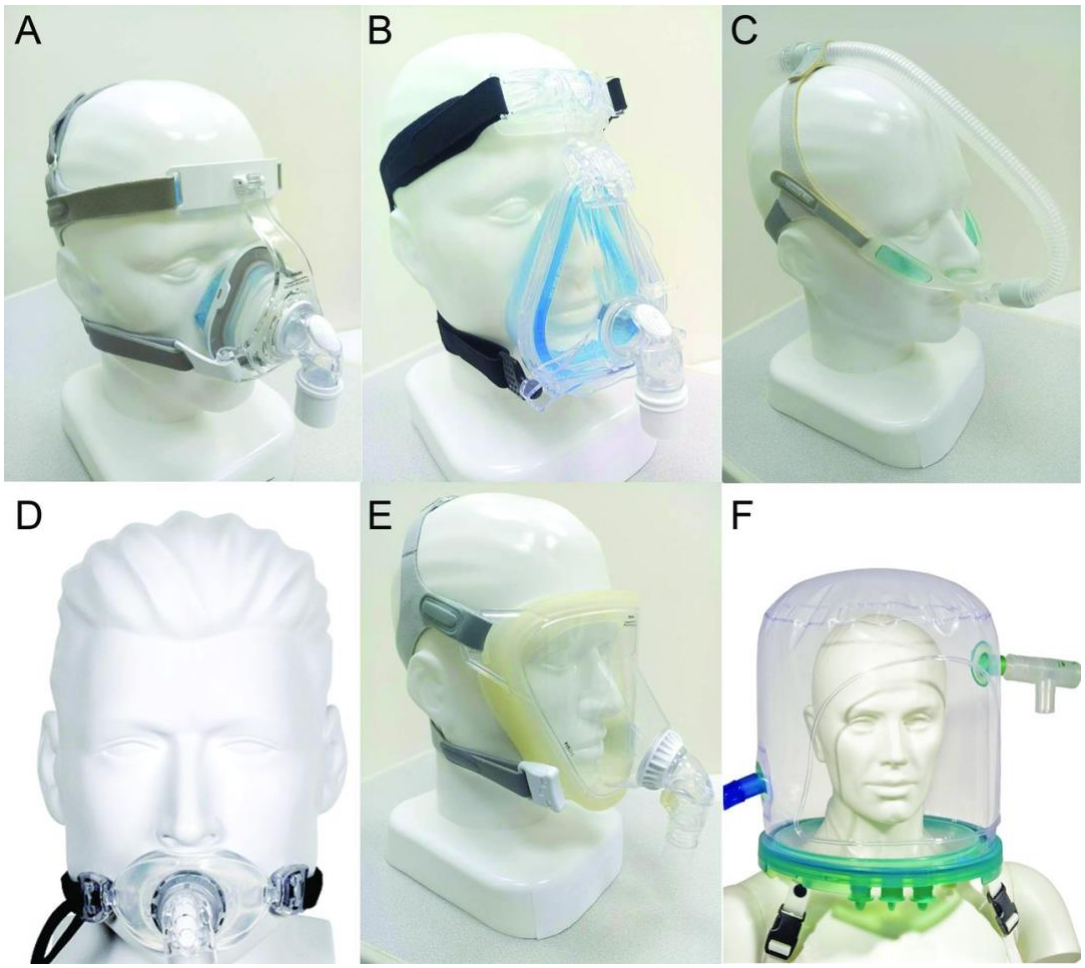

Figure 3.1 Interfaces for NIV. Reproduced with permission from [282].

Successful NIV treatment requires appropriate ventilator support in synchrony with the patient's breathing efforts. There is not a "one-size fits all" approach to NIV settings ,and treatment should be personalised with adjustment of ventilator settings tailored to each patient.

A common starting point for NIV treatment is pressure-support (or bilevel) with a low setting (e.g. inspiratory pressure 8-12 cmH_2O, expiratory pressure 4-5 cmH_2O), to maximise tolerance. Inspiratory pressure can then be gradually adjusted upward to alleviate persistent respiratory distress, within the patient's limit of tolerance. Expiratory pressure can be increased to improve triggering in the presence of auto-PEEP, or to improve oxygenation [285]. Oxygen supplementation is titrated to achieve the desired O_2 saturation. Humidification is usually not necessary for brief applications (such as for ACPE), but helps to avoid mucosal drying and enhances comfort for longer applications [281]. In the chronic setting, high-intensity ventilation is usually required to maximise the benefit from its application [273].

Paramount for a successful application of NIV treatment is its close monitoring, especially during the early adaptation period. The value of frequent checks to optimize

mask fit and ventilator settings and to encourage the patient is hard to overemphasise. Acutely ill patients should be monitored in an ICU or intermediate care unit until they are more stable. NIV can be administered on regular wards by experienced staff, but only for cooperative patients who are relatively stable and can call for help if needed [286]. Monitoring should include clinical examinations to look for signs of respiratory distress, assessment of mask-fit, and gas exchange through blood gas analysis and oxygen saturation.

3.1.3 Complications

NIV is usually a safe and well-tolerated technique. Its adverse effects are typically related to the chosen interface, which can cause skin reddening or ulceration especially over the nasal bridge, claustrophobia, sinus or nasal pain, and dryness of eyes and mouth due to air leaks. Uncommon complications reported in the literature, include pneumothorax, haemoptysis, painful gastric insufflation, and aspiration of gastric contents [259].

3.2 Nasal high-flow therapy

Nasal high-flow therapy is delivered by an apparatus consisting of a flow generator, an active humidifier, and nasal cannulae. The flow-generator, in conjunction with a gas blender based on the Venturi effect, can create flows up to 60 L/min of a gas blend with a variable F_IO_2 ranging from 0.21 to nearly 1.0 [258].

Given its high flows, the gas mixture needs to be humidified to avoid desiccation of the upper airways, and to improve patients' tolerance [287]. An in-line active humidifier is part of the circuit, and is used to heat the gas mixture to body temperature, and saturate it with water. The gas blend is then provided to the patient via loose-fitting nasal cannulae that are larger but softer than those used in conventional oxygen therapy.

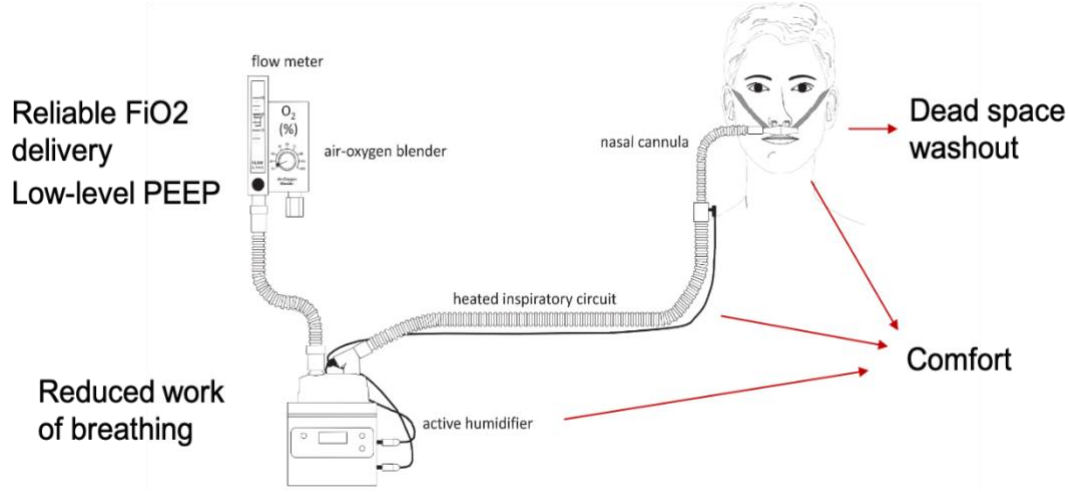

Figure 3.2 Schematics of NHFT and its effect, Reproduce and modified, under the Creative common license, from [288].

3.2.1 Physiology of NHFT

Several mechanisms underlie the beneficial effects of NHFT, with each effect being related to a different aspect of this therapy. NHFT has physiological effects on respiratory mechanics and oxygenation, and effects on muco-ciliary clearance.

3.2.1.1 Effects on respiratory mechanics and oxygenation

NHFT generates a variable level of positive airway pressure throughout the respiratory cycle, but particularly in the expiratory phase of the respiratory cycle, leading to an increase in the end-expiratory lung volume, and to improved oxygenation [289–293].

NHFT also promotes the washout of the dead-space, reducing work of breathing and respiratory rate [294–296]. While the effects on pressure and oxygenation are probably best achieved at high flows, the ones on work of breathing and respiratory rate are maximised at intermediated flow rates [297,298]. NHFT does not affect minute ventilation, but changes the ventilatory pattern thereby reducing respiratory rate and increasing tidal volume [299–302].

In addition, by matching the peak inspiratory flow-rate more closely than conventional oxygen therapy, NHFT reduces the entrainment of room air allowing for a more stable and reliable delivery of F_IO_2 compared to standard oxygen therapy [299,303,304].

3.2.1.2 Effects on muco-ciliary clearance

Muco-ciliary clearance is the first-line of defence in the bronchial tree. It effectiveness depends on the synchronous movement of the cilia, and on the presence of adequate water content in the mucus. Any deviation from the optimal conditions of temperature, absolute and relative humidity can negatively affect the muco-ciliary clearance [305–307].

In acute respiratory failure and in certain chronic pathological conditions, such as bronchiectasis and cystic fibrosis, muco-ciliary clearance is impaired, leading to a retention of mucus. Medical gases, traditionally delivered through various forms of respiratory support, contain only 6 part per million of water vapour, contributing therefore to the airways dehydrating. In addition, the delivery of high flows of gas through oxygen therapy or NIV causes unidirectional nasal flow, leading to the nasal mucosa to recover less moisture during expiration. As such, the upper airways cannot exert their heating and moisturizing effect, which needs to be taken over by the lower airways mucosa leading to increased inflammation and further impairment in cilia function and bronchial hyperreactivity [281,308,309].

NHFT provides the same level of absolute humidity found in the alveoli (44 mg/L). *In vitro*, this is associated with lower levels of inflammation and injury compared to

conditions of under-humidification [310]. It is conceivable that this leads to the restoration of the rheological properties of mucus, reducing the retention of secretions and the occurrence of atelectasis. Physiological studies *in vivo* have shown that patients with bronchiectasis treated with NHFT (20-25 L/min, F_IO_2 21%, 3 h/day for 6 days) improve their lung clearance with no significant change in cough frequency [311,312]. This could translate clinically in a reduced rate of exacerbations in patients with bronchiectasis and COPD [312,313].

3.2.2 Clinical indication of NHFT

NHFT has been shown to significantly improve the respiratory status of patients with *de novo* acute hypoxemic respiratory failure compared to standard oxygen therapy, in both observational studies and randomized control trials [258,314,315]. The use of NHFT is associated with a reduced rate of intubation, independent of the underlying cause of respiratory failure. Hence, in the absence of criteria for immediate intubation, in view of the poor outcomes of NIV for acute hypoxemic respiratory failure, a trial of NHFT is recommended to be offered to these patients, including if the underlying cause is severe acute respiratory distress syndrome [316].

Compared to standard oxygen therapy, NHFT reduces the rate of respiratory failure and re-intubation post-extubation in low-risk patients, and is non-inferior to NIV in high-risk patients. In this latter group, NHFT, by being better tolerated than NIV, could improve patients' adherence to treatment [317,318].

NHFT appears to reduce intubation rate, but not to improve mortality or comfort among immunocompromised subjects who present with acute hypercapnic respiratory failure in comparison to NIV [319]. Finally, despite NIV remaining the first choice ventilatory mode in hypercapnic respiratory failure, NHFT could have a role in the treatment of these patients either as a complementary therapy during breaks, or as an alternative to NIV or controlled oxygen therapy in mild acidosis [320].

There remains a paucity of evidence relating to the use of NHFT in the chronic settings. NHFT appears to be effective as part of the symptoms management in palliative care, and has been shown to improve the quality of life reduce dyspnoea in patients with COPD [313,321,322]. While the evidence to NHFT in chronic patients remains limited, the application of this technique in patients with chronic respiratory disease is now widespread in clinical practice.

3.3 Respiratory support in cystic fibrosis

Patients with cystic fibrosis, especially those with advanced lung disease, can develop hypoxemic and hypercapnic respiratory failure, both as an acute occurrence, or

chronically. While no formal guidance on the use of respiratory support in people with CF exists, a gradual, stepwise approach is advised whenever possible.

3.3.1 Oxygen therapy in CF

As discussed in Sections 1.4 and 2.4, the chronic changes to the parenchyma and airways that characterise patients with CF lead to hypoxaemia and potentially hypercapnia with secondary pulmonary hypertension. As such, it is conceivable that people with CF presenting with hypoxaemia could benefit from supplemental oxygen therapy similarly to patients with COPD [323–326].

Supplemental oxygen has been studied in patients with CF as part of short-term trials during sleep and exercise. No benefit on survival or lung function was shown in any of these studies [211,227,327–329]. Oxygen therapy has been shown to improve oxygen levels (pO_2 or S_pO_2) leading however to a greater increase in carbon dioxide, during both sleep and exercise. No significant benefits in quality of life and sleep, or on exercise tolerance were seen when using standard oxygen therapy [211].

Despite the lack of solid evidence supporting its use, oxygen therapy is widely prescribed for patients with CF with documented desaturation [211]. Blood gas analysis should be monitored in view of the increased risk of hypercapnia associated with the use of supplemental oxygen, and NIV should be considered as appropriate.

3.3.2 NIV in CF

NIV is used routinely in clinical practice to treat people with CF as an adjunct to airway clearance, in the acute or chronic setting and as a bridge to transplant [330–333]. NIV has been shown to reduce the load on the respiratory muscles, increase alveolar ventilation and gas exchange [279] and reverse the rapid and shallow breathing pattern commonly adopted by CF patients with advanced lung disease.

As discussed in Section 1.6, airways clearance is a key focus of symptomatic treatment of patients with CF. Effective airway clearance techniques can be tiring, and challenging for individuals with severe lung disease, due to increased work of breathing and dyspnoea. In these circumstances, NIV can be a valuable adjunct to chest physiotherapy (CPT) to reduce fatigue and respiratory rate and improve oxygenation [330,334–338].

As discussed in Section 3.1.1, NIV is a cornerstone therapy for acute hypercapnic respiratory failure, but there is only limited data on its use in this context in CF [229,262,339]. This notwithstanding, NIV is often used to treat acute hypoxemic and acute hypercapnic respiratory failure in patients with CF [229,332,333,340]. This is partly related to the historically poor outcomes of invasive mechanical ventilation among people with CF, and NIV can maximise the chances of recovery without pursuing the route of intensive care treatment [188].

NIV is also used in patients with chronic respiratory failure to reduce symptoms of hypercapnia, to improve quality of life and in those waiting for lung transplant as a bridge to lung transplantation [339,341–344].

As discussed in Section 2.4.1.1, oxygen desaturation during sleep, whether persistent or intermittent, is often the first sign of respiratory failure in patients with CF. This may occur prior to the development of daytime respiratory failure. Nocturnal oxygen therapy, despite being routinely prescribed in clinical practice, has not been shown to confer significant clinical benefit [211]. In contrast, small RCTs and observational studies suggest that NIV can improve sleep efficiency and alveolar ventilation [328,345–348]. In addition, it may overcome REM-related hypoventilation and delay the onset of ventilatory failure during daytime [328].

3.3.2.1 Contraindications and complications of NIV in CF

Spontaneous pneumothorax is a common complication in CF, especially among individuals with $FEV_1 < 40\%$. It has an annual incidence of 0.64%, affecting 1 patient in 167 every year, with 3.5% of individuals with CF suffering from at least one pneumothorax in their lifetime. More than half of the patients developing a first pneumothorax will have a recurrence, which often leads to the need of talc pleurodesis [66,92]. While an untreated acute pneumothorax is a contraindication to NIV, patients who have a chest drain in situ or have a history of pneumothorax can be treated with NIV.

Similarly, up to 9% of patients with CF will have haemoptysis once in their life, with only a minority having massive episodes [66].

3.3.2.2 Practical aspects

While the decision to start NIV as an adjunct to CPT is mainly driven and suggested by the physiotherapist, the use of NIV due to acute or chronic respiratory failure should follow a MDT discussion. Physiotherapists, in combination with dieticians and psychologists, need to be involved in the decision to start NIV from an early stage. The use of NIV can significantly impact on an individuals' perception of their health and interfere with nutritional input and overnight enteral feeding.

NIV as an adjunct to physiotherapy is usually started in hospital when patients are unwell or struggle to clear secretions due to increased work of breathing or severely compromised lung function. NIV is usually set in pressure-cycled modes. It can be delivered as IPPB (inspiratory positive pressure breathing - inspiratory pressure only), or as bilevel ventilation. Bilevel ventilation combines the advantage of reducing the risk of dynamic airway collapse by providing positive expiratory pressure, with the unload of inspiratory muscle and increased tidal volume.

The goal of using NIV in combination to airway clearance is to unload the respiratory muscles. It is therefore recommended that inspiratory pressure or pressure support is increased appropriately ensuring that levels are tolerated by the patient. Back-up rate can interfere with forced expiration, coughing and expectoration. The choice of interface should be chosen with the patient as some will prefer a mouth-piece or a nasal mask for the ease of expectoration without the need of removing the mask while others may prefer an oro-nasal mask.

In patients with raised nocturnal CO_2, or in conditions of respiratory failure, NIV is usually commenced following evidence that strategies aiming at delivering controlled oxygen therapy have not been effective. Studies have looked at the preferred way to start NIV in patients with CF, in terms of mode, settings, interfaces, but have provided equivocal results [330,349].

Bilevel ventilation in pressure-cycled mode is usually commenced with PEEP set at 4 or 5 cmH_2O and a pressure support as high as tolerated, to offload respiratory muscles and provide adequate alveolar ventilation. Patients with CF do not tend to have respiratory drive problems, therefore spontaneous or spontaneous/timed mode of ventilation are appropriate in this setting. Particular attention should be taken when setting the thresholds for the inspiratory triggering and the expiratory cycling. The inspiratory trigger should be sensitive enough to avoid these patients, who often have very low lung function, to feel suffocated at the beginning inspiration, but not too sensitive to cause auto-triggering in the event of coughing or leaks. Some ventilators allow to set the rise time, consisting in the interval time it takes to reach the inspiratory pressure at the beginning of inspiration. This should be carefully assessed based on patients' comfort and needs: a fast rise delivers more air by getting to the inspiratory pressure quickly, but it is often uncomfortable; on the other hand, a longer rise time might reduce the volume of air per cycle.

Whenever clinically possible, a gentle up-titration of the pressures and adjustment of the settings should be performed, to allow sufficient time for patients to get used to the ventilator. In order to facilitate the use of NIV, patients who are not acutely unwell might be advised to start using the ventilator initially during the day for short periods of time. As soon as the patient is comfortable to use NIV regularly, over-night treatment is also recommended to be started.

3.3.3 NHFT in cystic fibrosis

By virtue of the pathophysiological mechanisms described in Section 3.2.1, and in light of the positive outcomes of several clinical trials in other patients populations, it seems plausible that NHFT could successfully provide respiratory support to patients with CF, either as a complementary therapy to NIV or as an alternative to NIV or conventional oxygen treatment [350].

In the context of cystic fibrosis, NHFT could in fact improve gas exchange, reduce dead space and compensate for intrinsic PEEP, while facilitating mucus clearance. Despite NHFT being used routinely in CF clinical practice [351–353], the evidence base is very limited. A small, short term, prospective, randomised, crossover study assessed the physiological effects of NHFT in 15 patients with CF admitted to hospital following a pulmonary exacerbations [354]. Patients received supplemental oxygen via NHFT and NIV for 30 minutes each in a random order. NHFT had a similar effect on diaphragmatic activity compared to NIV, but was associated with reduction in respiratory rate and minute ventilation. This, however, was not associated with changes in carbon dioxide levels. In addition, NHFT did not lead to improvement in dyspnoea score, and comfort was similar to that of NIV, and lower than in baseline conditions [354].

These findings suggest that NHFT may result in physiological benefit in patients with CF, but further studies are required to assess if these translate into clinical benefits.

3.4 Conclusion

Several types of non-invasive respiratory support devices are currently available to improve oxygenation and ventilation, and therefore to correct acid-base disturbances of respiratory origin. These devices work by providing higher F_IO_2 (oxygen therapy and NHFT) or reducing the respiratory load (NHFT and NIV). Despite their widespread use in CF clinical practice, the clinical and physiological benefit is not fully established, This might be in part related to the unique physiology in CF, and to the need of better understanding gas exchange and acid-balance in this complex multisystem disease.

Chapter 4
Aim of the thesis

Metabolic alkalosis is a well described complication of cystic fibrosis (CF) which has conventionally been attributed to Pseudo-Bartter's Syndrome (PBS). Clinical experience suggests that isolated high concentration of serum bicarbonate is more frequent than previously reported in people with CF and can occur in the absence of Pseudo Bartter's syndrome or acid-base disturbance, effects which have not been previously described in the literature.

There remain a paucity of data as well as a lack of understanding of the mechanisms and clinical significance of increase serum bicarbonate levels in people with CF. It is also not clear if therapeutic options are needed to normalise levels and improve clinical outcomes.

4.1 Research questions, hypothesis and aim

The main goal of this research project was to answer the following questions regarding serum bicarbonate and blood gases in patients with CF:

1. Is the concentration of serum bicarbonate elevated in people with CF, even in absence of metabolic alkalosis?
2. If the concentration of serum bicarbonate is increased in people with CF, does it have clinical relevance?
3. Is the concentration of serum bicarbonate associated with changes in the acid-base status of the subject?
4. What is the cause of raised serum bicarbonate in patients with CF? In particular, is increased serum bicarbonate a consequence of:
    a. Ventilatory failure?, or
    b. CFTR disfunction in the kidney,?
5. If there is a ventilatory component leading to increased concentration of serum bicarbonate, can the use of respiratory support techniques affect serum bicarbonate levels, and the acid-base status of the subject?

4.1.1 Hypothesis

The working hypotheses of this research project, challenged the preconceived idea of acid base dysfunction in CF, and were as followed:

- Serum bicarbonate is raised in people with CF. Abnormally high levels of serum bicarbonate are more common among people with CF compared to the general population, and compared to individuals with other respiratory conditions.

- CF-specific comorbidities, such as pancreatic insufficiency, osteoporosis, recurrent courses of antibiotics, certain genotypes, and diabetes, are factors independently associated with acid-base and electrolyte abnormalities.
- Serum bicarbonate increases as a response to hypoventilation in individuals with respiratory conditions. I expect the same to be true for individuals with CF.
- The distribution of acid-base disturbances is different in patients with CF compared to individuals with other respiratory conditions. In particular, I expect metabolic alkalosis to be more prevalent in people with CF.
- Serum bicarbonate can be used as a prognostic marker of decline in people with CF. This would be similar to patients with obesity hypoventilation syndrome, who show increased levels of serum bicarbonate as a response to CO_2 retention, with bicarbonate playing a clinical role for ventilation similar to that of HbA1C for diabetes.
- Non-invasive ventilation and nasal high flow therapy, by affecting gas exchange and hypercapnia, can reduce serum bicarbonate levels, and play a role in the symptomatic treatment of patients with cystic fibrosis.

4.1.2 Aim

The aim of this research work was to improve the understanding of the role of serum bicarbonate and the distribution of acid-base disturbances in patients with CF, and to explore the role of serum bicarbonate as a prognostic marker.

This research project sought to:

- Characterise the levels of serum bicarbonate in the CF population and to see if serum bicarbonate levels are elevated in patients with CF, compared to the general population.
- Assess the levels of serum bicarbonate in clinically stable patients with CF, and explore independent factors associated with its concentration.
- Assess the levels of serum bicarbonate in patients with CF during pulmonary exacerbations, and explore any independent factors associated with its concentration.
- Evaluate if serum bicarbonate can be used as a prognostic marker, or as a new biomarker of severity of disease.
- Define the prevalence of acid-base disturbances in patients with cystic fibrosis, both in conditions of clinical stability, and during pulmonary exacerbations.

If the above primary hypotheses were confirmed, then I planned the following secondary aims, including:

- Exploring, directly or indirectly, the cause for the elevated levels of serum bicarbonate in patients with CF. This was to assess if the raised serum

concentration is the result of CFTR dysfunction in the kidney or compensates the increase in carbon dioxide resulting from hypoventilation.

- Determine if respiratory support techniques, such as non-invasive ventilation or nasal high-flow therapy, can control or reverse the elevation in serum bicarbonate in patients with CF.

This research project adds to the existing literature by establishing the prevalence of acid-base abnormalities in patients with CF, and comparing this with other cohorts of respiratory patients, as well as to the general population.

In addition, it furthers the understanding of the mechanisms underlying acid-base disturbances in CF, and attempts to clarify the relation between acid-base disorders and electrolytes disturbances in CF.

Finally, this research project explores the role of serum bicarbonate as a prognostic marker, as a biomarker of severity of the disease, and the impact of increased serum bicarbonate levels on prognosis.

4.2 Structure of this research project

To verify the validity of these hypotheses, and to achieve the aim of this research project, a collection of eight research studies, four retrospective, and four prospective, was planned.

The first phase consisted of four retrospective studies, focussing primarily on characterising serum bicarbonate levels and acid-base disturbance patterns in individuals with CF in conditions of clinical stability, and in those with end stage lung disease, prior to death. These studies also explored the effect of bicarbonate levels on ventilatory response, as well as the impact of non-invasive ventilatory support on bicarbonate concentration. At the time of writing this thesis, these single-centre studies using high-quality data and well characterised historical cohorts, remain some of the largest studies performed, to date, in this area of research.

The second phase consisted of four prospective studies focussing on confirming the results of the initial studies and on evaluating the clinical relevance of serum bicarbonate and further exploring the ventilatory component.

4.2.1 First phase: retrospective studies

The first study of this research project is presented in Chapter 5. The aim was to characterise the levels of serum bicarbonate, over a 14 year period, in a large cohort of clinically stable patients with CF who were under follow up at the Leeds Regional CF centre.

A large retrospective analysis of data, captured prospectively on the Unit EPRs was performed. This included all measures of serum bicarbonate collected at annual assessments, and contemporaneous clinical data including BMI, FEV_1, electrolytes and comorbidities. Thanks to the large dataset of over 2,800 annual assessments, I was able to characterise the average levels of serum bicarbonate in the CF population and identify a number of factors, including age, sex, and comorbidities, that were independently associated with raised serum bicarbonate levels.

In a subsequent study, presented in Chapter 6, the clinical relevance of raised bicarbonate, and its potential role as a prognostic tool or biomarker for disease severity were explored. To accomplish this, I performed a retrospective analysis of serum bicarbonate, collected in the 12 months preceding death of patients in over a 12 year time period. The data was used to characterise the pattern of variation in the levels of serum bicarbonate in patients with CF approaching death, and identified independent factors associated with these changes.

These studies demonstrated that serum bicarbonate is more frequently elevated in people with CF compared to the general population (Section 5.5). As such, a further retrospective study was undertaken to assess the distribution of acid-base disturbances in people with CF. The study, presented in Chapter 7, analysed over 500 arterialised capillary blood gases, obtained from patients with CF and other respiratory conditions during clinical stability. These ABGs were available as part of hypoxic altitude simulation tests (HASTs) performed in the Cardio-Respiratory Lab, a procedure commonly undertaken during clinical stability and prior to flying. In addition to assessing differences in the acid-base status in people with and without CF, this study enabled the characterisation of the impact of serum bicarbonate levels on the response to the hypoxic stimulus. This further allowed me to investigate whether the concentration of this anion could be representative of the ventilatory status, as previously hypothesised for individuals with OHS and COPD.

Finally, in Chapter 10, the role of ventilatory failure in determining serum bicarbonate concentration was further explored. A large-scale retrospective study, analysing NIV treatment episodes for all adults at the Leeds Regional CF centre over a 10 year period, and characterised individual response in a variety of conditions was performed. This allowed me to shed some light on the use of non-invasive respiratory support techniques in people with CF, a topic which has not been studied extensively in the literature despite being of significant clinical relevance.

4.2.2 Second phase: prospective studies

To confirm, validate and further explore the results of the first part of the thesis, four prospective studies were designed, three of which were completed and included in this

research thesis, and one was not performed after HRA approval due to the COVID-19 pandemic.

In Chapter 9, the aim was to confirm the results obtained in Chapter 7. A prospective external validation study on individuals attending the Cardio-Respiratory Lab for HASTs was performed. The aim of this study was to confirm the earlier result which demonstrated that metabolic alkalosis was the main acid-disturbance observed in clinically stable patients with CF, and that bicarbonate concentration could be used as a surrogate marker for ventilation, and a predictive marker of response to the hypoxic stimulus.

Having seen that the concentration of serum bicarbonate appears to be determined, at least partly, by changes in the patient's ventilatory pattern, I aimed to assess the role of the kidney in the handling of bicarbonate in patients with CF. A pilot case-control study was performed to assess if renal handling of bicarbonate was different in individuals with CF compared to those with other respiratory conditions (Chapter 8). I showed indirectly that mutated CFTR was playing a potential role in raising the serum bicarbonate concentration. These changes were likely co-exists with elevated bicarbonate secondary to metabolic compensation fduring hypoventilation.

Based on these outcomes, two further follow-up prospective studies were designed (Chapter 11 and Section 12.3). The first, was a prospective pilot trial assessing if transient hypercapnia occurred in people with CF during exercise, and whether nasal high flow therapy could affect the partial pressure of carbon dioxide and possibly the serum concentration of bicarbonate. This study is presented in Chapter 11.

In addition, I designed a further study, presented Chapter 12, aimed at evaluating the presence of sleep disturbances, and assessing respiratory variables during sleep in individuals with CF and abnormal concentrations of serum bicarbonate. The study was approved by the Research Ethics Committee (REC), Health Research Authority (HRA) and local Research and Innovation Department (R&I), but could not be started due to the COVID-19 pandemic. Therefore only the rationale and protocol are presented as part of this work.

4.3 General methodology

Each study within this research project followed its specific methodology, which is reported in detail as part of each Chapter. However, some methods of data collection, database creation and analysis were followed consistently throughout the project.

4.3.1 Data reporting and database creation

The Leeds Regional Cystic Fibrosis Centre has been recording clinical data on electronic records since 2007, using EMIS®, a system initially developed for primary care adapted for the Leeds CF Unit with *ad hoc* codes.

Patients attending the Leeds Regional CF Centre previously signed a consent form for their data to be stored on EMIS and used for research purposes (Appendix A).

EMIS allows to automatically generate reports based on specific search criteria based on pre-defined clinical codes. All data were extracted following information governance procedures, and consent agreement. Data can be extracted from EMIS as patient-identifiable, pseudo-anonymised or fully anonymised data.

The report tool on EMIS was extensively used in this research project to create the initial databases for the retrospective studies, and identify potentially eligible subjects for the prospective studies. Each automatically generated database was subsequently reviewed for accuracy, including full or random sample comparison with medical notes depending on the study.

The specific search criteria used for the generation of each database are described in each Chapter.

4.3.2 Automatic reporting of blood gas analysis

A fundamental part of the work of this thesis was to review and interpret a high volume of blood gas analyses, sometime in the order of hundreds (see e.g. Chapter 7).

ABG interpretation is an extremely time-consuming, error-prone, and potentially inaccurate process. Further, it ideally requires two independent clinicians to interpret results in order to guarantee consistency, by confirming concordance in the interpretation.

If I was to follow the standard approach of single- or double-clinician interpretation, a big part of the analyses performed in this project would have been impossible, due to the prohibitive amount of resources required to complete the interpretation of ABGs.

As part of the preliminary stages of this project, I developed an automated tool for the interpretation of blood gas analysis, and categorisation of each reading into pre-defined acid-base imbalance categories.

This tool can be used in two ways. The first and most useful integration is as an Excel formula. This can be quickly added to any automatically generated Excel database, such as those generated by EMIS. The Excel formula leads to an automatic interpretation, returning a categorical “string” variable. Such data can be further imported in IBM SPSS for further analysis.

A second version of this tool was also developed, and is available as an online web application (https://abg.giuliaspoletini.com/). This is useful for one-off interpretations, and cannot be used as part of a database.

The tool is based on the Boston interpretation approach for ABGs (Section 2.3.1.1), utilising readings of serum bicarbonate, pH and CO_2. It does not use a reading of Base Excess for interpreting a measure. The tool was initially tested for accuracy by myself. Further independent validation was performed by asking two respiratory physicians experienced in ABG interpretation (one Consultant and one registrar) to manually interpret a collection 50 blood gases.

The automated reporting tool and its validation are described in further detail in Appendix B.

Both versions of this tool, their source code and Documentation, are available online at https://abg.giuliaspoletini.com/, and are free to use subject to the MIT/BSD licence, and to the Creative Commons CC BY-NC-SA licence.

4.4 Ethical approval

Discussion with the R&I Department at Leeds Teaching Hospital NHS Trust took place prior to the definition and submission of each individual study.

Based on the outcome of these discussions, I sought full ethical approval via REC and HRA whenever necessary, or, whenever sufficient in view of the use of deidentified data within the clinical team, I sought a simpler local approval.

All studies obtained local R&I and CSU approval, and, when appropriate, HRA and REC approval. Further information regarding the ethical approval of each research protocol are provided in each individual Chapter.

4.5 Statistical analysis

The statistical analysis was performed by myself, using SPSS v26 as main statistical software. The support of Dr Lesley Smith (Leeds Centre for Personalised Medicine and Health, University of Leeds) was sought when necessary.

Part 2

Research studies

Chapter 5
Serum bicarbonate in clinical stability in CF: a retrospective study

5.1 Introduction

Bicarbonate is secreted and absorbed across several epithelia, including the respiratory, gastro-intestinal and renal epithelia, in a process facilitated by several anion exchangers and channels including CFTR. It is involved in a multitude of intracellular and extracellular processes in several systems [355].

CFTR is a chloride and bicarbonate channel, and, as such, its role is widely studied in basic CF research [15,239]. However, there is a paucity of meaningful data available on its clinical relevance [239,356].

The limited evidence to date suggests that people with CF frequently present with metabolic alkalosis, at a time of both clinical stability, and during pulmonary exacerbations [214,216,220,223,357–359]. Further to these data, personal communications and observations from clinicians in the Leeds CF Unit, seem to suggest that bicarbonate levels are frequently elevated in clinically stable patients with CF, especially those with severe lung disease. However, this data is very subjective and needs confirmation in formal studies investigating serum bicarbonate levels in clinically stable patients.

Understanding the effects of the patients' sex, age, comorbidities, and other related factors on the value of serum bicarbonate during clinical stability may help shed light on the pathophysiological mechanisms driving abnormal bicarbonate levels in CF and provide an indication of the normal range for this patient population.

Serum bicarbonate is not measured in routine clinical practice. However in Leeds, serum bicarbonate is included in the panel of the annual assessment, with bloods samples being taken yearly at a time of clinical stability.

A large-scale, single-centre retrospective study was performed looking at all available serum bicarbonate measures performed as part of the annual assessment at the Leeds Regional CF Unit over a period of 14 years.

In this Chapter, the results of this study, the largest of its kind on patients with CF, are reported attempting to characterise the average levels of serum bicarbonate, and any determining factors such as age, comorbidities, and genetic characteristics.

5.2 Aim

The aims of this study were:

- To characterise the distribution of serum bicarbonate across a large cohort of people with CF in conditions of clinical stability; and
- To identify any individual factor independently associated with any variation out of normal limits in serum bicarbonate concentration.

5.3 Methods

A retrospective analysis of data collected prospectively for standard clinical care on the Unit Electronic Patients Records (EPRs, EMIS) at the Leeds Regional Adult CF Centre between February 2007 and December 2020 was performed.

All patients included in the study previously consented for clinical data stored on the EPRs to be used for research purposes (Appendix A).

5.3.1 Study population

The EMIS reporting tool (Section 4.3.1) was used to identify all patients who met the following inclusion and exclusion criteria for the study population:

- A confirmed diagnosis of cystic fibrosis (defined as having two CF-causing mutations, and/or a sweat chloride test >30 mmol/L with clinical manifestation of CF);
- Age of 17 or older at time of the search;
- Under the care of the Leeds Regional Adult CF Centre throughout the study period (or for parts of the study period if they transferred out or transferred in);
- At least one measurement of serum bicarbonate at annual assessment bloods.
- Patients whose records of annual assessment bloods included measurements taken exclusively after lung transplantation were excluded;

A subsequent detailed review of patients records was performed to confirm that all patients meeting the inclusion and exclusion criteria were identified.

5.3.2 Data collection

The EPRs were searched for all annual assessment measures in the study period between February 2007 (the initial introduction of EPRs in the Unit) and December 2020 (the time of the analysis), which met the inclusion and exclusion criteria described above.

Serum bicarbonate, as well as any available contemporaneous measurements of electrolytes, C-reactive protein (CRP) and body mass index (BMI) were collected. Demographics, comorbidities, lung function results, requirement for respiratory support, transplant and microbiology status at the time of each annual assessment were also recorded.

5.3.3 Statistical analysis

No sample size calculation was required for this study. Data collection was limited to a time period identified *a priori*, starting from the original availability of electronic patient records (February 2007), and ending at the time of analysis (December 2020).

Multiple annual assessments measures are generally available for each subject. Although these assessments are normally performed annually, occasional occurrences of multiple assessment within a single year of age for the same subject were present. The dataset was thus reduced to one sample per subject, per year of age. No repeated measures analysis was therefore necessary, as all analyses included year of age as an independent factor.

Normal distribution of measured variables was assessed by visual inspection and using the Shapiro-Wilks test. Results are expressed as number (percentage), means (standard deviation), when normally distributed, or median (IQR, 25^{th}-75^{th} percentile) when not normally distributed.

In the first part of this study, the average levels of serum bicarbonate was inspected by age of the patient, and depending on one further individual determining factor (including sex, mutation, and use of CFTR modulators).

To do so, univariate Mixed Models were used to model the level of serum bicarbonate (dependent variable) based on age and a further subject-specific determining factor (as categorical factors, such as sex, genotype, use of modulators). Age was not modelled as a continuous covariate to avoid any assumptions on the linearity (or monotonousness) of the relationship between bicarbonate and age. In some analyses, age was linearised as a continuous covariate to provide an estimate of the change in average serum bicarbonate for each year of age.

Results are expressed as marginal means (standard deviation). A p-value <0.05 was considered statistically significant, both when evaluating a whole model, a covariant, or when comparing marginal means at different values of categorical factors.

In the second part of this study, the impact of comorbidities and lung transplant status on the levels of serum bicarbonate was explored. Co-morbidities and lung transplant status are re-assessed at each annual assessment, and it is expected that they increase in incidence with the age of each subject.

To model the impact of co-morbidities on serum bicarbonate levels, independently on age, univariate Mixed Models were used. Serum bicarbonate (dependent variable) was modelled based on age (as a categorical factor), and a collection of comorbidities (each as categorical factors). Similarly to the first part of the study, age was not modelled as a continuous covariate to avoid any assumptions on the linearity (or monotonousness) of the relationship between bicarbonate and age.

In the third party of this study, the impact of other continuous prognostic markers such as BMI and FEV_1, on the level of serum bicarbonate was investigated. These markers, particularly FEV_1, are expected to generally worsen over time, and will therefore decrease in correlation with age.

To identify the presence of general correlations between BMI or FEV_1 and serum bicarbonate, correlation analysis of variable pairs was performed by visual inspection of the scatter plot, and by using the Pearson correlation or the Spearman-rho coefficient, depending on the distribution of the variables, as appropriate. A p-value <0.05 was considered statistically significant.

Finally, to identify the main determinants of serum bicarbonate, a full factor model was developed allowing to extrapolate the independent role of each subject characteristic, co-morbidity, lung transplant status, BMI and FEV_1, on the average level of serum bicarbonate. Univariate Mixed Models were used to model serum bicarbonate (dependent variable) based on age (as a categorical factor), comorbidities and lung transplant status (each as a categorical factor), BMI and FEV_1 (as covariates). Again, age was not modelled as a continuous covariate to avoid any assumptions on the linearity (or monotonousness) of the relationship between bicarbonate and age.

A p-value <0.05 was considered statistically significant. All analyses were performed with IBM SPSS v26.

5.3.4 Ethics

This study was discussed with the LTHT R&I and deemed exempt from NHS REC and HRA. It was approved by the local R&I (RM17/93114) and the Cardio-Respiratory CSU at Leeds Teaching Hospital NHS Trust. All patients had previously consented for their clinical data to be used for research purposes.

5.4 Results

5.4.1 Study population and annual blood results

During the study period (February 2007-December 2020), 696 adult patients were under follow-up at the Leeds Regional Centre for Cystic Fibrosis. Of these, 4 did not provide consent for their data to be used for research purposes, leaving 692 to be considered for eligibility in the study.

Out of these, a total of 441 patients met all inclusions and no exclusion criteria, and were thus included in the study. Figure **5.1** shows the flow-chart of patients included.

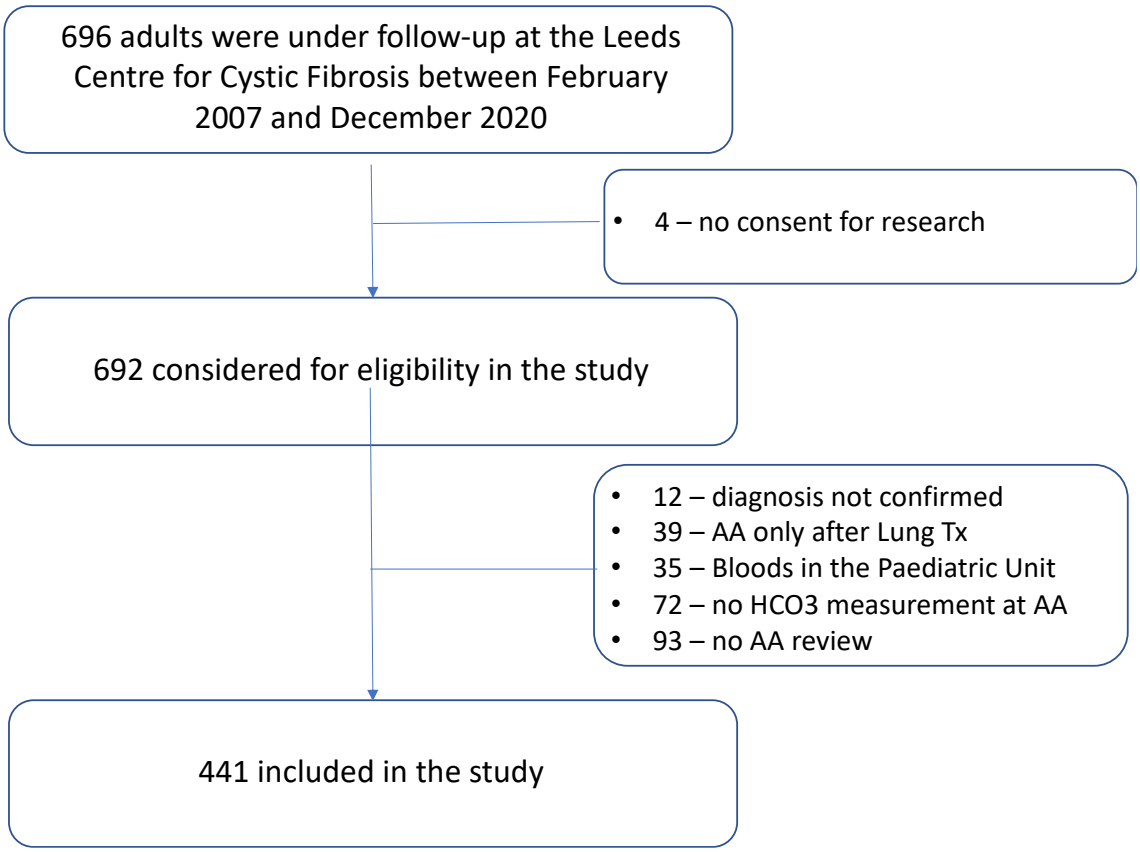

Figure 5.1 Flow-chart of inclusion in the study. HCO3-, bicarbonate; AA, annual assessment; Lung Tx, lung transplant.

A total of 2,876 annual assessment were recorded for the 441 included subjects within the study period, an average of 6.52 (2.96) for each subject (range 1 to 14 per subject).

The studied population was male predominant (59.7% vs 40.3%). Most patients were F508del homozygous (57.3%), or heterozygous (34.4%), with only a minority not carrying at least a F508del mutation (8.3%)

Table 5.1 presents the characteristics of the subjects at the time of each annual blood assessment.

Pooling all annual assessment data, serum bicarbonate concentration appeared to be within normal limits with a median at 27 [25.0-29.0] mmol/L (normal range 24-28 mmol/L). No significant abnormalities were observed in other blood results. Table 5.2 summarises the blood results at annual assessment.

Table 5.1 Characteristics of the population at annual assessment (AA). Results are expressed as number (%) or median (IQR).

	AA (n=2876)
N Subjects	441
Age at AA	28.5 [21.0-36.6]
Female sex, n (%)*	178 (40.3%)
Serum bicarbonate	27.0 [25.0-29.0]
BMI	22.8 [20.5-25.3]
FEV_1 % predicted	66 [44-84]
Transplant	
Referred/Active	151 (5.3%)
Post-transplant	62 (2.2%)
Not yet needed	2663 (92.6%)
Genotype*	
F508/F508	253 (57.3%)
F508/-	151 (34.3%%)
Other	37 (8.3%)
Modulator	
None	2464 (85.7%)
IVA	129 (4.5%)
LUM/IVA	56 (1.9%)
TEZ/IVA	143 (5%)
ELX/TEZ/IVA	84 (2.9%)
Comorbidities	
Diabetes	688 (23.9%)
Pancreatic insufficiency	2595 (90.2%)
Liver	
Normal	1418 (49.3%)
Liver disease	1306 (45.4%)
Cirrhosis	134 (4.7%)
Transplant	18 (0.6%)
Bone density	
Normal	1886 (65.6%)
Osteopenia	844 (29.3%)
Osteoporosis	146 (5.1%)
Microbiology status	
P.aeruginosa	
Free/Never	920 (32%)
Intermittent	398 (13.8%)
Chronic	1558 (54.2%)
B. cepacia complex	
Free/Never	2624 (91.2%)
Intermittent	8 (0.3%)
Chronic	244 (8.5%)

Table 5.2 Blood results at annual assessment (AA). Results are presented as median and interquartile range

	AA (n=2616)
Bicarbonate, mmol/L	27.0 [25.0-29.0]
Sodium, mmol/L	140 [139-141]
Chloride, mmol/L	104 [102-106]
Potassium, mmol/L	4.3 [4.1-4.5]
Urate umol/L	360 [299-418]
Albumin g/L	40 [36-44]
C-reactive protein mg/L	5 [5-8.8]

5.4.2 Serum bicarbonate and confounding factors

5.4.2.1 Part 1: Simplified models of serum bicarbonate by age

The mean concentration of serum bicarbonate changed by age ($p<0.001$), with a regular increase in serum concentration being observed with individuals getting older (Figure **5.2**). Serum bicarbonate levels grew substantially from 24.8 (2.5) at age 10, to 26.9 (3.1) at age 20, reaching 28.8 (3.0) by age 40, following a stable trend to reach 28.0 (2.4) by age 60.

On average, serum bicarbonate levels increased of 1.56 mmol/L for each 10 years of age.

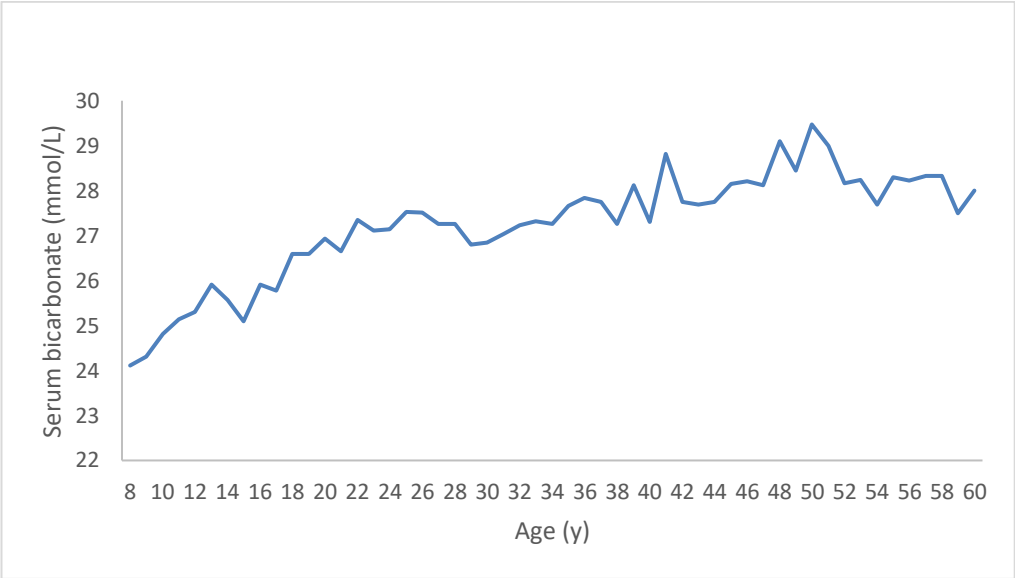

Figure 5.2 Mean bicarbonate concentration by age of the subject. The mean concentration of serum bicarbonate increased with age, by an average of 1.56 mmol/L every 10 years of age.

When modelling serum bicarbonate by age and sex of the subject, we observed that bicarbonate concentrations are generally higher in male subjects (marginal mean M vs. F 27.6 vs 26.7, $p<0.01$).

Concentration of serum bicarbonate increased with age in both male and female subjects ($p<0.01$). Serum bicarbonate appeared to be generally higher in male subjects from the age of 14 to the age of 50, compared to female subjects of the same age ($p<0.01$). No difference in serum bicarbonate levels was seen between males and females above the age of 50. Figure **5.3** shows the marginal means of serum bicarbonate by sex, and by age, in the male and female cohorts separately.

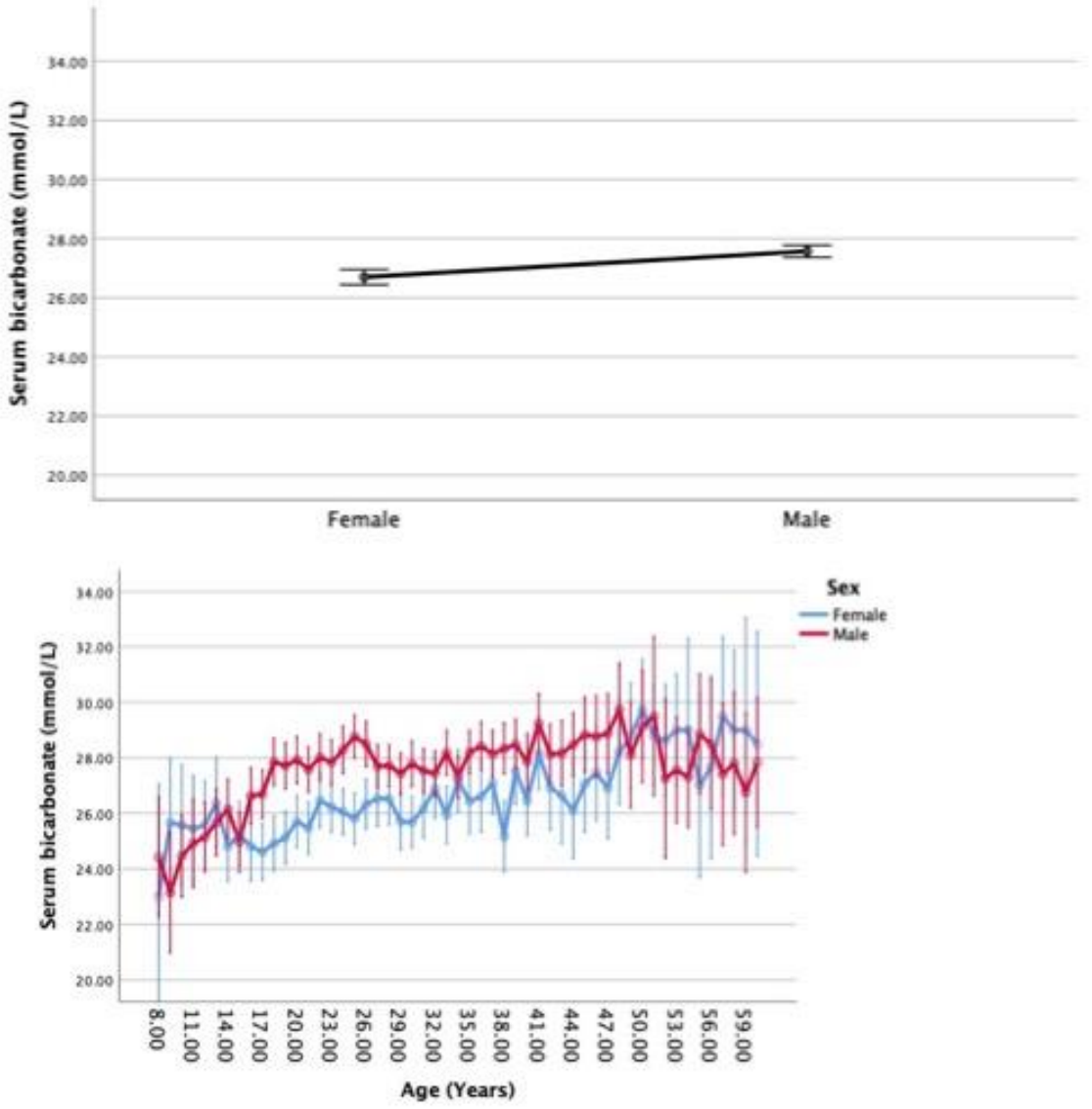

Figure 5.3 Mean bicarbonate concentration by age and sex. The left panel shows the marginal mean serum bicarbonate concentration in female and male patients. The right panel shows the mean serum bicarbonate concentration in each age group by sex (red is male and blue is female). Data are presented as mean and 95%CI.

When modelling serum bicarbonate by age and genotype of the subject, no difference was observed in the three genotype cohorts (F508del homozygous, heterozygous or not carrier of the F508del) (p=0.318). Figure **5.4** shows the marginal means of serum bicarbonate by genotype, and by age in each genotype cohort separately.

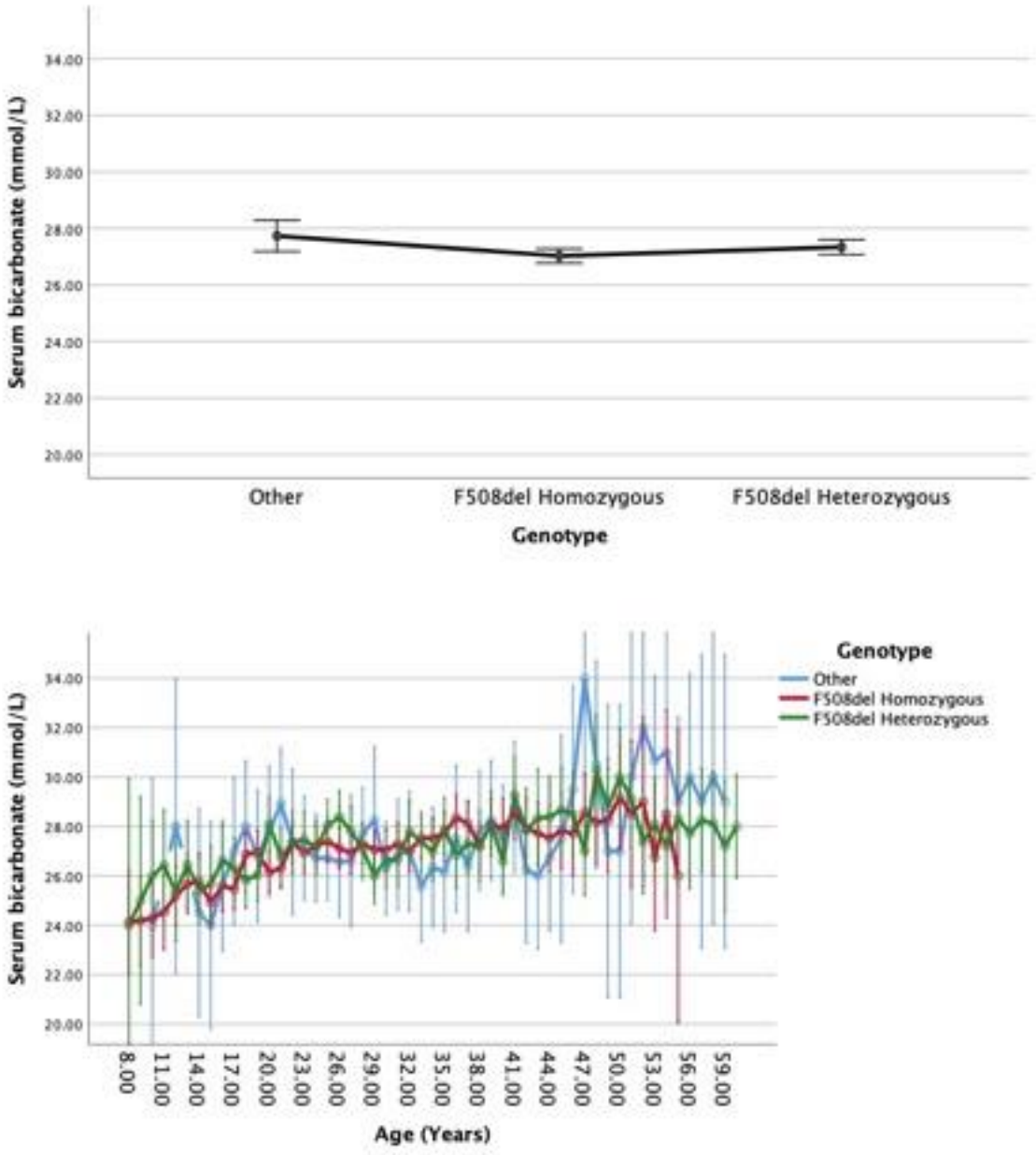

Figure 5.4 Mean bicarbonate concentration by age and genotype. The top panel shows the marginal mean serum bicarbonate across the three genotype cohorts (F508del homozygous, F508del heterozygous, other). The panel on the bottom shows the marginal mean bicarbonate in the three groups by age (red F508del homozygous, green F508del heterozygous and blue all others). Data are presented as mean and 95%CI.

When modelling serum bicarbonate by age and use of a CFTR modulator, we observed that serum bicarbonate concentration was higher in individuals with CF being treated with a CFTR modulator at the time of annual assessment (IVA, LUM/IVA, TEZ/IVA or ELX/TEZ/IVA), independently of age (28.2 vs 27.2, p=0.007). This difference appeared more pronounced in subjects over 40 years of age.

Figure **5.5** shows the marginal means of serum bicarbonate by use of CFTR modulators, and by age, in each CFTR modulator-based cohort separately.

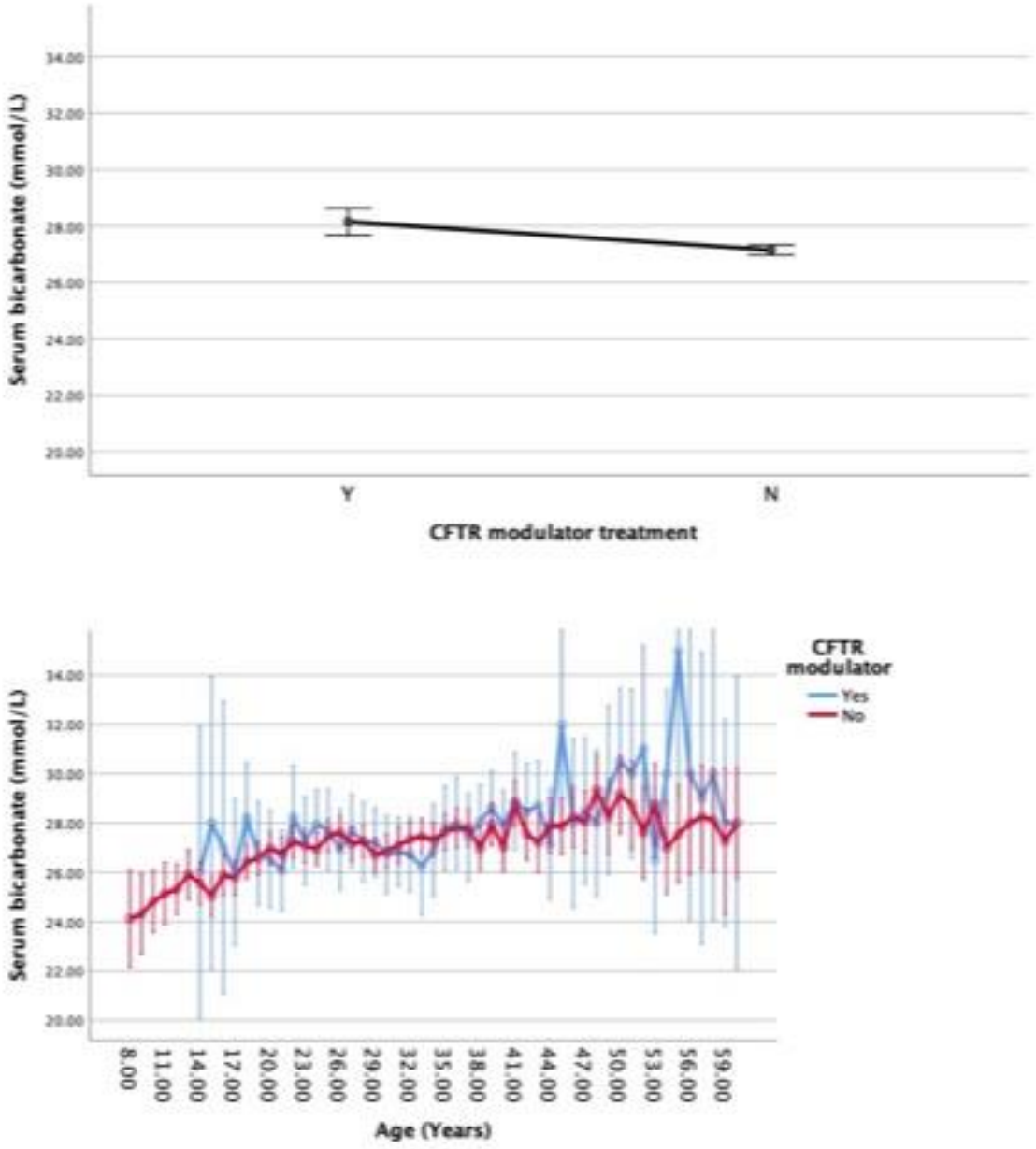

Figure 5.5 Mean bicarbonate concentration by age and treatment with CFTR modulators. The top shows the marginal mean serum bicarbonate concentration across the two groups (on modulators vs not on modulators). The bottom panel shows the serum bicarbonate in the two groups by age (blue on CFTR modulators, red not on treatment). Data are presented as mean and 95%CI.

5.4.2.2 Part 2: Simplified models of serum bicarbonate by age and comorbidities

When modelling serum bicarbonate by age and comorbidities, I observed that a diagnosis of diabetes did not affect serum bicarbonate concentration (p=0.7), but that osteoporosis was associated with higher levels of bicarbonate compared to osteopenia and normal bone density (marginal means 28.5 vs 27.4 vs 27.5, p=0.014) (Figure **5.6**).

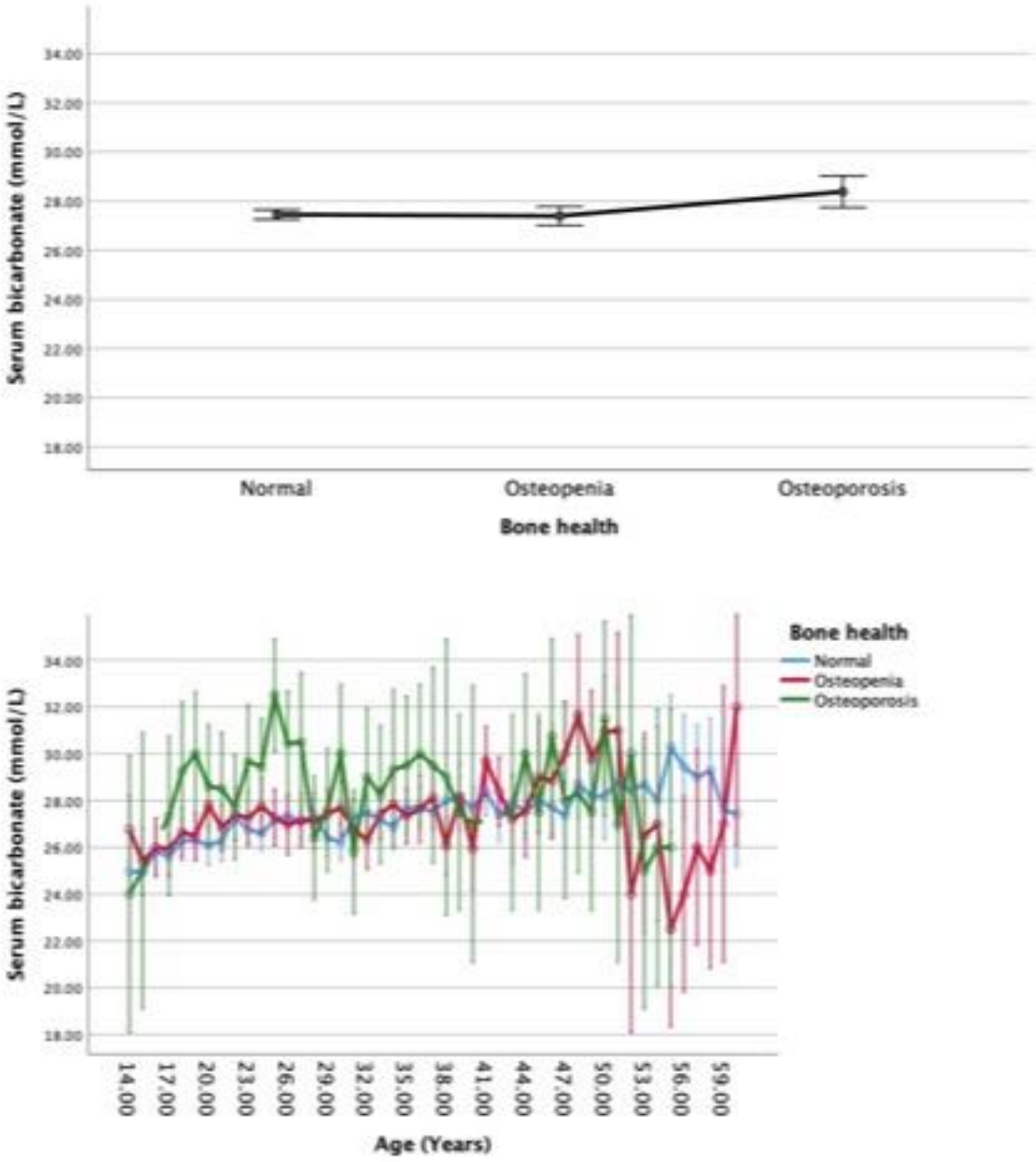

Figure 5.6 Mean bicarbonate concentration by age and bone health. The top panel shows the marginal mean serum bicarbonate depending on bone health overall (normal vs osteopenia vs osteoporosis). The bottom panel shows the marginal mean of serum bicarbonate by bone health and by age. Data are presented as mean and 95%CI.

Serum bicarbonate concentration was also more elevated in people with liver cirrhosis compared to those with CF-related liver disease, or no liver disease (marginal means 28.8 vs 27.2 vs 27.3, p<0.001) (Figure **5.7)**.

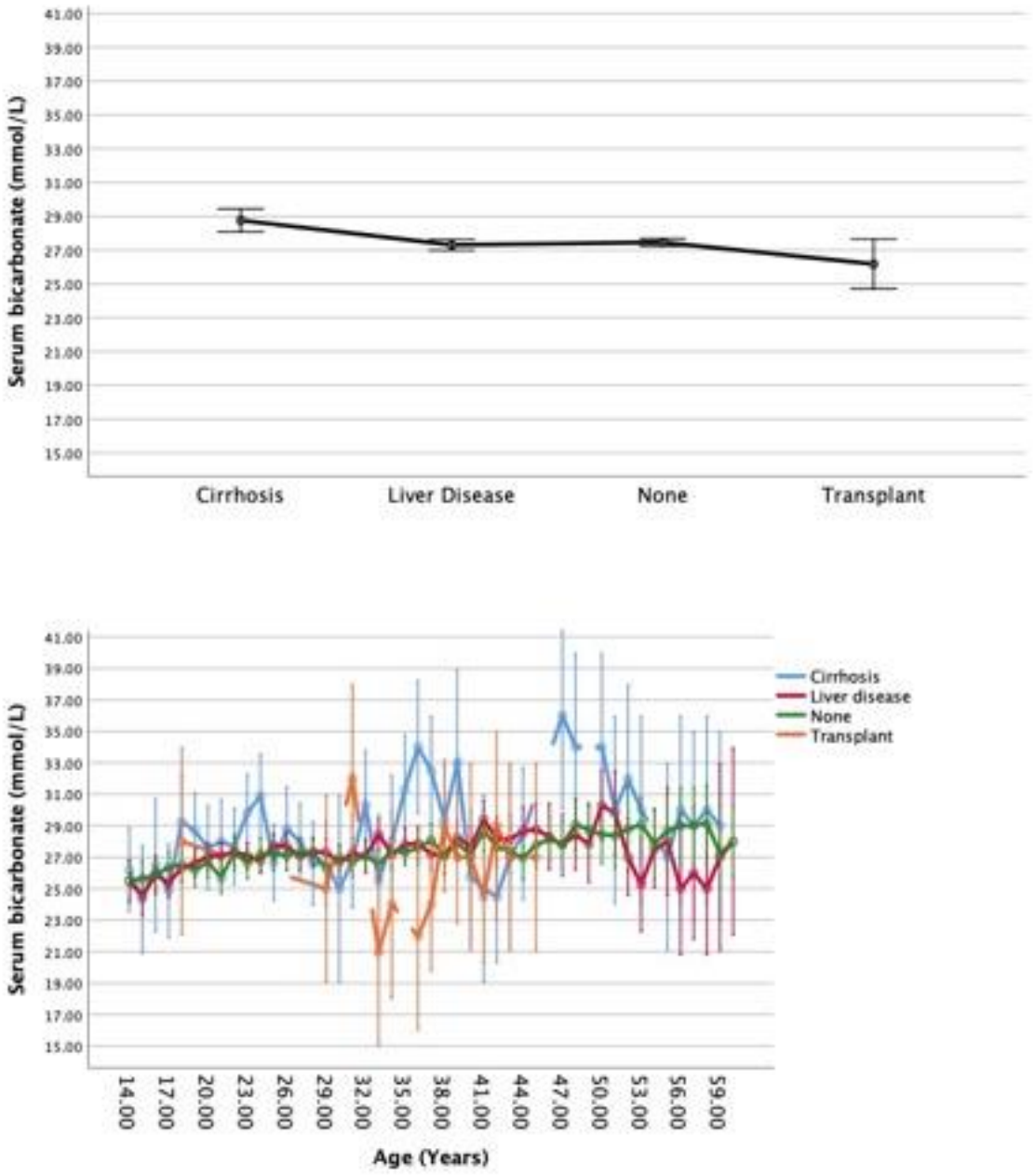

Figure 5.7 Mean bicarbonate concentration by age and liver disease. The top panel shows the marginal mean serum bicarbonate depending on liver disease. The bottom panel shows the marginal mean bicarbonate concentration by liver disease and age. Data are presented as mean and 95%CI.

Colonisation with *Pseudomonas aeruginosa* or the highly pathogenic BCC was not associated with any difference in serum bicarbonate levels.

When modelling serum bicarbonate by age and status on the lung transplant list, I observed that serum bicarbonate concentration is significantly different depending on whether annual assessment bloods were taken before or after lung transplantation (p<0.001).

Higher serum bicarbonate concentration was observed in individuals referred for assessment or on the active lung transplant list, compared to individuals that were not referred, or that were already recipients of a lung transplant (29.6 vs 27.5 vs 25.5, p<0.001).

Figure **5.8** shows the marginal means of serum bicarbonate concentration by status on the lung transplant list, and by age, in each cohort separately.

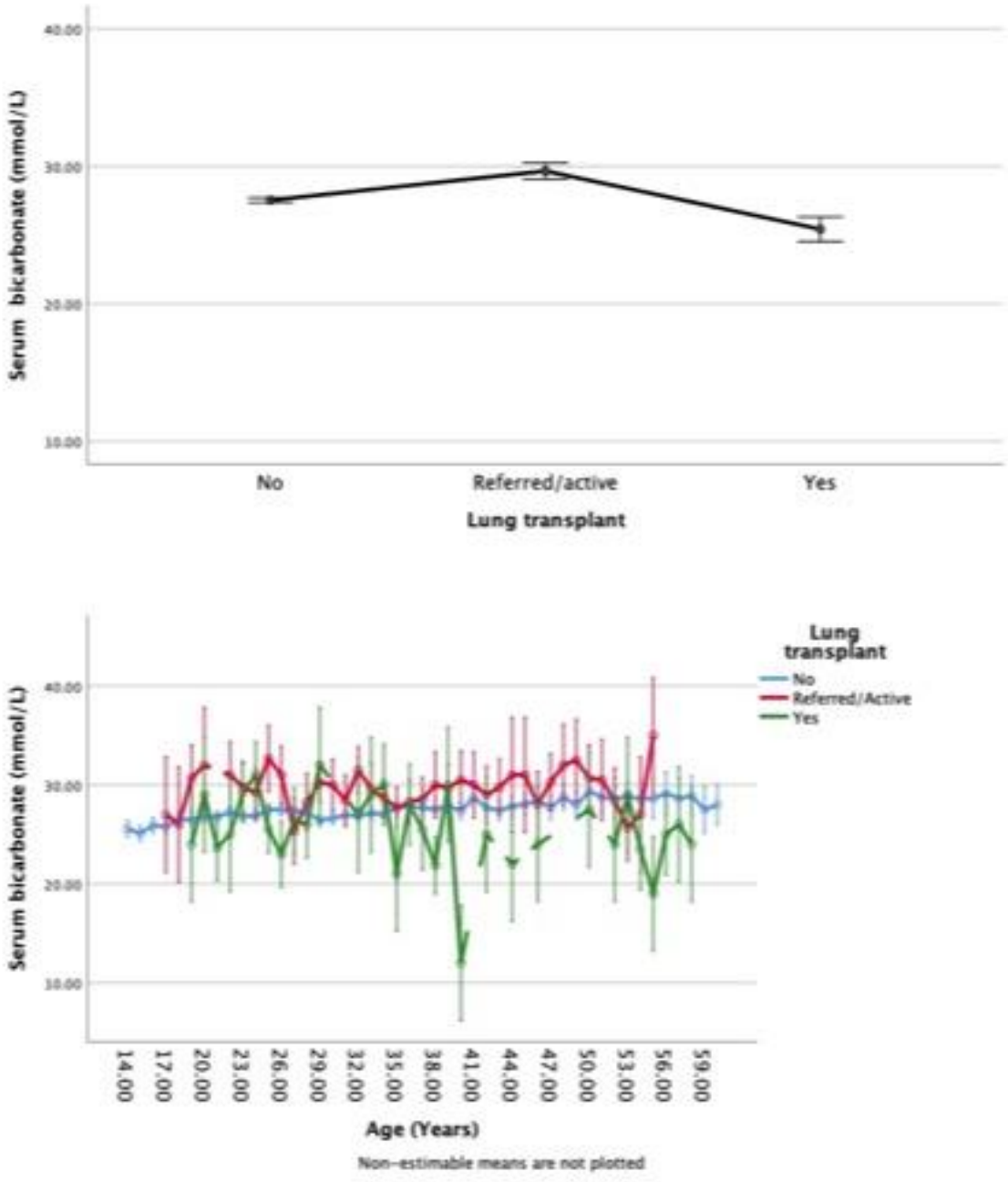

Figure 5.8 Mean bicarbonate concentration by age and status on the lung transplant list. The left panel shows the bicarbonate concentration across the three groups identified based on lung transplant (no vs referred/active vs transplant recipients). The panel on the right shows the bicarbonate levels in the three groups by age. Data are presented as mean and 95%CI.

When modelling serum bicarbonate by age, comorbidities, genotype, and status on the lung transplant list, I observed that the subject's genotype did not have a significant independent effect on serum bicarbonate levels. Similarly, bacterial colonisations had no independent effect on serum bicarbonate levels. Once accounting for all factors, the effects of being on treatment with CFTR modulators resulted to be not significant (p=0.194). Age, sex, pancreas, liver, bone and diabetes comorbidities, and colonisation with *P.aeruginosa* remained significant independent determinants of serum bicarbonate.

5.4.2.3 General correlations of serum bicarbonate

Pooling all data together, I explored general correlations between serum bicarbonate levels and other electrolytes.

Serum bicarbonate levels had a statistically significant, but extremely weak positive correlation with sodium and potassium concentration (r=0.072 and r=0.088, respectively, p<0.001). A very weak negative correlation was observed with urate (r=-0.049) (p<0.05). A strong negative correlation was shown with serum chloride (r=-0.475, p<0.001).

Figure **5.9** shows the overall correlation between serum bicarbonate and serum chloride.

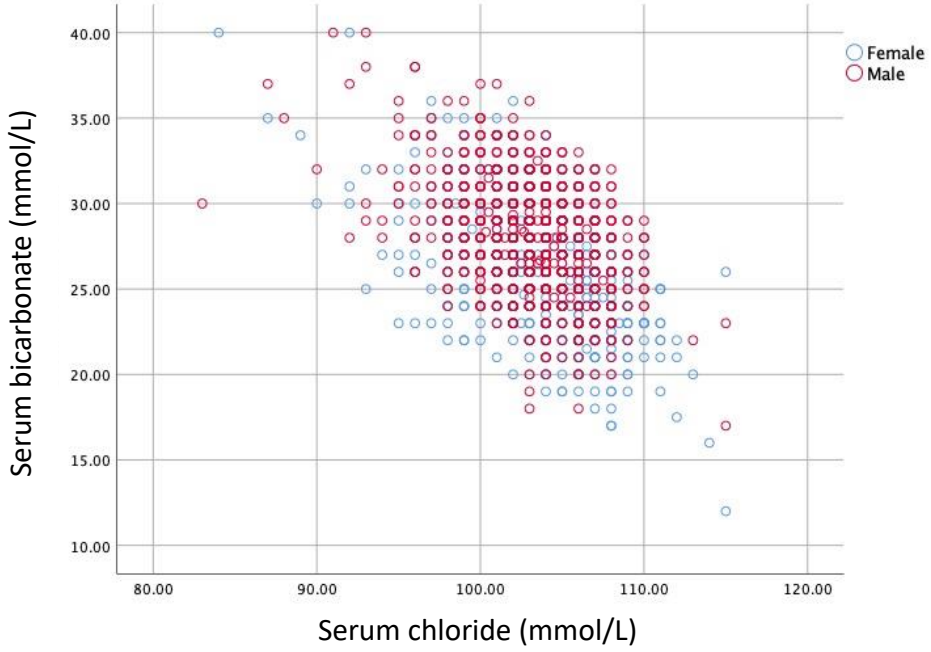

Figure 5.9 Correlation between serum bicarbonate and serum chloride. The scatter plot shows the moderate-strong negative correlation between these anions.

Pooling all data together, we explored general correlations between serum bicarbonate levels and other prognostic predictors, BMI and FEV_1 in particular.

Serum bicarbonate had a negative, weak correlation with BMI (r=-0.047, p<0.01). Serum bicarbonate concentration had a negative correlation with FEV_1 (r=-0.199, p<0.001).

An analysis of FEV_1 in relation with serum bicarbonate levels highlighted that a significant proportion of measurements (approximately 20%) showed high concentrations of

bicarbonate with relatively preserved lung function, with a FEV_1 greater than 40% predicted (Figure **5.10**)

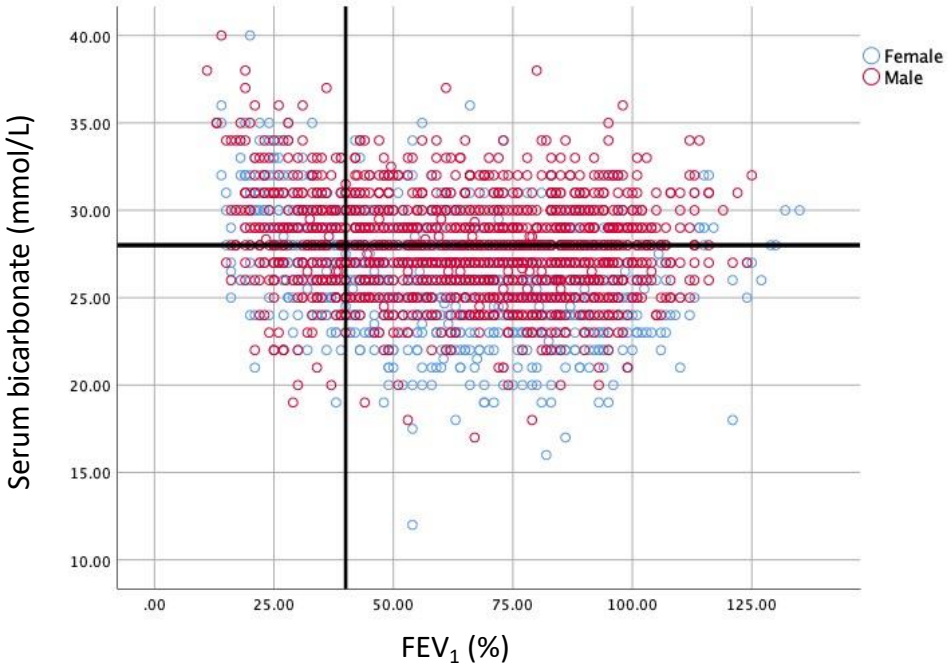

Figure 5.10 Correlation between serum bicarbonate and FEV1. The horizontal line represent the upper limit of normality for serum bicarbonate concentration at 28 mmol/L and the vertical line is set at 40% of FEV_1 as cut-off to define advanced lung disease.

The correlation between FEV_1 and serum bicarbonate concentration, when looking at each of the four quadrants separately, was only statistically significant among those with a FEV_1<40% and bicarbonate >28 mmol/L (r=-0.324, p<0.001). No significant correlation was present in the three other quadrants.

5.4.2.4 Part 2: Full model of serum bicarbonate by age, comorbidities, and prognostic factors

By incorporating all the previously explored factors (comorbidities, lung transplant status, FEV_1, BMI, sex and age) in a full-factor model, I observed that the interactions of comorbidities, age and lung function was different compared to that noted by looking at the factors independently. This is unsurprising given the nature of most of these factors being prognostic markers of decline, and worsening with age in a population of subjects with cystic fibrosis.

When modelling serum bicarbonate by age, comorbidities, genotype, status on the lung transplant list, FEV_1, and BMI, I observed that correlation between FEV_1 and serum bicarbonate was independent of age and remained statistically significant by incorporating the two factors in the same model. However, the clinical significance of the correlation of FEV_1 with bicarbonate was minimal, and lower than that of age.

By including in the model all the comorbidities, genotype and use of modulators, I observed that most factors maintained clinical significance with the exception of colonisation with BCC, genotype and use of CFTR modulators (Table 5.3).

Table 5.3 Parameter estimates and marginal means in the full model
BMD, bone mineral density; CF-LD, cystic fibrosis related liver disease; BMI, body mass index

	B	95%CI	Estimate marginal means (SD)	*p*
Sex				**<0.001**
Female	26.454	24.44-28.46	26.67 (0.57)	
Male	28.043	26.02-30.07	28.26 (0.56)	
Genotype				0.720
Homozygous	0.099	-0.361-0.558	27.51 (0.57)	
Heterozygous	0.116	-0.170-0.402	27.39 (0.56)	
Other	-		27.49 (0.59)	
CFTR modulators				0.194
Yes	0.223	-0.113-0.559	27.57 (0.58)	
No	-		27.35 (0.56)	
Transplant				**<0.001**
Yes	-		26.30 (0.68)	
No	1.036	0.223-1.849	27.34 (0.54)	
Referred	2.453	1.481-3.424	28.75 (0.60)	
P.aeruginosa				0.034
Free	0.071	-0.362-0.504	27.37 (0.57)	
Intermittent	-		27.30 (0.59)	
Chronic	0.408	0.002-0.814	27.71 (0.57)	
B.cepacia complex				0.477
Free	-		27.78 (0.29)	
Intermittent	-1.143	-3.94-1.65	26.64 (1.46)	
Chronic	0.195	-0.226-0.615	27.97 (0.34)	
Pancreas				**<0.001**
Pancreatic insufficiency	-1.389	-1.87 - -0.911	26.84 (0.47)	
Pancreatic sufficiency	-		28.14 (0.52)	
Bone health				**0.003**
Normal BMD	-0.604	-1.169 - -0.038	27.35 (0.56)	
Osteopenia	-0.874	-1.499 - -0.300	27.08 (0.56)	
Osteoporosis	-		27.96 (0.62)	
Liver				**0.009**
Normal	1.612	0.219-3.005	27.59 (0.53)	
CF-LD	1.736	0.345 – 3.126	27.72 (0.53)	
Cirrhosis	2.576	1.080-4.073	28.56 (0.59)	
Transplant	-		25.98 (0.89)	
Diabetes				**0.006**
Yes	0.305	0.008-0.601	27.62 (0.57)	
No	-		27.31 (0.57)	
Age	0.030	0.016-0.045		**<0.001**
FEV_1	-0.015	-0.021 - -0.009		**<0.001**
BMI	-0.062	-0.096 - -0.028		**<0.001**

5.5 Discussion

In this first of its kind, large-scale retrospective study of serum bicarbonate levels in clinically stable patients with cystic fibrosis, I showed that people with CF have serum bicarbonate levels at the upper end of normality, and often (>25% of measurements) above the normal range.

I pooled data from 2876 annual assessments performed in a period of 14 years at the Leeds Regional CF Unit. These assessments covered 441 patients and were performed in conditions of clinical stability. By combining all data recorded at annual assessment at the Leeds Regional CF Unit, together with demographics, and contemporaneous measures of BMI, FEV_1, and monitoring of comorbidities, I was able to study several factors possibly associated with the concentration of serum bicarbonate.

Overall, I observed that people with CF have a level of serum bicarbonate in the upper range of normality (median 27 [25.0-29.0] mmol/L). The serum concentration is higher in this cohort than that reported in the NHANES III cohort (24.9 mmol/L), a large longitudinal study following up a representative sample of the general population in the US [360]. Serum bicarbonate levels were above the upper limit of the normal range in over 25% of this dataset.

I demonstrated that serum bicarbonate levels are age dependent, and increase on average by 1.56 mmol/L for every 10 years of age. By the age of 30, levels of serum bicarbonate in the CF population tended towards the upper limit of the normal for healthy individuals. Serum bicarbonate levels were generally higher in men with CF, compared to women.

Interestingly, data from the NHANES III cohort reported the serum bicarbonate levels tended to be higher in older patients in the general population, in line with what I observed in the Leeds CF cohort, but the age related increase was significantly lower (0.24 mmol/L for each 10 years age) when compared to this CF cohort [360,361].

When analysing the relationship between serum bicarbonate and other key electrolytes, we observed a strong negative correlation between serum chloride and bicarbonate. This may suggest that the underlying mechanisms for hyperbicarbonataemia (and possibly metabolic alkalosis) could be linked to Pseudo-Bartter syndrome [222]. However, the lack of any clinically significant correlation between serum bicarbonate and sodium would point towards an alternative aetiology. In Chapter 8, the renal handling of bicarbonate and other electrolytes is further investigated, to clarify the potential aetiology of increased serum bicarbonate in CF.

In this cohort, I observed a general, albeit weak, correlation between higher serum bicarbonate levels and lower FEV_1. This is to be expected as respiratory dysfunction is likely to be influencing bicarbonate concentrations with both values potentially being negative prognostic predictors of clinical outcome.

While the correlation between serum bicarbonate and FEV_1 is stronger when values were lower than 40%, a sizeable proportion of measurement (20%) revealed that serum bicarbonate concentration were also significantly raised in individuals with relatively preserved or normal lung function. This may reflect that gas exchange abnormalities in CF are not exclusively seen in the presence of advanced lung disease [218] although it

is unlikely ventilatory failure is occurring in clinically stable individuals with preserved lung function.

Serum bicarbonate levels were significantly more elevated at the time of transplant assessment, when individuals are more likely to present with ventilatory failure. As such, increased serum bicarbonate concentration may reflect compensation for hypercapnia and potential hypoventilation, and may be further exacerbated by abnormal renal handling as explored in Chapter 8 and discussed in Chapter 13.

No differences in serum bicarbonate concentration were identified on the basis of CF genotype. While ELX/TEZ/IVA has recently been approved for the vast majority of adults with CF [36,135,362], this retrospective study was carried out prior to their approval in the UK. Individuals in this study who were treated with CFTR modulators were those with gating mutations receiving Ivacaftor, or patients with other genotypes who were deemed in critical need due to the severity of their condition and were granted early access to modulators via the compassionate use scheme sponsored by Vertex Pharmaceuticals. While CFTR modulators appeared to be associated with a higher serum bicarbonate concentration, this may simply reflect the fact that the compassionate use program entry criteria included low lung function and clinical deterioration. As such, it is conceivable that the higher concentration of serum bicarbonate noted in individuals on treatment with CFTR modulators is not secondary to the correction of CFTR function, but rather to the overall severity of their disease.

Using simplified models, I also assessed the role of comorbidities in impacting the average level of serum bicarbonate. Serum bicarbonate concentration was higher in patients with osteoporosis compared to osteopenia and normal bone density. Serum bicarbonate levels were also more elevated in people with liver cirrhosis and CF-related liver disease compared to those that have no liver complications. Colonisation with *Pseudomonas aeruginosa* or *Burkholderia cepacia* was not associated with any difference in serum bicarbonate.

In the final part of this study, I developed a full-factor model including age, subject characteristics (sex, genotype), treatments (modulators), lung transplant state, comorbidities, and FEV_1 and BMI. As expected, FEV_1 and BMI were inversely correlated with age and in part correlated with each other. Both parameters are recognised to correlate with disease severity and quality of life [363–366] .

In this model, the independent marginal impact of age diminishes as expected, to an average increase of 0.3 mmol/L for every 10 years of age. The independent correlation of FEV_1 with serum bicarbonate was still present, but was clinically insignificant (0.15 mmol/L for every 10% points of FEV_1 reduction). Higher levels of serum bicarbonate were independently associated with several co-morbidities, clear prognostic markers of advancement of disease, even when controlling for age, sex and other confounding

factors. Most CF-related comorbidities, such as diabetes, liver cirrhosis, and osteoporosis, and a subject being considered for lung transplantation, were associated with significantly higher levels of serum bicarbonate. These findings support the potential usefulness of serum bicarbonate as a prognostic marker of the worsening of disease.

In line with the interpretation of the impact of CFTR in the simplified model, no effect of CFTR modulator status on serum bicarbonate was seen in the full-factor model, when controlling for other markers prognostic of disease severity.

When comparing the results of this model with the available results from the general population, some differences emerge. In the general population, the presence of chronic-compensated liver disease has been associated with an increased concentration of serum bicarbonate [367]. This is in line with what I observed in the this cohort of individuals with CF. Conversely however, conflicting results were noted with regards to bone health and diabetes. In this cohort, osteoporosis appears to be associated with *higher* serum bicarbonate concentration, whereas in the general population hypobicarbonataemia and chronic metabolic acidosis are linked to low bone density, as bicarbonate promotes bone reabsorption and inhibits bone formation [368]. Similarly, in the general population, lower bicarbonate levels are associated with diabetes, and impaired glucose tolerance, not necessarily associated with chronic renal disease [360,369]. However, in this cohort I noted that diabetes mellitus is linked to a raised serum bicarbonate concentration.

To further explore the suitability of serum bicarbonate concentration, I conducted a further large-scale retrospective analysis, presented in Chapter 6, monitoring the variation in bicarbonate in the 12 months prior to death. I showed that bicarbonate levels increased significantly in the six months preceding death in patients with CF without a lung transplant, indicating that bicarbonate is associated with worsening respiratory status, and could be used as a prognostic predictors.

5.6 Conclusion

In this Chapter, I showed that the mean concentration of serum bicarbonate appears to be higher in clinically stable individuals with CF, compared to the general population. I also showed that the prevalence of isolated hyperbicarbonataemia is similar to that previously shown in large scale studies of the general adult population (as in the NHANES III cohort).

In patients with Cystic Fibrosis, serum bicarbonate levels in conditions of stability increase with age, and disease progression. Higher serum bicarbonate levels are independently associated with known prognostic markers of disease severity, such as lower lung function, osteoporosis, cirrhosis and diabetes.

The clinical relevance of high levels serum bicarbonate in people with CF is further explored in Chapter 6, where I investigate the trend in the twelve months preceding death, and the usefulness of serum bicarbonate as a potential prognostic marker.

Further multi-centre studies, including national registry studies, are warranted to better characterise the levels and independent determinants of serum bicarbonate in patients with cystic fibrosis in conditions of clinical stability.

Chapter 6
Serum bicarbonate as a prognostic marker in CF: A retrospective study

6.1 Introduction

When CF was first described in the late 1930s, the predicted survival for a newborn with the disease was only 6 months. Thanks to significant improvements in multidisciplinary care, as well as to the availability of life-changing treatments as described in Section 1.6, the survival rate and predicted age of death of patients with CF have improved dramatically in the recent decades [1,103,370,371]. Despite the improvement in life-expectancy, CF continues to be characterised by progressive lung disease which often culminates in ventilatory failure, requiring lung transplantation.

In the early 1990s, it was shown that patients with CF and severely impaired lung function had a 50% mortality rate at 2 years [372]. Since then, FEV_1 <30% has been considered one of the main criteria for a patient to be considered for lung transplantation [373–375]. The median waiting time for lung transplant is approximately 8.5 months in the UK and 12 months in the Euro-transplant zone, with up to 25% of patients waiting for more than 24 months [376,377].

More recently, conditions have improved for people with CF and advanced lung disease. Currently, patients with CF and a FEV_1 <30% have an average survival time longer than 5 years [103,370,371]. However, FEV_1-based criteria have not been changed, and the present criteria may not be optimal and have the potential of lead to an earlier than required referral for lung transplantation.

As such, alternative or complementary criteria for consideration for lung transplantation have been suggested, including the rate of decline in FEV_1, the presence of bacterial colonisations, nutritional status, and the need for respiratory support [378–382].

The criteria for allocating organs to patients who have been accepted on the lung transplant list differs by country, with the lung allocation score (LAS) being used by many healthcare systems to prioritise patients [383]. In the UK, a new system for lung allocation was introduced in mid 2017, dividing patients on the waiting list into three categories: normal priority, urgent listing, and super-urgent listing. Allocation for normal priority patients is at the discretion of each transplant centre, on the basis of clinical assessment and including waiting times.

Urgent and super-urgent lists are shared at a national level to prioritise lung transplantation to those patients at higher risk of imminent death. The current criteria for urgent and super-urgent listing in the UK for patients with CF are listed in Table 6.1.

Table 6.1 Criteria for urgent and super urgent lung allocation in the UK

	CF-specific criteria
Urgent listing	• Worsening hypoxia (pO_2 < 6.5 kPa) requiring increasing oxygen demand >10 L/min despite continuous NIV • pH <7.30 persistently despite continuous NIV • Refractory right heart failure despite all pharmacological interventions to support the right ventricle • Ongoing episodes of massive haemoptysis despite bronchial embolization
Super urgent listing	• vvECMO as a bridge to lung transplant • iLA as a bridge to lung transplant

While urgent listing requests can be resubmitted every 30 days, these criteria appear to be quite limiting. The actual viability of these criteria in identifying patients that require transplantation urgently, but not immediately, is debatable.

As such, new CF specific criteria are needed to allow the correct identification of patients who are rapidly progressing towards the terminal disease phase but prior to being at an imminent risk of death [381].

Serum bicarbonate is a known prognostic marker in a variety of health conditions [274,360,361,384–386]. Previous reports have suggested that serum bicarbonate levels are generally raised in patients with cystic fibrosis. I observed in Chapter 5 that many individuals with CF have higher than normal serum bicarbonate concentration, especially among those with advanced lung disease. I also identified a link between raised bicarbonate levels and several CF related comorbidities, and that levels increased with age. It remains unclear if the concentration and the rate of change of serum bicarbonate could be a useful prognostic factor in identifying patients with CF who are at a higher risk of death [381] and help prioritizing lung transplantation.

In this Chapter, I report the results of a single-centre retrospective study looking at serum bicarbonate levels of all patients who died in a time period of 12 years while under the care of the Leeds Regional Adult CF Centre. I analysed changes in serum bicarbonate concentration in the twelve months preceding death, in order to assess if this measure could be used as a meaningful prognostic marker.

6.2 Aims of the study

The aims of this study were:

- To assess if serum bicarbonate is elevated in patients with CF at their end of life, compared to similar patients with CF who are not near their end of life (Part 1)
- To assess the trend of serum bicarbonate over the 12 months preceding the death of patients with cystic fibrosis (Part 2);

- To assess if serum bicarbonate is a prognostic marker in patients with CF, after compensating the effect of known variables associated with decline of health and death (Part 2).

6.3 Methods

A retrospective analysis of data collected prospectively for standard clinical care on the Unit EPRs (EMIS) at the Leeds Regional Adult CF Centre between February 2007 and February 2019 was performed.

All patients previously consented for their clinical data to be used for research purposes (Appendix A).

6.3.1 Study population

The EMIS reporting tool (Section 4.3.1) was used to identify all patients who met the following inclusion and exclusion criteria for the study population:

- A confirmed diagnosis of cystic fibrosis (defined as having two CF-causing mutations, and/or a sweat chloride test >30 mmol/L with clinical manifestations of CF);
- Age 17 or older;
- Under the care of the CF Centre in Leeds at the time of death and for at least the 12 months preceding their death;
- Two or more serum bicarbonate measures in the 12 months preceding death, with one measurement taken within the last two months of life.
- Patients who died after having received a lung transplant were excluded from the analysis.

A subsequent detailed review of patients record was performed to confirm that all patients meeting the inclusion and exclusion criteria were correctly added to the cohort.

A 2:1 control cohort of patients matched for sex and age (with a one month precision) at the time of the index event was also identified. The EMIS reporting tool was used to identify all patients who met the following inclusion and exclusion criteria for the control population:

- A confirmed diagnosis of cystic fibrosis (defined as having two CF-causing mutations, and/or a sweat chloride test >30 mmol/L with clinical manifestations of CF);
- Age 17 or older;
- Alive 12 months after the index event (definition in the data collection Section);
- Not part of the case cohort.

The matching was performed in a randomised fashion using a true number generator, among all candidates matched for age and sex. No subject was included twice as part of the matched cohort to avoid over-representation.

6.3.2 Data collection

An index event was identified for each subject as the date of their death (case cohort), or the date when the subject's age was the same of their matched case at the time of their death (matched cohort). Data was collected for the 12 months preceding the index event of each subject.

Electronic patients records were searched for all subjects over the age of 17 who died between February 2007 and February 2019. Baseline demographics, comorbidities, requirement for respiratory support and microbiology status at the index date were recorded, as well as status on the lung transplant waiting list.

All available serum bicarbonate measurements in the twelve months before the index event were collected. Lung function, serum electrolytes, CRP and BMI at the time of each bicarbonate measurement were also collected.

All arterial or arterialised capillary blood gases collected in the year preceding the index event were also recorded. ABGs and CBGs were interpreted using an automated reporting tool developed as part of this thesis (Section 4.3.2 and Appendix B).

6.3.3 Statistical analysis

No sample size calculation was required for this study. Data collection was limited to a time period identified *a priori*, starting from the original availability of electronic patient records (2007), and ending to the closest round number of years at the time of analysis.

Normal distribution of measured variables was assessed by visual inspection and using the Shapiro-Wilks test. Results are expressed as number (percentage), means (standard deviation), when normally distributed, or median (IQR, 25^{th}-75^{th} percentile) when not normally distributed.

In Part 1 of the study, unpaired t-test for parametrical data or Mann-Whitney test for non-parametric data were used to compare each variable in the two cohorts (cases and matched cohort).

Correlation analysis of variable pairs was performed by visual inspection of the scatter plot, and by using the Pearson correlation or the Spearman-rho coefficient, depending on the distribution of the variables, as appropriate. A p-value <0.05 was considered statistically significant.

In Part 2 of the study, the trends of continuous variables (lung function, CRP and serum bicarbonate) were inspected over a period of 12 months approaching the date of death,

for each patient among the case cohort. To do so, data was grouped by subject, aligned at the date of death (month 0), and the time to index event was rounded to the closest month (1 month = 30 days).

Univariate Mixed Models were used to model the behaviour of continuous variables over time, compensating for confounding factors. Confounding factors modelled included a constant subject specific element (as a fixed random factor), selected continuous variables depending on the analysis (as covariates), and selected categorical variables depending on the analysis (as factors).

Each measure time (in months) was modelled as a categorical variable rather than a covariate to avoid any assumption of linearity in the trend over time. In some analyses, time was then linearised and treated as a co-variate to present an average rate of increase per month.

Results are expressed as marginal means (standard deviation). A p-value <0.05 was considered statistically significant, both when evaluating a whole model, a covariant, or when comparing marginal means at different values of categorical confounding factors.

All analyses were performed with IBM SPSS v26.

6.3.4 Ethics

This study was discussed with the LTHT R&I and was deemed exempt of needing approval by REC and HRA. The study was approved by the local R&I (RM18/110634, approval date 20/7/2018) and the Cardio-Respiratory CSU at Leeds Teaching Hospital NHS Trust. All patients had previously consented for their clinical data to be used for research purposes.

6.4 Results

6.4.1 Study population

During the study period (February 2007-February 2019), 948 patients (including both adults and children) were under follow-up at the Leeds Regional Centre for Cystic Fibrosis.

During the time span years considered for the study, 136 patients died, 133 of whom died as adults. Ninety-two adults died before receiving a lung transplant. Among these, 56 had serum bicarbonate measured at least once in the two months prior to their death, and were therefore included in the study (Figure 6.1).

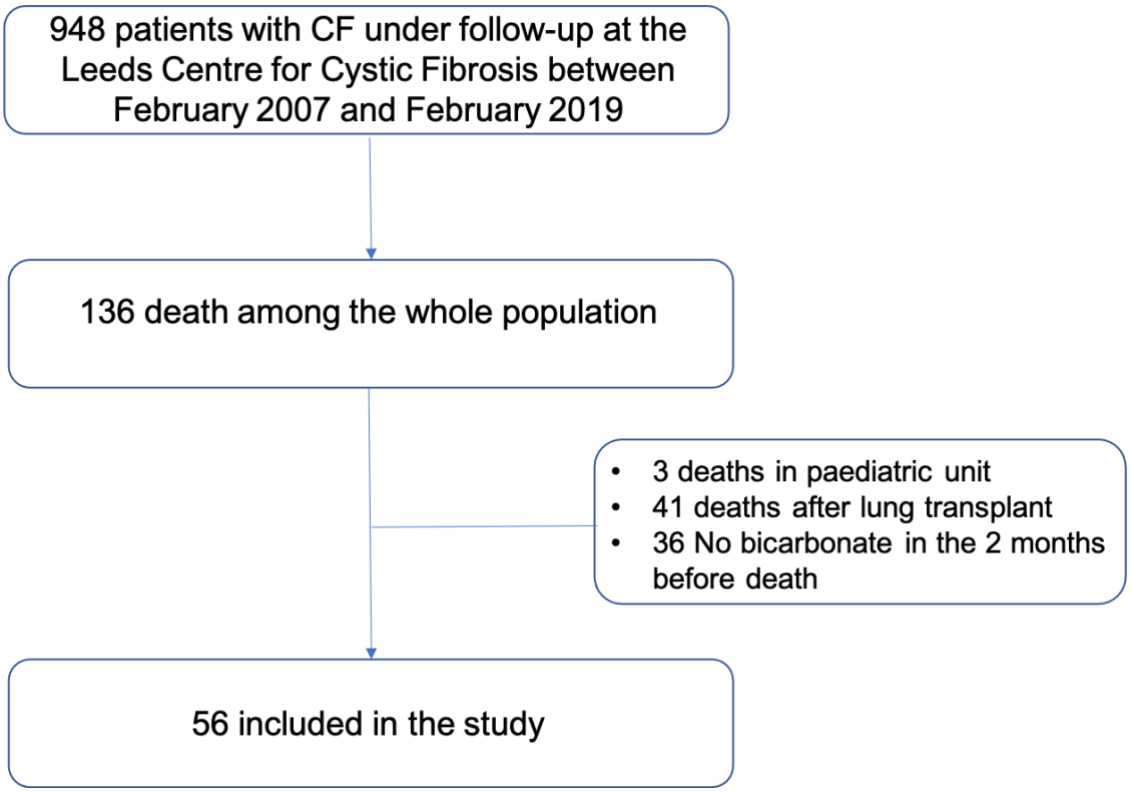

Figure 6.1 Flow-chart of participants inclusion to the study.

A 2:1 matched cohort (two controls for each case subject, total n=112) was identified based on the criteria described above. Table 6.2 summarises the demographics and baseline characteristics of the studied population (cases), as well as of the matched cohort.

The case cohort consisted of 56 subjects, and was female predominant (32 female, 57.1%). Median age at death was 30 (24-36) years of age. The matched control cohort consisted in 112 subjects, with the same distribution of sex (64 female, 57.1%) and age [30 (25-36)] by construction.

Distribution of genotypes was similar across the two cohorts (case vs control – F508del homozygous: 71.4% vs 59.8%; F508del heterozygous: 19.6% vs 28.6%; other 8.9% vs 11.6%, p=0.332). There was a higher prevalence of CFRD (48.2% vs 30.4%, p=0.023) and liver cirrhosis (12.5% vs 2.7%) among cases compared to controls, but not of liver disease (55.4% vs 69.6%). In addition, patients in the case cohort had been previously treated for pneumothorax more often (25% vs 1.8%, p<0.001).

More individuals in the case cohort were on the active list for lung transplantation or were under consideration for lung transplantation.

There was a higher prevalence of chronic *Pseudomonas aeruginosa* colonisation among the cases (91.1% vs 56.3%, p<0.001) compared to control. Chronic infections with *Achromobacter xylosoxidans* (7.1% vs 6.3%, p=0.825), *Stenotrophomonas maltophilia*

(16.1% vs 18.8, *p*=0.702), non-tubercolosis mycobacteria (16.1% vs 11.6% *p*=0.419) and BCC (5.4% vs 5.4%) were similar among the two groups. MSSA was more frequently isolated in the matched cohort (5.4% vs 25.6%, *p*<0.001).

Table 6.2 Baseline and demographics of the cases and matched cohort. PTX, pneumothorax; NTM, non-tubercolosis mycobacteria, MSSA, methicillin-sensitive *S.aureus*; LTOT, long-term oxygen therapy; NIV, non-invasive ventilation.

	Cases (n=56)	Control (n=112)	*p*
No Subjects	56	112	
Age at index event	30 (24-36)	30 (25-36)	0.647
Female sex, n (%)	32 (57.1%)	64 (57.1%)	1
Transplant			
Active list	11 (19.6%)	0 (0%)	**<0.001**
Under assessment	18 (32.6%)	6 (5.4%)	**<0.001**
Not yet considered	18 (32.6%)	106 (94.6%)	**<0.001**
Rejected	9 (15.2%)	0 (0%)	**<0.001**
Genotype			0.332
F508 homozygous	40 (71.4%)	67 (59.8%)	
F508 Heterozygous	11 (19.6%)	32 (28.6%)	
Other	5 (8.9%)	13 (11.6%)	
Comorbidities			
Diabetes	27 (48.2%)	34 (30.4%)	**0.023**
Liver disease/cirrhosis	38 (67.8%)	81 (72.3%)	**0.044**
Pancreatic insufficiency	55 (98.2%)	102 (91.1%)	0.078
Previous PTX	14 (25%)	2 (1.8%)	<0.001
Microbiology status			
P.aeruginosa	51 (91.1%)	63 (56.3%)	**<0.001**
A. xylosoxidans	4 (7.1%)	7 (6.3%)	0.825
B. cepacia complex	3 (5.4%)	6 (5.4%)	1
S. maltophilia	9 (16.1%)	21 (18.8%)	0.702
NTM	9 (16.1%)	13 (11.6%)	0.419
MSSA	3 (5.4%)	32 (25.6%)	<**0.001**
LTOT	45 (80.4%)	4 (3.6%)	**<0.001**
NIV	35 (62.5%)	1 (0.6%)	**<0.001**

6.4.2 Study Part 1: Serum Bicarbonate at the time of death

6.4.2.1 Index event

Table 6.3 summarises the subjects' characteristics closest to the date of the index event.

Subjects in the case group had a lower BMI [18.3 (16.7-19.9) kg/m^2 vs 22.6 (20.3-24.8) kg/m^2, *p* <0.001] and higher CRP [121 (66-189) mg/L vs 5 (5-13.8) mg/L, *p*<0.001] compared to the matched cohort.

Lung function as measured by FEV_1 was significantly lower in the cases cohort [20 (15-27) % vs 61 (41-77)%, *p* <0.001].

A high proportion of the case cohort subjects had an arterial or arterialised capillary blood gas collected at the same time as the serum bicarbonate [47 (83.9%)]. This was not the case in the control cohort, where only very few subjects had ABG data available close to

the index event [9 (8%)]. Therefore, analysis of ABG data was only performed for the case cohort.

Arterial blood gases were automatically interpreted, as discussed above, revealing that the vast majority of subject showed compensated or acute and/or partially compensated respiratory acidosis (39.1% and 22.7%, respectively). Metabolic alkalosis was also frequently observed (21.9%), and was less frequently fully compensated (10%). Metabolic acidosis was not observed in this cohort.

A higher proportion of patients in the case cohort was on IV antibiotics at the time of the index event (83.9% vs 51.8%, p<0.001) compared to the control cohort. No differences in the distribution of use of beta-lactams antibiotics was observed among the two cohorts, but tobramycin was used more frequently among controls whereas a trend towards a more frequent use of colomycin was noted among the cases (Table 6.3).

Table 6.3 Characteristics of the two cohorts at the index event. *indicates analysis on all ABG results (n=110)

	Cases (n=56)	**Control (n=112)**	***p***
No Subjects	56	112	
BMI	18.3 (16.7-19.9)	22.6 (20.3-24.9)	**<0.001**
C-reactive protein	121 (66-189)	5 (5-13.8)	**<0.001**
FEV_1, %	20 (15-27)	61 (41-77)	**<0.001**
IV antibiotics, Y			
Meropenem	14 (25%)	19 (17%)	0.217
Aztreonam	9 (16.1%	10 (8.9%)	0.168
Ceftazidime	12 (21.4%)	16 (14.3%)	0.243
Piperacillin/Tazobactam	8 (14.3%)	7 (6.3%)	0.085
Tobramycin	5 (8.9%)	33 (29.5%)	0.003
Colomycin	13 (23.2%)	14 (12.5%)	0.075
ABG, Y*	47 (83.9%)	9 (8%)	
pH	7.36 (0.088)		
pCO_2, kPa	9.38 (4.24)		
pO_2, kPa	8.13 (2.25)		
HCO_3^-, mmol/L	40.3 (13.6)		
BE	14.7 (13.68)		
ABG Results*			
Compensated respiratory acidosi	43 (39.1%)		
Respiratory acidosis	25 (22.7%)		
Metabolic alkalosis	24 (21.9%)		
Compensated metabolic alkalosis	11 (10%)		
Normal ABG	5 (4.5%)		
Mixed disorder	2 (1.8%)		

6.4.2.2 Serum bicarbonate at the index event

The concentration of serum bicarbonate was significantly higher in the case cohort compared to reference values. Furthermore, its concentration was more elevated in the case cohort compared to the control cohort [38 (33-41) mmol/L vs 27 (25-29) mmol/L, p<0.001] (Figure **6.2**).

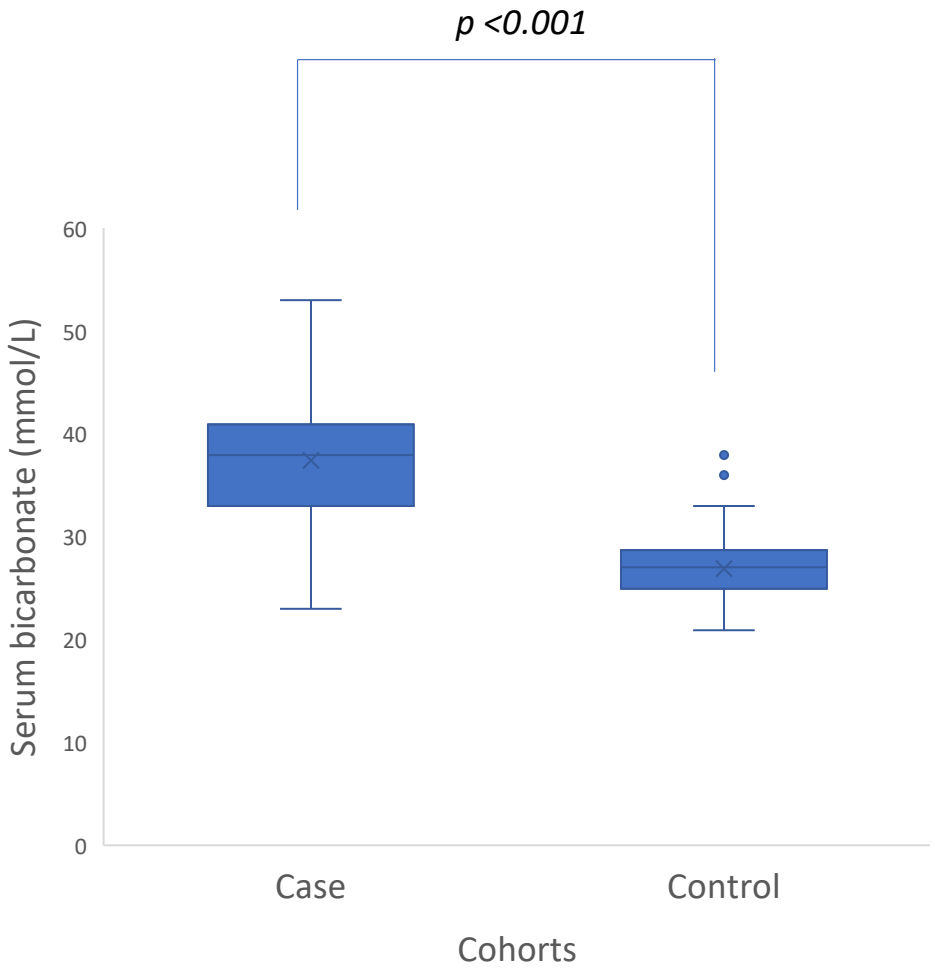

Figure 6.2 Serum bicarbonate concentration in the two cohorts. Subjects in the case cohort had higher serum bicarbonate concentration before death than a sex- and age-matched cohort not nearing death.

No statistically significant correlation was observed between age at the index event and serum bicarbonate concentration in either cohort (Figure **6.3**).

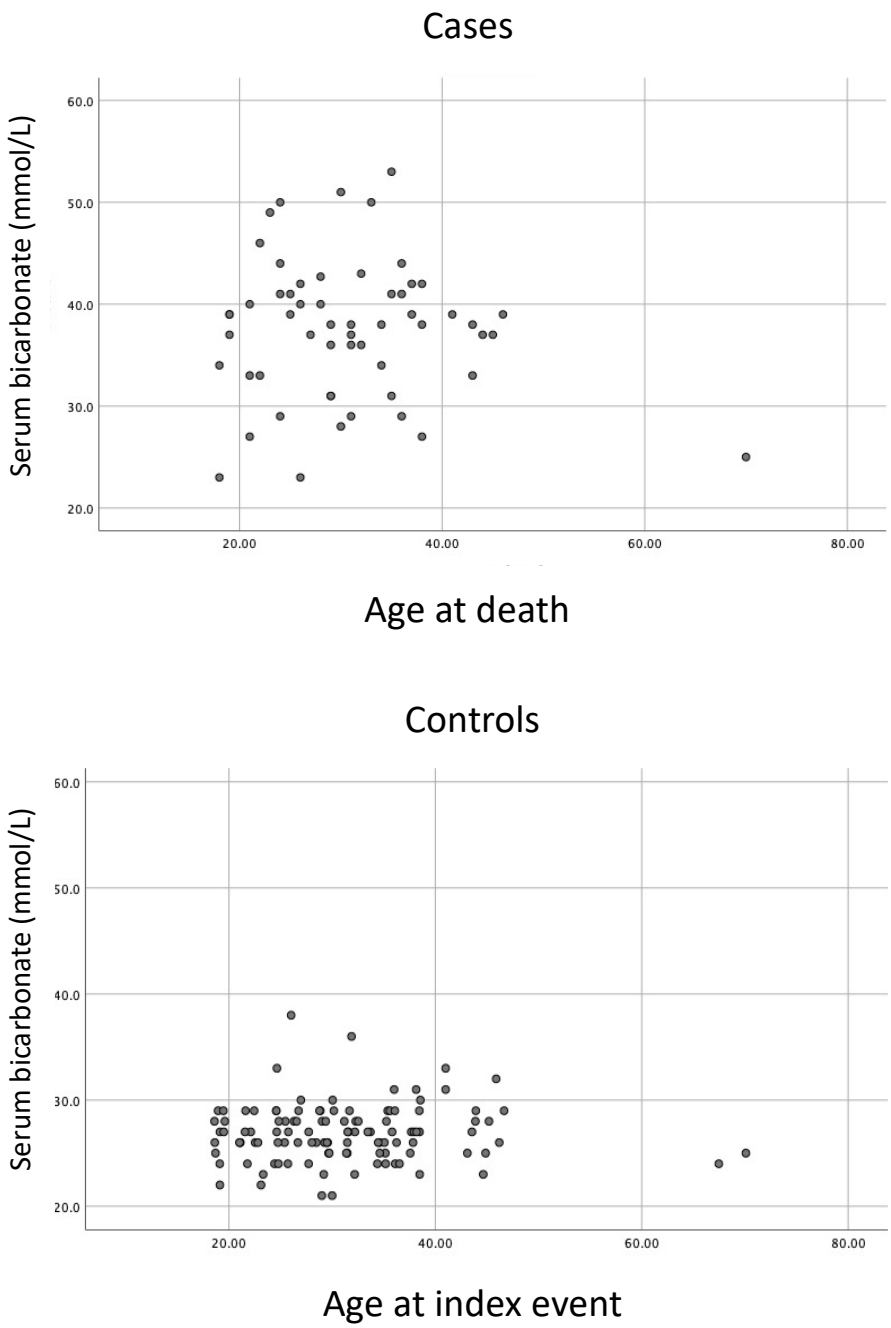

Figure 6.3 Correlation between serum bicarbonate and age. The scatter plot on the top panel represents the cases cohort and the one on the bottom the matched cohort. No correlation was noted between serum bicarbonate and age in either cohort.

Visual analysis of the scatter plots did not indicate any correlation between serum bicarbonate concentration and age at death or index event. Formal analysis of correlation confirmed this showing a Spearman-rho coefficient of -0.011 for the cases (p=0.937) and of 0.064 (p=0.505) for the controls.

No correlation was observed with BM (r=-0.061, p=0.095) and CRP (r=0.04, p=0.259). However, a significant correlation between serum bicarbonate concentration and FEV_1 was observed (r = -0.373, p=0.005) (Figure **6.4**).

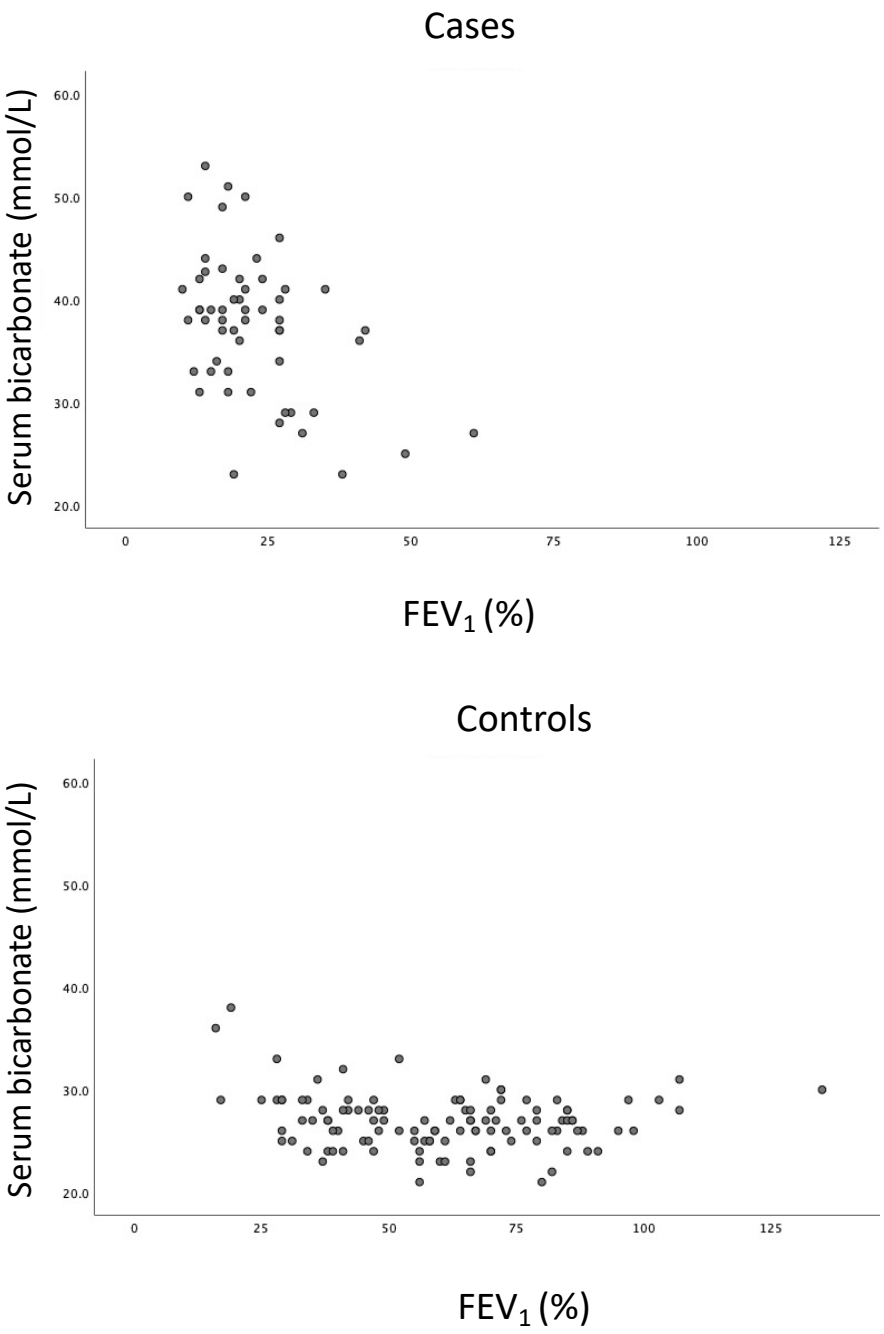

Figure 6.4 Correlation between serum bicarbonate and lung function. The scatter plot on the top panel represents the cases cohort and the one on the bottom the matched cohort. A negative correlation was observed in both cases with higher serum bicarbonate concentration at lower lung function levels.

A trend towards higher serum bicarbonate concentration in female subjects was observed in the cases cohort [39 (36-42) vs 37 (31-39), *p*=0.108]. In the control cohort, conversely, serum bicarbonates were higher among males [26 (24-28) vs 27.5 (26-29), *p*=0.02) (Figure **6.5**).

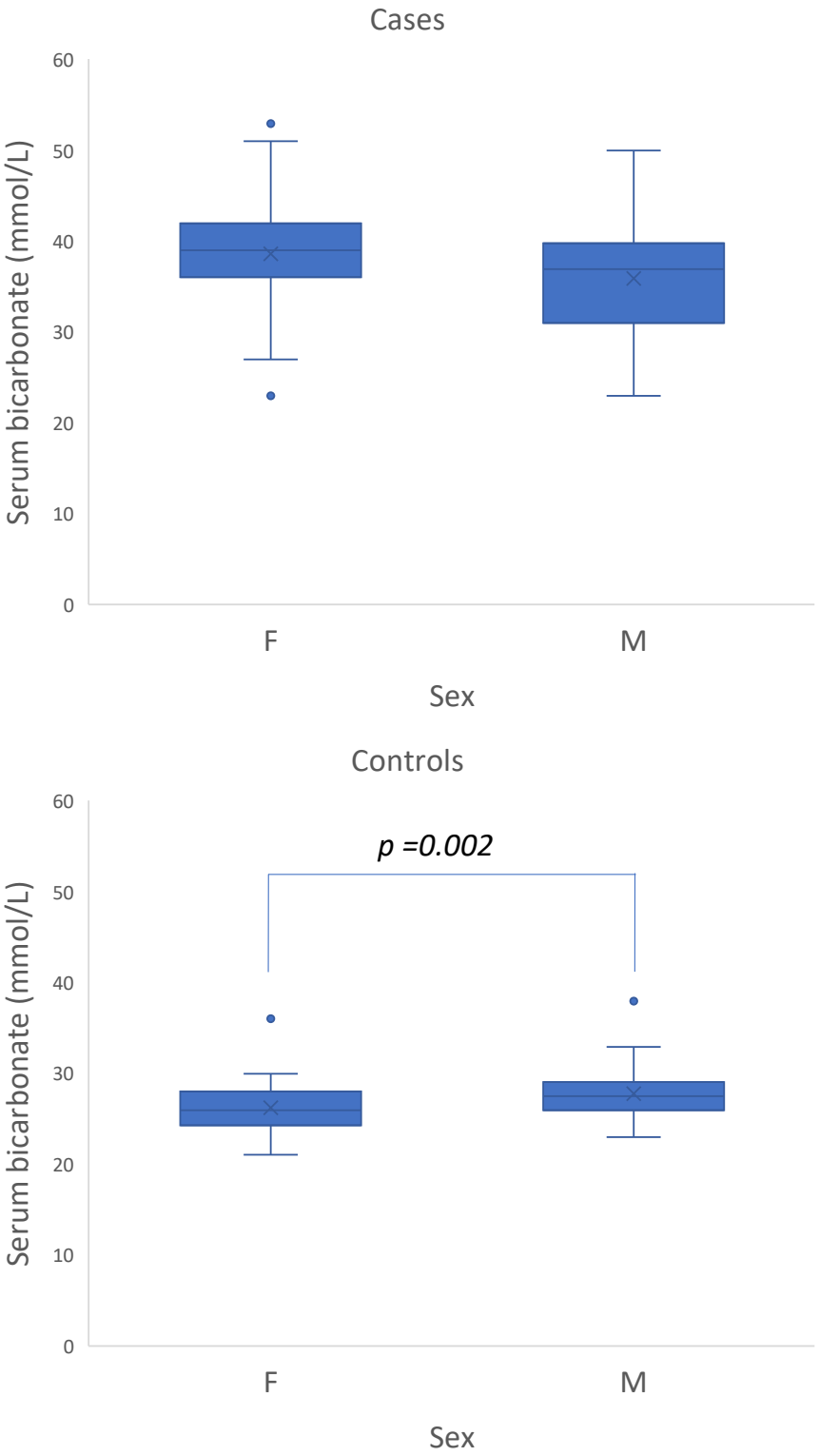

Figure 6.5 Serum bicarbonate at index event by cohort and by sex. Serum bicarbonate concentration tended to be higher among female before death, but is higher at the index event among males in the matched cohort.

Table 6.4 and Table 6.5 show the baseline characteristics of the case and control cohorts by sex, respectively.

Table 6.4 Baseline characteristics and demographics of the case cohort by sex BMI, body mass index; CRP, C-reactive protein; ABG, arterial blood gas; NIV, noninvasive ventilation, LTOT, long-term oxygen therapy. Results are expressed as number (%) or median (IQR).

	Female	**Male**	***p***
N	32	24	
Age at death	31 (11.75)	29 (10.75)	0.797
BMI	17.9 (2.79)	19.2 (4.65)	0.333
CRP	120 (146)	120 (123)	0.585
FEV_1, %	21 (10)	19 (14)	0.412
Transplant			0.078
Active list	7 (21.9%)	4 (16.7%)	
Under assessment	6 (18.8%)	4 (16.7%)	
Not yet considered	14 (43.8%)	12(50%)	
Rejected	5 (15.6%)	4 (16.7%)	
Genotype			0.109
F508/F508	26 (81.3%)	14 (58.3%)	
F508/-	5 (15.6%)	6 (25%)	
Other	1 (3.1%)	4 (16.7%)	
Comorbidities			
Diabetes	15 (46.9%)	12 (50%)	0.395
Liver disease/cirrhosis	18 (56.3%)	20 (83.3%)	0.054
Pancreatic insufficiency	32 (100%)	23 (95.8%)	0.078
Previous PTX	6 (18.8%)	8 (33.3%)	0.212
Microbiology status			
P.aeruginosa	29 (90.6%)	22 (91.7%)	0.395
A. xylosoxidans	2 (6.3%)	2 (8.3%)	0.825
B. cepacia complex	1 (3,1%)	2 (8.3%)	0.392
S. maltophilia	6 (18.8%)	3 (12.5%)	0.529
NTM	4 (12.5%)	5 (20.8%)	0.401
MSSA	2 (6.3%)	1 (4.2%)	0.732
IV antibiotics, Y			
Meropenem	6 (18.8%)	8 (33.3%)	0.212
Aztreonam	8 (25%)	1 (4.2%)	**0.036**
Ceftazidime	4 (12.5%)	8 (33.3%)	0.06
Piperacillin/Tazobactam	7 (21.9%)	1 (4.2%)	0.06
Tobramycin	1 (3.1%)	4 (16.7%)	0.079
Colomycin	7 (21.9%)	6 (25%)	0.784
ABG			
pH	7.37 (0.093)	7.355 (0.093)	0.568
pCO_2, kPa	8.82 (4.33)	10.4 (4.5)	0.607
pO_2, kPa	7.75 (1.93)	8.41 (2.04)	0.125
HCO_3^-, mmol/L	38.3 (10.7)	44.64 (16.8)	0.543
BE	13.6 (10.81)	20.35 (15.53)	0.496
NIV, Y	1 (3.1%)	3 (12.5%)	0.192
LTOT	21 (65.6%)	14 (58.3%)	0.475

Among the cases, demographics, lung function, inflammatory markers and comorbidities were similar comparing male and female subjects, with the exception of prevalence of liver disease. A lower proportion of female patients had liver disease compared to male

patients (56.3% vs 83.3%, p=0.054). No differences in arterial blood gas analyses results were observed.

A similar proportion of male and female patients were on antibiotics, and in the majority of cases patients were on a combination of beta-lactams and tobramycin or colomycin. No differences in the use of meropenem or colomycin were observed among male and female patients, but more female patients were aztreonam.

In the matched cohort (Table 6.5), there were no difference in demographics, comorbidities, lung function and inflammatory markers at the index event comparing males and females. The use of antibiotics was similar across the two cohorts.

Table 6.5 Baseline characteristics and demographics of the matched cohort by sex. BMI, body mass index; CRP, C-reactive protein. Results are expressed as number (%) or median (IQR).

	Female	**Male**	***p***
N	64	48	
Age at index event	31.4 (11.12)	29.6 (10.9)	0.647
BMI	22.1 (5.55)	22.9 (3.86)	0.692
CRP	6.1 (9.2)	5 (7.8)	0.072
FEV_1, %	59 (34)	63 (40)	0.988
Transplant			0.716
Active list	0 (0%)	0 (0%)	
Under assessment	3 (4.7%)	3 (6.3%)	
Not yet considered	61 (95.3%)	45 (93.7%)	
Rejected	0 (0%)	0 (0%)	
Genotype			0.672
F508/F508	36 (56.3%)	31 (64.3%)	
F508/-	20 (31.3%)	12 (25%)	
Other	8 (12.5%)	5 (10.4%)	
Comorbidities			
Diabetes	23 (35.9%)	11 (22.9%)	0.138
Liver disease/cirrhosis	45 (70.3%)	36 (75%)	0.182
Pancreatic insufficiency	58 (90.6%)	44 (91.7%)	0.848
Previous PTX	(3.1%)	0 (0%)	0.506
Microbiology status			
P.aeruginosa	39 (60.9%)	24 (50%)	0.512
A. xylosoxidans	4 (3.1%)	3 (6.3%)	0.507
B. cepacia complex	2 (3,1%)	3 (8.3%)	0.392
S. maltophilia	14 (21.9%)	7 (14.6%)	0.408
NTM	8 (12.5%)	5 (10.4%)	0.733
MSSA	17 (26.6%)	15 (31.3%)	0.587
IV antibiotics, Y			
Meropenem	14 (21.9%)	5 (10.4%)	0.110
Aztreonam	5 (7.8%)	5 (10.4%)	0.632
Ceftazidime	12 (18.8%)	4 (8.3%)	0.119
Piperacillin/Tazobactam	2 (3.1%)	5 (10.4%)	0.115
Tobramycin	20 (31.3%)	13 (27.1%)	0.632
Colomycin	8 (12.5%)	6 (12.5%)	1.0
NIV	0 (0%)	1 (2.1%)	
LTOT	2 (3.125%)	2 (4.16%)	

6.4.3 Study Part 2: Serum bicarbonate in the 12 months preceding death

Within the case cohort, I extended the analysis by exploring any changes in lung function, CRP and serum bicarbonate over the 12 months preceding each subject's death. To do so, I modelled each variable over time, as described in Section 6.3.3.

6.4.3.1 Lung function

There were 281 measures of lung function available in the 12 months before death for the 56 subjects included in the study. FEV_1 did drop over the time 12 months ($p<0.01$). On average FEV_1 dropped from 27.6% [95%CI (23.47-31.81)] twelve months before death, to 21.8% [95%CI (16.9-26.6)] in the month preceding death (p=0.138) (Figure 6.6)

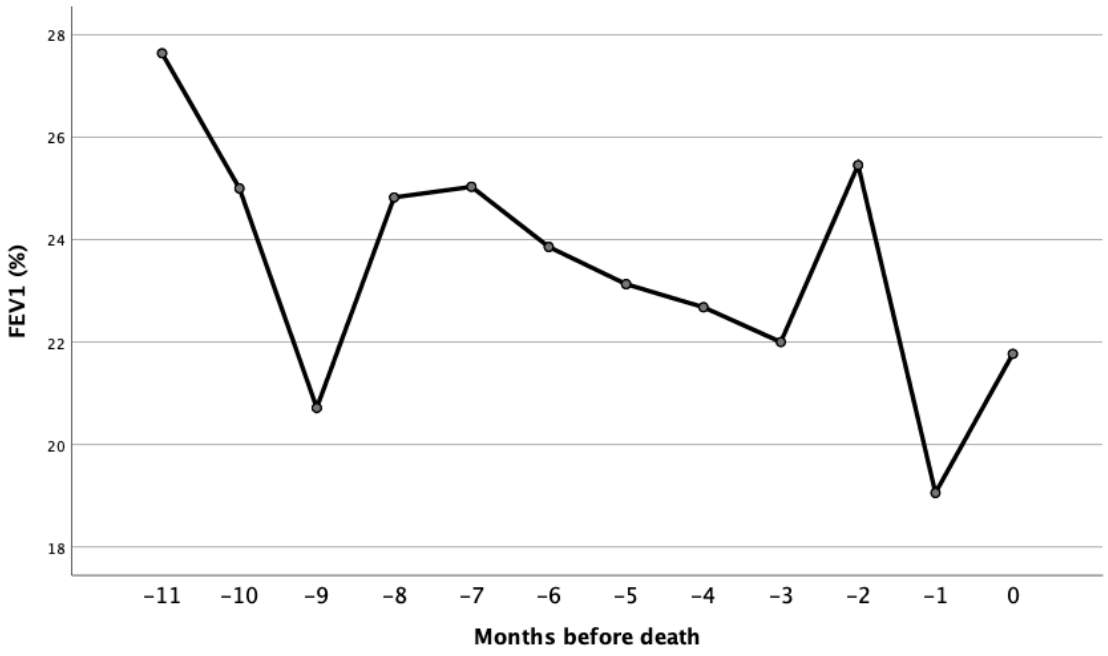

Figure 6.6 Variation of mean FEV_1 (%) in the year before death.

6.4.3.2 CRP

A total of 781 measures of CRP were available in the 12 months before death for the 56 subjects included in the study.

Overall CRP was clinically elevated, with a very limited number of normal results for any subject within the year before death. Overall an increase from 45.7 (95%CI 28-63) mg/L to 121.5 (95%CI 107.5-137.5) mg/L was observed (p <0.001). In the last four months before death, a steeper increase in average CRP was seen, with the mean CRP at -3 months being 50.8 (95% CI 39.9-61.1) mg/L (Figure 6.7).

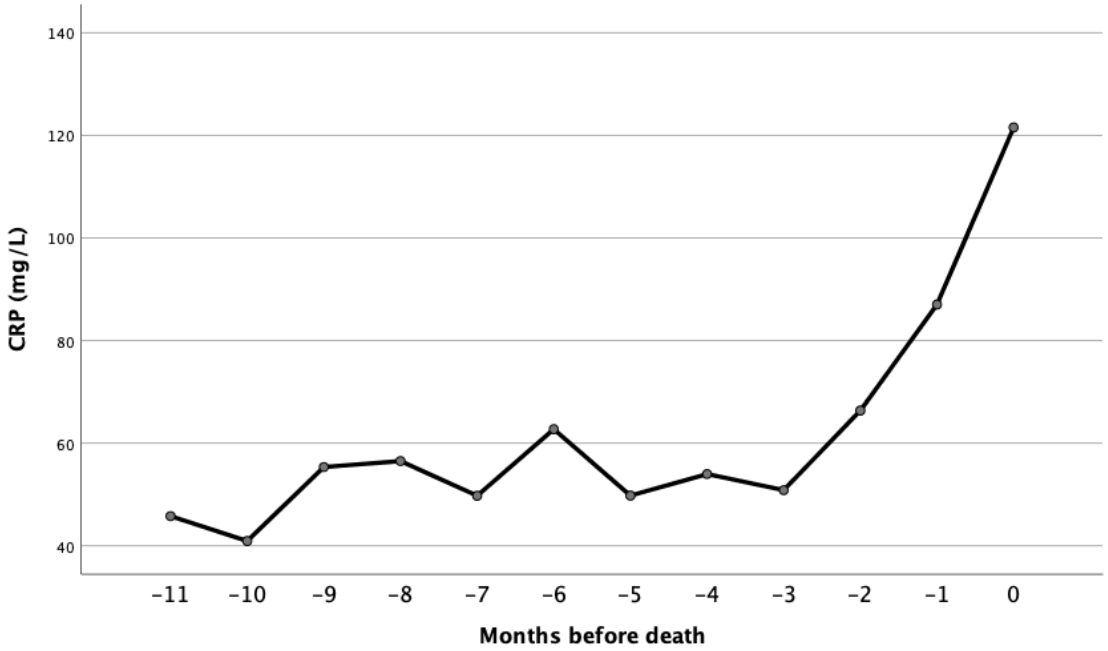

Figure 6.7 Variation in mean CRP in the year before death.

6.4.3.3 Bicarbonate

A total of 789 serum bicarbonate measures within the 12 months before death were available for the 56 subjects of the case cohort.

Serum bicarbonate was not constant over the 12 months ($p<0.01$), increasing progressively over time from a mean of 29.6 twelve months before death [95%CI (28.35-30.93)] to a mean of 37.57 [95%CI (36.43-38.72)] in the month preceding death.

The average marginal rate of increase was 0.79 mmol/L/month [95%CI (0.70-0.88)], $p<0.001$ (Figure **6.8**).

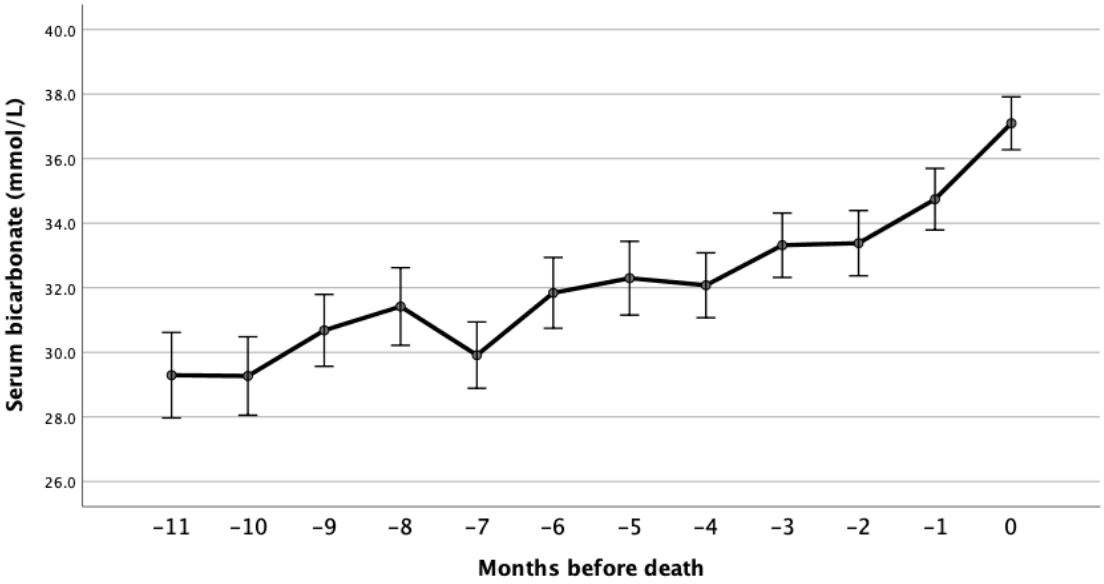

Figure 6.8 Variation of serum bicarbonate over time in the year before death. Data are presented as mean and 95% CI.

After visual inspection of the variation of bicarbonate in the 12 months preceding death, a post-hoc analysis was performed, splitting the dataset between months 0-6 and months 7-11.

Between month 11 and 6, the average rate of increase is serum bicarbonate was 0.39 mmol/L/month [95%CI 0.19-0.59]. Between months 6 and death, the average rate of increase was 1.27 mmol/L/month [95%CI 1.0-1.55].

When comparing female and male subjects, the profile of serum bicarbonate in the 12 months preceding death was different ($p<0.01$) (Figure **6.9**). A steeper increase in bicarbonate levels over time was seen in women compared to men.

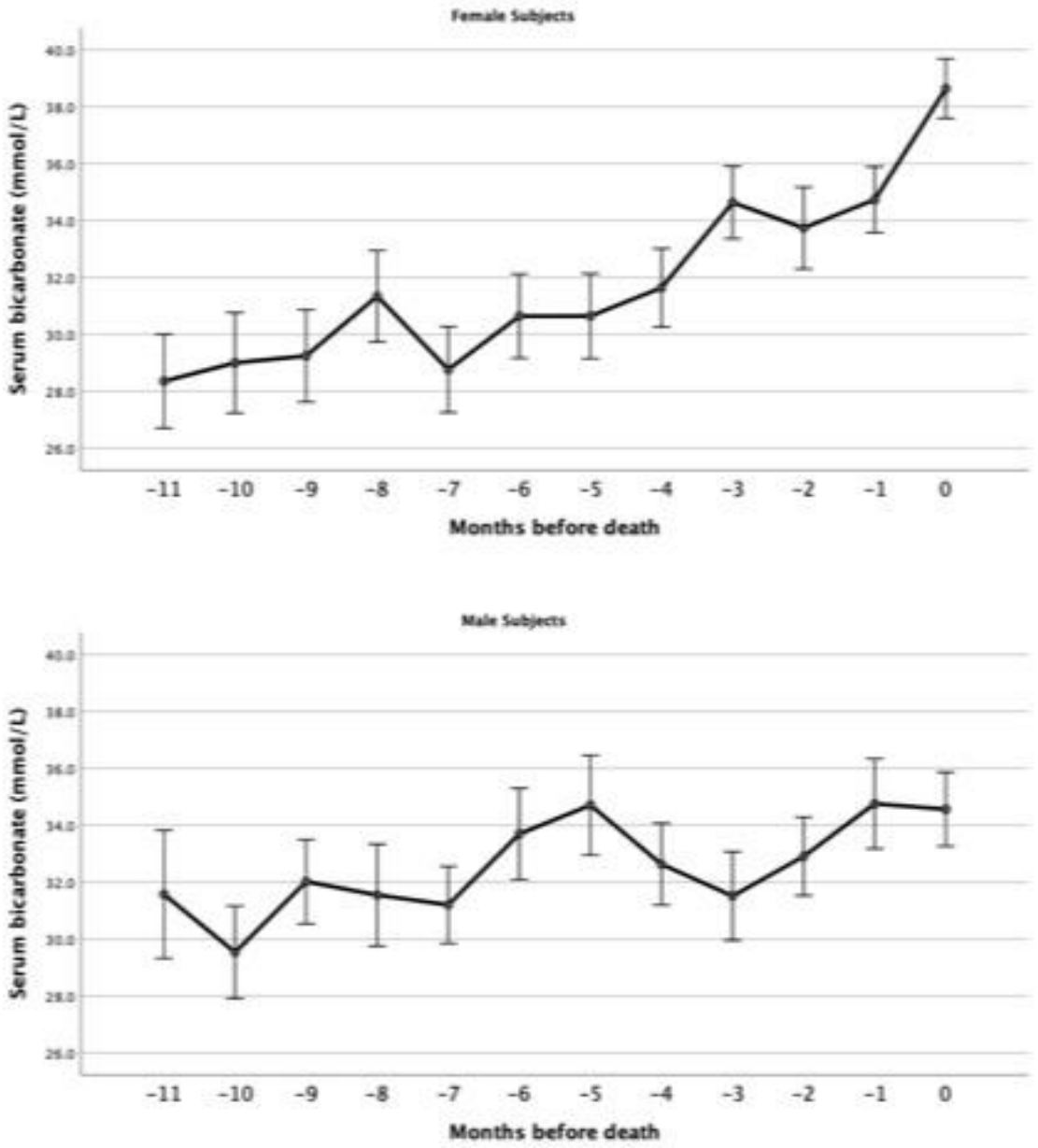

Figure 6.9 Variation of serum bicarbonate in the year before death. The top panel shows the variation of serum bicarbonate in female subjects, and the bottom panel in male subjects. Data are presented as mean and 95% CI.

When comparing subjects with different genotypes, I observed an increasing trend of bicarbonate over the 12 months preceding death regardless of genotype ($p<0.01$).

However, the average rate of increase was different, being 0.856 mmol/L/month in F508del homozygous, 0.66 mmol/L/month in F508del heterozygous, and 0.29 mmol/L/month in those with other mutations (Figure **6.10)**.

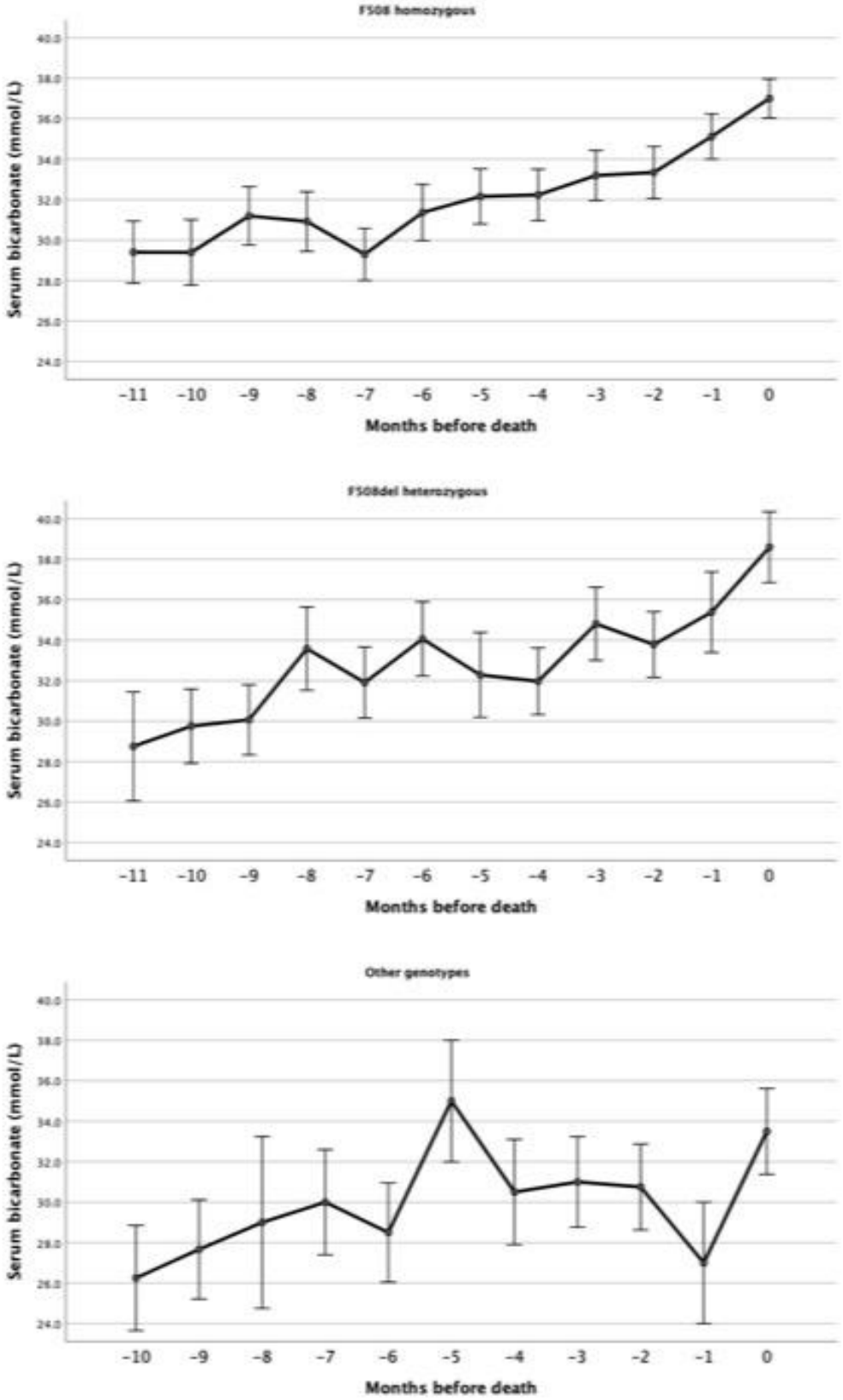

Figure 6.10 Variation of serum bicarbonate in the year before death, based on genotype. The top panel shows the trend in subjects who are F508del homozygous, the middle panel in F508del heterozygous and the bottom one those with other genotypes. Data are presented as mean and 95% CI.

No differences were observed in rate of increase depending on comorbidities, including diabetes.

Long-term oxygen therapy did not affect the rate of increase in bicarbonate, but the use of NIV did. The rate of increase in bicarbonate among subjects that did not receive NIV was 0.62 mmol/L/month [95% CI 0.52-0.71], whereas it was 0.39 mmol/L/month [95% CI 0.07-0.71] (Figure **6.11**)

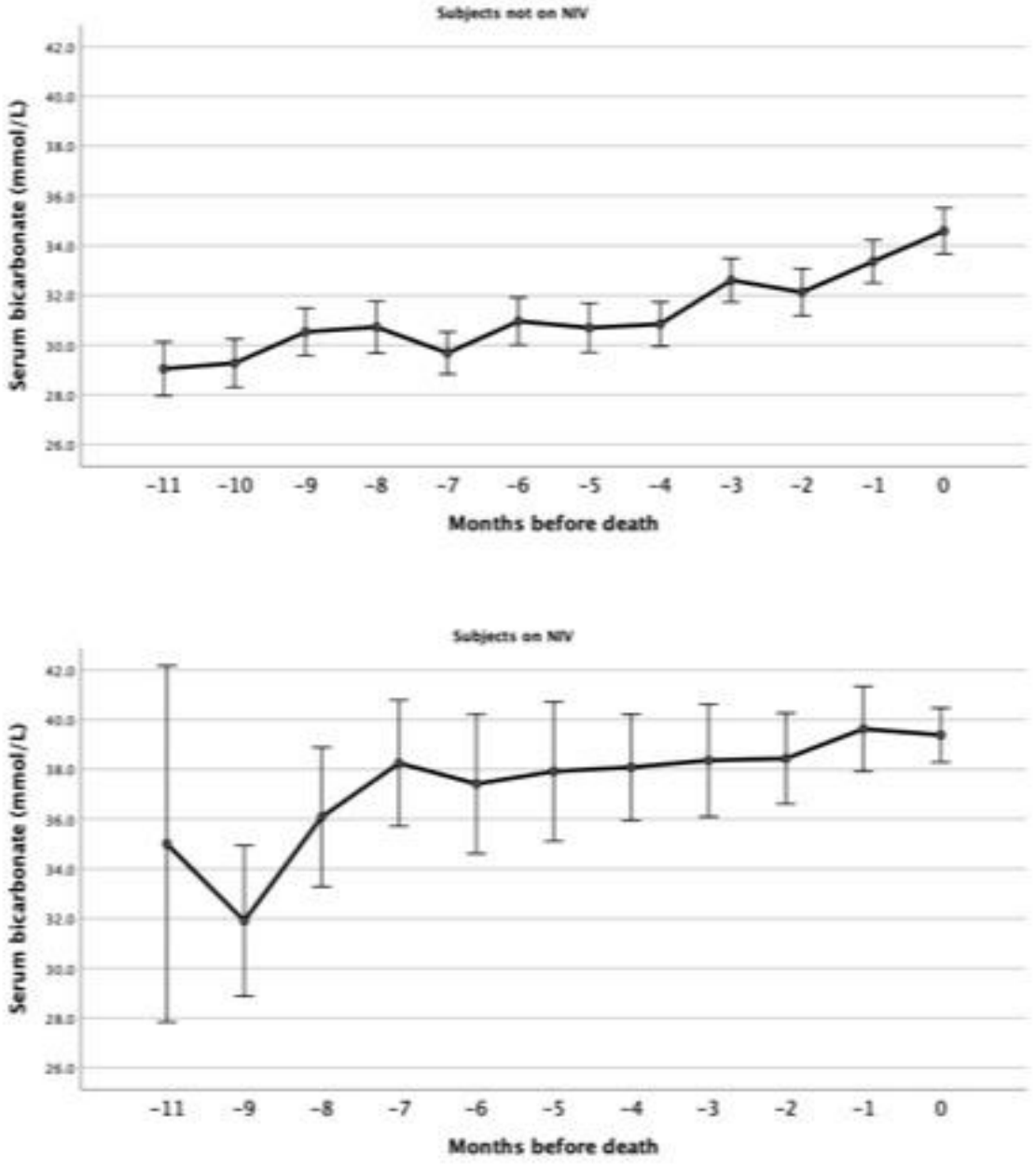

Figure 6.11 Variation of serum bicarbonate in the year before death depending on the use of NIV. Top panel: not on NIV; bottom panel: on NIV. Data are shown as mean and 95% CI.

6.5 Discussion

The level and rate of change in serum bicarbonate have been known to be good prognostic markers in several respiratory conditions, including COPD and neuromuscular diseases [274,384–386]. In these conditions, the increase in bicarbonate is secondary to episodes of hypoventilation, and can be used as a good prognostic biomarker, as it predicts respiratory failure.

I previously showed that, among people with CF, serum bicarbonate concentration is frequently increased above the upper limits of normality, particularly among patients with advanced lung disease (Chapter 5). Multiple factors appear to be associated with raised serum concentrations of bicarbonate, all of which have been previously linked to worse prognosis for individuals with CF. This suggests that serum bicarbonate has the potential to be used as a marker of severity of the condition.

To the best of my knowledge, this is the first study looking at the changes in serum bicarbonate in individuals with cystic fibrosis before death, in order to ascertain if serum levels could be useful prognostic biomarkers.

In Part 1 of this study, I showed that the concentration of serum bicarbonate is higher in people with CF who were close to death, compared to a matched cohort of CF patients who were stable.

This was undertaken by analysing the last available serum bicarbonate measure before death of all patients who died during the study period. For any patient who had a valid serum bicarbonate measure within the last two months prior to their death, I randomly identified two CF controls to create a 2:1 matched cohort of CF patients with the same age and sex characteristics of the case cohort, but not nearing their death.

Serum bicarbonate concentration was significantly increased [38 (33-41) mmol/L] in people with CF approaching death. These values were significantly higher than the controls [27 (25-29) mmol/L]. Serum bicarbonate at death was also higher than the median serum bicarbonate observed for the previous cohort described in Chapter 5 (median 27 mmol/L), which included all adults with CF who had at least one measurement of bicarbonate at a time of clinical stability.

I showed in Chapter 5 (Section 5.4.2.1) that serum bicarbonate concentration increases with age. However, there was no correlation in this study between the last available serum bicarbonate measurement before death and age at death.

No correlation was observed between the last available bicarbonate, BMI or CRP. However, prior to death, higher serum bicarbonate concentration was associated with a lower FEV_1, whereas no correlation was seen in the matched cohort. This is in keeping with what previously observed in Chapter 5 (Section 5.4.2.1) where a correlation

between serum bicarbonate and FEV_1 was shown overall, but was proved to be stronger when measurements were taken in the presence of advanced lung disease.

Interestingly, the last serum bicarbonate prior to death tended to be higher among females compared to males [F = 39 (36-42) vs 37 (31-39) mmol/L], while the opposite was true in the control cohort [26 (24-28) vs 27.5 (26-29) mmol/L), confirming the results of Chapter 5.

In Part 2 of this study, I focused my attention to the trend of serum bicarbonate and other measures of clinical stability in the 12 months preceding death of the patient.

I showed that serum bicarbonate levels rise gradually (0.79 mmol/L per month) in the year prior to death. This rate of increase is significantly more pronounced in the last six months preceding death (1.27 mmol/L per month), compared to months 7-12 (0.39 mmol/L per month). Conversely, lung function tends to remain very low but stable (FEV_1 <30%) throughout the 12 months.

Similarly to serum bicarbonate, CRP was observed to gradually rise in the 12 months prior to death, with the rate becoming steeper in the 3 months before death occurred.

At 12 months before death, the mean serum concentration of bicarbonate was mildly elevated at 29.6 mmol/L, and higher than what observed in the assessment performed in clinical stability (median 27 mmol/L) in Chapter 5. This gradually grew further, reaching an average of 37.57 mmol/L in the month preceding death.

The only available study [381] in the literature exploring the levels of serum bicarbonate in people with CF at time of their referral to lung transplantation reported a variable level between 24 and 40 mmol/L. Elevated values of serum bicarbonate appeared to be equally distributed in individuals with a survival greater than two years, or lower than one year. It was therefore concluded that serum bicarbonate has a negligible role in assessing prognosis and survival on the lung transplant waiting list [381].

This contrasts with the present study which was performed on a larger cohort of patients. I found that the rate of change of bicarbonate has a clear upwards trend in the 12 months preceding death, and that this trend is even more evident in the six months approaching death. This discrepancy is most likely due to the difference in observing a trend over time (which I did in this study), rather than a punctual measure in time (which was done in the literature).

When looking at the trend of serum bicarbonate levels in the twelve months preceding death, I confirmed the results obtained on the last available bicarbonate reading. Overall bicarbonate levels, as well as rate of change, were higher among female patients with CF nearing death, compared to males. At baseline, this is reversed, with stable female patients having a lower concentration of bicarbonate compared to males.

Similarly to serum bicarbonate levels, I analysed the trend of CRP over the 12 months preceding death. While an overall raised CRP was observed among individuals across this time span, levels increase more steeply in the 3 months before death occurred. This is suggestive that out-of-control infection and inflammation are negative prognostic factors in CF, irrespective of their comorbidities and ventilatory status.

On the other hand, no significant changes in FEV_1 were noted in the year before death. This is agreement with the literature, which demonstrates that FEV_1 is a poor predictor of decline in individuals with CF and advanced lung disease particularly in the last year of life.

In this study I did not explore the mechanisms underlying the increase in bicarbonate in people with CF prior to death. In this cohort, I observed no difference in the rate of increase in serum bicarbonate concentration in individuals on long term oxygen therapy. This contrasted with non-invasive ventilation (NIV), which was associated with stabilisation of serum bicarbonate concentration.

The increase in serum bicarbonate is likely to be partially due to a response to hypercapnic respiratory failure, as previously demonstrated in COPD and OHS [274,384,387–389]. Previous studies have concluded that LTOT increases the partial pressure of oxygen in individuals with CF and can raise end-tidal CO_2 [211] while NIV can improve alveolar ventilation thereby reducing CO_2. In CF, NIV appears to stabilise rather than reduce CO_2 [262,330,339].

The lack of contemporaneous arterial blood gases, preventing us from assessing the acid-base balance and ventilation and drawing a definitive conclusion. By analysing the last blood gas prior to death (Part 1), most of the results were in keeping with hypercapnic respiratory failure, showing either compensated or acute respiratory failure (Table 6.2) and therefore supporting the hypothesis that elevated serum bicarbonate is in part due to a compensation of hypoventilation. However, a fifth of these blood gases showed compensated or acute metabolic alkalosis.

This would suggest that the increased serum bicarbonate might be in fact the *primum movens* of acid-base changes rather than a compensatory effect. Therefore, a potential role of CFTR dysfunction in the kidney with reduced or different response to an increase concentration of bicarbonate, as discussed in Chapter 2 Section 2.5.2 and further assessed in Chapter 8, cannot be excluded.

6.6 Conclusion

Serum bicarbonate is elevated in people with CF in the 12 months prior to death, compared to to sex- and age-matched CF controls.

This increase is already visible 12 months prior to death, but becomes particularly pronounced in the last 6 months before death. Monitoring the levels and trend of this anion, could provide an adjunct prognostic biomarker to prioritise patient for lung transplantation.

Further multi-centre studies are needed to assess if a similar trend is noted in individuals with CF who are on CFTR modulator therapy.

Chapter 7
Hypoxic altitude simulation test in respiratory disease: a retrospective study

7.1 Introduction

As discussed in Section 2.5, small-scale reports have highlighted that patients with CF present with metabolic alkalosis in conditions of stability at a prevalence higher than those with other respiratory conditions [215,216]. In addition, chronic respiratory acidosis is also frequently observed in patients with CF in stable conditions [218,223].

One of the hypotheses of this research (see Chapter 4, Section 4.1.1) is that acid-base disturbances, and metabolic alkalosis specifically, are more frequent in patients with CF compared to those with other respiratory conditions, not only during exacerbations but also in conditions of clinical stability. I also hypothesise that, in the absence of raised pCO_2, raised bicarbonate (or base excess) could be a biomarker of ventilatory failure and be associated with reduced ventilatory drive, similarly to what previously described for OHS [387].

The hypoxic altitude simulation test (HAST) is a readily available and widely performed test to predict the effects of altitude on a patient's gas exchanges and assess if an individual is safe to fly with or without supplemental oxygen.

To further characterise the acid-base balance status in people with CF, I analysed the differential response to HAST, in comparison to people with other respiratory conditions. Due to its routine use in clinical practice, I was able to compare a large dataset and assess the distribution of acid-base disturbances and the response to an hypoxemic stimulus, within these different patient cohorts.

7.2 Aim of the study

The aims of this study were to:

- Describe the prevalence and degree of gas exchange and acid-base abnormalities in patients with CF and to compare this to other respiratory conditions, at a time of clinical stability;
- Assess the response to the hypoxic stimulus in patients with CF and other respiratory conditions;
- Develop a predictive model of the outcome of HAST in patients with CF and other respiratory conditions, to understand the main factors driving the response to the hypoxic stimulus.

7.3 Methods

A retrospective analysis of all HASTs performed over a five-year period (February 2012-January 2017) at the Cardio-respiratory Department at Leeds Teaching Hospital NHS Trust (LTHT) was performed.

7.3.1 Study population

The database of the Cardio-respiratory Department (Cardiobase) at Leeds Teaching Hospital NHS Trust was audited to extract all the HASTs performed during the study period. The inclusion criteria for the study were:

- Age 16 years or older, and
- Background of chronic respiratory disease.

No exclusion criteria were defined for this study.

7.3.2 Data collection and measurements

Electronic medical records (EMIS for patients with CF, PPM+ for all other individuals) were searched to collate demographics and primary respiratory diagnosis. Demographics, and clinical baseline information were collected in anonymised form for each subject. In addition, best FEV_1, overnight oximetry and 6-minute walking test (6MWT) performed on the closest date within the 12 months prior the HAST were recorded, whenever available.

7.3.2.1 Hypoxic altitude simulation test

A hypoxic altitude simulation test is a readily available, cheaper alternative to the gold standard test to assess the effects of altitude on gas exchange, which needs to be performed in an hypobaric chamber. A HAST consists of a patient breathing a gas mixture containing 15% oxygen in nitrogen, simulating the maximum cabin altitude of 8000 ft [178,182,390–392].

Several methods can be used to deliver the required F_iO_2. Oxygen and nitrogen can be mixed in adequate proportions in a Douglas bag, or pre-filled cylinders can be purchased. This mixture can be administered via a mouthpiece, or via a tight-fitting mask with a non-rebreathing valve. Alternatively, a modified body pletismograph can be filled with the mixture creating a hypoxic environment. Finally, the Venturi mask method allows to deliver similar levels of gas mixture using a 40% mask with 100% nitrogen as driving gas. The air entraining dilutes the nitrogen and produces a 15% oxygen mixture. The Venturi mask method is the most widely used in clinical practice [391] as it is well tolerated and inexpensive and was the chosen method used in the laboratory at Leeds Teaching Hospital NHS Trust.

Patients are asked to breathe a 15% oxygen gas mixture for 20 minutes in order to allow for gas equilibrium, even though this is likely to be reached sooner. Arterialised capillary bloods gas (CBG) is performed at baseline and at the end of the test. Oxygen saturation is measured with a pulse-oximeter throughout the test. The test terminates at the end of the 20 minutes, or in case of desaturation or onset of symptoms. The test is considered positive (i.e. in-flight oxygen recommended) when pO_2 drops below 6.6 kPa at the end of the test.

Baseline and final blood gases were also collected as part of the data collection protocol, and analysed for the study. The automated reporting tool to interpret blood gas analysis, described in Section 4.3.2 and detailed in Appendix B, was used to interpret the baseline CBGs for the HASTs.

7.3.3 Statistical analysis

The statistical analysis of these data was performed in two phases.

A first phase of statistical analysis was conducted to evaluate the prevalence of baseline gas exchange and acid-base abnormalities in the whole population, and in the group of people with CF compared to those with other respiratory diagnoses.

A second phase of statistical analysis focussed on the development of a logistic model of the response to the hypoxic stimulus from baseline measured data. Such model was used to identify the main baseline determinants of the response to the hypoxic stimulus.

7.3.3.1 Descriptive statistics

Data were tested for normality by visual inspection and using the Shapiro-Wilks test. Data are presented as mean and SD for normally distributed data, median and IQR for data not distributed normally. Number and percentage was used to describe the frequency of distribution of acid-base status as defined by blood gas analysis interpretation.

Parametric and non-parametric analyses were conducted as appropriate. The chi-square test was performed to assess different distribution in prevalence of acid-base abnormalities.

Further, four groups were identified in each cohort (CF and non-CF), based serum bicarbonate and partial pressure of carbon dioxide:

- Normal daytime pCO_2 and bicarbonate
- Normal daytime pCO_2 and raised bicarbonate
- Elevated daytime pCO_2
- All others.

Differences between groups were assessed using ANOVA with post-hoc assessment of any significant intergroup differences using Duncan post-hoc comparison. Any non-normally distributed data were first rendered normal via a logarithmic transformation, after which the ANOVA test was performed.

7.3.3.2 Model development

The development of a model of response to the hypoxemic stimulus was conducted following the TRIPOD statement and results are reported following TRIPOD checklist (Appendix D) which provides state-of-the-art guidance for the development of predictive diagnostic tests.

A binary logistic model was selected to predict the binary outcome of each HAST from a combination of baseline physiological variables and characteristics, including both continuous and categorical variables.

For the development and validation of the model, two cohorts were identified *a priori* by partitioning the dataset based on when each HAST was conducted: a “training” group, which included one test per patient performed between February 1, 2012 and July 31, 2016 (66% of tests, or approximately two thirds of the total available data), and a “validation” group, including all other tests (34% tests, or approximately one third of the total available data). If more than one test was available for a patient within the pre-defined time period for the training group, only the first one was included and all the subsequent ones were used as part of the validation group. This was in accordance to the Type 2b validation according to TRIPOD statement and avoids the over-representation of subjects with multiple HASTs conducted in the ”training” time window.

Within the training set, differences in baseline variables between positive and negative HASTs were assessed using univariate analysis: one-way ANOVA for parametric data, Kruskal-Wallis for non-parametric data and Chi-square for categorical variables.

Continuous variables which were significantly different were plotted on Receiver Operating Characteristic (ROC) curves to test the hypothesis that these variables could be good univariate predictors of HAST results. This univariate analysis was used to select all the variables that would be used to develop a multivariate predictive model of HAST results.

Variables, selected from the univariate analysis, were entered in the binary logistic regression analysis using a backward conditional approach. In addition to continuous variables, categorical baseline variables where also entered in the binary logistic regression and used as indicators. During model building, the criterion for selecting a variable as a predictor was $p<0.05$, with the criterion for rejection being $p>0.1$. The goodness of fit of the final model was evaluated using the Hosmer-Lemshow test, and models with a $p<0.05$ were rejected.

Predicted probabilities resulting from each of the models were plotted on ROC curves against positive HAST outcomes. ROC AUCs were compared, to assess if any difference between the discriminatory powers of the models was observed, and the area under their curve was used to select the best multivariate model [393]. The same analyses were also conducted against the best univariate model, in order to assess whether a multivariate model had indeed a higher discriminatory power to predict the output of the HAST.

Starting from the output of the resulting model, two cut-off values were determined to convert the predicted probability of a negative HAST (the output of the binary logistic model) into one of three results:

1. Prediction of a negative HAST result (predicted probability > first cut-off value);
2. Prediction of a positive HAST result (predicted probability < second cut-off value);
3. Indeterminate (any other predicted probability).

These cut-off values convert the continuous probability result of the predictor into a categorical prediction. If the model proved accurate, the first two outcomes would represent cases in which a HAST would no longer be necessary, as its result could be predicted from baseline data.

Cut-off values were chosen at 95% and 97.5% specificity to maximise the correct identification of patients with negative and positive HAST. The traditional approach of compromising between sensitivity and specificity using the Youden-J coefficient was considered not appropriate in this context as it would not be helpful in identifying either outcome with accuracy, and would be associated with a high false positive rate. This concluded the development of the model.

The model was validated on the second set of data (validation cohort). The multivariate models were computed on the validation set, and new ROC curves were generated. The ROC AUC of the validation set was compared with that of the derivation set, to verify the consistency of the results.

IBM SPSS statistics version 26 (IBM Corp, Armonk, NY, USA) was used for all the analyses.

7.3.4 Ethical approval

This study was discussed with LTHT R&I and deemed exempted from NHS Research Ethics Committee and Health Research Authority approvals.

It received cardio-respiratory CSU approval and was approved by the local R&I (RM17/97475).

7.4 Results

7.4.1 Study population

Over the study period (February 2012-January 2017), 365 patients underwent a total of 519 hypoxic challenge tests (range 1-6 per patient), with a trend towards an increase number of HAST in more recent years (Figure 7.1).

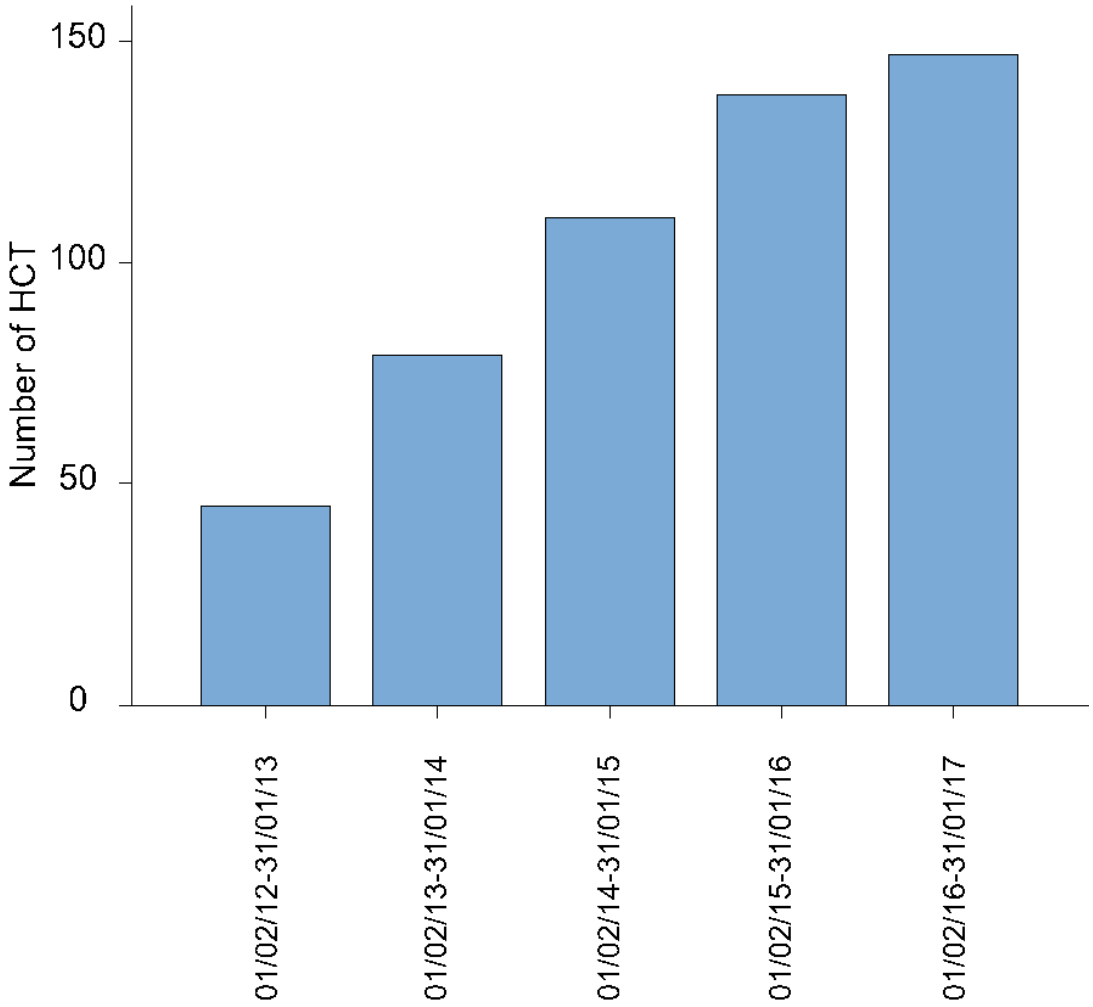

Figure 7.1 Number of HAST performed each year in the cardio-respiratory department at Leeds Teaching Hospital NHS Trust.

One-hundred and twenty-four subjects had CF, representing the 33.9% of the whole population.

Table 7.1 shows the baseline characteristics of the whole population, subjects with CF and those with other respiratory diagnoses. Subjects with CF were younger, had a lower BMI and had a lower prevalence of cardiovascular comorbidities.

Table 7.1 Baseline characteristics for the whole population and the CF cohort. *indicates analysis completed on the whole population of 365 patients (124 CF and 241 non-CF); **indicates analysis completed on the population considering each individual test (total 519, CF 225 and non-CF 294).BMI, body mass index; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; NMD, neuromuscular disorders; CBG, arterialised capillary blood gas. Results are expressed as number (%) or median (IQR).

Baseline characteristics of population	Whole population (n=365)	CF cohort (n=124)	Non-CF cohort (n=241)	*p*
Male gender, n(%)*	181 (49.6%)	64 (51.6%)	117 (48.5%)	0.579
Age at test in years**	51.6 [29-69]	28.5 [22.6-34]	67.6 [60.2-72.9]	**<0.001**
BMI, (kg/m^2)**	24.7 [21.6-28.7]	22.2 [20.6-24.3]	27.4 [24-31.9]	**<0.001**
Ethnicity*				0.198
Caucasian	331 (90.7%)	117 (94.4%)	214 (88.8%)	
Asian	29 (7.9%)	7 (5.6%)	22 (9.1%)	
Black	5 (1.4%)	-	5 (2.1%)	
Diagnosis*				**<0.001**
CF	124 (34%)	124 (100%)	-	
COPD	75 (20.5%)	-	75 (31.1%)	
ILD	86 (23.6%)	-	86 (35.6%)	
NMD	16 (4.4%)	-	16 (6.5%)	
Other	65 (26.9%)	-	65 (26.8%)	
Baseline CBG**				
pH	7.43 [7.41-7.46]	7.44 [7.42-7.46]	7.43 [7.41-7.46]	**0.026**
pCO_2, kPa	5.1 [4.7-5.5]	4.9 [4.7-5.3]	5.2 [4.7-5.7]	**0.001**
pO_2, kPa	9.3 [8.3-10.4]	10.0 [9.2-10.7]	8.7 [7.7-9.7]	**<0.001**
HCO_3^-, mmol/L	25.7 [23.5-27.8]	25.6 [23.5-27.2]	25.9 [23.7-28.5]	0.097
BE	1.7 [-0.5-3.6]	1.65 [-0.5-3.1]	1.7 [-0.6-4.2]	0.161
SO_2, %	94 [92-96]	95 [94-96]	93 [90-95]	**<0.001**
Blood gas interpretation**				**0.003**
Normal CBG	226 (43.5%)	104 (46.2%)	122 (41.5%)	ns
Metabolic alkalosis	91(17.5%)	48 (21.3%)	43 (14.6%)	**<0.05**
Metabolic acidosis	5 (1.0%)	-	5 (1.7%)	ns
Respiratory alkalosis	44 (8.5%)	18 (8.0%)	26 (8.8%)	ns
Respiratory acidosis	2 (0.4%)	3 (1.3%)	2 (0.7%)	ns
Mixed disorders	4 (0.8%)	12 (5.%)	1 (0.3%)	ns
Compensated disorders	117 (22.5%)	35 (15.6%)	82 (27.9%)	**<0.05**
Indeterminate	24 (4.6%)	16 (7.1%)	8 (2.7%)	
Missing	6 (1.2%)	1 (0.4%)	5 (1.0%)	

7.4.2 Acid-base status

Each blood gas results was interpreted using the automatic tool as described in Section 4.3.2 and Appendix B. When the automatic tool provided an “unable to determine” result, the blood gas was interpreted manually following the Boston and the Steward approaches.

The distribution of ABG results differed significantly when comparing subjects with CF with those with other respiratory conditions (Table 7.1) (p=0.014). In both cohorts, blood gas results were most often within normal limits. There was a higher prevalence of metabolic alkalosis among people with CF compared to the control cohort (21.3% vs 14.6%, p<0.05). In addition, metabolic alkalosis was the most common disturbance of the acid-base status among people with CF, whereas subjects with other respiratory conditions more often presented with compensated acid-base disorders (Figure 7.2).

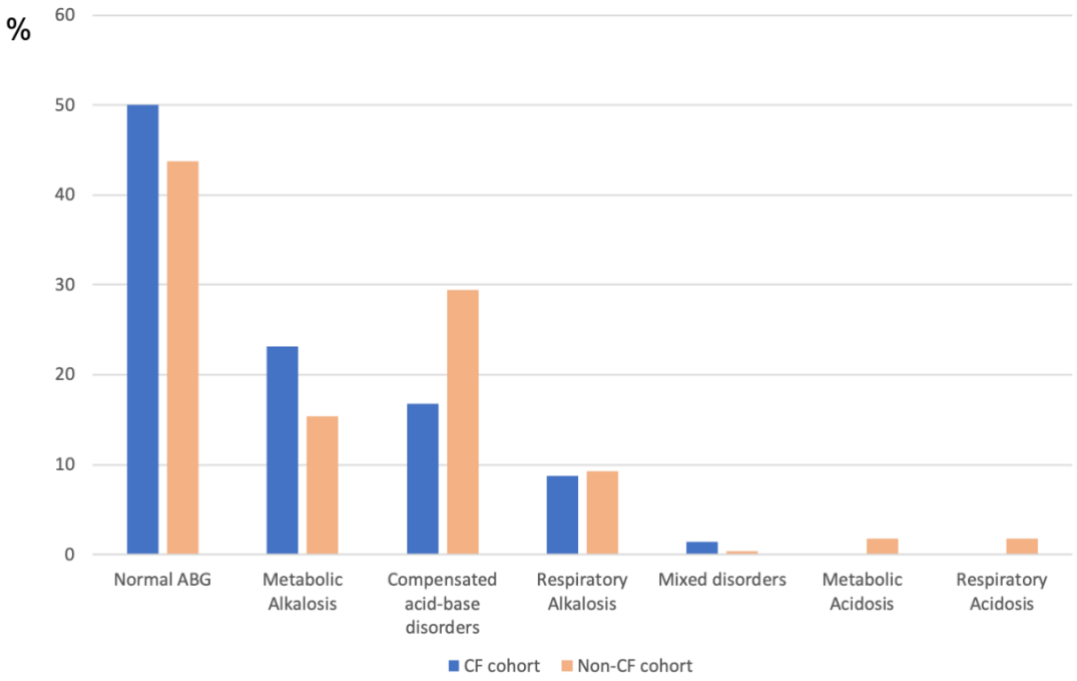

Figure 7.2 Frequency of distribution of blood gas analysis results in the CF cohort and among individuals with other respiratory conditions.

No cases of uncompensated metabolic acidosis or respiratory acidosis were noted among patients with CF, whereas these were observed in the non-CF cohort.

No significant difference in age, sex or BMI were noted in either cohort depending on blood gas results. No difference was observed in the relative response to the hypoxic stimulus as measured by relative change in blood gas variables, depending on baseline acid-base status in the whole population and in either cohort.

However, the overall response to the HAST, according to BTS recommendation, differed depending on baseline acid-base balance and the prevalence of each acid-base disorder was different comparing negative and positive test (Table 7.2).

In particular, among those who had a negative HAST, there was a higher prevalence of normal ABG (53.3%). This was significantly different compared to the most common disorders, consisting of compensated disturbances (18%) and metabolic alkalosis (15.7%). Conversely, among those requiring oxygen in flight following a positive HAST, normal ABG at baseline were observed in 35% of cases, with a higher prevalence of compensated disorders (33.9%) and metabolic alkalosis (23.9%).

Similar results were observed within each cohort separately (Table 7.2). However in patients with CF, the only differences noted in the prevalence of blood gases results were for normal ABG and metabolic alkalosis. Subjects with normal ABG were more frequent among those with a negative HAST and those with metabolic alkalosis represented the higher proportion of positive tests.

Table 7.2 Response to the HAST depending on acid-base status in the whole population, and in the two cohort (CF and non-CF)

Acid-base	Negative HAST	Positive HAST	*p*
Whole population			
Normal CBG	163 (53.3%)	63 (35%)	**<0.05**
Compensated disorder	55 (18%)	61 (33.9%)	**<0.05**
Metabolic Alkalosis	48 (15.7%)	43 (47.3%)	**<0.05**
Metabolic Acidosis	3 (1%)	2 (1.1%)	ns
Respiratory Alkalosis	35 (11.4%)	9 (5%)	**<0.05**
Mixed disorders	2 (0.7%)	2 (0.7)	ns
Cystic Fibrosis			
Normal CBG	94 (52.8%)	10 (33.3%)	**<0.05**
Compensated disorder	32 (18%)	3 (10%)	ns
Metabolic Alkalosis	33 (18.5%)	15 (50%)	**<0.05**
Respiratory Alkalosis	17 (9.6%)	1 (3.3%)	ns
Mixed disorder	2 (1.1%)	1 (3.3%)	ns
Non-CF			
Normal CBG	69 (53.9%)	53 (35.3%)	**<0.05**
Compensated disorder	23 (18%)	58 (38.7%)	**<0.05**
Metabolic Alkalosis	15 (11.7%)	28 (18.7%)	ns
Respiratory Alkalosis	18 (14.1%)	8 (5.3%)	**<0.05**
Metabolic Acidosis	3 (2.3%)	2 (1.2%)	ns

7.4.3 Raised bicarbonate

Four groups were identified based on serum bicarbonate and partial pressure of carbon dioxide, as follows:

1. Group 1: normal pCO_2 and normal bicarbonate;
2. Group 2: normal pCO_2 and elevated bicarbonate;
3. Group 3: elevated pCO_2;
4. Group 4: all others.

Table 7.3 Baseline characteristics for the groups based on arterial bicarbonate concentration. *indicates analysis completed on the whole population of 365 patients (124 CF and 241 non-CF); **indicates analysis completed on the population considering each individual test. Group 1: normal pCO2 and bicarbonate; group 2: normal pCO2 and elevated bicarbonate; Group 3: elevated pCO2; group 4: all others. BMI body mass index; CF, cystic fibrosis; ILD, interstitial lung disease; NMD, neuromuscular disease; OSA, obstructive sleep apnoea; OHS, obesity hypoventilation syndrome; CBG capillary blood gas.

Baseline characteristics	**Group 1**	**Group 2**	**Group 3**	**Group 4**	***p***
Male gender, n(%)*	90 (52.6%)	99 (55%)	17 (35.4%)	52 (45.6%)	0.065
Age at test in years**	48.4 [28-68.3]	39 [27.5-65.6]	67.3 [58.9-71.8]	53.4 [29.3-70.7]	**<0.0001**
BMI, (kg/m^2)**	24.7 [21.6-27.6]	24.3 [21.6-28.5]	26.8 [22.3-33.1]	24.5 [21.1-28.9]	0.632
Diagnosis*					<**0.0001**
CF	78 (45.6%)	90 (50%)	6 (12.7%)	50 (43.9%)	
COPD	20 (11.7%)	41 (22.8%)	17 (35.3%)	10 (8.8%)	
ILD	44 (25.7%)	20 (11.1%)	3 (6.2%)	39 (34.1%)	
NMD	6 (3.5%)	10 (5.6%)	4 (8.2%)	2 (1.8%)	
OSA/OHS	1 (0.6%)	8 (4.4%)	9 (18.8%)	1 (0.9%)	
Other	22 (12.9%)	11 (6.1%)	9 (18.8%)	12 (10.5%)	
Baseline CBG**					
pH	7.42 [7.41-7.44]	7.45 [7.44-7.47]	7.42 [7.40-7.43]	7.44 [7.40-7.47]	**<0.0001**
pCO_2, kPa	4.9 [4.8-5.2]	5.3 [5.1-5.6]	6.5 [6.2-6.9]	4.4 [4.2-4.5]	**<0.0001**
pO_2, kPa	9.5 [8.7-10.5]	9.2 [8-10.1]	7.7 [7-9]	9.9 [8.7-10.8]	**<0.0001**
HCO_3^-, mmol/L	24.6 [23.5-25.4]	27.8 [26.9-29.2]	31.2 [28.8-33.3]	22.5 [21.4-23.5]	**<0.0001**
BE	0.3 [-0.7-1.1]	3.5 [2.8-4.9]	5.9 [3.9-7.8]	-1.6 [-3.3 - -0.1]	**<0.0001**
SO_2, %	95 [93-96]	94 [92-96]	90 [87.5-93.1]	95 [93.1- 96]	**<0.0001**

7.4.3.1 Whole cohort

Considering the whole population, most subjects fell in groups 1 and 2 (171 and 180, respectively), whereas only 48 had elevated daytime pCO_2. The groups were similar for age and BMI, for the exception of group 3 which included older patients with a higher BMI. No differences in sex distribution were noted. Baseline blood gas was different across the four groups as excepted with the definition being based on partial pressure of carbon dioxide and bicarbonate concentration (Table 7.3).

The response to the hypoxic stimulus represented by the HAST was different in the four groups, with more subjects in group 2 and 3 having a positive HAST, and conversely more subjects in cohort 1 and 4 having a negative response (Table 7.4).

Table 7.4 Response to the HAST in the groups identified based on bicarbonate concentration across the whole population and the two cohorts (CF and non-CF). Group 1: normal pCO2 and bicarbonate; group 2: normal pCO2 and elevated bicarbonate; Group 3: elevated pCO2; group 4: all others.

Acid-base	Negative HAST	Positive HAST	*p*
Whole population			**<0.001**
Group 1	126 (38.9%)	45 (23.9%)	**<0.05**
Group 2	99 (30.6%)	80 (42.6%)	**<0.05**
Group 3	10 (3.1%)	38 (20.2%)	**<0.05**
Group 4	89 (27.5%)	25 (13.3%)	**<0.05**
CF cohort			**0.005**
Group 1	70 (36.6%)	8 (24.2%)	ns
Group 2	6 (36.1%)	21 (63.6%)	**<0.05**
Group 3	4 (2.1%)	2 (6.1%)	ns
Group 4	48 (25.1%)	2 (6.1%)	**<0.05**
Non-CF cohort			**<0.001**
Group 1	56 (42.1%)	37 (23.9%)	**<0.05**
Group 2	30 (22.6%)	59 (38.1%)	**<0.05**
Group 3	6 (4.5%)	36 (23.2%)	**<0.05**
Group 4	41 (30.8%)	23 (14.8%)	**<0.05**

The relative change in partial pressure of oxygen was similar in the four groups (p=0.869), but the change in pCO_2, HCO_3^- and pH differed. In particular, the response to the hypoxic stimulus in terms of ventilation as acid-base balance was different among subjects in group 3 compared to the other. In addition, those with raised bicarbonate but normal daytime pCO_2 (group 2) had a similar response to group 1 for relative change in pCO_2, and to group 3 for the relative change in HCO_3^- (Figure 7.3, Table 7.5).

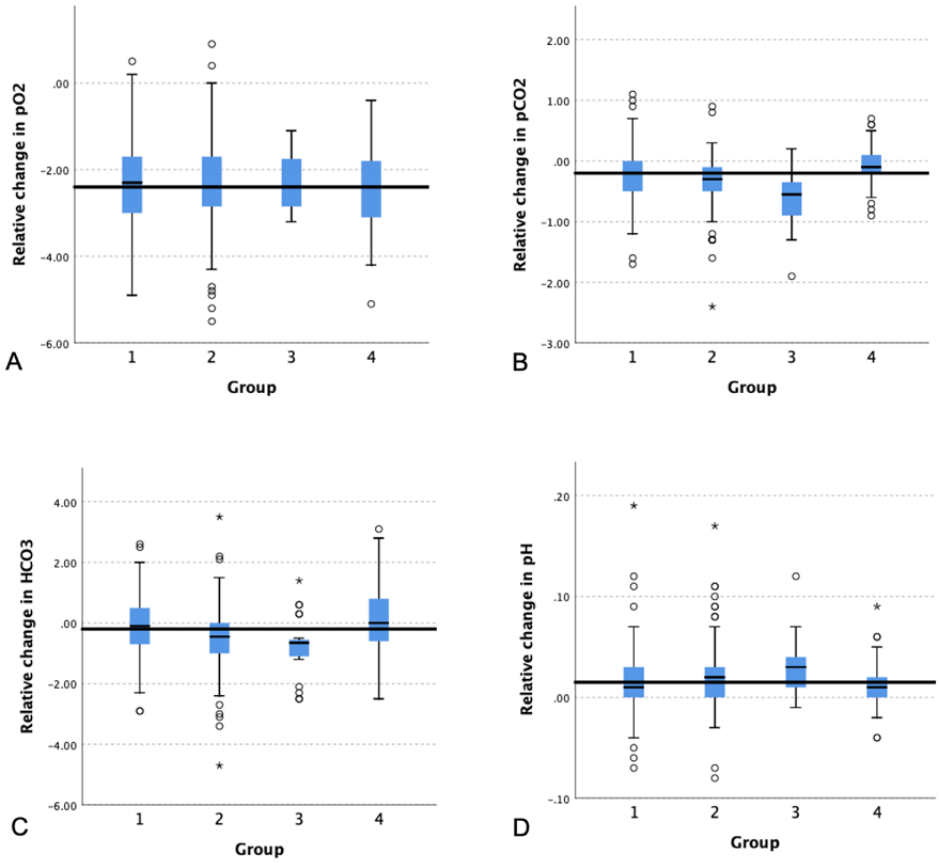

Figure 7.3 Relative change in blood gas variables after the HAST in groups identified based on arterial bicarbonate.

Table 7.5 Relative change in blood gas results during HAST in groups identified based on arterial bicarbonate. Group 1: normal pCO2 and bicarbonate; group 2: normal pCO2 and elevated bicarbonate; Group 3: elevated pCO2; group 4: all others.

	Group 1	Group 2	Group 3	Group 4	*p*
Whole population					
Delta pO_2	-2.3	-2.4	-2.4	-2.4	0.869
Delta pCO_2	-0.2	-0.3	-0.55	-0.1	**<0.001**
Delta pH	0.01	0.02	0.03	0.01	**0.004**
Delta HCO_3^-	-0.1	-0.45	-0.65	0	**<0.001**
Delta S_pO_2	-5.8	-5.0	-8.0	-4.9	**0.002**
CF cohort					
Delta pO_2	-2.5	-2.4	-2.4	-2.5	0.647
Delta pCO_2	-0.2	-0.3	-0.5	0	**<0.001**
Delta pH	0.02	0.02	0.03	0.01	0.318
Delta HCO_3^-	-0.15	-0.6	-0.7	0.15	**0.019**
Delta S_pO_2	-5	-5	-5	-4	0.051
Non-CF cohort					
Delta pO_2	-2.3	-2.5	-2.4	-2.1	0.994
Delta pCO_2	-0.2	-0.3	-0.6	-0.1	**0.009**
Delta pH	0.01	0.02	0.03	0.01	0.263
Delta HCO_3^-	-0.1	-0.3	-0.6	0	**0.072**
Delta S_pO_2	-6	-7	-9	-5.7	**0.020**

7.4.3.2 CF and non-CF cohorts

The distribution of the four groups across the two cohorts was different, with less individuals in the CF cohorts presenting with elevated pCO_2 compared to the non-CF cohort. Age and BMI distribution was similar across the four groups in the two cohorts.

Conversely to what was observed in the whole population, the distribution of sex was different in the groups across the cohorts. In particular in the CF cohort a higher proportion of male was included in group 2 (elevated bicarbonate and normal partial pressure of carbon dioxide), and female in group 4. On the other hand, in the non-CF cohort, more male were included in group 1 and female in group 3.

The response to the hypoxic stimulus represented by the HAST was different in the four groups among subjects with CF and the non-CF cohort. Subjects in group 2 represented a higher proportion of those with a positive test among patients with CF compared to the non-CF cohort (63.6% vs 36.1%) (Table 7.4)

Within the CF cohort, no difference in the relative change of pO_2 and SpO_2 was observed across the four groups (p=0.752 and p=0.094, respectively). The relative change in pCO_2 was similar comparing group 2 with group 1 and 3, but differed in all other comparisons. Conversely, the change in bicarbonate differed in group 2 compared to group 1 and 4 (Figure **7.4**).

Within the non-CF cohort, partial pressure of oxygen was similar across the groups (p=0.992), but SpO_2 differed when comparing group 3 with the others. The relative change in pCO_2 differed in all comparison, except for groups 1 and 2 (p=0.623). Similarly, the change in bicarbonate was similar in comparing group 2 with groups 1 and 3, but otherwise differed (Figure **7.4**).

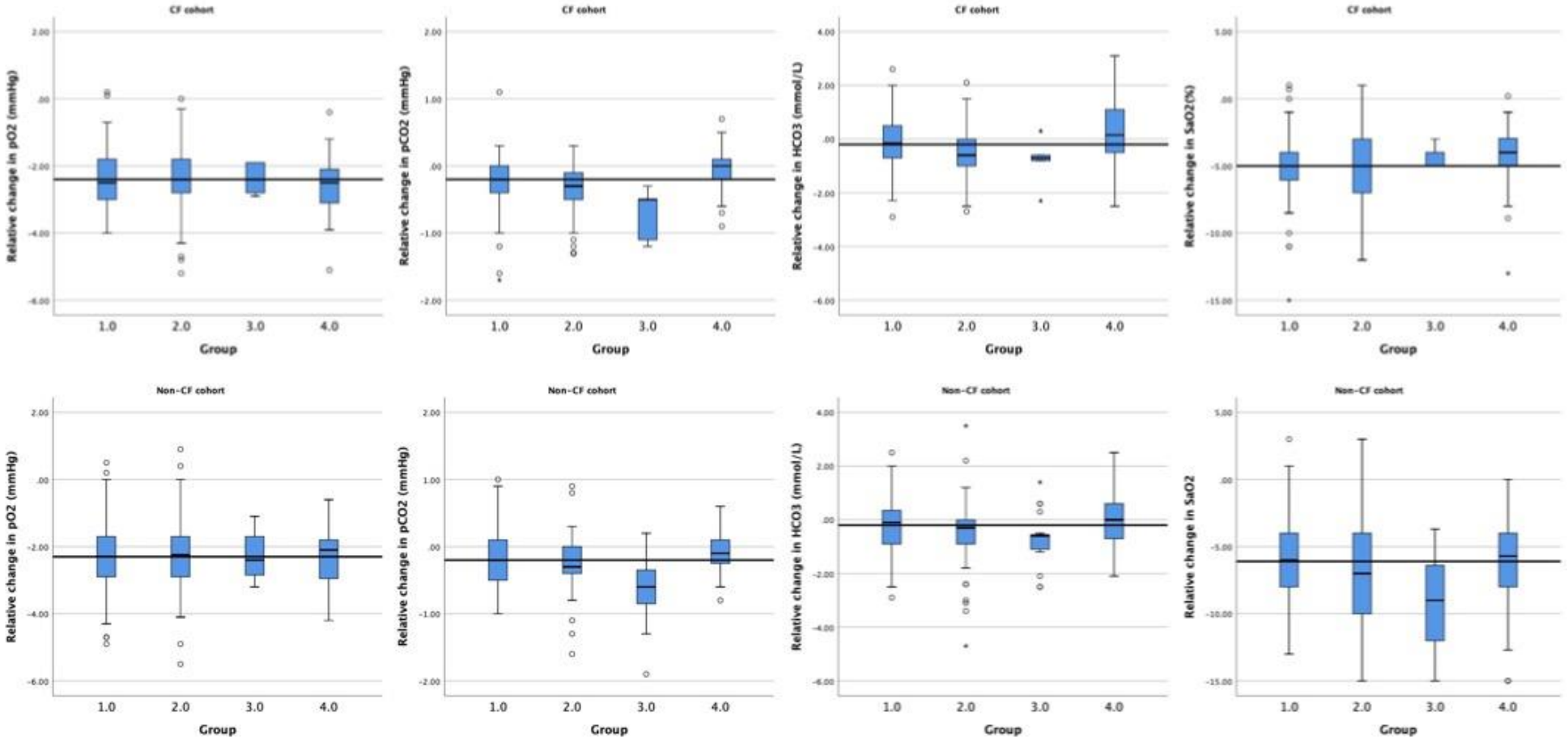

Figure 7.4 Relative change in blood gas variables across groups in the CF and non-CF cohorts. Boxplot represent median and IQR in each group, horizontal lines represent the median across the cohort.

7.4.4 HAST results

Over the 5-years study period, 365 patients underwent a total of 519 hypoxic challenge tests. Of these 519 tests, 188 (36.6%) gave a positive result, as defined by a drop in pO2 below 6.6 kPa, indicating the need for oxygen during air travel, whereas the rest (63.4%) were negative (pO2 at the end of the test >6,6 kPa).

7.4.4.1 Model development

As described in Section 7.3.3.2, a training and a validation set were identified *a priori* based on a temporal definition using the cut-off date of July 31, 2016 so that approximately 66% of tests were included in the derivation cohort.

Table 7.6 shows the baseline characteristics of the training and validation sets, which included 335 and 184 tests, respectively. Sex and ethnicity distribution were comparable in the two sets . Subjects in the training set were older (59 vs 37 years), had a lower prevalence of cystic fibrosis (CF, 47.3% vs 33.3%, $p<0.01$). Capillary blood gas analysis results were different in the training and validation set for all parameters, except for pH.

42.6% and 26.4% of tests were positive (pO_2 <6.6 kPa at the end of the test) in the training and validation set, respectively ($p<0.001$).

Table 7.6 Baseline characteristics in the training and validation cohorts. BMI, body mass index; CF, cyrstic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; NMD, neuromuscular disorder; OSA, obstructive sleep apnoea; OHS, obesity hypoventilation syndrome. Data are expressed as number (%) or median (IQR).

	Training (n=335)	**Validation (n=184)**	***p***
Age	59.2 [30.6-70.3]	36.9 [27.4-67.2]	**0.001**
BMI	24.9 [21.7-29.3]	24.5 [21.5-27.7]	0.470
Ethnicity			0.151
Caucasian	304 (90.7%)	171 (92.9%)	
Asian	26 (7.8%)	7 (3.8%)	
Black	5 (1.5%)	6 (3.3%)	
Male sex	164 (49%)	97 (52.7%)	0.412
Diagnosis			**0.015**
CF	124 (37%)	101 (54.9%)	
COPD	69 (20.6%)	22 (12.1%)	
ILD	68 (20.3%)	38 (20.7%)	
NMD	16 (4.8%)	6 (3.3%)	
OSA/OHS	15 (4.5%)	4 (2.2%)	
Other	43 (12.8%)	13 (7.1%)	
Baseline CBG			
pH	7.44 [7.42-7.46]	7.43 [7.41-7.45]	0.092
pCO_2, kPa	5.2 [4.8-5.6]	4.9 [4.6-5.4]	**0.003**
pO_2, kPa	9.1 [8-10.1]	9.7 [8.8-10.5]	**0.001**
HCO_3^-, mmol/L	26.2 [24-28.4]	25 [23-26.9]	**<0.001**
BE	2.0 [0-4.1]	0.9 [-1.5-2.9]	**<0.001**
SO_2, %	94 [92-96]	95 [93-96]	**0.024**

7.4.4.1.1 Single variable predictors

In the training cohort, patients with a positive HAST were older; more frequently had COPD or ILD and had worse lung function. Their baseline CBG showed lower oxygenation, higher pCO_2 and bicarbonate (Table 7.7).

Table 7.7 Characteristics for positive and negative HAST in the training set. BMI, body mass index; CF, cyrstic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; NMD, neuromuscular disorder; OSA, obstructive sleep apnoea; OHS, obesity hypoventilation syndrome. Data are expressed as number (%) or median (IQR).

	Negative	**Positive**	***p***
Age	40.5 [26.3-67.9]	64.8 [50.2-71.9]	**<0.001**
BMI	24 [21.7-27.5]	26.3 [21.7-31.8]	**0.001**
Ethnicity			0.213
Caucasian	175 (91.1%)	126 (90%)	
Asian	16 (8.3%)	10 (7.1%)	
Black	1 (0.5%)	4 (2.9%)	
Male sex	104 (54.2%)	58 (41.4%)	**0.022**
Diagnosis			**<0.001**
CF	98 (51%)	26 (18.6%)	
COPD	17 (8.9%)	50 (35.7%)	
ILD	37 (19.3%)	31 (22.1%)	
NMD	14 (7.3%)	2 (1.4%)	
OSA/OHS	1 (0.5%)	13 (9.3%)	
Other	25 (13%)	18 (12.8%)	
Baseline CBG			
pH	7.44 [7.42-7.46]	7.44 [7.41-7.47]	0.641
pCO_2, kPa	4.9 [4.7-5.3]	5.3 [4.9-6]	**<0.001**
pO_2, kPa	9.9 [9.1-10.7]	8 [7.2-8.8]	**<0.001**
HCO_3^-, mmol/L	25.2 [23.4-27.1]	27.5 [25.3-29.9]	**<0.001**
BE	1.15 [-0.5-3.1]	3.2 [0.9-5.2]	**<0.001**
SO_2, %	95 [94-96]	92 [89-93]	**<0.001**

Table 7.8 reports the ROC AUC of each variable to predict HAST results. The ROC curves were compared using the methods described by Delong et al [393], with pO_2 showing the highest single-variable discriminatory power.

Table 7.8 ROC AUC for potential single variable predictors for HAST results.

Variable	**ROC AUC**	**95% CI**	***p***
Age at test (yr)	0.661	0.602-0.719	**<0.001**
BMI	0.586	0.522-0.650	**0.008**
Best FEV_1 (L)	0.688	0.623-0.753	**<0.001**
Best FEV_1 (%)	0.604	0.533-0.675	**0.004**
Baseline CBG			
pCO_2	0.684	0.625-0.724	**<0.001**
pO_2	**0.877**	**0.839-0.915**	**<0.001**
HCO_3^-	0.697	0.639-0.756	**<0.001**
S_aO_2	0.859	0.817-0.902	**<0.001**
BE	0.686	0.628-0.745	**<0.001**

Baseline pO_2 was the best single variable predictor for negative HAST (ROC AUC 0.877 [95% CI 0.839-0.915], p<0.001) (Figure 7.5).

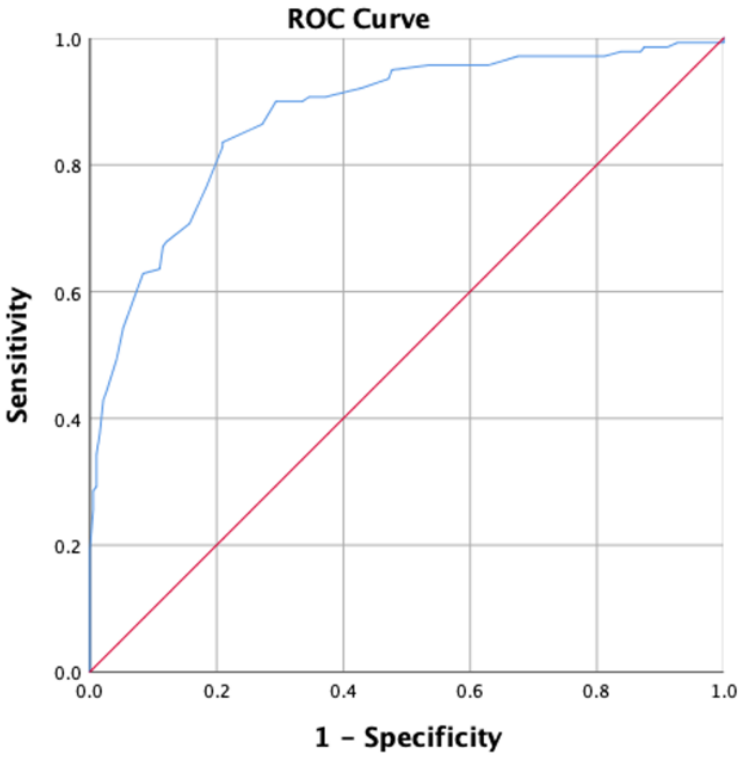

Figure 7.5 ROC curve for pO_2 to predict results of HAST in the training set.

7.4.4.1.2 Training set: regression analysis and multivariate model

Statistically significant continuous variables were tested for multicollinearity prior to being entered in the binary logistic regression. pCO_2, HCO_3^- and BE had a high variance inflation factor (VIF) score, so as pO_2 and S_aO_2. Therefore, no collinear variable was entered contemporaneously in the same multiple binary regression analyses.

Table 7.9 shows the final regression coefficients, and odds ratios with 95% CI for each of the models, which passed the goodness of fit test.

Each model was plotted in a ROC AUC against the HAST outcome (Figure **7.6**, Panel A). ROC AUC were 0.888 (95%CI 0.851-0.924), 0.882 (95%CI 0.844-0.919) and 0.887 (95%CI 0.850-0.950), respectively. No differences were observed between the three ROC AUC, and comparing the ROC AUC of the multivariable models with the pO_2 univariate model. Thresholds of predicted probability at 95% and 97.5% specificity for positive and negative outcomes were identified for each model, to then be applied in the validation cohort.

Table 7.9 Model derivation with binary logistic regression in the training set. HCO3-, bicarbonate, SaO2 arterial oxygen saturation, COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease.

Models and variables	*β*	*p*	OR (95% CI)
Model 1			
Baseline HCO_3^-	0.219	**<0.001**	1.245 [1.124-1.378]
Baseline S_aO_2	- 0.589	**<0.001**	0.555 [0.474-0.650]
COPD, N	-0.917	**0.032**	0.400 [0.173-0.924]
ILD, N	-1.315	**<0.001**	0.269 [0.124-0.584]
Sex, F	0.713	**0.025**	2.039 [1.095-3.797]
Model 2			
Baseline pCO_2	0.930	**<0.001**	2.535 [1.529-4.204]
Baseline S_aO_2	-0.550	**<0.001**	0.577 [0.493-0.675]
COPD, N	-1.002	0.19	0.367 [0.159-0.848]
ILD, N	-1.204	**0.002**	0.300 [0.140-0.643]
Sex, F	0.799	**0.012**	2.223 [1.196-4.132]
Model 3			
Baseline BE	0.232	**<0.001**	1.261 [1.136-1.401]
Baseline S_aO_2	-0.613	**<0.001**	0.542 [0.461-0.636]
COPD, N	-0.885	**0.039**	0.413 [0.178-0.955]
ILD, N	-1.283	**0.001**	0.277 [0.128-0.600]
Sex, F	0.659	**0.038**	1.932 [1.037-3.599]

7.4.4.2 Model validation

The validation cohort included 184 HAST, 26.4% of which were positive. The validation cohort differed in comparison to the training cohort as previously described (Table 7.6. section 7.4.4.1).

ROC curves for pO_2 and the 3 multivariable models were plotted against the HAST outcomes (Figure **7.6**, Panel B). The ROC AUC were 0.888 (95%CI 0.824-0.951), 0.916 (95%CI 0.871-0.961), 0.907 (95%CI 0.856-0.958) and 0.914 (95%CI 0.868-0.959) for the baseline pO_2, Model 1, Model 2 and Model 3, respectively. No significant differences between the four curves were observed within the validation cohort. Similarly, no differences were observed with the ROC curves plotted for the training cohort (Figure **7.6**).

Previously identified cut-off values at 95% and 97.5% were applied to each model. The best performing model was Model 1 with the following thresholds (Figure 7.7):

- 97.5% thresholds
    - Output > 0.77: predicted positive HAST (in-flight oxygen requirement);
    - Output < 0.092: predicted negative HAST (no in-flight oxygen requirement);
    - Any other output: indeterminate prediction (in which case an actual HAST would need to be performed).
- 95% thresholds
    - Output > 0.715: predicted positive HAST (in-flight oxygen requirement);

- Output < 0.195: predicted negative HAST (no in-flight oxygen requirement);
- Any other output: indeterminate prediction (in which case an actual HAST would need to be performed).

These thresholds were applied to the validation cohort to assess how many HASTs could have been avoided, and the associated error rate.

The 95% thresholds would have allowed a 65% reduction in number of HAST, with 6 misclassifications. These would have been distributed as 3 wrongly identified as not requiring oxygen (3.5%) and 3 as needing in-flight oxygen (13%).

The 97.5% thresholds would have reduced the HAST performed by 40.9%, with only 3 misclassification, all as requiring in-flight oxygen (Figure 7.7).

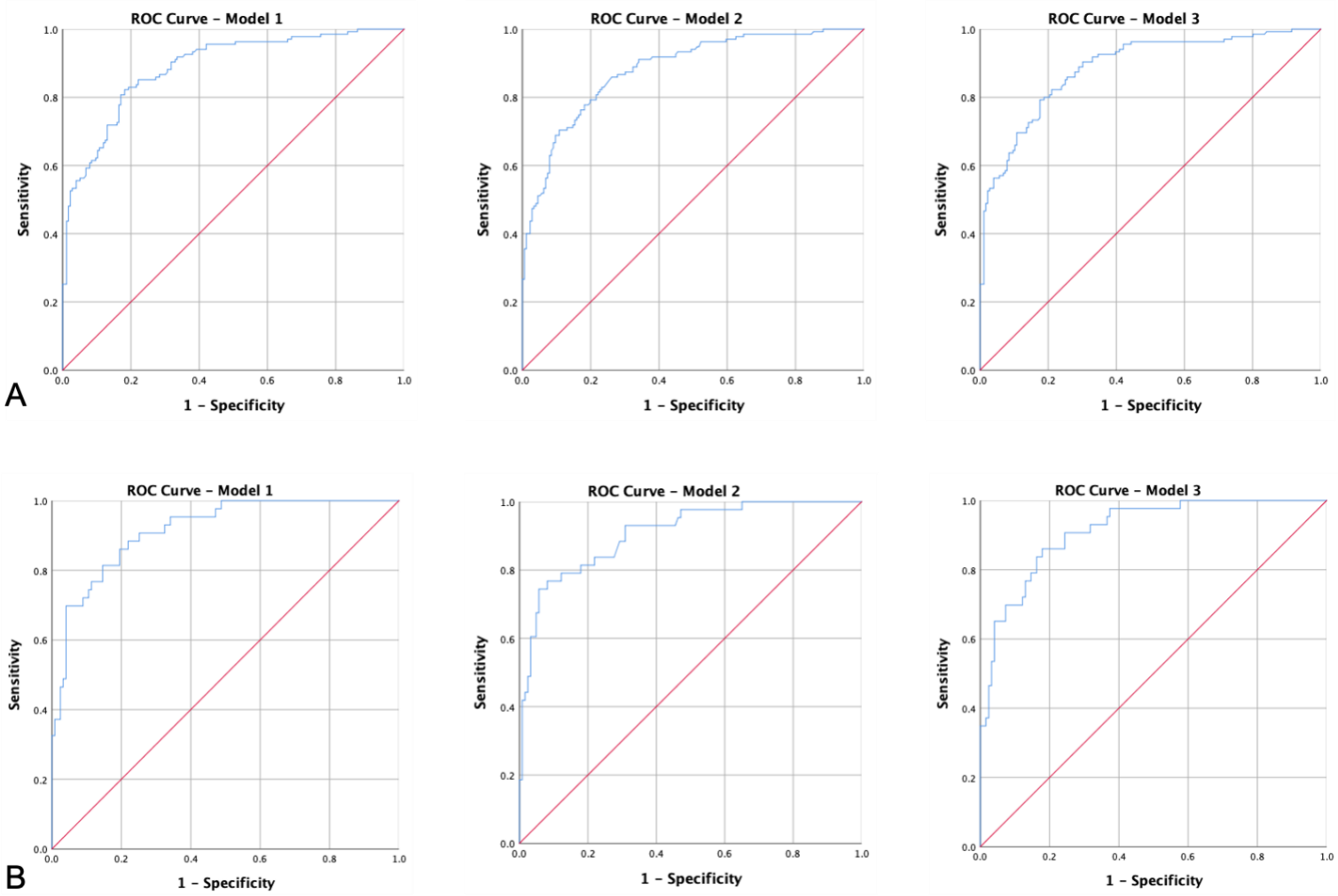

Figure 7.6 ROC Curve of the three models against a positive outcome of the HAST. Panel A shows the ROC curves in the training cohort and Panel B represents the ROC curves in the validation cohort.

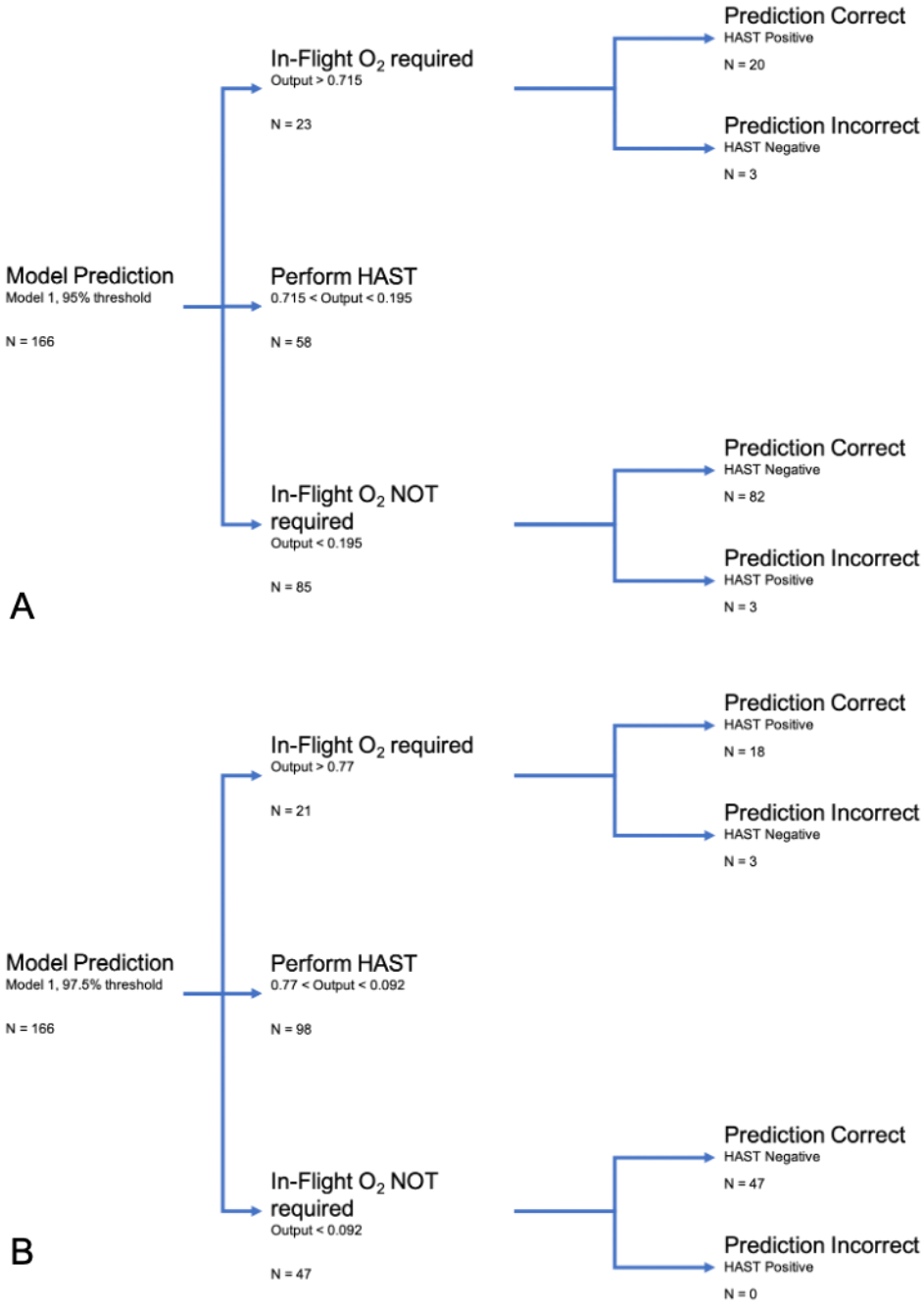

Figure 7.7 Result of model prediction in the validation cohort.

7.5 Discussion

Recent data from University-affiliated hospitals and tertiary centres, including Leeds (Figure 7.1), have shown a progressive increase in number of HAST performed every year on patients with cystic fibrosis and other respiratory conditions [391,394].

This provided me with an invaluable opportunity to explore the acid-base status in a large cohort of patients with underlying respiratory conditions, and to compare the distribution of acid-base disturbances between patients with CF and those with other lung diseases.

Over 500 blood gas samples were analysed in this study, including 225 measurements in patients with CF. The majority of blood gases showed a normal acid-base status in people with CF. This may reflect the fact that the criteria of referral for CF patients was not restricted to those with severe disease but included a diverse group including those with relatively good lung function and exercise tolerance. This notwithstanding, acid-base disturbances were observed with metabolic alkalosis being the most frequently reported among people with CF. Metabolic alkalosis occurred more frequently in subjects with CF compared to those with other respiratory conditions (21.3% vs 14.6%), as previously described [216,217,223]. In addition, I confirmed that people with CF can present with elevated bicarbonate, and normal daytime pCO_2 and pH.

Metabolic alkalosis is a well-recognised CF complication, and is historically often attributed to Pseudo-Bartter syndrome [221,222,395], as previously discussed (Section 2.5.1). In this retrospective study, data on serum electrolytes or renal function were not available. As such, in this cohort, association of metabolic alkalosis with electrolytes disturbances, dehydration and pseudo-Bartter syndrome cannot be ruled out. However, Pseudo-Bartter syndrome has, traditionally, been identified as a complication occurring during pulmonary exacerbations or in a hot climate. The requirement for clinical stability prior to a HAST, the climate at our latitude and data collected as part of a parallel study discussed in my thesis (Chapter 8) would suggest that Pseudo-Bartter is unlikely to be the cause of metabolic alkalosis in this context.

Previous studies in patients with obesity hypoventilation syndrome suggest that isolated elevation of bicarbonate is on the spectrum of ventilatory failure [387]. Individuals with elevated bicarbonate and BE have a similar response to hypoxic and hypercapnic stimulus to individuals with daytime hypercapnia. In addition, bicarbonate has been shown to be an independent predictor of respiratory muscle weakness, hypercapnia, and survival in patients with neuromuscular disorders and COPD [274,384–386]

While I did not assess the response to a hypercapnic stimulus, HAST is a variation of a hypoxic test. In the whole population, the response to HAST was different in the four groups identified on the basis of arterial bicarbonate, with those with high bicarbonate concentration showing an intermediate response compared with individuals with normal pCO_2 and HCO_3^- and people with elevated daytime pCO_2. A similar trend was observed among patients with CF. In addition, more patients with elevated bicarbonate, with or without elevation of daytime pCO_2, had a clinically significant drop in pO_2 and a positive response to the HAST (Table 7.4), in both cohorts. This suggest that elevation of arterial bicarbonate, even in the presence of normal daytime pCO_2, might be on the spectrum of chronic ventilatory failure.

Despite the international recommendation and wide use of HAST in the assessment of patents with cardio-respiratory conditions prior to flying [178,182–184], there is a paucity

of data on the correlation between anticipated in-flight hypoxaemia and hypoxia-related symptoms during air travel [177,179,181]. HASTs need to be performed during clinical stability to mimic as closely as possible the time of travel, are time-consuming and expensive tests which require a dedicated-hospital appointment.

As a result of this analysis, I combined oxygenation and ventilation variables to develop and independently validate a simple, clinically feasible algorithm (Figure 7.7) for pre-flight assessment in patients with any chronic lung disease, based on baseline CBG, diagnosis and sex. This predictive model shows how a combination of baseline oxygenation and ventilation variables (HCO_3^- and S_aO_2), together with sex, and underlying respiratory disease, can reliably predict the response to the hypoxic stimulus in a high percentage of cases. This predictive model could be used as a practical clinical application to triage patients prior to referral for HAST, reducing the number of tests performed, with close to a nil error rate.

It is well established that patients with hypercapnia have a blunted response to the hypoxic drive, and in this cohort, as discussed above, patients with raised bicarbonate appear to have a response to the hypoxic stimulus, at least in part, similar to that of patients with daytime hypercapnia. It is, therefore, conceivable that HCO_3^- and BE could reflect the ventilatory component of gas exchange.

Single variables, measured at sea-level, have been shown to correlate with in-flight oxygen saturation [396], but to be inadequate predictors of in-flight oxygen as they can under-detect individuals at risk of hypoxaemia at altitude [397,398]. In contrast, these results demonstrated that baseline capillary pO_2 had good predictive value (Figure 7.5). It can however lead to an overestimation in the need for inflight oxygen likely because of the lower accuracy of capillary pO_2 compared to arterial pO_2 for mild hypoxaemia at rest conditions [163].

Crucially, by combining oxygen saturation - the accuracy of which has recently been confirmed [399] - with measurements of HCO_3, the predictive value of baseline pO_2 alone was significantly improved. This approach further supports that hypothesis that arterial bicarbonate is a marker of ventilation irrespective of daytime pCO_2 or pH.

Compared to previous algorithms [400,401], the multivariable model proposed here depends solely on a single blood gas measurement, which can be performed during a clinic appointment, and appeared to be valid for respiratory patients irrespective of diagnosis.

In view of the correlation between the aerobic capacity and in-flight hypoxaemia [402–405], Edvardsen et al [400] proposed an algorithm which included S_pO_2 at rest and during the 6MWT to reduce the number of HASTs performed. Despite 6MWT distance, desaturation and cardio-pulmonary exercise test variables being good predictors of HAST outcomes in other respiratory conditions [401,402,406], the algorithm was only

validated in patients with COPD. The usefulness of 6MWT in discriminating the need for inflight oxygen remains unclear with many centres not routinely undertaking both procedures as part of a pre-flight assessment [182,394]. In the present study I was unable to include the 6MWT in the regression models as only 15.9% of patients had undergone the test.

Some authors suggested that an advantage of HAST compared to predictive equations and pre-assessment algorithms is the possibility to titrate oxygen during the HAST [186]. However, international recommendations consider acceptable to prescribe supplemental oxygen at 2 L/min, or 2 L/min over usual flow rates for patients on long term oxygen therapy, for individual at risk of in-flight hypoxaemia [182,407]. In this context, the model proposed here can be used to identify a proportion (>50%) of patients who are fit to fly without oxygen and of those who need oxygen without the need to perform a HAST.

7.5.1.1 Strengths and limitations

The main strength of this study is the inclusion of a large number of consecutive blood gases collected during the HASTs performed by patients with a variety of respiratory conditions. This amount of data is invaluable to provide a large-scale assessment of the acid-base imbalance among patients with CF in comparison with those with other respiratory conditions.

This large amount of data also allowed me to develop and independently validate a multivariate predictive model, with training and validation cohorts defined *a priori*, in line with the TRIPOD statement. In this study, this algorithm appears to be applicable to patients irrespective of underlying primary respiratory diagnosis. The different characteristics of training and validation cohorts suggest, in fact, that the algorithm has the potential to be generalizable to respiratory patients in real-life setting.

This study is limited by its retrospective and single-centre design. This led to the inability of retrieving contemporaneous serum electrolytes and bicarbonate measurements, and of accounting for comorbidities. In addition, with the Leeds Respiratory department being a tertiary centre for ILD and a regional referral centre for CF, there was overrepresentation of these two conditions in the present cohort, and a lower than expected frequency of COPD. Therefore, external prospective cross-sectional studies is needed to confirm these results.

7.5.2 Conclusion

In this large-scale retrospective study, metabolic alkalosis appears to be the most frequent disturbance in acid-base balance among people with CF in conditions of stability. In addition, it has a higher prevalence among individuals with CF compared to those with non-CF respiratory conditions.

More patients with CF are in the subgroup with raised serum bicarbonate and normal daytime CO_2. In this group, the response to hypoxic stimulus is closer to that of people with daytime hypercapnia suggesting a blunt respiratory drive.

To my knowledge, this is the first study combining variables of ventilation and oxygenation together with underlying diagnosis to create a simple score for the pre-flight evaluation of patients with respiratory conditions, irrespective of their diagnosis. The model proposed in this study could potentially reduce the number of hypoxic challenge tests that need to be performed, by correctly identifying the majority of patients with chronic lung diseases as fit to fly with or without oxygen on one blood gas test alone.

Further external prospective cross-sectional studies will be needed to confirm these results, and their applicability using less invasive measurements, and to assess if different criteria could be chosen for specific diagnosis.

Chapter 8
Renal involvement in acid-base balance in CF: a case-controlled pilot study

8.1 Introduction

Metabolic alkalosis is well recognised as a feature in infants and children with CF, who frequently present with Pseudo-Bartter syndrome (PBS) (Section 2.5.1). Over the last two decades, an increasing body of evidence has developed, suggesting that metabolic alkalosis is also commonly observed in adults with CF [216–218,223,408–411].

As observed in previous studies and confirmed in the Leeds CF cohort (as discussed in Chapter 7), the prevalence of this acid-base disorder appears to be higher in people with CF compared to other lung diseases, both during pulmonary exacerbations as well as in conditions of stability. Metabolic alkalosis has also been suggested to be a contributing factor to the development of hypercapnic respiratory failure, due to hypoventilation in response to metabolic alkalosis itself [223].

Cystic fibrosis is a multi-system disease with CFTR being expressed throughout the body including the kidneys, where it interacts with multiple ion channels. While the handling of bicarbonate in the kidney may be an important co factor in driving metabolic alkalosis in CF, the aetiology remains unclear [241,242].

We performed a case-controlled pilot study to further explore the role of the kidney in acid-base balance in CF.

8.2 Aim of the study

The aims of this study were:

- To perform a preliminary, non-invasive assessment of renal involvement in acid-base balance of people with CF.
- To assess the feasibility of larger prospective trials assessing the acid-base status in people with CF compared to other chronic respiratory conditions;

8.3 Methods

A prospective, pilot, case-control study was performed in the Leeds Regional CF Unit and Respiratory Department.

8.3.1 Study population

Eligible participants were recruited on admission to the Department of Respiratory Medicine.

The inclusion criteria to be enrolled in this study included:

- A baseline diagnosis of either:
    - cystic fibrosis (two confirmed mutations and/or sweat chloride >30 mmol/L with clinical manifestation of CF) for the cases, and
    - pneumonia, exacerbation of non-CF primary respiratory disorder (bronchiectasis, COPD, asthma, ILD), or other chest infection for control subjects;
- Admission to the Department of Respiratory Medicine following a pulmonary exacerbation or lower respiratory tract infection;;
- Being able to provide a urinary sample;
- Having an age of 55 years or lower.

The exclusion criteria were:

- Primary reason for hospital admission being non-respiratory;
- Being treated with diuretics;
- Having uncontrolled diabetes,
- Inability to provide consent.

8.3.2 Data collection

Blood gases, either arterial or arterialised capillary, and contemporaneous urine samples for urinary electrolytes were collected on admission (day 1), on day 7 and on day 14 or discharge if sooner.

Urine samples for pH, HCO_3^- and chloride were transferred immediately after collection in vacuum tubes, and hand-delivered to the laboratory for immediate analysis [412].

Baseline demographic data of comorbidities, use of supplemental oxygen and use of non-invasive ventilation, were collected from electronic medical notes (EMIS for patients with CF and PPM+ for controls), and collated with the remainder of the data set.

The average lung function in the year preceding enrolment was also recorded whenever available.

All arterial or arterialised capillary blood gases were interpreted using an automated reporting tool developed as part of this thesis (Section 4.3.2 and Appendix B).

8.3.3 Study outcomes

The study was designed to preliminary evaluate the renal handling of bicarbonate. Outcomes of the studies were distribution of acid-base disturbances and urinary concentration of bicarbonate and other electrolytes in people with and without CF.

The study also assessed the feasibility of performing a larger trial to evaluate the role of the kidney in acid-base status in people with CF, compared with those with other lung conditions.

8.3.4 Statistical analysis

No sample size calculation was performed for this pilot study. The aim was to to enrol at least 15 participants in each arm, over an expected study duration of 3 months.

Normal distribution of measured variables was assessed by visual inspection and using the Shapiro-Wilks test. Results are expressed as number (percentage), means (standard deviation), when normally distributed, or median (IQR, 25^{th}-75^{th} percentile) when not normally distributed.

Unpaired t-test for parametrical data or Mann-Whitney test for non-parametric data was used to compare each variable in the two cohorts (CF and other diagnoses). Mean (95% CI) difference is presented. A p-value <0.05 was considered statistically significant.

All analyses were performed with IBM SPSS v26.

8.3.5 Ethics

The study was approved by REC and HRA (14/NE/1197) and local R&I (RM14/11390), and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

8.3.6 Contribution

This study was designed by Dr Giles Fitch and Prof Daniel Peckham. Dr Fitch and the research nurses in the Leeds Regional Adult CF Unit enrolled research participants. My contribution to the study consisted in the collection of data, auditing of participants' notes and records to confirm accuracy, statistical analysis and critical interpretation.

8.4 Results

8.4.1 Study population

Between February and September 2015, a total of 31 participants were enrolled to this study, of which 15 in the case cohort and 16 in the control cohort. Table 8.1 summarises the demographics of the two groups of patients. Among controls, the most common underlying respiratory diagnosis was asthma (n=9, 56.3%), followed by bronchiectasis (n=3, 18.8%) and pneumonia (n=3, 18.8%). One subject in the control group had ILD.

Individuals with CF were younger and had lower lung function than those in the control arm. They also had a higher prevalence of liver disease, and a lower prevalence of

cardiovascular comorbidities, compared to the control group. Requirements for respiratory support at home were similar in the two cohorts.

Table 8.1 Baseline characteristics of the CF and control cohorts. Data are presented as number (%) and median (IQR).

	Cases (n=15)	Controls (n=16)	*p*
Age	31 [26-35]	45 [33-59]	**0.002**
Male Sex, n (%)	10 (66.7%)	7 (43.8%)	0.2
BMI	22.5 [17.4-24.9]	27.8 [23-32]	**0.007**
FEV1 %	36 [27-35]	61 [55-99]	**<0.001**
A&E admission, n(%)	0	13 (81.3%)	**<0.001**
Comorbidities			
Cardiovascular	1 (6.7%)	5 (31.3%)	0.083
Liver disease	10 (66.7%)	1 (6.3%)	**0.002**
Diabetes	4 (26.7%)	1 (6.3%)	0.061
Respiratory support			
Oxygen therapy	2 (13.3%)	1 (6.3%)	0.505
NIV	0	2 (12.5%)	0.157

8.4.2 Trial feasibility outcomes

The average recruitment rate was 3.75 subjects per month, but differed between participants both cohorts (3 subjects per month with CF vs 5 subject per month in controls). There were no dropouts from the study.

Data completion rate was 100% for data collected on day 1, but dropped to 50% for data collected on day 7 and 14 or on discharge. As a consequence of this, only baseline data could be analysed for the exploratory outcomes of interest.

8.4.3 Exploratory outcomes of interest: blood gas analysis

Table 8.2 summarises the arterial blood gas analyses of the participants in the CF and control group. Baseline blood gas was performed on room air in 13 (86.7%) subjects with CF, and in 9 (56.7%) in the control groups. All other blood gases were performed on supplemental conventional oxygen (nasal cannula or Venturi mask). No blood gas was done on NIV.

Table 8.2 Blood gases results in the two cohorts. Data are presented as median (IQR).

	Cases (n=15)	Control (n=16)	*p*
ABG			
pH	7.46 [7.41-7.49]	7.43 [7.42-7.49]	0.953
pCO_2, kPa	5.25 [4.88-5.73]	4.49 (0.42)	**<0.001**
pO_2, kPa	9.6 [9-10.2]	11.29 [8.19-13.87]	0.281
HCO_3^-, mmol/L	27.8 [26.3-30]	23.4 [22.1-24.5]	**<0.001**
BE	3 [2-7]	-0.85 [-2.75-0.17]	**<0.001**

Oxygenation was similar across the two groups [mean difference -1.62 (95%CI -4.13-0.88], whereas ventilation was significantly different as shown in Table 8.2 [mean

difference pCO_2 1.25 (95%CI 0.47-1.95)]. Arterial bicarbonate was significantly higher among subjects with CF [difference of +5.33 (95%CI 3.55-7.11].

Interpretation of the blood gas analyses showed that metabolic alkalosis occurred more frequently in individuals with CF (46.7% vs 7.1%).

8.4.4 Exploratory outcomes of interest: serum bicarbonate

Serum bicarbonate concentration was higher in subjects with CF compared to the control cohort [28 (25.5-28.5) vs 24 (23-24.75), *p*=0.004] (

Figure 8.1). In the cohort of subjects with CF, serum bicarbonate tended to be at the upper limit of normal, whereas in the control cohort the concentration was on average lower than the reference values, despite normal creatinine and renal function (Table 8.3).

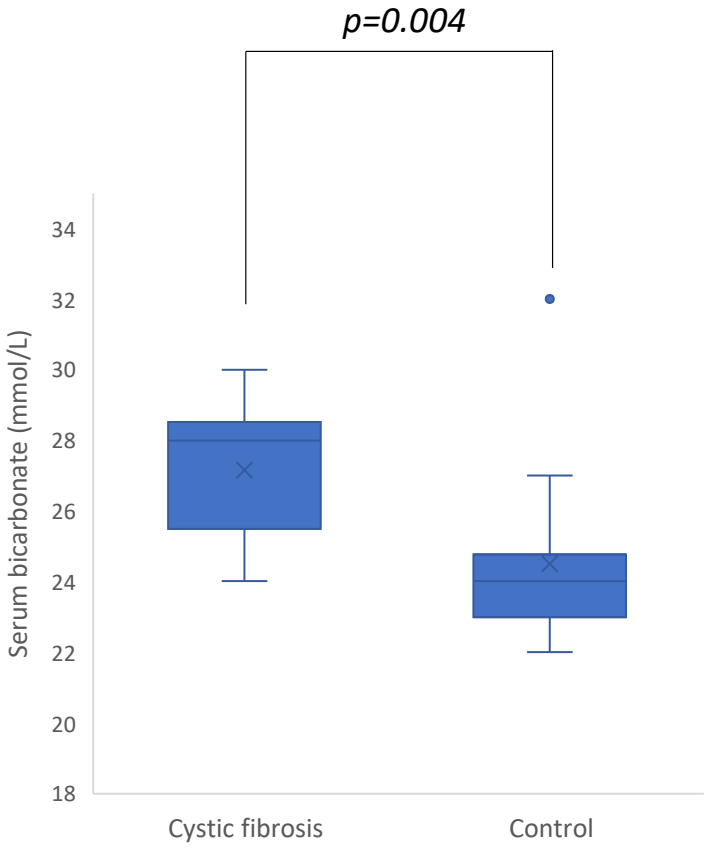

Figure 8.1 Serum bicarbonate concentration based on underlying diagnosis

Table 8.3 Serum electrolyte concentration across the two cohorts.

	Cases (n=15)	**Control (n=14)**	***p***
Serum bicarbonate, mmol/L	28 (3)	24 (1)	**0.001**
Serum chloride, mmol/L	101 (7)	105 (5)	0.067
Serum sodium, mmol/L	138 (3)	141 (4)	0.108
Serum potassium, mmol/L	4.1 (0.4)	4.0 (0.5)	0.892
Urea, mmol/L	4.2 (3.2)	4.9 (2.2)	1.0
Creatinine, umol/L	61 (46)	58 (15)	0.650

Serum bicarbonate correlated well with the results of the blood gas analysis (Figure 8.2), with a Pearson correlation coefficient of 0.494 (p=0.016).

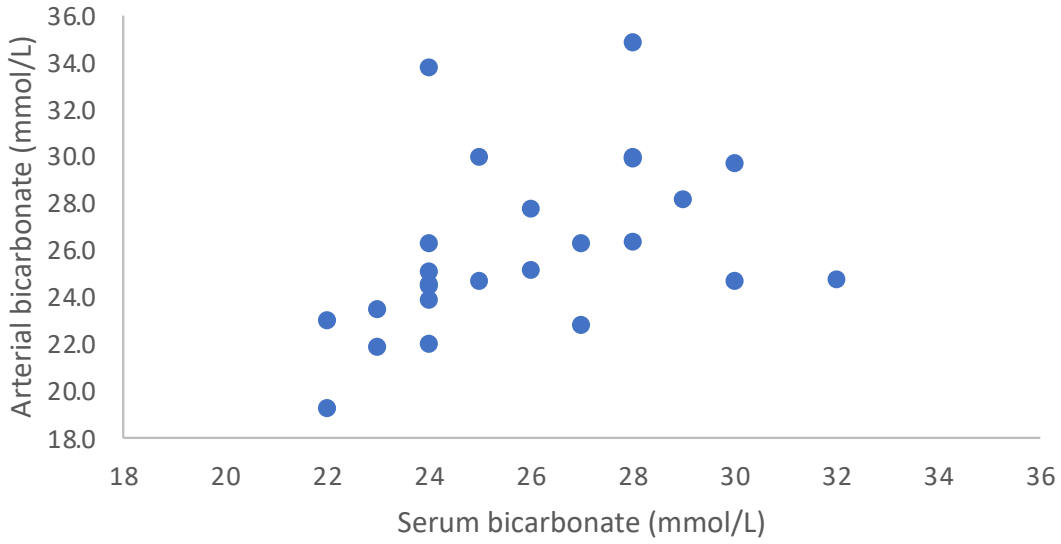

Figure 8.2 Scatter plot of serum bicarbonate and arterial bicarbonate.

As subjects with CF were younger, had a lower BMI and FEV_1 than those in the control cohort, correlation analyses were performed to assess the relationship of serum bicarbonate with these variables.

Overall, there was a significant negative correlation between age and bicarbonate (Pearson coefficient -0.45, p=0.023). When assessing the two cohorts independently, a trend was observed among patients with CF (Pearson coefficient for CF -0.523, p=0.07), but no correlation was noted for other diagnosis (Pearson coefficient -0.117, p=0.717). No correlation was observed between serum bicarbonate and FEV_1 in the whole population and in the two cohorts assessed separately.

Serum chloride was within normal limits in both cohorts, but there was a trend towards a lower concentration among subjects with CF compared to the control cohorts. All other electrolytes showed a similar serum concentration in the two cohorts, and within reference range (Table 8.3).

No correlation was observed between the concentration of serum bicarbonate and any other serum electrolytes (Figure 8.3), either in the overall population or in the separate cohorts.

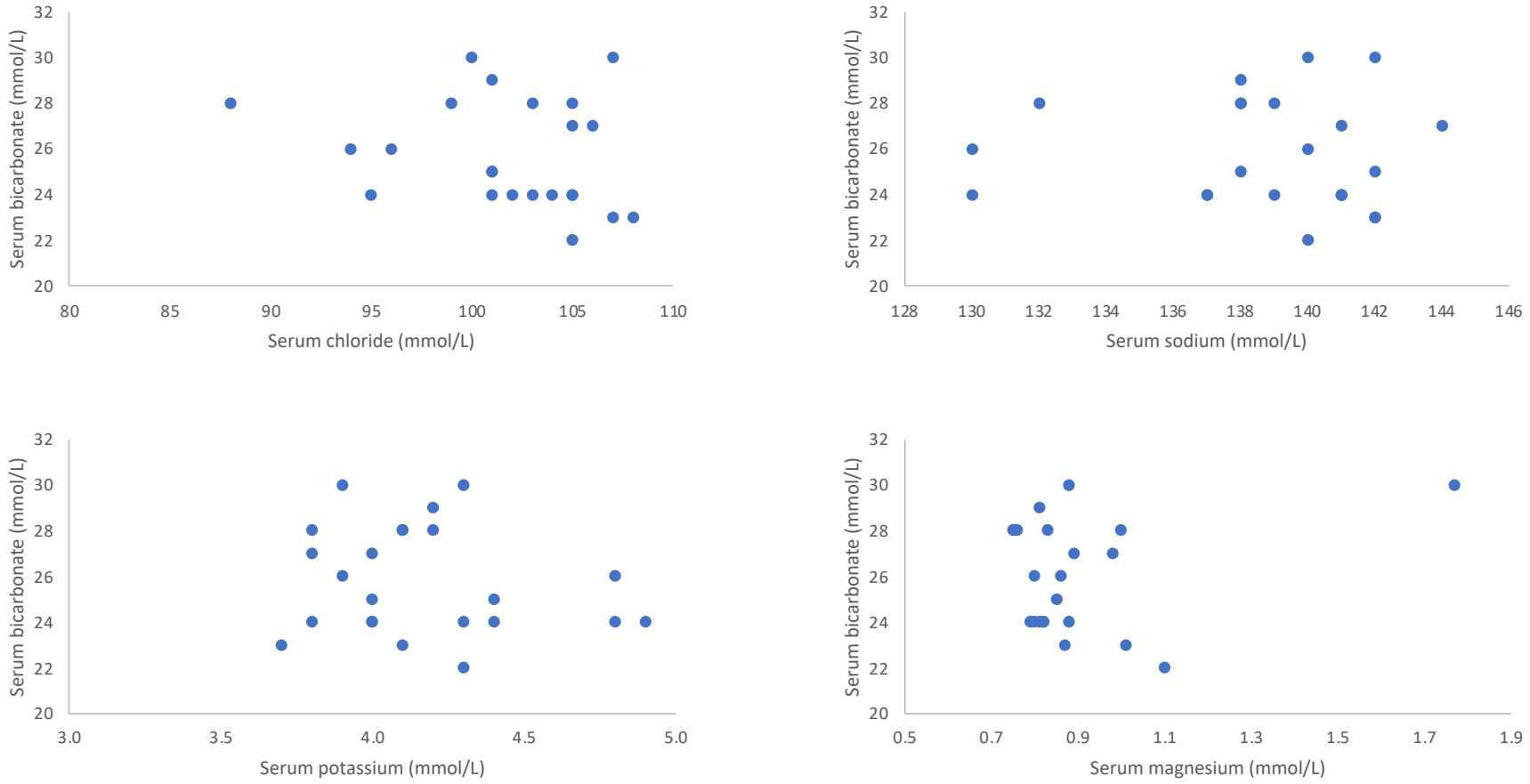

Figure 8.3 Scatter plots of bicarbonate with serum electrolytes in the whole population.

8.4.5 Exploratory outcomes of interest: urinary electrolytes

Table 8.4 reports the urinary electrolytes and pH in the CF and control cohorts.

Table 8.4 Urinary electrolytes and analysis across the two cohorts. Data are presented as median (IQR).

	Cases (n=15)	Control (n=16)	*p*
Urinary pH (lab)	6 [5.75-6.5]	6 [5.25-6.87]	0.918
Urinary pH (dipstick)	6 [5.38-6.13]	6 [5-7.75]	0.687
Urinary bicarbonate mmol/l	3.5 [1.22-5]	2.5 [0-9.75]	0.982
Urinary chloride, mmol/L	133 [65.25-174.75]	49.5 [37.5-117]	*0.070*
Urinary sodium, mmol/L	99 [65.75-157.75]	51.5 [29-91]	*0.056*
Urinary potassium, mmol/L	64 [25.75-80]	46.5 [24-66.5]	0.654
Urinary urea, mmol/L	286.5 [199.75-345.75]	260.5 [125-392]	0.740
Urinary creatinine, mmol/L	11 [5.27-12.25]	10.7 [4.72-16.95]	0.711
Urinary magnesium, mmol/	3.2 [1.82-6.12]	2.35 [1.4-4.64]	0.137
Urinary calcium, mmol//L	4.8 [1.68-6.76]	2.6 [1.7-4.9]	0.412
Urinary phosphate, mmol/L	22.6 [15.94-26.37]	10.7 [2.24-23.52]	**0.033**

No differences were observed in renal excretion of bicarbonate, but there was a trend towards a higher urinary chloride and sodium concentration among subjects with CF (Figure **8.4**).

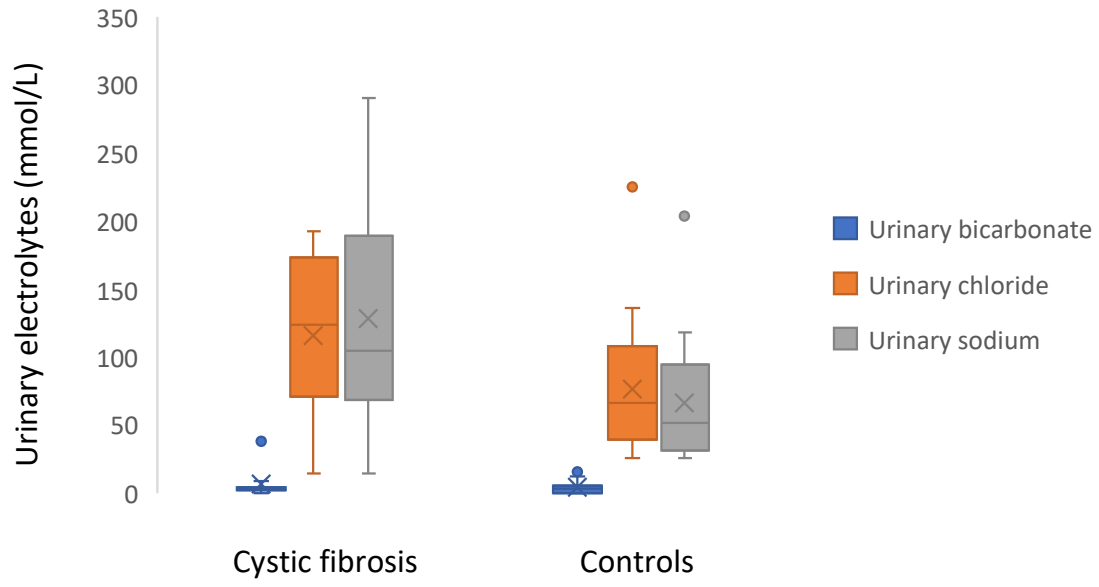

Figure 8.4 Urinary electrolytes in the two cohorts. No difference in the urinary excretion of bicarbonate was observed in CF (left) and control (right) cohorts. A trend towards a higher excretion of sodium and chloride was noted among subjects with CF.

No correlation between serum concentration and urinary excretion of bicarbonate was observed in the overall group and in the two cohorts (Figure 8.5). The correlation coefficient was 0.077 (p=0.833) for the subjects with CF and 0.046 (p=0.899) for subjects in the control cohort.

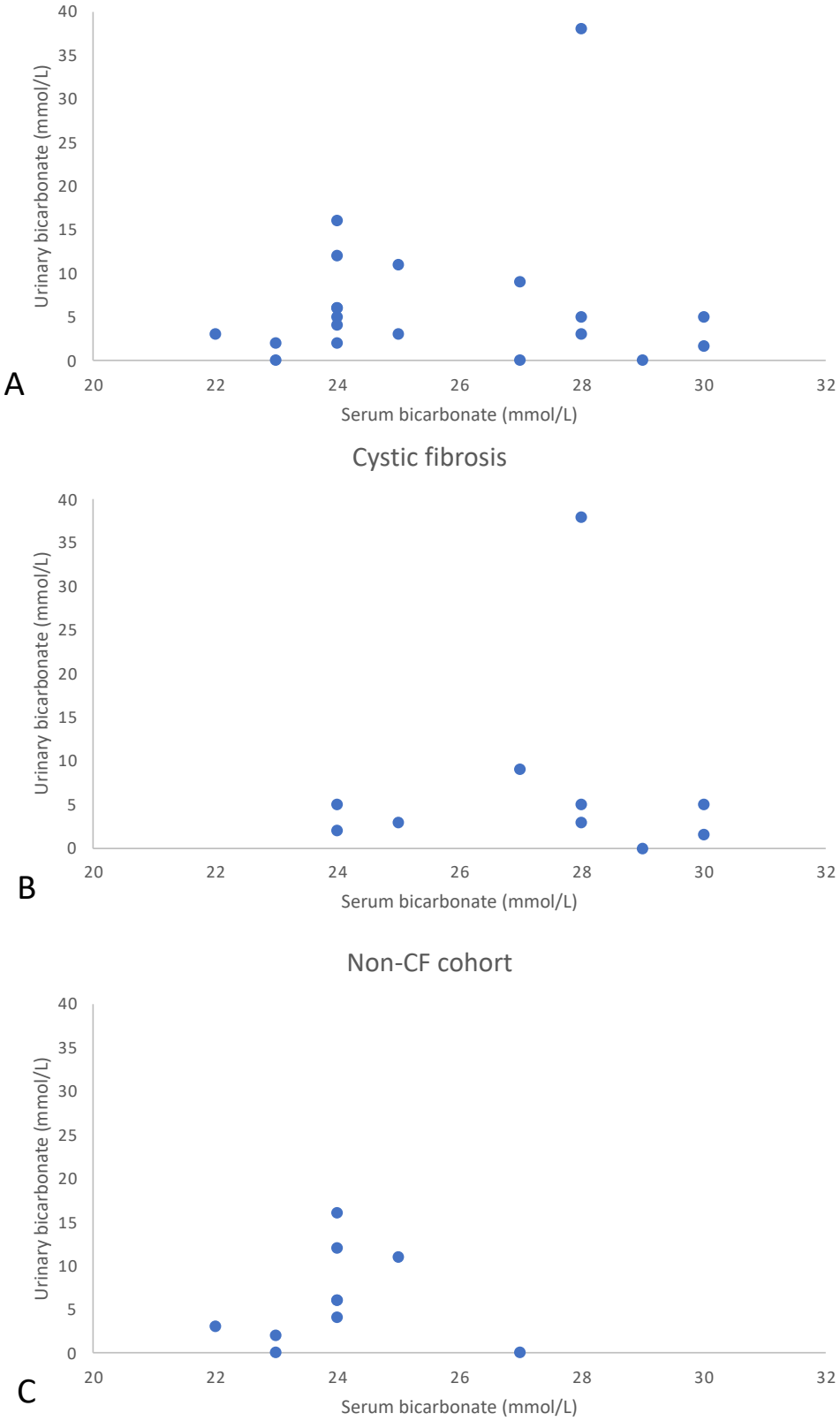

Figure 8.5 Scatter plot of urinary and serum bicarbonate in the whole population and across the cohorts.

Similarly no correlations between urinary excretion of chloride or sodium and serum bicarbonate were observed, both by visual inspections of the scatter plots and formal statistical analysis of the correlation coefficients.

8.5 Discussion

The increased prevalence of metabolic alkalosis in adults with CF compared to the general population is well known [216,218,223]. However, the aetiology of this acid-base imbalance in the CF population is not clear.

To clarify the underlying mechanisms to this imbalance, this study assessed the renal handling of HCO_3^- electrolytes in relation to acid-base status during pulmonary exacerbations. To the best of my knowledge, this is the first study exploring the contribution of the kidneys to the metabolic status in people with CF, by non-invasively assessing the urinary excretion of anion and cations involved in the process.

This pilot study highlighted the complexity of having a procedure in place to collect and promptly process urinary samples for the assessment of bicarbonate and other electrolytes, while avoiding loss of CO_2 for bicarbonate analysis [412]. This requires sample collection and processing to be completed within the stringent timeframes of one hour.

Despite concerns around this element of the protocol, a larger-scale study seems feasible. However, design and recruitment strategies should be revisited. In our population, we had excellent data completion rate at day 1, including for urinary bicarbonates, but a poor one for data collected on day 7 and 14 or at discharge. This discrepancy cannot be attributed to the procedure of collecting urinary samples, but rather to the recruitment strategy. Patients enrolled in the control cohort were often discharged prior to the follow-up data collection dates, preventing data collection within the time frame of an admission.

In a larger-scale, confirmatory study, follow-up reviews at day 7 and day 14 should be arranged in dedicated clinics, for all patients discharged prior to the date of follow up. Care should be taken for these clinics to be arranged within the same hospital where the laboratory for processing urinary samples is located, rather than in the community, where a laboratory would not be readily available.

In addition, the distribution of data in our exploratory analysis suggests that a large sample size would be required to properly power a confirmation trial.

Metabolic alkalosis and isolated elevation of serum bicarbonate occurred more frequently in people with CF compared to controls, but this did not appear to be associated with Pseudo-Bartter syndrome or to a post-hypercapnic status. The normal

serum electrolytes and increase in urinary sodium and chloride excretion all point to the presence of an alternative mechanism at play, including in particular defective HCO_3^- handling secondary to CFTR dysfunction.

In the two cohorts, patients with CF presented with metabolic alkalosis more frequently than the control group (46.7% vs 7.1%), as previously described [223]. Compared to previous studies however, the control group in our trial included mostly patients with asthma, and no patients with COPD. While this might affect the acid-base profile of the control cohort, as patients with exacerbations of asthma frequently present with respiratory alkalosis, it should not affect the considerations related to CF cohort.

Among patients with CF in this study, metabolic alkalosis does not appear to be associated with PBS. Serum electrolytes and renal function were in fact normal among subjects with CF, as well as in controls. In addition, within the CF cohorts electrolytes were within normal range irrespective of the acid-base status.

Similarly, no differences in the urinary excretion of electrolytes were observed when comparing patients with metabolic alkalosis and those with a different metabolic status.

An elevation in sodium and chloride urinary excretions usually reflects a mineralocorticoid excess, or the presence of a disorder that mimics it, including primary activation of ENaC [413]. Changes in ion transport in the renal tubules could be due to CFTR dysfunction and possible elevation in ENaC activity [414].

Previous studies on patients with obesity-hypoventilation syndrome suggest that raised HCO_3^- and BE may be part of the spectrum of chronic ventilatory failure even in the presence of normal pH and daytime pCO_2 [387]. If the same were to apply to people with CF, transient hypoventilation with raised pCO_2 levels may be the cause of the increased serum HCO_3^- noted in the CF cohort. While the potential role of this compensatory mechanisms was not investigated in the present study, a post-hypercapnic status would usually be associated with low urinary excretion of chloride (<20 mmol/L) [413].

Furthermore, increased serum concentration of HCO_3^- should result in increased urinary excretion, via process mediated by the hormone secretin. In this study, urinary excretion of HCO_3^- was similar in the CF and control cohorts. In CF, serum HCO_3^- levels do not correlate with urinary excretions as might have been expected.

Renal handling of HCO_3^- in CF appears to be defective, in line with earlier small studies which showed that people with CF excreted less HCO_3^- than controls, despite having comparable serum levels and pCO_2 [241]. This might well be a direct consequence of CFTR dysfunction, in view of the recent link proposed between CFTR, renal pendrin and metabolic alkalosis in knock-out murine models [243].

8.5.1 Limitations

None of the participants in the CF group were retested on CFTR modulators to asses if treatment led to changes in urinary bicarbonate excretion.

If we speculate a direct role of defective CFTR in determining the acid-base status in people with CF, a control group of patients treated with small molecule therapy able to restore the function of CFTR across all systems would be an ideal control cohort. This study was however carried out prior to the introduction of the newer modulators.

In addition, the lack of sleep and/or exercise studies in patients with CF meant that we were unable to assess if subjects were experiencing any hypoventilation, albeit transient, that could affect their acid-balance status. In addition, the high proportion of patients with exacerbations of asthma in the control cohort could have led to an increase prevalence of hypocapnia and subsequent lower concentration of bicarbonate among the control subject.

Finally, despite the inclusion criteria for the control cohort being quite narrow in terms of age, the two groups were different in demographics and baseline characteristics, with patients with CF being younger and having a more severe lung disease and systemic complications.

8.6 Conclusion

This study demonstrates that acid-balance differs in people with CF compared to individuals with other respiratory conditions at time of pulmonary exacerbations. Patients with CF tend to present more often with metabolic alkalosis during exacerbations compared to individuals with other respiratory conditions.

The aetiology of these changes in acid-base and metabolic status of people with CF appears to be complex. Based on these exploratory outcomes, we speculate that respiratory and metabolic dysfunction are co-determinant for the altered metabolic status of people with CF.

Patients with CF might experience transient hypoventilation with subsequent increase in partial pressure of carbon dioxide and bicarbonate concentration as a compensatory mechanism. CFTR dysfunction in the kidneys leads to loss of inhibition of ENaC with subsequent high excretion of chloride and sodium. Similarly, CFTR dysfunction at tubular level leads to defective upregulation of pendrin and lack of increase in urinary excretion of bicarbonate in response to high serum concentration or base load.

A larger prospective study including subjects on highly effective CFTR modulators is needed to clarify if these drugs can modify urinary electrolyte excretion and hyperbicarbonatemia during exacerbations.

Chapter 9
Hypoxic altitude simulation test in respiratory disease: a prospective external validation study

9.1 Introduction

Metabolic alkalosis appears to be the most frequent acid-base disturbance in patients with cystic fibrosis, both in clinical stability and during pulmonary exacerbations (Chapter 7 and Chapter 8) [215–217,223,359]. Similarly, isolated elevation of serum bicarbonate has been noted across the Leeds cohort of patients with CF during regular annual assessments (Chapter 5), where it appears to not to be exclusively associated with severe lung disease.

I hypothesised (Chapter 4, Section 4.1.1) that isolated elevation of serum bicarbonate in patients with CF could either be the result of defective handling of bicarbonate in the kidney, or be on the spectrum of chronic ventilatory failure, as previously described for obesity hypoventilation syndrome [387,389].

As previously described in Chapter 7, patients with raised bicarbonate had a response to the hypoxic stimulus similar to that of people with daytime hypercapnia. This was shown on a large cohort of patients with CF and other respiratory conditions, attending the Cardio-respiratory Department for a hypoxic altitude simulation test (HAST).

Further, I showed that bicarbonate is a significant determinant of the response to the HAST, which allowed me to develop a predictive algorithm of HAST outcome, based solely on easy-to-measure variables of oxygenation and ventilation.

To further validate the results presented in Chapter 7, we performed a prospective external validation study of the predictive value of this model.

9.2 Aim of the study

The aims of this study were to:

- Confirm that metabolic alkalosis is the main acid-base disturbance observed in people with CF in a prospective cohort.
- Further assess the level of serum bicarbonate in people with CF in comparison with those with other respiratory conditions, and evaluate the level of agreement between serum and arterial bicarbonate.
- Validate the model previously developed in an external prospective cohort (Chapter 7, Section 7.4.4).

- Assess whether a simplified version of the model, that uses pulse oximetry and serum bicarbonate instead of arterial oxygen saturation and bicarbonate, is feasible and has comparable predictive value to the original model.

9.3 Methods

A single-centre, prospective observational study for external validation was performed at the Cardio-Respiratory Department of Leeds Teaching Hospital NHS Trust.

9.3.1 Study population

All patients attending the Department for a HAST were considered for eligibility to take part in the study.

The inclusion criteria for the study were:

- Age 18 years or older;
- Conditions of clinical stability;
- Background of chronic respiratory disease.

No exclusion criteria were defined for this study. Patients unable to provide informed consent were excluded.

9.3.2 Study procedures

Patients who agreed to take part in the trial received clinical care in keeping with recognised clinical practice. HASTs were performed as per standard BTS guidance at the Cardio-Respiratory department at LTHT by qualified physiologists, and was considered positive if pO_2 at the end of the test was lower than 6.6 kPa, or if a drop in oxygen saturation <85% with or without symptoms was noted during the test [182,391].

In addition to what is standard procedure for HAST, subjects taking part in the study agreed to the following:

- Measurement of respiratory rate (RR) and heart rate (HR) before and after the test;
- Venepuncture to collect a 4 ml blood sample for serum bicarbonate;
- Review of their medical notes by the study team.

9.3.3 Data collection

Results of HAST, RR and HR, as well as serum bicarbonate concentration were recorded.

Electronic medical records (EMIS for patients with CF, PPM+ for all other individuals) were searched to collate demographics, and primary respiratory diagnosis. Demographics, and clinical baseline information were collected in anonymised form for

each subject. In addition, best FEV_1, overnight oximetry and 6-minute walking test (6MWT) performed on the closest date within the 12 months prior the HAST were recorded, when available.

9.3.4 Statistical analysis

The statistical plan of this study, as well as the sample size calculation, were based on the validation of the previously defined model (Chapter 7).

Based on the prevalence of positive HASTs observed in the retrospective cohort (36.2% during a 5-year period), and considering that previous studies have concluded that at least 100 event and 100 non-event (positive HAST) outcomes are required to confirm that a predictive model is well calibrated, a sample size of 280 subjects was calculated.

However, in view of recent studies showing that smaller samples are often sufficient to externally validate scoring systems based on logistic regression models, multiple interim analyses were pre-planned (100, 150, 200 and 280 patients) with the aim to terminate the study early upon successful validation of the model.

Demographics and baseline characteristics of patients were analysed on the whole population and by cohorts depending on the underlying diagnosis, the outcome of the HAST and the four groups previously identified based on bicarbonate levels (Chapter 7).

Data distribution was assessed by visual inspection and using the Shapiro-Wilks test. Data are presented as mean and SD if normally distributed, median and IQR if distribution is non normal. Number and percentage was used to describe the frequency of distribution of acid-base status as defined by blood gas analysis interpretation.

Parametric and non-parametric analyses were conducted as appropriate. The chi-square test was performed to assess different distribution in prevalence.

The binary logistic regression model, developed as in Chapter 7, was applied to the external validation cohort. ROC curves were plotted to test the hypothesis that the proposed model is a good predictor of HAST outcomes. The accuracy of the results and error rate were subsequently computed, using the pre-identified cut-off criteria (Section 7.4.4.1) against the results of the HAST.

IBM SPSS statistics version 26 (IBM Corp, Armonk, NY, USA) was used for all the analyses.

9.3.5 Ethical approval

The study was approved by HRA (18/WS/0117) and local R&I (RM18/109075), and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

9.4 Results

9.4.1 Study population

Between November 2018 and March 2020, 114 patients underwent a HAST of which 96 were enrolled in the study. The study was paused in March 2020 due to the beginning of the COVID-19 pandemic. Because the number of enrolled patients at the time was really close to the first pre-planned interim analysis (n=100), a full interim analysis was conducted to assess if it was possible to terminate the study early as pre-planned.

Table 9.1 shows the baseline characteristics of the study population, and of the cohorts of subjects with CF and other respiratory diagnoses. Thirty-six subjects had CF, representing the 37.5% of the whole population. Subjects with CF were younger, had a lower BMI and had a lower prevalence of cardiovascular comorbidities and pulmonary hypertension, but more frequently had diabetes and liver disease ($p < 0.05$).

Table 9.1 Baseline characteristics for the whole population and the CF cohort
BMI, body mass index; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; CBG, capillary blood gas. Data are expressed as number (%) or median (IQR).

Baseline characteristics of population	Whole population (n=96)	CF cohort (n=36)	Non-CF cohort (n=60)	*p*
Male gender, n(%)	54 (56.3%)	19 (52.8%)	35 (58.3%)	0.595
Age at test in years	60 [31.5-72.4]	31.2 [26.1-37.3]	70.9 [62.8-75.6]	**<0.001**
BMI, (kg/m^2)	25.6 [21.8-29.8]	22 [20.7-25.6]	27.6 [24.3-29]	**<0.001**
Ethnicity				0.710
Caucasian	89 (92.7%)	33 (91.7%)	56 (93.3%)	
Asian	7 (7.3%)	3 (8.3%)	4 (6.7%)	
Diagnosis				**<0.001**
CF	36 (37.5%)	36 (100%)	-	
COPD	22 (22.9%)	-	22 (36.7%)	
ILD	30 (31.3%)	-	30 (50%)	
Other	8 (8.3%)	-	8 (13.3%)	
Comorbidities				
Diabetes	25 (26%)	15 (41.7%)	10 (16.7%)	**0.014**
Liver diseases	35 (36.5)	31 (86.1%)	4 (6.7%)	**<0.001**
Pulmonary hypertension	8 (8.3%)	0 (0%)	8 (13.3%)	**0.035**
Cardiovascular	23 (24%)	2 (5.6%)	21 (35%)	**0.002**
Baseline CBG				
pH	7.42 [7.4-7.44]	7.42 [7.4-7.44]	7.42 [7.4-7.44]	0.643
pCO_2, kPa	4.7 [4.4-5.1]	4.7 [4.3-5.1]	4.7 [4.4-5.2]	0.421
pO_2, kPa	9.3 [8.5-10.4]	10.1 [9.3-10.1]	8.8 [7.8-9.4]	**<0.001**
HCO_3^-, mmol/L	23.8 [22.6-25.1]	23.6 [22.4-25]	23.8 [22.7-25.1]	0.655
BE	-0.8 [-2.3-0.8]	-1.2 [-2.5-0.8]	-0.8 [-2.1-0.8]	0.660
SO_2, %	93.6 [91.5-94.6]	94.8 [94-96]	93 [90-94]	**<0.001**
Serum HCO_3^-, mmol/L	27 [24-29]	26.5 [24-28]	27 [24.25-29]	0.420
Baseline SpO_2, %	94 [92-95]	95 [94-96]	92 [91-94]	**<0.001**
Blood gas interpretation				ns
Normal CBG	51 (53.1%)	19 (52.8%)	32 (53.3%)	
Metabolic alkalosis	2 (2.1%)	-	2 (3.3%)	
Metabolic acidosis	-	-	-	
Respiratory alkalosis	11 (11.5%)	6 (16.7%)	5 (8.3%)	
Respiratory acidosis	1 (1.0%)	-	1 (1.0%)	
Mixed disorders	2 (2.1%)	2 (5.6%)	-	
Compensated disorders	23 (23.9%)	9 (24.9%)	14 (23.3%)	
Missing	6 (6.3%)	-	6 (10%)	

9.4.2 Acid-base status

Each blood gas results was interpreted using the automatic tool described in Section 4.3.2 and Appendix B.

The distribution of acid-base status was similar comparing subjects with CF and those with other respiratory conditions (Table 9.1). In both cohorts, blood gas results were most often within normal limits. The most common acid-base disturbance observed in both cohorts was compensated acid-base disorders. Metabolic alkalosis and acute uncompensated respiratory acidosis were not observed among people with CF, and appeared to be a rare occurrence within the control cohort (Figure 9.1).

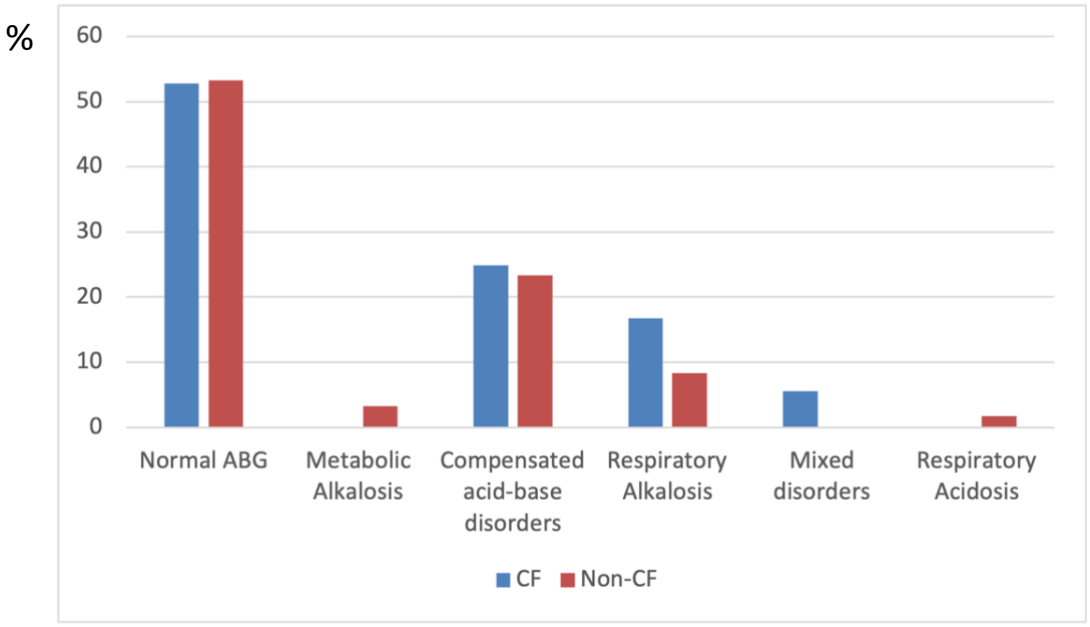

Figure 9.1 Frequency of distribution of blood gas analysis results in the CF cohort and among individuals with other respiratory conditions.

In view of the small numbers of tests across the different groups based on the acid-base assessment, analysis on the response to the hypoxic stimulus depending on acid-base status was not performed.

9.4.3 Arterial and serum bicarbonate

As in the retrospective study (Chapter 7), four groups were identified based on serum bicarbonate and partial pressure of carbon dioxide, as follows:

1. Group 1: normal pCO_2 and normal bicarbonate;
2. Group 2: normal pCO_2 and elevated bicarbonate;
3. Group 3: elevated pCO_2;
4. Group 4: all others.

Within the whole population, most subjects were included in groups 1 and 4 (23 and 59, respectively), whereas only 7 had elevated daytime pCO_2 and 1 had raised arterial

bicarbonate with normal pCO_2. Within the CF cohort, no subject had daytime hypercapnia or isolated raised arterial bicarbonate. Considering this and the low number of subjects in the four subgroups, analysis of the different response to the hypoxic stimulus was not performed.

While no difference overall was noted in arterial or serum bicarbonate concentration comparing people with CF and those with other respiratory conditions, a direct comparison of serum and arterial bicarbonate in both cohorts showed that serum bicarbonate level tended to be more elevated than arterial (Figure 9.2).

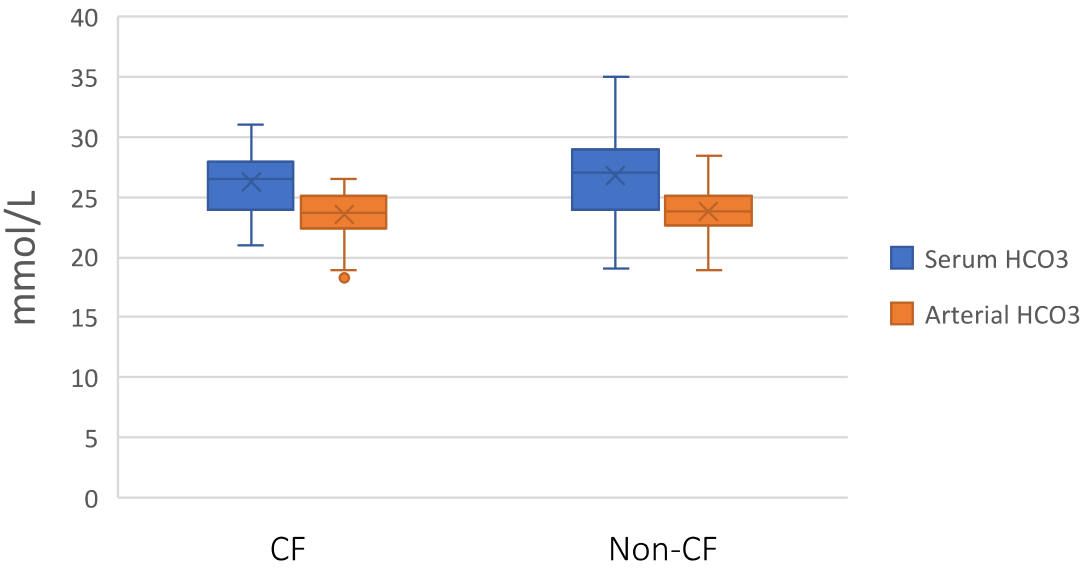

Figure 9.2 Arterial and serum bicarbonate in the CF and non-CF cohort

A good correlation was, however, noted in the overall population and in each cohort separately. This correlation was stronger in the CF cohort (Spearman rho 0.679, $p<0.01$) than in the non-CF cohort (Spearman rho 0.538, $p<0.01$) (Figure **9.3**).

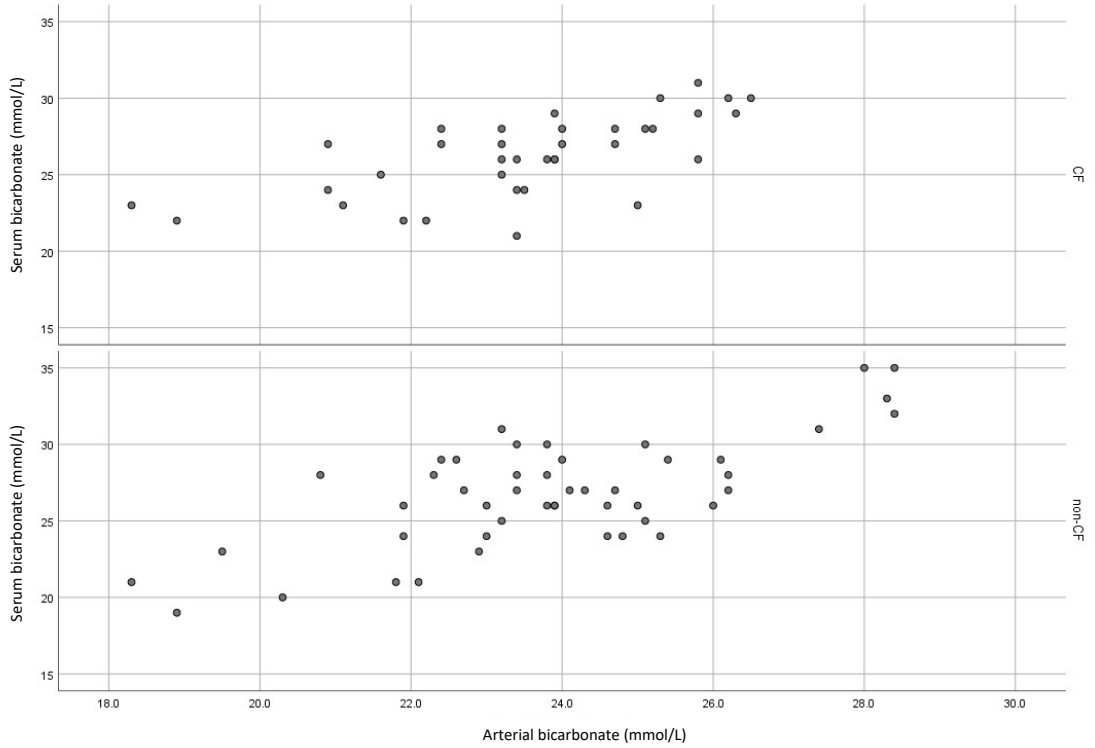

Figure 9.3 Correlation between serum and arterial bicarbonate in the CF and non-CF cohorts. The top panel represents the CF cohort with a Spearman rho=0.679, the bottom panel shows the non-CF cohort with a Spearman rho=0.538.

A stronger correlation was observed between pCO_2 and serum bicarbonate, but this was more significant in the non-CF cohort (Spearman's rho 0.703, p<0.01) than in the CF group (Spearman's rho 0.566, p<0.01) (Figure **9.4**).

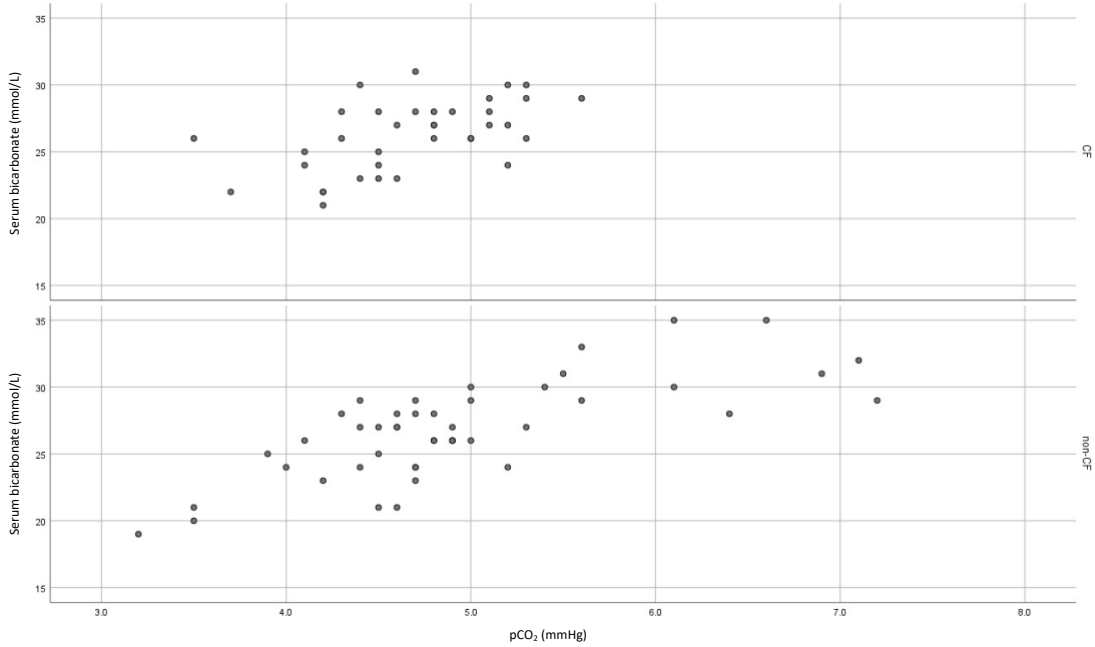

Figure 9.4 Correlation between serum bicarbonate and pCO2 in the CF and non-CF cohorts. The top panel represents the CF cohort with a Spearman rho=0.566, the bottom panel shows the non-CF cohort with a Spearman rho=0.703.

9.4.4 Predictive model validation

The cohort included in this study was used to validate the model developed and previously retrospectively validated in Chapter 7.

The validation cohort included 96 HAST, 29.2% of which were positive. The validation cohort was different compared to the previous training cohort in terms of underlying diagnoses and baseline CBG (Table 9.2).

Table 9.2 Baseline characteristics of the training and external validation cohorts. BMI, body mass index; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; NMD, neuromuscular disorder; OSA, obstructive sleep apnoea; OHS, obesity hypoventilation syndrome. Data are expressed as number (%), and median (IQR).

	Training	**External validation**	***p***
Age	59.2 [30.6-70.3]	60.1 [31.5-72.4]	0.466
BMI	24.9 [21.7-29.3]	25.6 [21.8-29.8]	0.792
Ethnicity			0.502
Caucasian	304 (90.7%)	88 (91.7)	
Asian	26 (7.8%)	7 (7.3%)	
Black	5 (1.5%)	-	
Male sex	164 (49%)	54 (56.3)	0.247
Diagnosis			**0.022**
CF	124 (37%)	36 (37.5%)	
COPD	69 (20.6%)	22 (22.9%)	
ILD	68 (20.3%)	30 (31.3%)	
NMD	16 (4.8%)	-	
OSA/OHS	15 (4.5%)	-	
Other	43 (12.8%)	8 (8.3%)	
Baseline CBG			
pH	7.44 [7.42-7.46]	7.42 [7.40-7.44]	**0.001**
pCO_2 kPa	5.2 [4.8-5.6]	4.7 [4.4-5.1]	**<0.001**
pO_2, kPa	9.1 [8-10.1]	9.3 [8.5-10.4]	0.235
HCO_3^-, mmol/L	26.2 [24-28.4]	23.8 [22.6-25.1]	**<0.001**
BE	2.0 [0-4.1]	-0.8 [-2.3-0.8]	**<0.001**
SO_2, %	94 [92-96]	93.6 [92.2-95.2]	0.634
Serum HCO_3^-, mmol/L	n/a	25.6 [24-29]	

9.4.4.1 ROC Analyses in the prospective validation cohort

ROC curves for pO_2 and the 3 multivariable models were plotted against the HAST outcomes (Figure **9.5**). The ROC AUC were 0.849 (95%CI 0.753-0.944), 0.854 (95%CI 0.765-0.943), 0.842 (95%CI 0.750-0.933) and 0.840 (95%CI 0.742-0.937) for the baseline pO_2 model, Model 1, Model 2 and Model 3, respectively.

No significant differences between the four curves were observed within the validation cohort. Similarly, no differences were observed with the ROC curves plotted for the training cohort (Figure **9.5**).

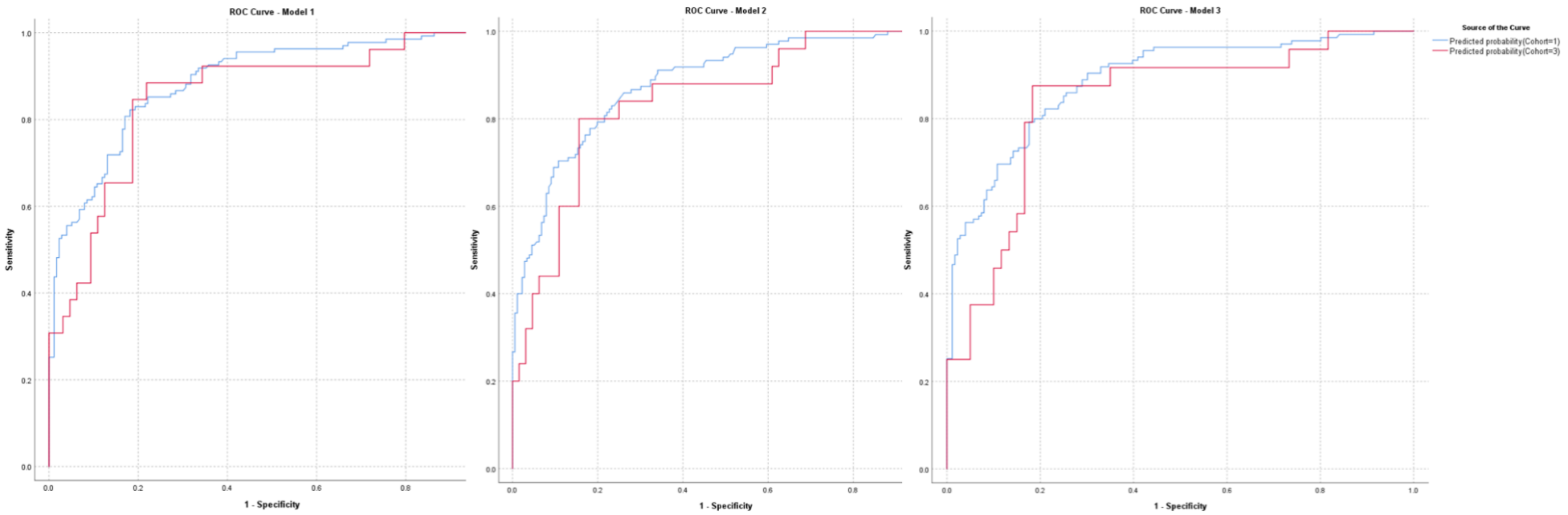

Figure 9.5 ROC Curve of the three models against a positive outcome of the HAST in the training and external validation cohort. Each panel present the ROC curve for one of the models in the training (blue) and validation (red) cohorts. No differences were observed between the ROC AUC for the training and validation cohorts.

9.4.4.2 Predicted probability in the external validation cohort

The cut-off values at 95% and 97.5% (Chapter 7, Section 7.4.4.2) for Model 1, previously identified as the best performing, were applied to the output of this validation cohort.

The 95% thresholds would have allowed a 56.3% reduction in number of HAST, with 7 (7.3%) misclassification. These would have been distributed as 2 wrongly identified as not requiring oxygen (2.1%) and 5 as needing in-flight oxygen (5.2%).

The 97.5% thresholds would have reduced the HAST performed by 32.3%, with 5 misclassification. Of these, one subjects would have been wrongly identified as not requiring in-flight oxygen (1.0%), and 4 (4.2%) as requiring in-flight oxygen (Figure 9.6).

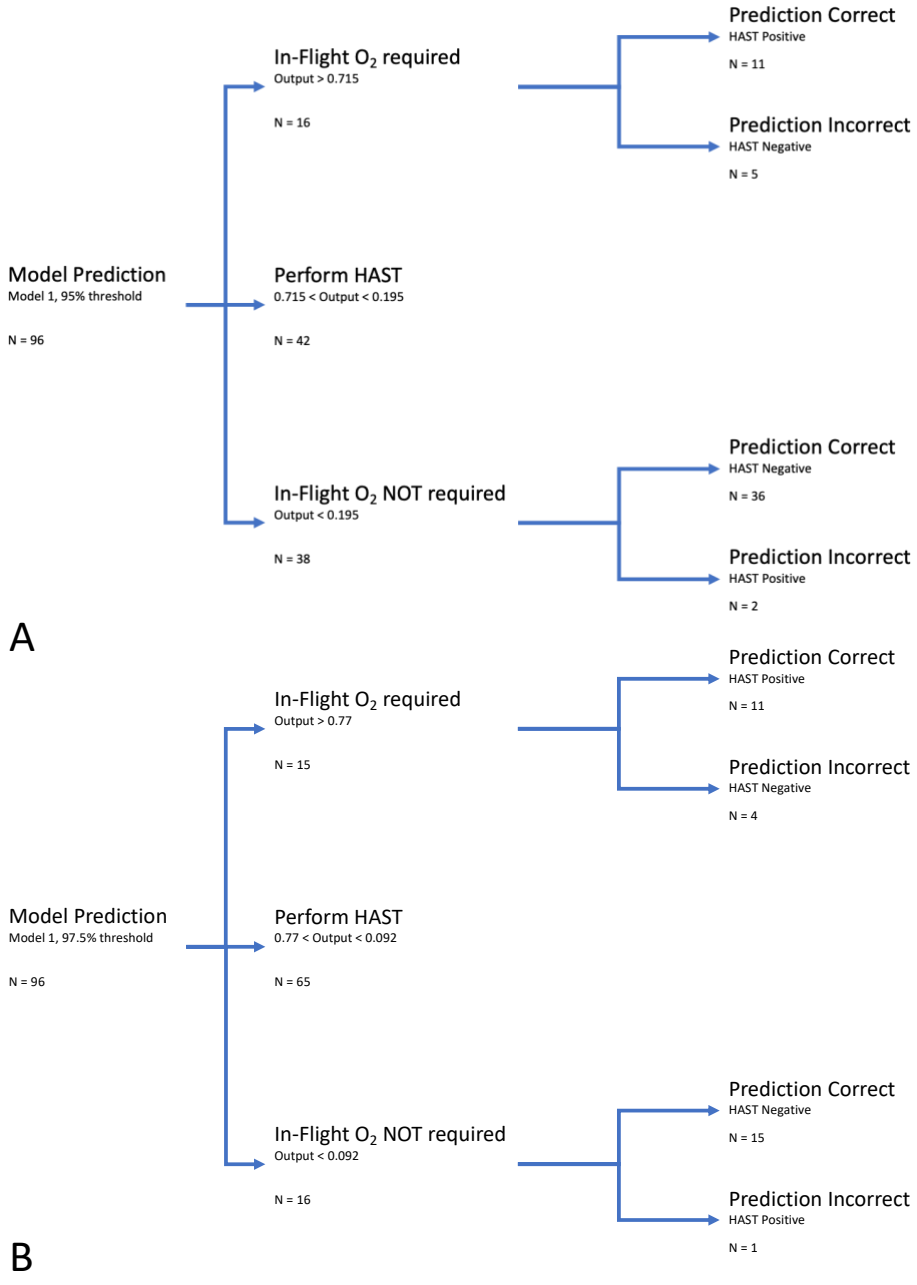

Figure 9.6 Results of model prediction in the prospective validation cohort.

9.4.5 Simplified predictive model

The output of Model 1 was recalculated on this validation cohort by replacing arterial oxygen measures with pulse-oximetry measures, and measures of bicarbonate with measures of serum bicarbonate. These are in theory one-for-one replaceable measures, thanks to the good correlation between arterial and serum bicarbonate, and arterial SO_2 and SpO_2, although known to be less precise and less accurate than their counterparts.

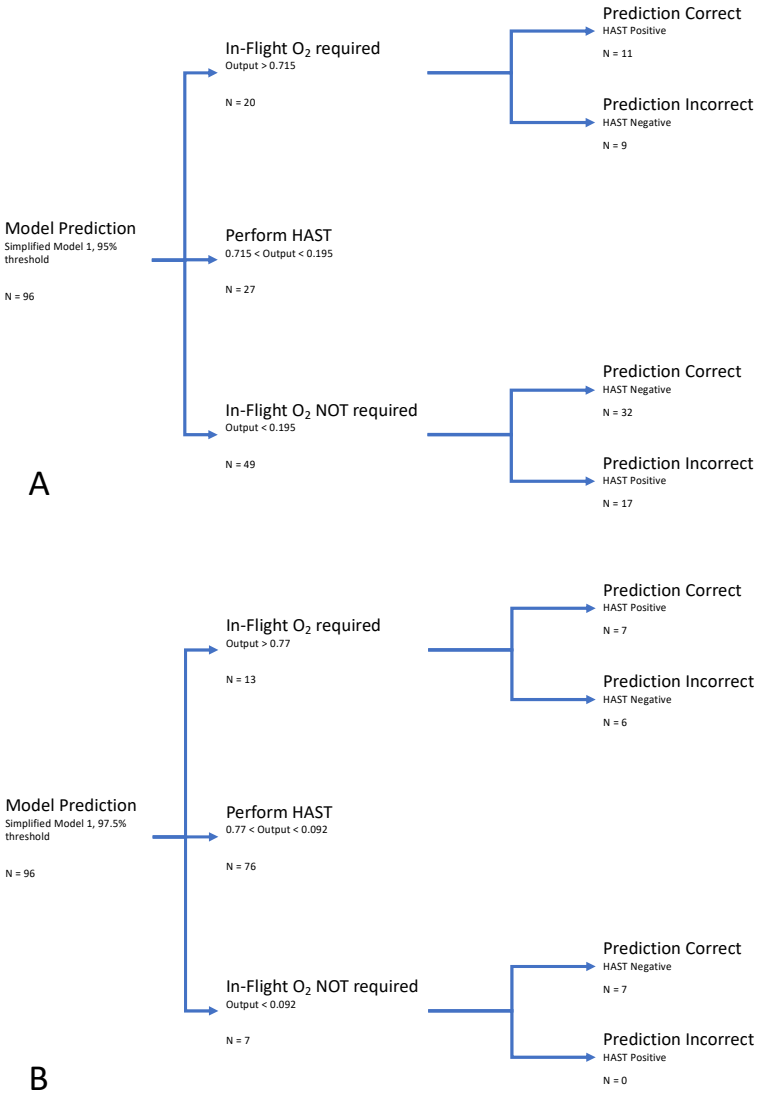

Figure 9.7 Results of model prediction with the simplified version of model 1in the prospective validation cohort.

While the simplified model result in a ROC AUC of 0.836, the use of the previously identified threshold for the computation of this simplified model resulted in a significant loss of accuracy compared to the original model outputs, with a major increase in the number of misclassifications. In particular, the 95% thresholds would have allowed a 71.8% reduction in number of HAST, but leading to 26 (27.1%) misclassification. The

97.5% thresholds would have reduced the HAST performed by 20.1%, with 6 misclassification (6.25%).

9.5 Discussion

In this prospective study, the distribution of acid-base disturbances in people with CF is substantially different compared to what previously described in my large-scale cohorts (Chapter 7), and in the literature [216–218,359].

Blood gases were in fact normal in the majority of cases, with metabolic alkalosis not being observed among subjects with CF. Similarly, levels of serum bicarbonate, which I had expected to be raised in people with CF based on my previous results (Chapter 5 and Chapter 8), were similar in the two cohorts.

While this could be a result of the small sample size of this prospective cohort (n=36 patients with CF), it could also reflect the improved clinical stability and better overall health of patients with CF that attend for HASTs, compared to that of the historical Leeds CF cohort (Chapter 7). This improved stability and health is, in big part, due to the wider availability of better treatment, particularly CFTR modulators. Approximately a third (n=10) of the subjects with CF included in this study were on treatment with CFTR modulators (IVA n=1; TEZ/IVA n=6; or ELX/TEZ/IVA n=3).

Recent studies suggest that individuals with CF might suffer from an impaired handling of bicarbonate in the kidneys as a result of defective CFTR [243]. The results presented in Chapter 8 are in accord to these recent findings, and support a contributing role of the kidney in causing metabolic alkalosis and raised bicarbonate in patients with cystic fibrosis. An increased expression of CFTR in renal tubules, caused by CFTR modulators, could therefore affect the acid-base status and serum bicarbonate levels, bringing them closer to normality. Further studies are certainly warranted to understand the impact of highly effective CFTR modulators, now the gold standard in the treatment of CF, on the distribution of acid-base disturbances.

In this study, HAST yielded negative results in the most cases, with only a third of the individuals undergoing the test requiring in-flight oxygen. This further supports the potential significant benefit of introducing an algorithm-based triage system to reduce the number of tests performed. HASTs are time-consuming, heavy on resources and require additional hospital appointments, and a significant reduction of tests performed would be very welcome, particularly in these times of high pressure to any healthcare system.

The multivariable model developed and validated retrospectively in Chapter 7 based on a single blood gas combining variables of oxygenation and ventilation, was proven to be valid and accurate in this external cohort.

Were the predictor applied to this cohort of patients as a triaging algorithm, it would have resulted in a reduction of a least 30% in the number of HAST performed, with a minimal error rate, mostly consisting of over-conservative recommendations for inflight oxygen. Although the model was validated on a smaller than anticipated sample size, the results satisfy the pre-determined criteria for early termination.

The three cohorts of derivation, retrospective and prospective validation were significantly different in terms of baseline characteristics of the patients and their underlying diagnosis. This notwithstanding, the predictive value of the multivariate score was unaffected. This confirms that the results are robust and widely generalisable.

In view of the good correlation shown for arterial and serum bicarbonate and arterial SO_2 and SpO_2, I attempted to use these simpler-to-measure pulse-oximetry variables in lieu of the original to compute the predictive score. The simplified version of the model proved, however, not viable due to reduced accuracy and increased number of misclassifications.

9.6 Conclusion

This study supports and confirms my previous results, that combining variables of ventilation and oxygenation with underlying diagnosis could allow for a simple, but effective triage of people with respiratory conditions who are planning to fly. Despite a good correlation of serum and arterial bicarbonate, and arterial saturation with pulse oximetry, these are not interchangeable in the model.

Chapter 10
Non-invasive ventilation in CF: a retrospective study

10.1 Introduction

Approximately 20% of individuals with Cystic Fibrosis develop severe lung disease, defined as FEV_1 <40%, by the age of 30 years [2,415]. As previously described in Chapter 2 (Sections 2.4 and 2.5) patients with CF and advanced lung disease can develop gas exchange abnormalities, presenting with nocturnal hypoxaemia and hypercapnia. These often precede daytime respiratory failure [66,416]. Chronic respiratory failure is associated with a poorer outcome, a more rapid decline in lung function, and is an indication for referral for lung transplantation [374,375].

By slowing the rate of progression of respiratory failure, contributing to control of inflammation, and favouring expectoration thereby reducing the risk of atelectasis, NIV has the potential of improving prognosis in individuals with CF [330,417]. In addition, NIV is used where appropriate to treat acute respiratory failure during episodes of pulmonary exacerbations.

By virtue of these, as well as of all the physiological effects described in Chapter 3, NIV could play a significant role in CF.

Despite a strong pathophysiological rationale for using NIV in CF, a recent Cochrane review concluded that evidence on the use of this technique in CF is limited, with no data on its impact on disease progression [330]. This notwithstanding, NIV is used routinely in everyday clinical practice, and up to 10% of patients with CF have received NIV at least once in their life [229,332]. This is especially true in the context of advanced lung disease and chronic ventilatory failure where NIV can play a role in bridging patients to transplant and controlling pCO_2 [339,341,343,418–420].

To increase the understanding of how NIV is used in patients with CF, to evaluate its impact on their outcomes, and to assess its impact on acid-base balance and serum bicarbonate, I conducted a single-centre, large-scale retrospective study of all patients with CF who received NIV in a 10-year period within the Leeds CF centre.

10.2 Aim of this study

The aims of this study were:

- To assess the indications of NIV use in adults with cystic fibrosis attending the Leeds CF centre;
- To assess any changes in blood gas and serum bicarbonate in response to the application of NIV in patients with CF;

- To describe the outcomes associated with NIV treatment;
- To assess if any baseline factor could predict the patient's response to NIV.

10.3 Methods

A retrospective analysis of data collected prospectively for standard clinical care on the Unit Electronic Patients Records (EPRs, EMIS) at the Leeds Regional Adult CF Centre between January 2008 and December 2018 was performed.

All patients included in the study previously consented for clinical data stored on the EPRs to be used for research purposes (Appendix A).

10.3.1 Study population

The EMIS reporting tool was used to identify all patients who met the following inclusion and exclusion criteria for the study population:

- A confirmed diagnosis of cystic fibrosis (defined as having two CF-causing mutations, and/or a sweat chloride test >30 mmol/L with clinical manifestations of CF);
- Age of 17 years or older at time of the search;
- At least one application of NIV for a duration of at least 12 hours.
- Individuals who had NIV after having received lung transplantation, or specifically for obstructive sleep apnoea (OSA) or chest physiotherapy (CPT) only, were excluded from the study.

A subsequent detailed reviewed of patients record allowed to confirm that all patients meeting the inclusion and exclusion criteria were correctly identified.

To assess the medium-term outcomes of treatment with NIV, two groups were identified *a priori* based on the length of their follow up with the Unit:

- Group 1: patients who had a follow up longer than 6 months;
- Group 2: patients who had a follow up shorter than 6 months.

Of the patients who had a follow up longer than 6 months, two sub-groups were identified based on the duration of NIV treatment:

- Subgroup 1A: subjects who remained on NIV throughout their follow-up period;
- Subgroup 1B: subjects who declined to continue treatment with NIV because of discomfort, despite continued clinical and/or blood gas indications for NIV.

Subjects were considered part of the study for the whole duration of their follow-up with the CF unit, or until December 2019, depending on which occurred earlier.

10.3.2 Data collection

The EPRs were searched for all the occurrences, starting dates and ending dates of each application of NIV. Baseline demographics, comorbidities and microbiology status at the starting date of each NIV session were recorded, as well as status on the lung transplant waiting list, date of termination of treatment with NIV, date of transplant and/or death.

All available lung function results in the year before and after start of each NIV session were retrieved. FEV_1 decline (slope) was computed in correspondence with each data point, through linear regression in the year preceding and in the year following start of treatment with NIV. Similarly, the rate of change in serum bicarbonate (bicarbonate slope) was computed for the period before and after the initiation of NIV.

Number of days on IV antibiotic treatment and in-hospital stay in the year preceding and following NIV were also extracted. Electronic medical notes were reviewed to record self-reported side effects and complications of NIV.

Arterial or arterialised capillary blood gases collected prior to the start of NIV and for the month after treatment initiation were recorded, together with all serum bicarbonates in the three months preceding and following the start of NIV. ABG and CBG were reported automatically using the tool described in Appendix B.

Computerised tomography (CT) scans performed within 12 months of the NIV start date were also retrieved and anonymised. The images were independently reviewed by two Consultant Chest Radiologists according to the modified Bhalla Score (Figure **10.1**) (Appendix C) [421].

	Score			
CT Abnormalities	0	1	2	3
Severity of bronchiectasis	Absent	Lumen slightly greater than adjacent vessel	Lumen 2 to 3 × adjacent vessel	Lumen > 3 × adjacent vessel
Peribronchial thickening	Absent	Airway wall thickness equal to adjacent vessel	Airway wall thickening ≤ 2 × adjacent vessel	Airway wall thickening > 2 × adjacent vessel
Extent of bronchiectasis (BPS)	Absent	1–5	6–9	> 9
Extent of mucous plugging (BPS)	Absent	1–5	6–9	> 9
Sacculations/abscesses (BPS)	Absent	1–5	6–9	> 9
Generations of bronchial divisions	Absent	Up to fourth generation	Up to fifth generation	Up to sixth generation
No. of bullae	Absent	Unilateral	Bilateral	> 4
Emphysema (BPS)	Absent	1–5	> 5	
Collapse/consolidation	Absent	Subsegmental	Segmental/lobar	
Mosaic perfusion*	Absent	1–5	> 5	
Air trapping*	Absent	1–5	> 5	
Acinar nodules*	Absent	Subsegmental/segmental	Lobar	
Thickening of intralobular septae*	Absent	Subsegmental/segmental	Lobar	Diffuse (> 1 lobe)
Ground glass*	Absent	Subsegmental/segmental	Lobar	Diffuse (> 1 lobe)

*Modifications to the original Bhalla score. BPS = bronchopulmonary segments.

Figure 10.1 Modified Bhalla score, reproduced with permission from [421].

10.3.3 Statistical analysis

Comparison of variables before and after NIV sessions were performed with paired t-tests for normally distributed data and with Wilcoxon Signed Ranks tests if data was not distributed normally.

Unpaired t-test or Mann Whitney test were used for between-group comparisons of normally and non-normally distributed data, respectively. The chi-square test was used to assess differences in frequency distributions between groups.

All tests were two-sided and significance level was set at $p<0.05$. Data are reported as mean and SD, if normally distributed, and as median and IQR if not.

IBM SPSS statistics version 23 (IBM Corp, Armonk, NY, USA) was used for all the analyses.

10.3.4 Ethics

This study was discussed with the LTHT R&I and was deemed exempt from NHS Research Ethics Committee and Health Research Authority approval. It was approved by the local R&I (RM17/99996) and the Cardio-Respiratory CSU at Leeds Teaching Hospital NHS Trust. All patients had previously consented for their clinical data to be used for research purposes.

10.4 Results

10.4.1 Study population

Over the study period, 68 out of a total of 629 adults with CF under follow up at the Unit were treated with NIV on at least one occasion. Eligibility criteria were met in 56 cases (Figure 10.2) with NIV being initiated on 64 occasions, with seven subjects receiving NIV more than once. Four subjects who recovered from a first episode of acute hypercapnic respiratory failure presented again with acute or chronic respiratory failure. Three subjects declined to continue with NIV but re-tried it during a subsequent admission.

Table 10.1 summarises the baseline characteristics of patients at the time of starting NIV, overall and considering the two groups identified depending on duration of follow-up. In 31 (48.4%) instances of NIV initiation, patients were followed up longer than 6 months (FU>6 months). In the remaining 33 (51.6%) occurrences, follow-up was shorter than 6 months (FU<6 months).

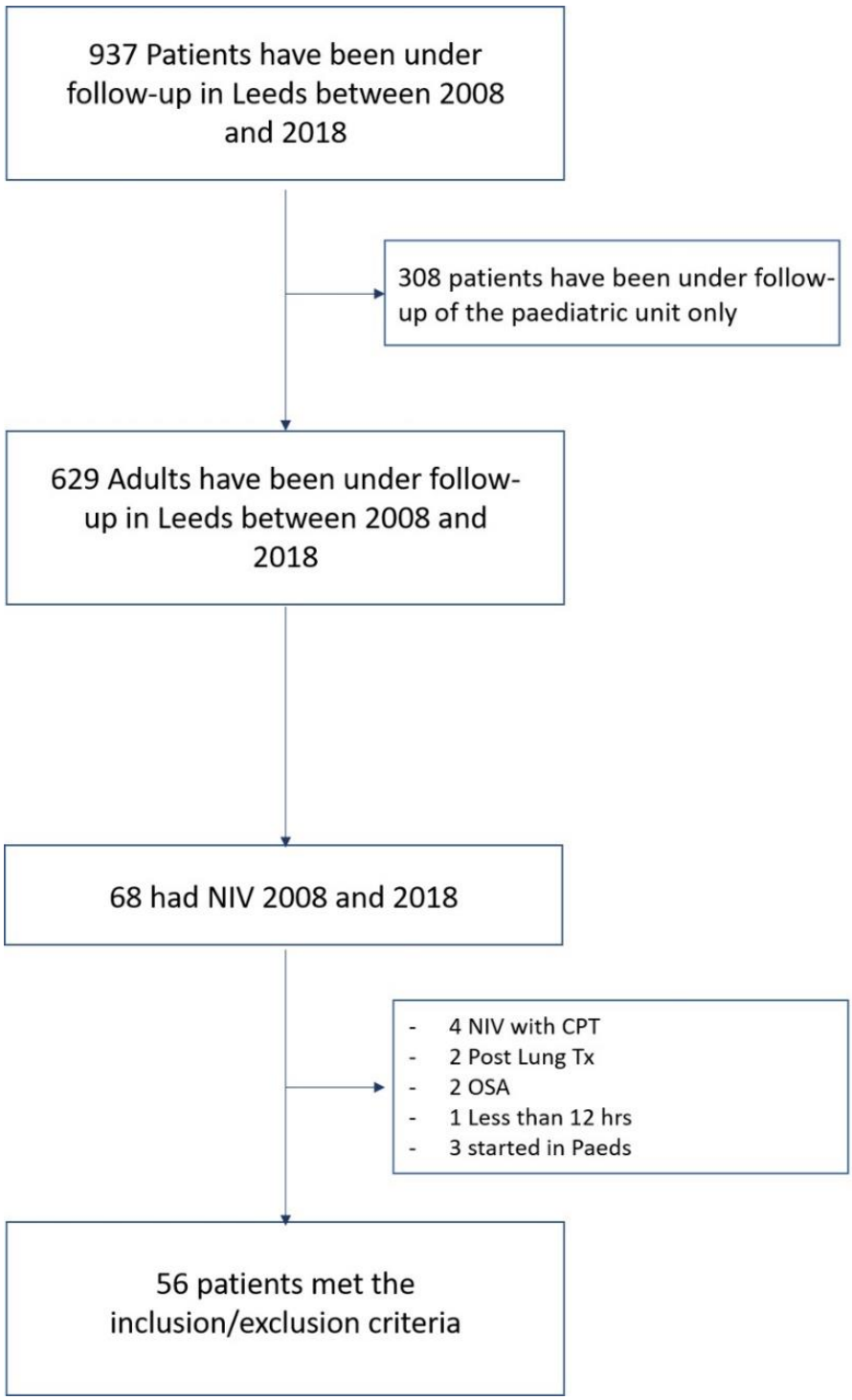

Figure 10.2 Flow-chart of patient selection.

Table 10.1 Baseline characteristics of patients included in the study. NIV, noninvasive ventilation; PTX, pneumothorax; NTM, non-tubercolosis mycobacterial; LTOT, long-term oxygen therapy; IV, intravenous antibiotics. Data are expressed as number (%) and median (IQR) and refer to number of subjects. As subjects, might have had more than one episode during which NIV was initiated, the number of episodes is also presented.

	All	**FU>6 months**	**FU<6 months**	***p***
No Subjects	56	28	33	n/a
NIV episodes	64	31	33	n/a
Age at start	28.8 [24.1-34.9]	28.3 [24.4-33]	29.3 [24-34.7]	0.664
Male Sex, n (%)	25 (44.6)	14 (50)	15 (45.5)	0.723
FEV_1, %				
Best in the 12 mo	29.5 [23-35.7]	30 [27-39.5]	29 [23-35]	0.516
Lowest in the 12 mo	17.0 [14-20]	17 [14-21.5]	17 [13.7-20]	0.435
FEV_1 slope prior to NIV		-0.16 [-0.47 - 0]	-0.18 [-0.21- -0.15]	0.917
FVC, %				
Best in the 12 mo		57 [42.5 - 66]	54.5 [46 - 61]	0.793
Lowest in the 12 mo		30 [24 – 39.5]	30 [23 - 35]	0.446
$FEF_{25-75\%}$				
Best in the 12 mo		0.46 [0.33 – 0.68]	0.41 [0.28 – 0.61]	0.176
Lowest in the 12 mo		0.22 [0.16 – 0.26]	0.19 [0.16 – 0.25]	0.426
Transplant				**0.024**
Active list	15 (26.8)	4 (14.3)	12 (36.4)	
Under assessment	22 (39.3)	9 (32.1)	15 (45.5)	
Not yet considered	17 (30.4)	14 (50)	5 (15.2)	
Rejected	2 (3.6)	1 (3.6)	1 (3)	
Genotype				0.156
F508/F508	35 (62.5)	14 (50)	24 (72.7)	
F508/-	18 (32.1)	11 (39.3)	8 (24.2)	
Other	3 (5.4)	3 (10.7)	1 (3)	
Comorbidities				
Diabetes	26 (46.4)	12 (42.9)	17 (51.5)	0.448
Liver disease	36 (64.3)	20 (71.4)	20 (60.6)	0.628
Sinus disease	6 (10.7)	3 (10.7)	4 (12.1)	1.0
Previous PTX	9 (16.1)	3 (10.7)	6 (18.2)	0.488
Previous Haemoptysis	6 (10.7)	1 (3.6)	5 (15.2)	0.205
Microbiology status				
P.aeruginosa	46 (82.1)	23 (82.1)	28 (84.8)	0.776
A. xylosoxidans	4 (7.1)	1 (3.6)	3 (9.1)	0.618
B. cepacia complex	5 (8.9)	2 (7.1)	3 (9.1)	1.0
S. maltophilia	10 (17.9)	4 (14.3)	7 (21.2)	0.483
NTM	4 (7.1)	3 (10.7)	1 (3)	0.325
LTOT	10 (17.9)	3 (10.7)	10 (30.3)	0.115
IV days, days	98 [51-174]	72 [41.5-124]	112 [71-265]	**0.013**
Hospital stay, days	45 [25-74]	43 [14-73]	57 [33-96.5]	0.109

The two groups were similar for demographics, comorbidities and microbiology. However, lung function tended to be lower among subjects in the group with follow-up <6 months, who required more IV antibiotic treatment in the year preceding the start of NIV treatment and appeared to be more often on long-term oxygen therapy.

More subjects in the group with follow-up <6 months were on the active transplant waiting list or under consideration for lung transplantation (Table 10.1).

The inter-observer agreement for the Bhalla score appeared to be fair or good for the majority of the data scored, with the exception of sacculation (Kappa value 0.094), mosaic perfusion (Kappa 0.072) and air trapping (Kappa 0.095). No differences in the radiological patterns according to the Bhalla score were observed among the two groups (total score 17 vs 14, *p*=0.9).

Baseline blood gases at start of treatment among the two cohorts appeared similar with no significant difference in oxygenation or ventilation (Table 10.2).

Table 10.2 Baseline blood gas analyses and serum bicarbonate in the two groups identified based on duration of follow-up.

	FU>6 months	**FU<6 months**	***p***
No Subjects	28	33	
Blood gas results			
pH	7.37 [7.33-7.43]	7.39 [7.32-7.45]	0.337
pCO_2, kPa	8.23 [7.29-9.50]	8.51 [7.54-9.71]	0.639
pO_2, kPa	8.15 [7.52-9.65]	8.05 [6.91-8.83]	0.263
HCO_3^-, mmol/L	32.5 [28.9-42.2]	37.35 [30.1-43.6]	0.184
BE	8 [4.9-16]	13 [6.4-18.8]	0.145
Serum bicarbonate, mmol/L	32.5 [29.3-36]	34.5 [31.0 -40.5]	*0.07*
Slope bicarbonate, pre	9.49 [-0.93 – 21.48]	13.32 [0.2 – 33.8]	0.296

Serum bicarbonate was overall high, and tended to be lower at start of NIV among patients who had a longer follow-up [32.5 (IQR 7) vs 34.5 (IQR 10), *p*=0.07] (Figure 10.3).

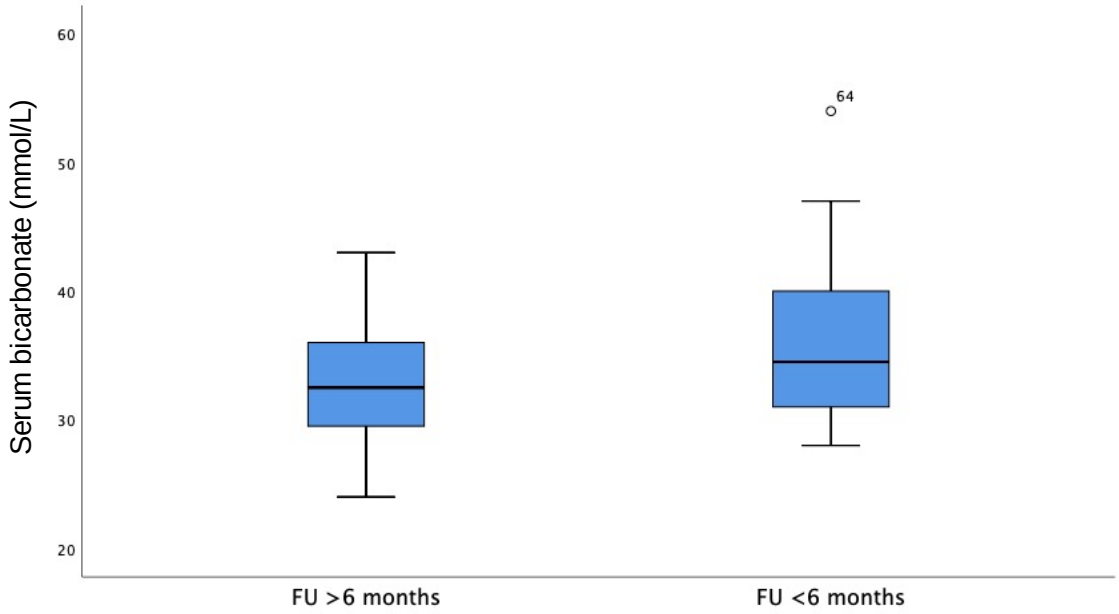

Figure 10.3 Serum bicarbonate concentration before starting NIV in the two groups identified based on duration of follow-up.

The rate of change in serum bicarbonate concentration, computed through linear regression as a slope, did not differ across the two groups identified based on the duration of follow-up (Table 10.2). The concentration of serum bicarbonate showed a high degree of variability in the studied population.

10.4.2 NIV courses: indications to start

NIV was started during a hospital stay in all but one case. Symptoms suggestive of CO_2 retention, such as morning headaches, lethargy, and drowsiness or dyspnoea, and signs of increased work of breathing (tachypnoea and use of accessory muscle) were assessed on admission and daily. Blood gas analyses were performed in presence of such symptoms or signs, and NIV was started accordingly.

Chronic type-II respiratory failure was the most common indication to start NIV (n=31/64, 48.5%), followed by acute hypercapnic respiratory failure (n=21/64, 32.8%). Other indications to start NIV treatment were symptomatic nocturnal hypoventilation (n=4/64, 6.25%), hypoxemia (n=5/64, 7.8%) and increased work of breathing (n=3/64, 4.7%).

At the time of starting NIV, most subjects complained of increased dyspnoea (67.2%) and morning headaches (64.1%). Table 10.3 summarises the distribution of symptoms, results of baseline arterial blood gases, and inflammatory markers on admission depending on the indication for initiation of NIV. Arterial carbon dioxide and pH were significantly different depending on the indication to commence NIV, whereas arterial and serum bicarbonate did not show any significant difference (Table 10.3 and Figure **10.4**).

Headaches, lethargy, and drowsiness were most common among those subjects who commenced NIV due to acute or chronic hypercapnic respiratory failure.

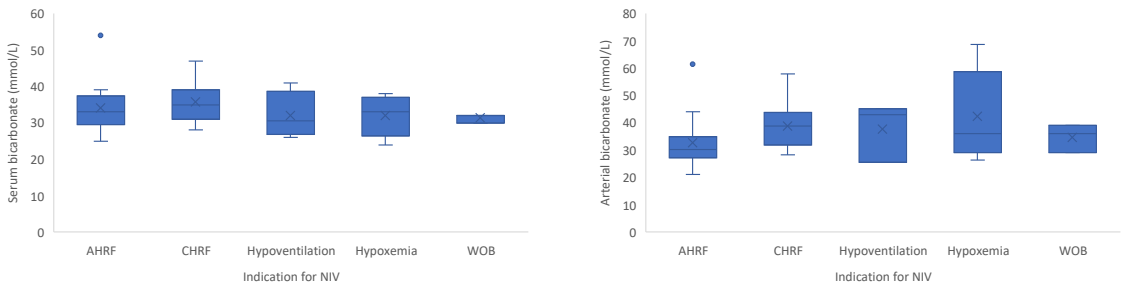

Figure 10.4 Serum and arterial bicarbonate concentration depending on the indication to start NIV. AHRF, acute hypercapnic respiratory failure (n=21); CHRF, chronic hypercapnic respiratory failure (n=31); hypoventilation (n=4); hypoxia (n=4); WOB, increased work of breathing (n=3).

The interval time between admission and initiation of NIV treatment was variable: less than 12 hours in 11 cases (17.2%), between 12 and 24 hours in 12 cases (18.8%), and more than 24 hours [6.5 (1.5-88) days] in 40 cases (62.5%). No differences were

observed in the interval time to start NIV depending on the indication for treatment. However, subjects who had a follow-up longer than 6 months

Table 10.3 Indication for NIV and baseline blood gas analysis AHRF, acute hypercapnic respiratory failure; CHRF, chronic hypercapnic respiratory failure; WOB, increased work of breathing. Data are expressed as number (%) and median (IQR).

	AHRF	CHRF	Hypoventilation	Hypoxemia	WOB	*p*
NIV episodes	21	31	4	5	3	
Male sex	9 (42.9%)	17 (54.8%)	1 (25%)	2 (40%)	1 (33.3%)	0.734
Age at start	29.6 [24.0-37.4]	29.5 [23.4-34.4]	29.3 [22.3-40]	22.4 [20.1-27.4]	27.6 [27-29.6]	0.249
Baseline ABG						
pH	7.31 [7.28-7.33]	7.39 [7.37-7.45]	7.44 [7.43-7.52]	7.53 [7.47-7.58]	7.49 [7.40-7.54]	**0.000**
pCO_2, kPa	9.5 [8.1-11.1]	8.47 [7.77-9.26]	7.23 [5.14-8.28]	5.89 [5.22-8.32]	6.18 [6.0-6.2]	**0.001**
pO_2, kPa	8.1 [6.9-9.7]	8.2 [7.4-8.85]	7.84 [7.3-8.1]	7.6 [6.65-7.95]	10.3 [9.6-11]	*0.083*
HCO_3^-, mmol/L	30.3 [27.3-35]	38.9 [32-43.7]	43 [25.4-45.1]	36.1 [29-58.8]	36 [29-39.2]	*0.077*
Headaches	14 (66.7%)	22 (71%)	4 (100%)	1 (20%)	0 (0)	**0.006**
Dyspnea	14 (66.7%)	20 (64.5%)	2 (50%)	5 (100%)	2 (66.7%)	0.333
Sleep disturbances	3 (14.3%)	13 (41.9%)	4 (100%)	1 (20%)	0 (0)	**0.031**
Lethargy/drowsiness	9 (42.9%)	10 (32.3%)	0 (0)	2 (40%)	1 (33.3%)	0.386
Increased WOB	7 (33.3%)	13 (41.9%)	0 (0)	4 (80%)	3 (100%)	**0.010**
C-reactive protein	82 [29-147]	61.5 [46.3-107.5]	23.5 [15-28.5]	176 [84.5-288.5]	149 [34-232]	**0.019**
$sHCO_3^-$	36.5 [32.2-39]	36 [33-39]	30.5 [26.7-38.7]	37.5 [27-47.25]	31 [30-32]	0.386

were started on NIV earlier during their admission compared to those who had a shorter follow up (Group with follow-up<6 months) (*p*=0.004).

10.4.3 NIV setting

NIV treatment was set up by senior respiratory physiotherapists with the support of the sleep and NIV service as required. Treatment was started using a number of ventilators, all of which were designed for home treatment.

NIV was set either in pressure control or pressure support mode. Median IPAP at start was 14 cmH_2O, then gradually increased to 17 cmH_2O, median EPAP remained stable at 5 cmH_2O. The process of pressure titration was performed over variable time (from a few hours to a few days) to facilitate tolerance. In 61 cases, supplemental oxygen was required through the NIV at a median flow-rate of 2L/min (range: 0-8L/min) to target oxygen saturation depending on the baseline diagnosis at 88-92% for subjects with hypercapnia and >94% for hypoxemic subjects. Pressure settings were similar in the two groups identified based on follow-up duration, but subjects with a follow-up shorter than 6 months required higher flow rates of oxygen (Table 10.4).

No difference in setting was observed depending on baseline bicarbonate concentration or indication to start NIV.

Table 10.4 NIV setting in the two groups identified based on follow-up duration IPAP, inspiratory positive airway pressure; EPAP, expiratory positive airway pressure. Data are reported as median (IQR).

	FU>6 months	**FU<6 months**	***p***
IPAP (cmH_2O), at start	15 [12-16]	14 [11-16]	0.394
IPAP (cmH_2O), final	20 [16-23]	17 [14-20]	0.873
EPAP (cmH_2O), initial	4 [4-5]	5 [3.5- 5]	0.675
EPAP (cmH_2O), final	5 [5-6]	5 [4-6]	0.749
O_2 (L/min) at start	2 [1-3]	2 [2-4.5]	**0.023**
O_2 (L/min) final	2 [1-3]	3 [2-4]	**0.027**

Commercially-available full-face masks were the interface of choice in 56 out of 64 cases with nasal mask or nasal pillows being preferred in 3 patients. In 5 subjects a rotational strategy was used, with the patient alternating between face mask and nasal pillow. Active humidification was added in 50 cases (78.1%) using either an external (n=28, 43.7%) or an integrated (n=22, 34.3%) humidifier. No differences in the choice of interface or use of humidification were noted between groups.

10.4.4 Follow-up and outcomes

Median duration of treatment with NIV was 58 [7-266] days over a follow-up period of 156 [26-531] days. No differences in duration of treatment were noted depending on the status on transplant waiting list at NIV initiation (Figure 10.5).

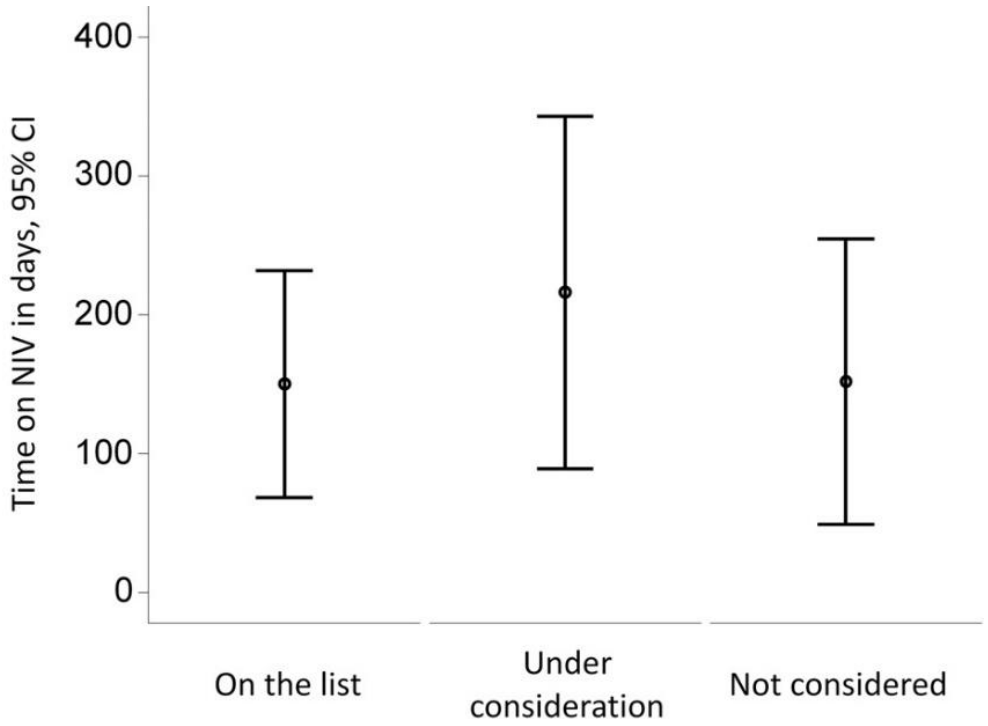

Figure 10.5 Duration of treatment with NIV depending on status on transplant waiting list.

A total of 18 patients received a lung transplantation, with NIV being discontinued at time of transplant; 31 patients died without transplant and NIV was continued until the day of death in 17 cases.

Among those with a longer follow-up, the median duration of follow up was 537 [302-728] days. In this group, duration of NIV treatment varied very significantly ranging from 1 to 1145 days (median: 268 [13-601] days). Thus, two sub-groups were identified: nineteen patients continued using NIV for the whole duration of follow-up (Subgroup 1A), while 12 subjects stopped using it within 6 months from start of treatment due to poor tolerance (Subgroup 1B).

The two subgroups were similar for demographics and comorbidities, but those in Subgroup 1A had lower baseline FEV_1 and spent longer time on IV antibiotics at baseline. No differences in the modified Bhalla score were observed. In the year preceding the start of NIV, best FEV_1 was lower (0.87 [0.79-1.21] L vs 1.34 [1.07-1.49] L, p=0.048 and 28 [22-31]% vs 38 [20.3-49.5]%, p=0.036) and IV antibiotic requirements higher (90 [50-154] vs 55 [25-77] d/yr, p=0.048) among those who continued NIV throughout their follow up. No differences in setting, interface and use of humidification were observed in the two subgroups.

In the group with a follow up shorter than 6 months (33/64, 51.6%), the median duration of observation was 36 [16-102] days, and duration of NIV treatment was 24 [4.5-80] days. In this time frame, 24 (72.8%) patients died, 8 (24.2%) received lung transplantation and one (3%) relocated and transferred to another CF Unit.

10.4.4.1 Blood gases and bicarbonate

Only a minority of patients included in the study had serial blood gases or assessment of ventilation through overnight oximetry or TOSCA performed in the first three months after starting treatment. As such, analysis of these variables was not possible.

The rate of change in serum bicarbonate concentration, as computed by the slope of bicarbonate concentration through linear regression, showed that after NIV initiation there was a reduction in serum bicarbonate concentration (Figure **10.6**).

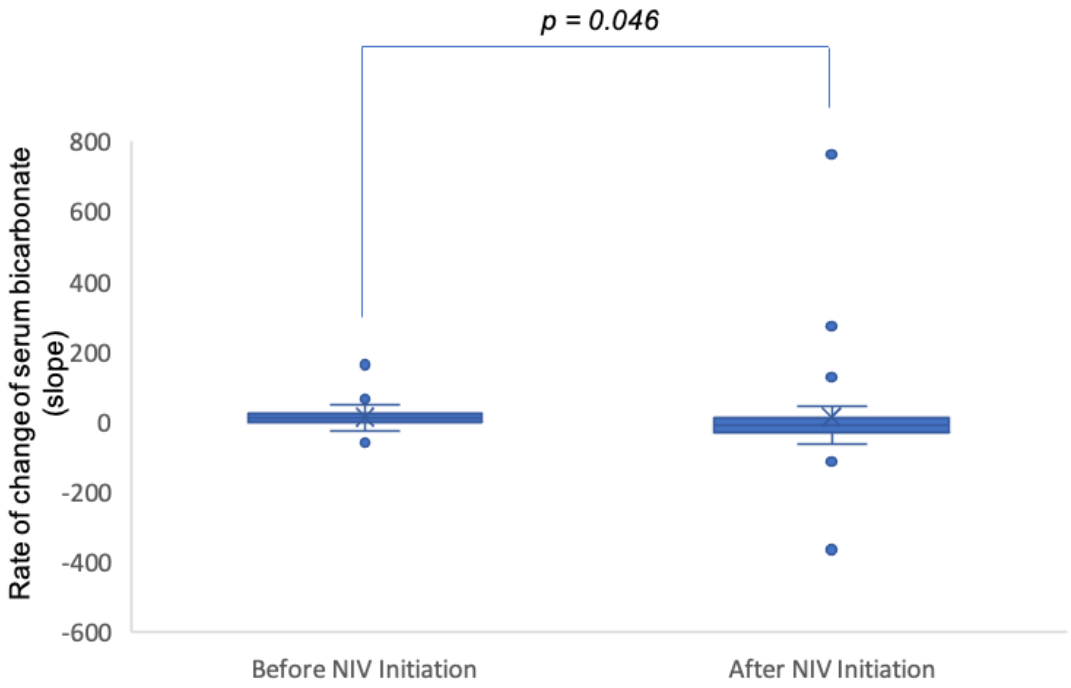

Figure 10.6 Comparison of bicarbonate slope before and after NIV initiation in the whole population. The slope of bicarbonate concentration became negative after NIV initiation in the overall population suggesting a reduction in the serum concentration of bicarbonate compared to before NIV set-up.

The rate of change of bicarbonate was +10.8 mmol/L/y before starting NIV, and declined to –8.39 mmol/L/y after NIV (p=0.046). This change was driven mainly by the group with a longer follow-up, as among those who had a shorter period of observation no change in rate of change of bicarbonate was observed (Figure **10.7**)

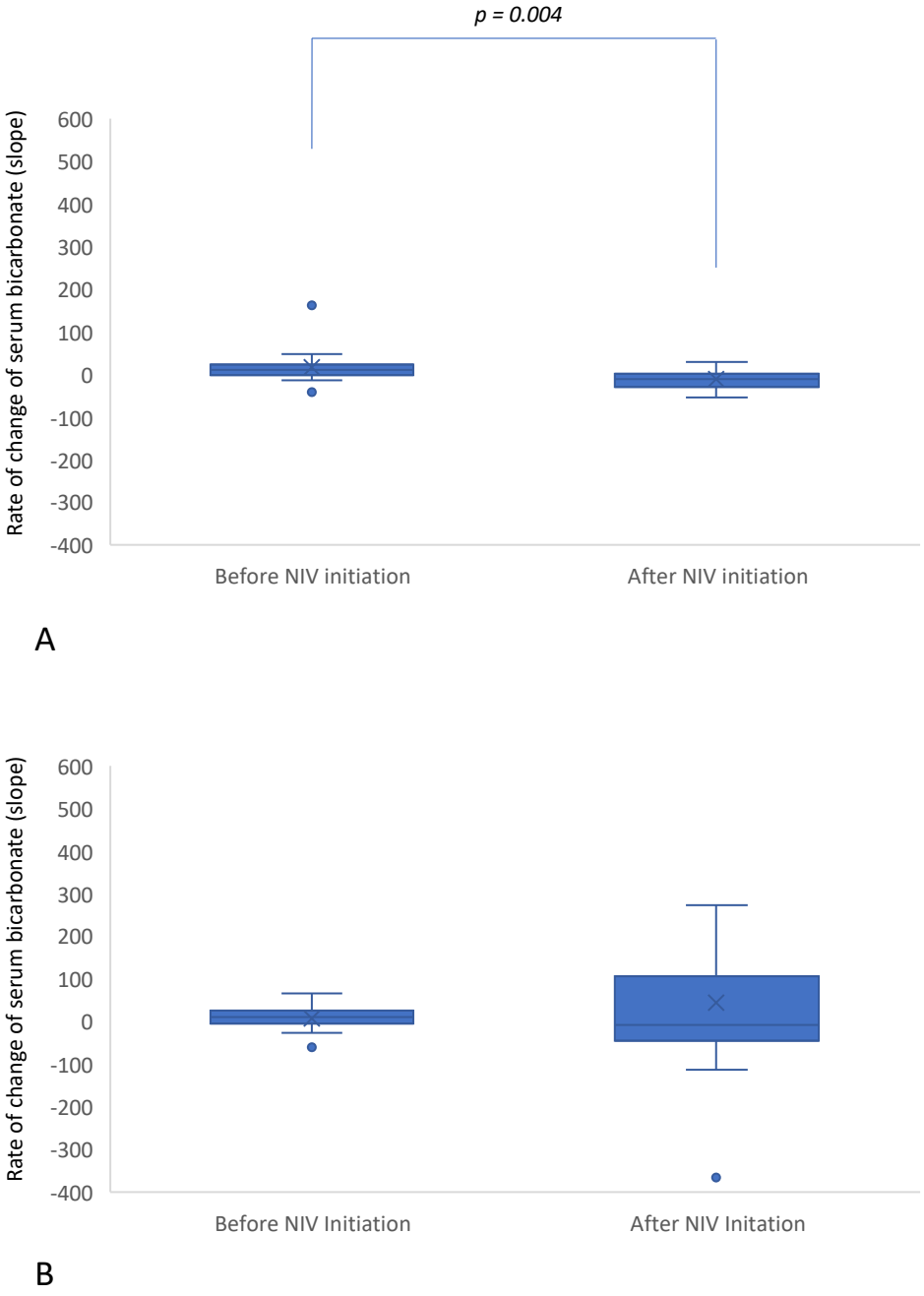

Figure 10.7 Comparison of bicarbonate slope before and after NIV initiation in the groups identified based on follow-up duration. Panel A shows the slope of bicarbonate concentration becoming negative after NIV initiation in the group with longer follow-up. Panel B represents the slope of bicarbonate before and after NIV initiation in the group with follow-up less than 6 months: no difference was observed.

Among patients with longer follow-up, those who used NIV for the whole duration of follow-up (Subgroup 1A) had a significant reduction in the rate of change of bicarbonate concentration (Figure 10.8). A meaningful analysis of the rate of change of bicarbonate in Subgroup 1B was not possible due to the high percentage of missing data (60%).

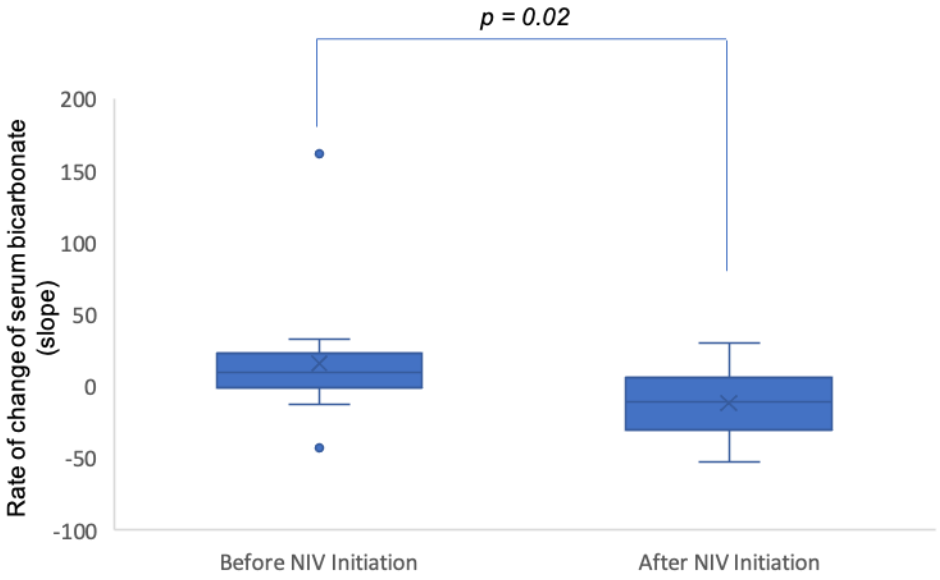

Figure 10.8 Comparison of bicarbonate slope before and after NIV initiation in the subgroup using NIV throughout follow-up.

10.4.4.2 Lung function

Within the first 12 months of starting treatment, FEV_1 decline did not change in those who stopped using NIV (Subgroup 1B, pre: - 0.04 [-0.35 - +0.03] L/y vs post: -0.07 [-0.35 - +0.01] L/y, *p*=0.508), but it improved in the other subgroup (Subgroup 1A, pre: -0.25 [-0.52 - -0.02] L/y vs post: -0.07 [-0.13 - +0.16] L/y, *p*=0.006) (Figure 10.9).

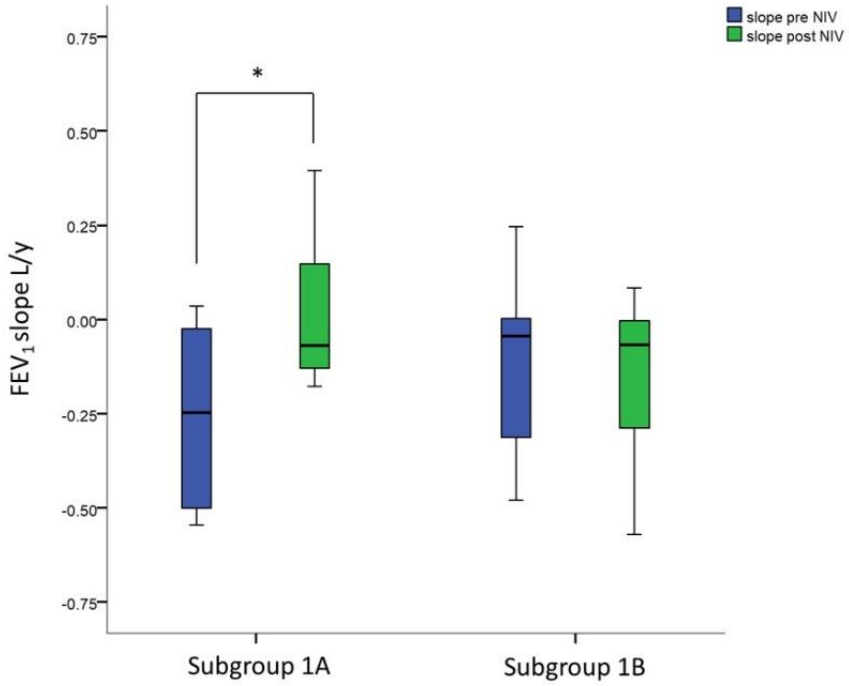

Figure 10.9 FEV_1 decline before and after NIV treatment depending on its use.

In subgroup 1A, no difference in number of hospital day was observed before and after NIV initiation (Subgroup 1A, pre 47.5 [11.75-80.26] d vs post 62 [39-100] d, *p*=0.107), but IV antibiotic requirement increased after NIV Initiation (156 [113-252]d) compared to

before (90 [50.25-154.75] d, *p*=0.014). In subgroup 1B both hospital stay (pre: 42 [16.75-70]d vs post 82[47.75-109.25]d, *p*=0.008) and IV requirement increased after NIV initiation (pre 54.5 [25.5-77] d vs 76 [52.75-103.25], *p*=0.012).

10.4.5 Complications

Ten subjects had complications after using NIV. In particular, pneumothorax requiring the insertion of chest drain while on NIV occurred in 4 cases (4/64, 6.3%), one of whom had a known history of PTX. Similarly, moderate-massive haemoptysis requiring temporary interruption of treatment was observed in 4 occurrences (6.3%), two with previous history. No exacerbations of sinus disease or pressure ulcers were recorded.

Failure of NIV was observed in 10 patients. This led to early termination of treatment in 6 cases, the remaining 4 underwent unsuccessful endotracheal intubation and invasive mechanical ventilation.

10.5 Discussion

Over the last three decades, non-invasive ventilation has been routinely used in clinical practice to treat individuals with CF. While the use of NIV has been based on a sound pathophysiological rationale, clinical evidence to support its use so far has been scarce, especially with regards to outcomes of treatment.

At the Leeds Regional Adult CF centre, over a 10-year period, approximately 10% of adults received NIV on at least one occasion, with 60% of episodes occurring over the last 5 years. This frequency of use is in line with UK CF registry data collected between 2007 and 2015 (10.0%), but is significantly higher than French surveys collected in 2008 (5.0%) [229,332].

Presently, there is insufficient data to define clear criteria for initiation of treatment, as only a small number of randomized controlled trials of NIV have been undertaken in the CF population. In clinical practice, the rationale and indications for using NIV in CF often rely on evidence borrowed from other chronic respiratory diseases, such as COPD and chest wall restrictive disorders.

In this cohort, the indications for starting NIV were consistent with those previously reported in the literature [331,333], and the criteria for using NIV were similar those used for a wide range of other respiratory conditions (Chapter 3). The most common indications were chronic (48.5%) and acute (32.8%) hypercapnic respiratory failure, associated with symptoms typical of CO_2 retention such as early morning headaches and drowsiness. A significant minority of individuals were treated with NIV due to hypoxaemia, in keeping with previous reports [229,333]. This use is in contrast with the latest guidelines from the American Thoracic Society and the European Respiratory Society on the use of NIV in the acute setting, which do not recommend NIV in

hypoxaemic patients other than as a trial in the ICU under close monitoring to avoid delaying intubation [263].

Individuals with CF and advanced lung disease can be difficult to ventilate invasively due to severe airflow obstruction and high burden of secretions [188]. Endotracheal intubation and IMV are in fact historically associated with poor outcomes, adding justification to the use of non-invasive treatment in their lieu. In this analysis only a handful of patients who experienced NIV failure underwent subsequent care in ITU with IMV and they all had a fatal outcome, in keeping with the literature [422,423]. As a result, severely hypoxaemic patients with CF who do not improve with conventional oxygen treatment have limited options for escalation of respiratory support. Recent advances proposing the use of nasal high flow therapy in severe hypoxaemia provide a potential alternative to NIV, but evidence is still insufficient to support its routine use in CF [258,315,354].

The majority of individuals with CF receiving NIV were on the transplant waiting list or under consideration for lung transplantation. In the era of ECMO [424], treatment with NIV remains a successful means to bridge CF patients to lung transplantation [339,341,343,425], as its long-term use contributes to the stabilisation of lung function [328,339,341,343,425,426]. Access to ECMO remains limited, and this technique is associated with risks and complications [373,427].

These data support the use of NIV as a bridge to lung transplantation, as 18 subjects successfully underwent transplant while receiving long-term NIV support. However, in this cohort NIV was started in most cases in patients who had not yet been considered, or had been declined transplant due to comorbidities. This highlights how the approach to the use of NIV has been changing over time.

The follow-up period was relatively short as lost individuals had either received a lung transplant or died within 6 months of starting NIV. This begs the question as to whether or not earlier intervention with NIV could have been more beneficial and improved survival for those awaiting lung transplantation.

In this cohort, individuals who had a shorter follow-up were similar in baseline characteristics and comorbidities to those who remained under follow-up in the unit for a longer period of time. Baseline arterial blood gas analysis or indication to initiate treatment with NIV did not differ either. However, among those with a shorter follow-up, baseline serum bicarbonate tended to be more elevated with no difference in the rate of change in serum bicarbonate concentration.

Following NIV initiation, serum bicarbonate concentration decreased significantly among those individuals with a longer follow-up, especially those who use NIV throughout the period, whereas no difference in the rate of change (increase) was noted among those with a shorter follow-up. This suggest that serum bicarbonate can be used as a marker

of the ventilatory response to NIV treatment, and supports the hypothesis that bicarbonate concentration is a negative prognostic marker among individuals with CF (Chapter 5 and Chapter 6).

Individuals using regular NIV for a period longer than 6 months showed significant benefit from it, with a clear stabilisation of lung function. A significant reduction in the rate of FEV_1 decline after NIV was seen compared to that before to NIV among subjects compliant with long-term treatment. This was despite patients having lower lung function and increased exacerbations prior to starting treatment. The use of NIV did not however lead to reductions in IV antibiotic requirements or frequency of hospitalisations.

One advantage of using EPR data was being able to include as many clinical data points as available, in contrast to sporadic yearly measurements recorded by registries [332]. Data extraction from registries, however, has the benefit of including larger populations, and diverse clinical care settings. These results are limited by the retrospective and single centre nature of the study. Despite all data being collected prospectively in real time, the data collection was driven by clinical need, resulting in a lack of systematic collection of blood gas analysis at specific time points and of data on quality of life.

With the recent introduction of highly-effective CFTR modulator therapies, the predicted outcomes of NIV, and in general the natural evolution of the CF disease may significantly change from what assessed in this study. In any case, improving quality of life and controlling symptoms remains an essential part of treatment. In this cohort, NIV was also used successfully as part of the end of life care to reduce the burden of symptoms, an indication recommended by Society of Critical Care Medicine [269].

The use of NIV in certain clinical scenarios including pneumothorax, haemoptysis and severe sinus disease can be challenging, especially when options are limited. It was reassuring to find that the occurrence of these complications was low over the 10 year period and that a prior history of pneumothorax and haemoptysis resulted in low recurrence rate. None of the patients included in this analysis experienced significant mucus impaction, atelectasis or exacerbation of sinus disease, most likely as a result of the use of active humidification in most cases. In France, many centres reports using a combination of custom-made and commercially-available interfaces to optimise patients' tolerance of the treatment [229]. In the Leeds CF centre, only commercially-available interfaces are used. These are carefully selected by experienced respiratory physiotherapists to optimise comfort. A rotational strategy was adopted in a handful of cases, mostly in patients who required NIV for prolonged period of time during the day as well. With this practice, no pressure ulcers were recorded.

Changes on chest CT could not predict the likely benefit of NIV with the modified Bhalla score showing no correlation with outcomes, tolerance and complications in this cohort of patients.

10.6 Conclusions

NIV is used as an adjunct therapy in the management of people with CF and advanced lung disease in a manner similar to other chronic respiratory conditions. The long term use of NIV leads to a stabilisation in serum bicarbonate concentration and lung function, but does not affect the frequency of exacerbations and intravenous antibiotics use.

Further large scale studies are needed to define disease-specific criteria for initiation of NIV and appropriate settings, and to assess effectiveness of treatment. Critically, because this intervention is predominantly used by patients with severe and end-stage lung disease, an assessment of the impact on quality of life, symptoms relief and improvements in clinical outcomes is needed.

Chapter 11
Gas exchange and use of NHFT as an adjunct during exercise: a pilot cross-over randomized controlled trial

11.1 Introduction

Aerobic exercise is an integral part of the management of people with cystic fibrosis, and is associated with a reduction of the rate of decline in lung function, and with improved bone mineralisation and quality of life [248–252]. However, progression of the CF disease and its resulting structural damage to the lungs (Section 1.4) result in increased airway resistance, static and dynamic hyperinflation, hypoventilation and ventilation-perfusion mismatch.

All these factors contribute to reduce the tolerance to physical exercise in patients with CF [202,203,205,428]. Patients can be easily fatigued and dyspnoeic during exercise, with exercise-induced hypoxaemia or desaturation occurring in 15 to 30% of cases [429,430]. This severely limits the amount of physical exercise that patients with CF can incorporate in their routine, and therefore its benefits.

Short-term oxygen has been shown to improve S_pO_2 during or immediately after an exercise bout, with a subsequent increase in end-tidal CO_2 when compared to not receiving short-term oxygen. However, it has also been shown not to improve exercise tolerance, dyspnoea or fatigue [211].

As presented in Chapter 3 Section 3.2, nasal high-flow therapy (NHFT) delivers heated, humidified, and oxygenated gas with flow rates up to 60 L/min with a controllable F_IO_2 between 0.21 and 1.0. Gases are provided to the patient via soft, loose fitting, large-bore nasal prongs.

NHFT has been shown to wash out nasopharyngeal dead space, thereby minimizing CO_2 rebreathing and providing a reservoir for fresh air. In addition, NHFT has been shown to reduce respiratory rate and increase tidal volume and, by delivering high flow of humidified gas, it more closely matches the patient's inspiratory flow rate and reduces airway dryness [258,350]. By virtue of all these pathophysiological mechanisms, NHFT has been proven to be an effective alternative to NIV or conventional oxygen therapy in a variety of clinical scenarios [258,350].

NHFT was recently shown to increase exercise tolerance in patients with COPD, improving oxygen saturation and dyspnoea [431,432]. In view of the pathophysiological mechanisms underlying exercise limitation in people with CF, described above and in Section 3.2.1, it is reasonable to hypothesise that NHFT could be beneficial in this context.

In this pilot cross-over randomized controlled trial, gas exchanges during exercise in patients with CF and severe lung disease and the viability and effects of NHFT as an adjunct therapy to exercise in this cohort are assessed.

11.2 Aims of the study

The aims of this study were:

- To describe gas exchanges during exercise in patients with CF and severe lung disease;
- To assess the feasibility of using NHFT as an adjunct therapy during exercise in patients with CF and advanced lung disease;
- To evaluate the effects of this therapy on respiratory and subjective variables during a treadmill 6-minute walking test (TWT).

This trial was designed as a pilot study to investigate gas exchanges during exercise in patients with CF and to assess the feasibility of performing larger trials. Evaluation of trial feasibility included study recruitment rate, data completeness for exploratory outcome measures, drop-out rate, and participants' feedback regarding participation in future trials.

Exploratory outcomes of interest were the 6-minute walking distance (WD), mean and nadir oxygen saturation during exercise, mean transcutaneous carbon dioxide ($tcCO_2$), SpO_2 recovery time, dyspnoea, fatigue and comfort.

11.3 Methods

An open, single-centre, short-term, pilot feasibility trial with a randomized cross-over design comparing NHFT to baseline conditions during a TWT was performed in the Leeds Regional Adult CF Centre (Leeds, UK).

11.3.1 Study population

All patients attending the Leeds CF Unit were considered for eligibility to take part in the study.

The inclusion criteria for the study were:

- Age 18 years or older;
- A confirmed diagnosis of cystic fibrosis (defined as having two CF-causing mutations, and/or a sweat chloride test >30 mmol/L with clinical manifestations of CF);
- Known advanced lung disease (defined as FEV_1 <40% in the 6 months prior the admission.

The exclusion criteria for the study were:

- Acute viral illness with positive viral PCR on nose and throat swab in the 5 preceding days prior to the participation,
- Need for 6 L/min or more of oxygen at rest;
- Decompensated type-2 respiratory failure;
- Pneumothorax in the 6 preceding weeks;
- Any of the usual contraindications to a 6 min walking test (as defined in [255])
- Inability to provide consent.

Consecutive patients admitted to the Unit who met the inclusion and exclusion criteria were approached to assess eligibility in study participation during hospital admission for pulmonary exacerbation, and if they agreed to take part, were recruited during the second week of treatment once deemed clinically stable by the clinical team.

11.3.2 Study procedures

Upon enrolment, all subjects underwent a baseline arterialised capillary blood gas (CBG).

Subjects performed two treadmill walking tests (TWT), one with NHFT (NHFT-test) and one without (control-test). To minimize bias due to the study unblinded design, and to reduce the potential effects to the recovery from a pulmonary exacerbation, the two TWTs were performed in a random order at an interval time of 24 to 48h. Randomization was done in a masked fashion and performed in permuted blocks of 10 with a computer generated random-number sequence, with a 1:1 allocation.

Subjects were familiarized on how to use the treadmill and instructed prior to beginning study procedures. No warm-up period before the test was performed, but subjects were asked to rest sitting in a chair for 30 min (acclimation period).

During the treadmill walking test, participants were directed as per American Thoracic Society (ATS) guidelines [255]. The initial speed of the treadmill was set at 2.5 km/h, and subjects were subsequently allowed to adjust the speed as they wished. The control test was performed on room air or conventional supplemental oxygen, delivered via nasal cannulae or Venturi mask, according to each subject's habitual prescription. NHFT was delivered through nasal cannulae (Optiflow+, Fisher&Paykel Healthcare NZ) using the Airvo 2 (Fisher&Paykel Healthcare, NZ), with size chosen based on manufacturer recommendations.

On the day of the NHFT-test, as part of the acclimation period, subjects were first started on NHFT for 15 min at 30 L/min, 37 C, and subsequently increased at 45 L/min for 15 more minutes. During the TWT, flow-rate was maintained at 45 L/min and F_IO_2 was set to match what usually prescribed during exercise for each subject. During the resting period, subjects were asked to continue using the NHFT for 30 min.

The distance walked (WD) on the treadmill was recorded, as well as the highest and lowest speed reached. Throughout the study, subjects were continuously monitored for oxygen saturation and transcutaneous carbon dioxide using a Sentec Digital Monitor V-sign sensor (Sentec AG, Switzerland). Furthermore, blood pressure (BP), respiratory rate (RR), Borg scale for dyspnoea (Borg-D), and leg fatigue (Borg-F) and comfort score were recorded every 15 min during the acclimation period, and every two minutes both during the TWT and in the 10 min after its end.

Spirometry was performed according to the ERS guidelines, 30 min before and after the TWT, to allow for a rest period, using an Alpha Touch Spirometer (Vitalograph, UK).

Figure **11.1** schematises the study procedures.

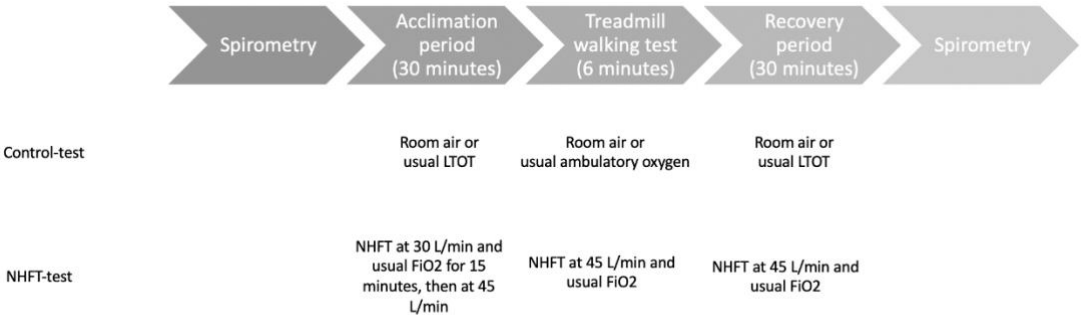

Figure 11.1 Schematics of the methodology during each session of the trial. The order the two conditions were delivered was randomized. Interval time between tests was 24–48 h.

11.3.3 Data collection

Baseline demographic data, comorbidities, use of supplemental oxygen or non-invasive ventilation, and status on the lung transplant waiting list were collected from medical notes. Lung function on admission and baseline blood gas results were also recorded.

11.3.4 Patient and public involvement

The study design and protocol were discussed in a face-to-face meeting with a patient representative of the group included in the study. The research questions were deemed of interest for individuals with CF and the methods used during the study were considered not too intrusive or burdensome.

11.3.5 Statistical analysis

Due to the pilot nature of the study, no formal sample size calculation was performed. Based on expected capability of recruitment within the unit and previous studies on respiratory support during exercise [433] and on NHFT in CF [354], I planned to enrol up to 25 subjects, in order to achieve a full data set on 20 subjects, expecting a drop-out rate of up to 20%.

Normal distribution of each measured variable was assessed by visual inspection and using the Shapiro-Wilks test. Results are expressed as number (percentage), means (standard deviation) when normally distributed, or median (25th-75th percentile) when not normally distributed.

Paired t-test for parametrical data or Wilcoxon test for non-parametric data was used to compare each variable in the two walking tests (NHFT- and control-test). Estimates of effect size are provided with 95% confidence interval (95% CI). A p-value < 0.05 was considered statistically significant.

All analyses were performed with IBM SPSS v26.

11.3.6 Ethical approvals

The study received approval by NHS Research Ethics Committee and Health Research Authority (19/LO/0571), and local R&I (RM19/121,917). It was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

The study was included on NIHR Portfolio and was registered on clinicaltrials.gov (NCT03965832).

11.3.7 Funding

The study was partially funded by Fisher&Paykell Healthcare (Auckland, NZ). The funder also provided all the equipment used in the study, but had no involvement in the planning or conduct of the trial and in the data analysis.

11.4 Results

11.4.1 Subjects

Between June 2019 and February 2020, 155 patients were admitted to the Cystic Fibrosis Unit at Leeds Teaching Hospital, thirty of whom met the inclusion criteria for the study and were screened for participation. Twenty-three patients gave consent to take part in the study, one was withdrawn by the study team as FEV_1 had transiently increased above 40%. Figure 11.2 provides a flow chart of the patients' screening and recruitment.

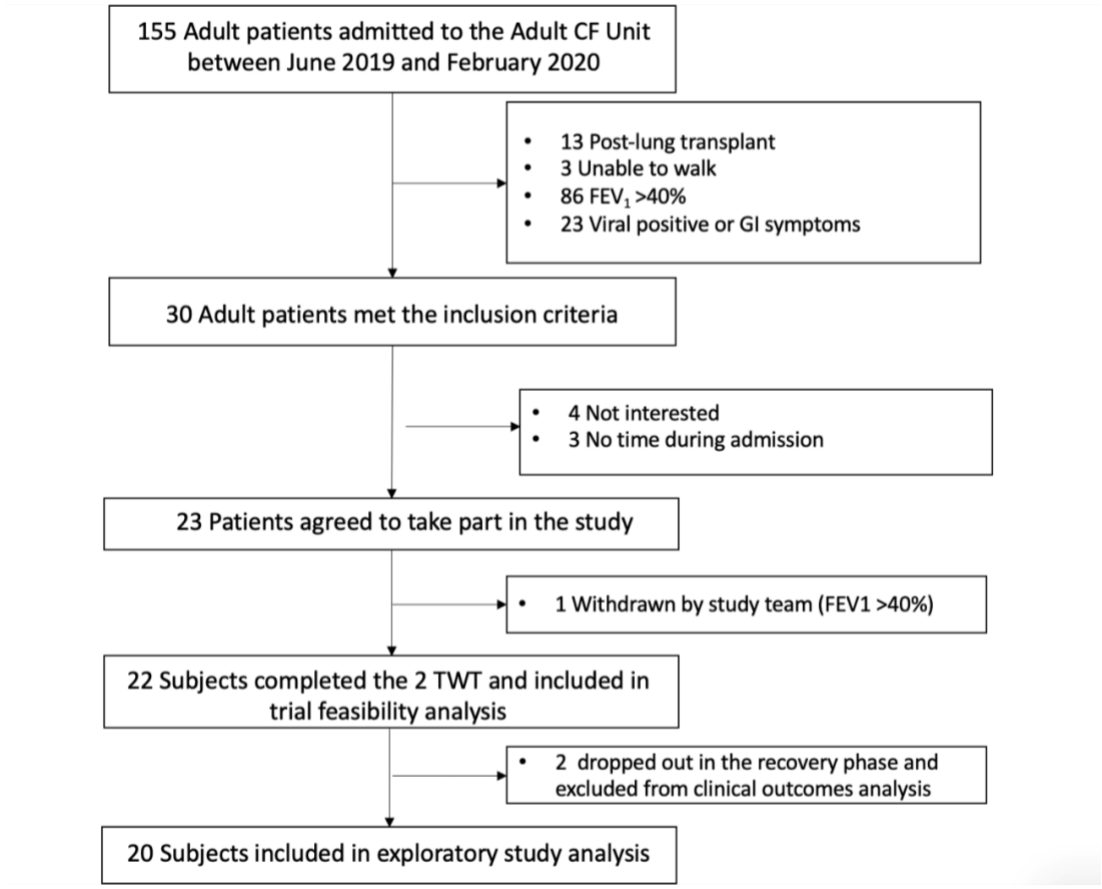

Figure 11.2 Flow-chart of patient recruitment and inclusion in the study and analysis.

Despite the initial plan to enrol up to 25 subjects, in view of the start of the COVID-19 pandemic, enrolment was closed after enrolling 23 patients.

The study population was slightly female predominant (n = 12, 54.5%), with a median age at 34 years. Most participants (n = 15, 68.2%) were on CFTR modulator therapy and were under consideration for lung transplantation (n = 8, 36.4%) or on the active waiting list for lung transplant (n = 7, 31.8%) (Table 11.1).

Table 11.1 Baseline characteristics of study population. LTOT, long term oxygen therapy; NIV, noninvasive ventilation; CBG, capillary blood gas. Data are reported as number (%) and median (IQR).

	N=22
Age	34 [29.5-39]
Male sex, n (%)	10 (45.5%)
BMI	20.9 [18.6 -22.1]
Genotype	
F508del/F508del	12 (54.5%)
F508del/-	9 (41%)
Other	1 (4.5%)
CFTR modulator	
Ivacaftor	3 (13.6%)
Double therapy (LUM/IVA or TEZ/IVA)	11 (50%)
Triple therapy (ELX/TEZ/IVA)	1 (4.5%)
none	7 (31.8%)
Transplant	
Not yet considered	7 (31.8%)
Under consideration	8 (36.4%)
Declined	4 (18.2%)
Active list	3 (13.6%)
Comorbidities	
Diabetes	4 (18.2%)
Liver disease	9 (41%)
Low bone mineral density	14 (63.6%)
Arthritis	4 (18.2%)
Microbiology	
Chronic P.aeruginosa	16 (72.7%)
MRSA	2 (9.1%)
M. Abscessus	4 (18.2%)
B. cepacian complez	2 (9.1%)
Respiratory support	
LTOT	5 (22.7%)
Nocturnal oxygen	3 (13.6%)
Ambulatory oxygen	3 (13.6%)
NIV	5 (22.7%)
FEV_1 on admission, l	0.83 [0.65-1.06]
FEV_1 on admission, %	25 [26-31]
CBG at baseline	
pH	7.43 [7.41-7.46]
pCO_2, kPa	5.48 [4.8 – 6.03]
pO_2, kPa	9.2 [8.6 – 9.7]
HCO_3^-, mmol/L	27 [23.7 – 28.5]

11.4.2 Respiratory variables during exercise in CF

Respiratory variables, and in particular respiratory rate (RR), oxygen saturation and carbon dioxide, were assessed during the TWT on baseline conditions.

All subjects had an increase in RR during the test [22 (20-24) vs 30 (26-34), $p < 0.05$]. A drop in oxygen saturation was observed. Mean S_pO_2 during the TWT was lower than that recorded before the test, although the difference was not statistically significant

(p=0.095). The nadir of S_pO_2 during the TWT was significantly lower than mean oxygen saturation before and after the test, but the drop was not always clinically significant. Similarly, an increase in CO_2 was noted during and immediately after exercise. The highest CO_2 levels were always reached within 2 minutes of exercise termination, before CO_2 started dropping to baseline, and was significantly different compared to mean $tcCO_2$ before and after the walking test (Figure **11.3**).

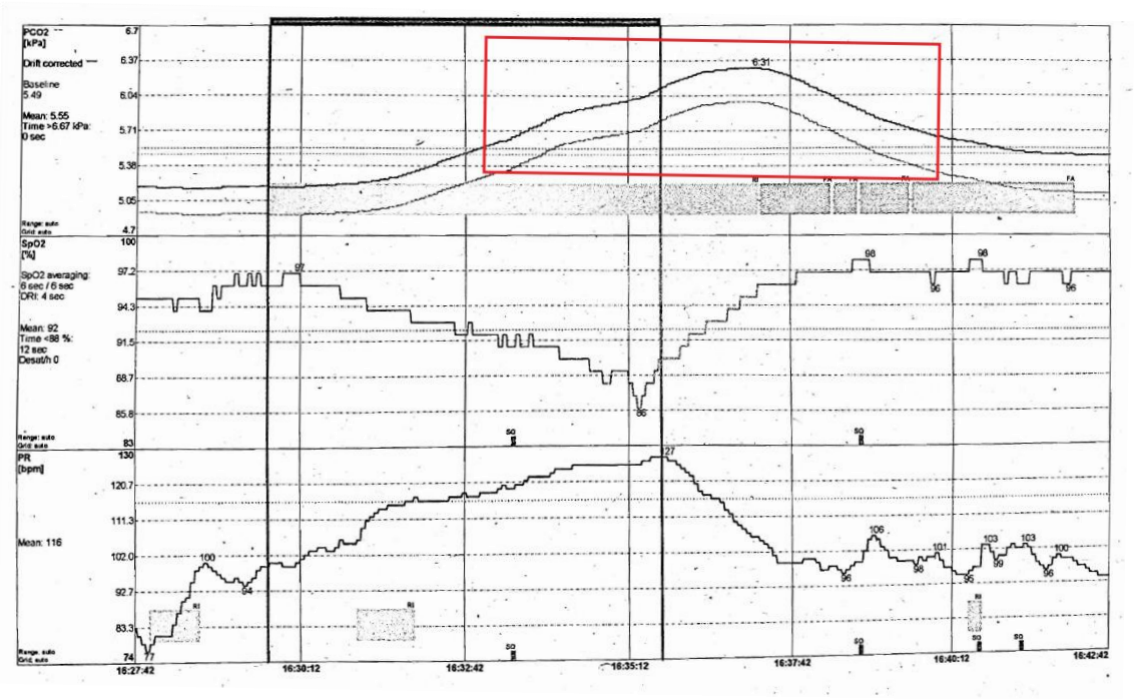

Figure 11.3 TOSCA during study visit. The TOSCA recording during a full study visit at baseline shows that CO_2 (top row) remains stable at baseline, and starts rising during the treadmill walk test, to reach the peak in the few minutes after its completion, before returning to baseline. Oxygen saturation (second row) drops during exercise and returns to baseline at rest.

11.4.3 Trial feasibility

The recruitment rate was on average 2.4 subjects per month, with screening to randomization ratio of 1.3:1 (76.7%). One subject was withdrawn as no longer meeting the inclusion criteria on day 1 of the study (baseline lung function transiently increased above 40%). Two subjects completed the walking tests on both conditions but dropped out before the recovery phase of the NHFT-test as they did not tolerate the device. Data completion rate was 100% for the twenty subjects who completed the study.

Tolerability was good. All but two subjects expressed a positive experience about participating in this research study and indicated that they would take part in longer term studies on the use of HFNT during exercise. The two subjects who reported negative experience required reduction in the temperature during NHFT-test and asked to stop using the device after completion of data collection of the walking test 15 min in the resting period.

One subject, who did not tolerate the device, reported chest pain during the TWT on NHFT. This was not associated with any change in vital signs and, on history review, was not deemed to be caused by NHFT.

Lung function was not adversely affected when using NHFT, with no episode of bronchoconstriction secondary to the use of NHFT.

11.4.4 Exploratory clinical outcomes of interest

To meet the exploratory aims, clinical outcomes of interests were assessed on the 91% of subjects (n = 20 out of 22) who tolerated the device and completed the study in full. Table 11.2 summarises the results of the exploratory outcomes of interest.

Table 11.2 Exploratory clinical outcomes of interest of the pilot trial.

	Control test (n=20)	**NHFT-test (n=20)**	***p***
Walking distance, m	430 [352-537]	450 [360-550]	**0.013**
Recovery time, sec	51.5 [0-114]	54.5 [10-75]	0.7
SpO2, %			
Mean	93 [91-95]	92.75 [90-95]	0.138
Nadir	89 [86-92.75]	88 [83-93]	0.255
Mean tcCO2, kPa	5.35 [4.99-5.44]	4.89 [4.56-5.47]	**0.03**
Respiratory rate			
At start	22 [20-24]	16 [13-18]	**<0.001**
At end	30 [26-34]	26 [20-30]	**0.003**
Borg – Dyspnea			
At start	0 [0-0.875]	0.25 [0-1.75]	*0.07*
At 2 minutes	2 [0.5-2.875]	1.5 [1-2.875]	0.88
At 4 minutes	3 [1-3.875]	3 [1.25-3]	0.38
At end	3 [1.275-4.75]	3 [1.25-4]	0.33
Borg – Fatigue			
At start	0 [0-0]	0 [0-1]	0.246
At 2 minutes	0.75 [0-2]	1 [0.5-2]	0.916
At 4 minutes	1.5 [0-2.375]	1.25 [0.125-2.375]	0.759
At end	2 [0.125-3.625]	2 [0.5-4]	0.905
Comfort score			
At start	10 [9-10]	8 [6.125-10]	**0.003**
At 2 minutes	9 [8-9.75]	8 [7-8.75]	**0.03**
At 4 minutes	8 [6-9]	7.75 [6.25-8]	0.426
At end	8 [5-9]	7 [5.25-8]	0.566

All subjects completed both the NHFT- and control-tests with no interruptions. WD was significantly higher on NHFT than on baseline conditions (mean difference = 19 m [95% CI 4.8 - 33.1], p = 0.01). No differences in highest or lowest speed recorded during the test were observed.

Mean and nadir S_pO_2, as well as recovery time for S_pO_2 were similar for the control and NHFT conditions both before and during the TWT (Table 11.2).

Respiratory rate at the end of the TWT was significantly lower on NHFT than during the control test (mean difference = −3.9 breaths/min [95% CI −5.9 - −1.9], p = 0.001). Mean

transcutaneous CO_2 was also lower on NHFT compared to the control test (mean difference = −0.22 kPa [95% CI −0.4 – 0.04], p = 0.019).

Comfort score was better at the start of the test on control condition, but Borg-D was similar. When comparing the same scores at the end of each TWT, no differences were observed in comfort, Borg-D and Borg-F across the whole dataset (Table 11.2).

Figure **11.4** shows the relative change in comfort and dyspnoea during the TWT. A lesser reduction in comfort was observed during NHFT-test compared to control test (mean difference in delta comfort = 1.3 [95% CI 0.2 – 2.5], p = 0.024).

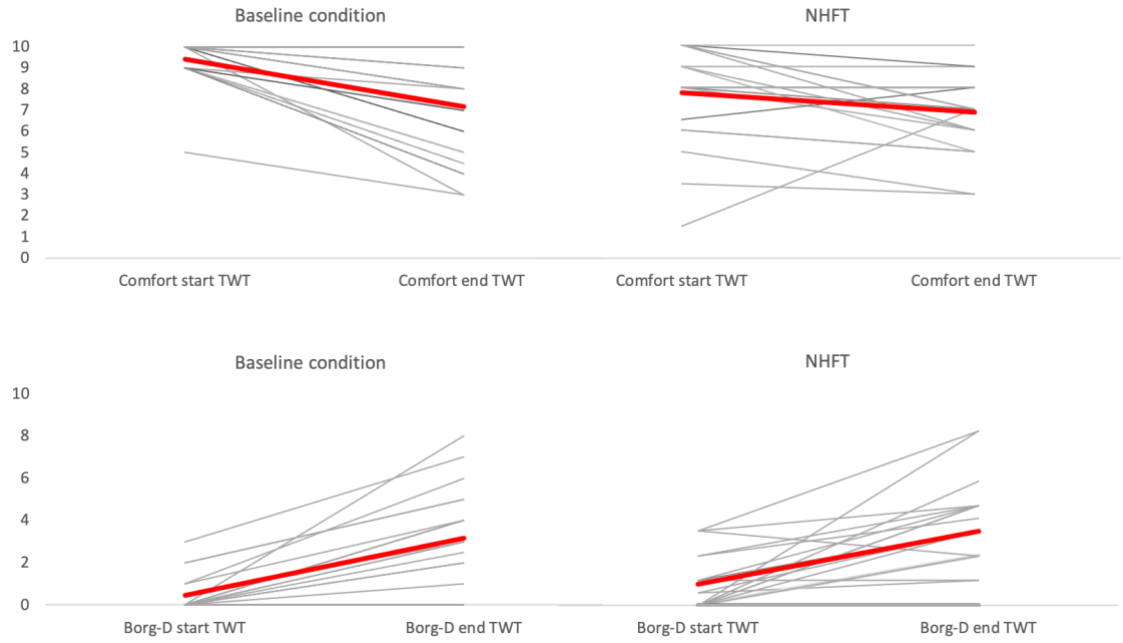

Figure 11.4 Change in comfort and dyspnoea score during the TWT on baseline conditions and on NHFT. Individual data are presented in grey lines, mean data are presented in red lines.

11.5 Discussion

People with CF, especially those with severe lung disease, often have a limited exercise capacity due to dyspnoea and desaturation. Ventilatory response to exercise in people with CF and severe lung disease appears to be differ from people with other respiratory conditions and healthy controls. The participants in this study retained CO_2 during exercise despite an increase in respiratory rate (Figure **11.3**), a feature previously reported in children [416,434] and in a small study undertaken in adults [435,436], This elevation in CO_2 is likely to reflect the inability to maintain adequate alveolar ventilation due to excessive dead space ventilation and a failure to increase tidal volume appropriately [204,428]. As CO_2 retention during exercise is a negative prognostic marker [416], and exercise is an integral part of the treatment, a fine balance is needed to ensure that individuals continue to exercise but that the impact does not result in clinical deterioration in people with CF and severe lung disease. The use of respiratory support techniques to improve ventilation can help support exercise in this patient cohort.

In this pilot study, the use of NHFT in the context of clinical trials during exercise in people with CF appears feasible. Exploratory outcomes of interests for efficacy indicated that NHFT might improve 6MWD amongst individuals with CF and advanced lung disease, and reduce respiratory rate and carbon dioxide during exercise. Dyspnoea, comfort and oxygen saturation were stable throughout exercise on NHFT.

Recruitment target was achieved within 9 months from starting the study, 10 months ahead of scheduled time. Screening failure rate was low, and screening to randomization rate was good, suggesting suitability of the recruitment strategies and eligibility criteria. Two subjects dropped out during the recovery phase of the NHFT-test, providing a 9% of drop-out rate, much lower than the planned 20%.

Participants provided positive feedback and remained interested in participating in future trials. This highlighted that the study procedures were acceptable to the majority of patients, and that recruitment to future trials exploring the role of NHFT during longer training programmes would be feasible. Such trials could also be performed remotely with participants exercising in their home environment to reduce their risk of cross-infection.

Two participants (9%) were unable to tolerate the device and were therefore unwilling to participate in similar trials in the future. On visual inspection of their characteristics, these patients had marked reduction of the FVC (< 1 L) and were significantly underweight. As FVC correlates with peak inspiratory flow, it is conceivable that the flow-rates delivered by NHFT are disproportionate for patients with significant reduction in FVC.

In planning future trials, inclusion criteria should be adapted in light of this finding. In particular, a dropout rate of at least 10% should be considered to account for participants who might not tolerate the device, decide to stop participation due to the longer nature of a study in the home environment and/or experience clinical deterioration. In addition, subjects with FVC < 1 L who, in this pilot study, reported significant discomfort and disliked the device, could be excluded or, alternatively, a pre-specified stratification based on FVC should be planned to further explore tolerability in the subgroup with significant reduction in FVC.

This pilot study was not designed to assess intervention efficacy. Exploratory outcomes, however, showed improvement in distance walked during the TWT. While CPET is the gold standard to assess globally the response to exercise [253], its use in CF is limited and most patients are routinely assessed with 6-minute walking test, which has been shown to have good correlation with the peak oxygen uptake in patients with severe lung disease, to be reliable and repeatable [437–439]. In this study, a treadmill walking test was preferred to a standard 6MWT, as commonly done in previous trials in this population [402,433], in view of infection control policies to reduce the risks of cross-infection.

Currently, no threshold for minimally clinically important difference (MCID) for change in treadmill or over-grounds 6-minute walking distance is defined for people with CF [255]. Previous studies on patients with COPD and pulmonary hypertension showed a variable correlation between the TWD and 6MWD, but consistently more favourable results when the walking test was performed on grounds rather than on the treadmill [440–442]. A recent systematic review proposed that any change in 6MWD on grounds between 14 and 30.5 m should be considered a MCID across multiple patients' group [443]. The improvement in WD observed in this study is statistically significant and within this proposed range (19 m mean difference) and well above the mean difference observed for repeated test (8 m) in people with CF [439].

The intra-test comparison showed that respiratory rate before and after the TWT increased at similar magnitude during both the NHFT and the control tests. However, respiratory rate was lower on NHFT compared to baseline conditions across the whole study, starting from acclimation period to the end of the walking test. The change in RR was of the same magnitude of what observed in a recent crossover trial comparing NHFT to NIV and baseline conditions in stabilised patients with CF admitted with pulmonary exacerbation [354]. This, in association with the observed reduction in carbon dioxide level, suggest the NHFT could contribute to a change in breathing pattern and minute ventilation as previously observed during exercise on NHFT in people with severe COPD, and in patients with CF recovering from exacerbations [354,431].

NHFT has been reported to improve comfort in various scenarios compared to both baseline conditions and NIV [258,316,320]. In keeping with what reported in [354], participants reported lower comfort when started on NHFT at rest. However, comfort at end of exercise was comparable in the two conditions, and decreased more during the control test. Similarly, dyspnoea measured with the Borg scale was similar at the start of the test in both conditions but increased to a lesser extent during the NHFT test. These findings suggest that relatively stable patients might not benefit from NHFT delivered at flowrates at 30 L/min or higher, as they are not in any respiratory distress. However, once their respiratory demands increased during exercise, the additional support provided by NHFT might be beneficial.

The main limitation of this study, similarly to any trial using NHFT, is the lack of blinding, as no sham device is available for the control-test. However, none of the participants had used NHFT ahead of their participation in the trial. In addition, minute ventilation during exercise could not be measured accurately due to the inability to use a pneumotachograph on NHFT, and inductive plethysmography would lead to artefacts by movement. However, while the absence of measurements of breathing pattern can limit the interpretation of some of the exploratory outcomes with regards to ventilation, it does not affect the effect observed for this treatment.

Finally, while the exploratory outcome of interest provided positive results, the study was not powered for efficacy outcomes given the pilot nature and changes in WD are best observed after a training programme. As such, these findings need to be confirmed and further investigated in adequately powered trials to assess the longer-term effects of NHFT during exercise training programme in people with CF.

11.6 Conclusion

This pilot study showed that individuals with CF and advanced lung disease retain CO_2 even during short exercise bout such as a treadmill walking test. This increase reaches the peak after completion of exercise before returning to baseline. NHFT appears to be beneficial in improving gas exchange, while increasing walking distance, reducing respiratory rate, and achieving a better control of dyspnoea and comfort compared to baseline conditions.

Further studies are proved to be feasible and are needed to explore the use of NHFT during a longer-term physical training programme in people with cystic fibrosis and severe lung disease are warranted by the results of this pilot.

Chapter 12
Sleep disordered breathing and bicarbonate

12.1 Introduction

As discussed in Chapter 2 Section 2.2, the HCO_3^-/CO_2 buffer is the main driver to maintain acid-base homeostasis. As such, I hypothesised that serum bicarbonate could be a viable marker of hypoventilation, and could act in clinical practice as the equivalent of an HbA1C for diabetes, but for ventilation. This concept has previously been investigated in obesity hypoventilation syndrome (OHS), where the diagnosis of OHS could be excluded in a cohort of subjects with normal serum bicarbonate levels [388,444].

Patients with CF, including children, experience nocturnal hypoxemia with hypoventilation during REM sleep, irrespective of age, weight and lung function (Section 2.4.1.1). It is therefore conceivable that increased bicarbonate levels in patients with cystic fibrosis could be secondary to increased nocturnal CO_2, compensated by changes in ventilation pattern when awake.

To establish whether bicarbonate could be used as a marker of sleep-disordered breathing and nocturnal hypoventilation, I designed a retrospective study to analyse the level of serum bicarbonate in patients referred to the sleep clinic, followed by a second prospective study to investigate if raised serum bicarbonate was associated with increased CO_2 overnight in people with CF.

12.2 Serum bicarbonate in the sleep clinic: a retrospective study

12.2.1 Background and rationale

Sleep-disordered breathing (SDB) encompasses a range of conditions characterised by abnormal breathing during sleep, ranging from intermittent obstruction of the airways (obstructive sleep apnoea, OSA) to cessation of breathing without airways obstruction (central sleep apnoea, CSA) and reduction of breathing during sleep associated with obesity (obesity hypoventilation syndrome, OHS) [445].

Obstructive sleep apnoea and OHS affect up to the 25% and 0.3%-2.3% of the population respectively with approximately 20% of people with OSA having OHS. Patients with OHS have higher mortality and morbidity, lower quality of life and higher

healthcare-associated expenses and usage compared to eucapnic obese subjects with and without OSA.

The majority of patients with OHS are diagnosed in their 40s or 50s, with the most common presentation being acute admission for decompensated type II respiratory failure. More rarely, diagnosis of OHS follows a routine assessment in a sleep centre due to symptoms such as snoring, morning headaches and daytime sleepiness. With the global obesity epidemic, the prevalence of OHS is expected to rise further.

Currently, the diagnosis of OHS follows a daytime ABG demonstrating hypercapnia in obese patients. However, ABG can cause discomfort and routine testing is not always available in sleep laboratories. In addition, a one-off ABG does not account for the variability of pCO_2 and could be an overly restrictive diagnostic criterium [389].

Further, overnight oximetry output, such as nadir SpO_2, mean SpO_2 or time spent with SpO_2 <90%, have all been proven to be good predictors of OHS. To gather such data however, referral to a sleep clinic and an overnight assessment are required.

Based on all these considerations, there is scope for a simpler, more readily available alternative diagnostic tool to facilitate the screening and diagnosis of OHS.

12.2.2 Hypothesis and aim

An increase in bicarbonate reabsorption and production in the kidney is likely to happen in response to an increase in carbon dioxide (Section 2.2.2). As such, I hypothesised that serum bicarbonate could be used as a simple screening tool for OHS, acting as a predictor of increased overnight CO_2.

The aim of this retrospective study was to assess if serum bicarbonate could be used as a screening tool to identify patients to be referred to sleep centre for suspected OHS.

12.2.3 Methods

I performed a retrospective analysis of data collected prospectively as part of a service development project in the Leeds Sleep Centre.

The service development project included all obese patients (BMI ≥ 30) who attended the Leeds Sleep Centre over a 15-month period (July 2016-December 2017) and underwent a sleep study. A venepuncture was performed to measure serum bicarbonate and renal function.

Demographic data, blood results and overnight oximetry variables were collected from the medical notes, and anonymised for analysis.

12.2.4 Statistical analysis

Continuous data are presented as mean and standard deviation if normally distributed and median and IQR if not normally distributed. Categorical data is presented as number and percentages.

ROC curves were constructed to determine if $sHCO_3^-$ could predict three overnight oximetry variables indicating OHS: ODI>15, mean SpO_2 < 90%, and time spent with SpO_2 <90% being >10%. Thresholds were selected to optimise the Youden's J index combining sensitivity with specificity. A subsequent cohort analysis was performed including only subjects with moderate-severe obesity (BMI >35).

All analyses were performed using SPSS v26.

12.2.5 Results

Over the 15-month period of this study, 162 eligible patients attended the Sleep Centre and underwent a sleep study.

Table 12.1 summarises the baseline characteristics of the whole population, and of those with a BMI >35 (n=100, 61.7%). The two groups were similar for age, smoking history and comorbidities. Median serum HCO_3^- was 29 in both cohorts, with normal renal function and electrolytes.

Table 12.1 Baseline characteristics of the whole cohort and patients with moderate-severe obesity. COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; CAD, coronary artery disease; AF, atrial fibrillation; T2DM, type 2 diabetes. Data are expressed as number (%), mean (SD), median (IQR).

Characteristic	BMI ≥30 (n=162)	BMI ≥ 35 (n=100)
Age at test, yrs	50±12	48±12
BMI	38.2±6.3	41.7±5.5
Female sex, n (%)	58 (35.8%)	43 (43%)
Smoking history		
Current smoker	29 (17.9%)	20 (20%)
Ex-smoker	31 (19.1%)	19 (19%)
Never smoker	102 (63%)	61 (61%)
Comorbidities		
COPD	9 (5.6%)	7 (7%)
ILD	1 (0.6%)	1 (1%)
Asthma	25 (15.4%)	18 (18%)
Hypertension	49 (30.2%)	31 (31%)
CAD	8 (4.9%)	4 (4%)
AF	8 (4.9%)	5 (5%)
T2DM	27 (16.7%)	17 (17%)
Blood results		
Bicarbonate, mmol/L	29 [3]	29 [4]
Potassium, mmol/L	4.5 [0.5]	4.5 [0.4]
Creatinine, umol/L	76 [18]	76 [22]
Urea, mmol/L	5.5 [2]	5.5 [1.95]

Overnight oximetry variables showed a mean oxygen desaturation index of 35.8±29.3, with 130 (62.8%) patients having a ODI>15. Mean S_pO_2 was 91±3.5%, being <90% in 53 (25.6%) cases. Time spent with S_pO_2<90% was 26±28% and was >10% in 94 (45.4%) studies (Table 12.2).

Table 12.2 Sleep studies variable in the whole cohort and patients with moderate-severe obesity. AHI, apnoea-hypopnoea index; ODI, oxygen desaturation index; TST, time with SpO2 <90%.

Variable	BMI ≥30 (n=162)	BMI ≥ 35 (n=100)
AHI, events/hr	36.4±31.3	45.3±34.2
ODI, events/hr	35.8±29.3	43.1±31.5
ODI>15, n (%)	130 (62.8%)	73 (73%)
Mean S_pO_2	91±3.5	90.3±4
Mean S_pO_2<90%, n (%)	53 (25.6%)	41 (41%)
TST<90%	26.3±28.8	31.7±29.5
TST90 >10%	94 (45.4%)	60 (60%)

The area under the ROC curve for $sHCO_3^-$ to predict ODI>15, mean S_pO_2<90% and time with S_pO_2<90% >10% were 0.643, 0.650 and 0.655 (all p<0.01), respectively (Figure 12.1).

ROC AUC in patients with BMI>35 (n=100) were higher, but not significantly different, at 0.684, 0.710 and 0.662 (all p<0.01), respectively. Optimal threshold was 29 mmol/L for all three measures in both cohorts.

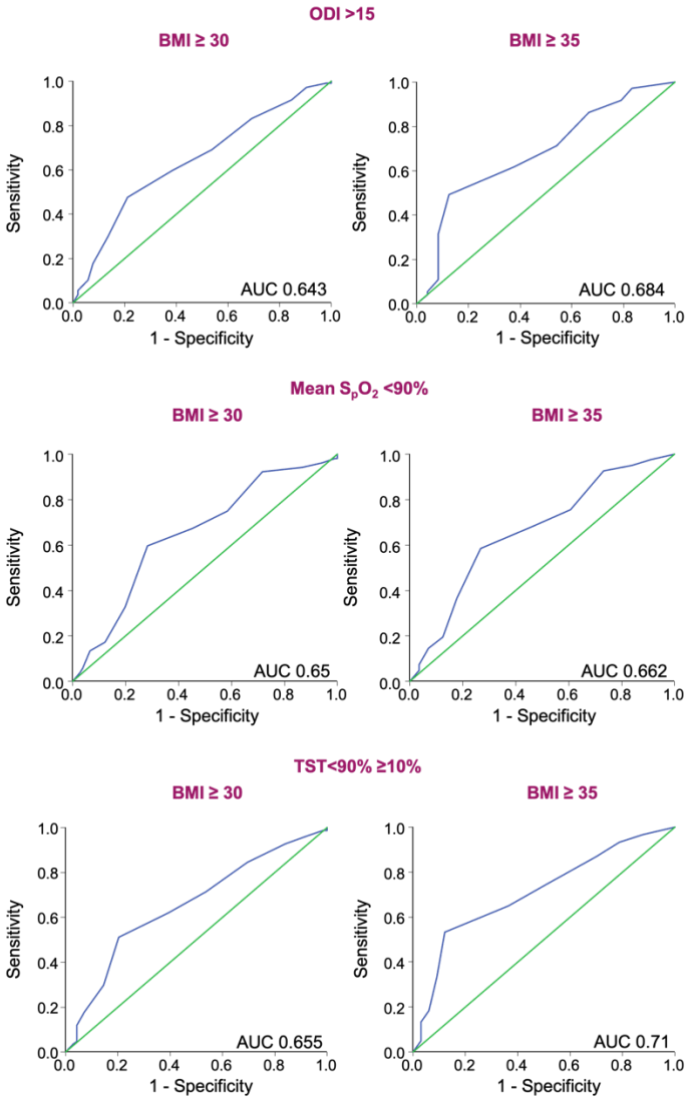

Figure 12.1 ROC curve analyses of serum bicarbonate to predict sleep study variables in obese patients.

12.2.6 Discussion

In this study, serum bicarbonate appears to be a predictor of nocturnal hypoxaemia. I showed that serum bicarbonate can predict mean oxygen saturation <90%, ODI >15, and time with SpO_2 <90% greater or equal than 10%. All of these sleep variables have been associated with OHS. In this analysis, I identified as optimal cut-off level a serum bicarbonate concentration of 29 mmol/L. This was established using the Youden-J to optimize sensitivity and specificity.

Serum bicarbonate being a predictor of nocturnal hypoventilation is in keeping with previous studies. In an observational study, Mokhlesi and colleagues determined and validated a threshold for arterial bicarbonate to identify people with OHS among patients referred for evaluation for OSA. Serum bicarbonate within normal limits can practically rule out OHS in obese patients, and increased serum bicarbonate is associated with daytime hypercapnia in 50% of cases [388]. These results were replicated by Macavei

and colleagues in a retrospective analysis on a large number of patients attending the sleep clinic. In their cohort a cut-off at 27 mmol/L for arterial bicarbonate had over 85.7% sensitivity and 89.5% specificity with a negative predictive value at 95.9% for OHS [446].

These studies identified a lower cut-off value compared to what I found in my study. However, in this study the 29 mmol/L threshold was established by using the Youden-J index, which optimises a combination of both sensitivity and specificity. As OHS is associated with significant morbidity, a different approach could be taken in optimising only sensitivity. This different index would have led to the cut-off of 27 mmol/L in this cohort, exactly in line with previous studies.

An attempt at validating these results in a prospective study in unselected obese subjects was also performed. However, despite an initial screening of over 1000 subjects, the combination of a significant number of drop-outs and the lower than expected prevalence of OHS, reduced the analysis to 114 identified OHS subjects where serum bicarbonate levels were 27 mmol/L or greater in all patients with OHS [444].

Based on these results, the current practical definition of OHS based on a single measurement of CO_2 appears to be restrictive. As such, a call to change such definition to add serum bicarbonate concentration as a marker for ventilation was recently made [389]. Many patients with nocturnal hypoventilation in OHS have normal daytime CO_2, but raised arterial bicarbonate and/or BE, and behave in response to hypoxic and hypercapnic stimulus similarly to hypercapnic obese patients [387]. It is therefore conceivable that measuring serum bicarbonate could allow for a screening of patients with hypoventilation, and specifically OHS, as bicarbonate could be the equivalent for ventilation as HbA1C is for diabetes[389].

This study, as well as those previously investigating the role of bicarbonate as a screening tool for OHS, was limited to patients already under assessment or referred for assessment in the Sleep Clinic. Furthermore, dataset in this service development project did not include an arterial blood gas which currently is the gold standard to diagnose OHS. Conversely, previous results were based on arterial rather than serum bicarbonate [388,446].

To overcome these limitations, a prospective cohort study to identify the best threshold to screen for OHS in obese patients in unselected setting is needed. I estimated that serum bicarbonate level greater than 28 mmol/L would be an appropriate threshold for screening OHS in obese patients. Based on an estimated prevalence of OHS in this population of 3 to 5%, a sample size of 1475 patients would be needed.

A feasibility assessment for such a study was conducted and concluded that it would not have been feasible to perform this prospective study as a single-centre trial in the context of this PhD programme.

12.2.7 Conclusion

Serum bicarbonate appears to be a good predictor of OHS, and could potentially act as a long-term marker for ventilation.

Prospective studies would be required to assess the use of serum bicarbonate as a screening tool for OHS in obese patients in settings other than sleep centres. Such studies would require large sample sizes and should be performed as multi-centre due to the anticipated difficulties in enrolling subjects, as previously demonstrated by others [444,446].

12.3 Hypoventilation and bicarbonate in CF – a pilot study

People with CF often present with sleep fragmentation and daytime fatigue and/or sleepiness [189,200,447,448]. However, sleep disordered breathing is rarely diagnosed in people with CF [190,197,449]. These disorders would normally be treated using oxygen therapy or NIV. Both these techniques are widely used also in patients with CF, but evidence on their effectiveness in reducing pulmonary exacerbations, disease progression and mortality remains unclear [211,330]. It is possible that the lack of significant response to treatment is due to respiratory support being initiated too late. If this was confirmed, starting respiratory support earlier on in the progression of disease could contribute to more stabilisation and/or improvement.

I hypothesized that raised serum bicarbonate levels in CF are partly a consequence of transient nocturnal hypercapnia, as I (Section 12.2) and others described in people with OHS.

Therefore, I planned a pilot study to investigate if increased serum bicarbonate levels could be explained with sleep disturbances, and in particular hypoventilation or flow-limitations.

12.3.1 Aim and objectives

The aims of this study are:

- To assess if there is any correlation between daytime concentration of serum bicarbonate, and overnight CO_2 in patients with CF.
- To explore if the results of an overnight TOSCA correlate with oximetry, or whether the use of TOSCA could provide any advantage over standard monitoring in patients with CF.
- To rule out that respiratory events during sleep (such as central or obstructive sleep apnoea, hypopnoea, flow-limitation, etc.) play any role in the nocturnal levels of CO_2 and daytime serum bicarbonate in patients with CF.

- To explore if there is any correlation between the respiratory rate overnight and the results of the TOSCA comparing patients with normal and raised serum bicarbonate.

Further exploratory outcomes of interests are:

- To assess if any correlation between CO_2 monitoring overnight and morning blood gas is present;
- To assess the frequency of respiratory events overnight;
- To explore differences between overnight oximetry, TOSCA and respiratory polygraphy.

12.3.2 Methods and study design

A single-centre, short-term, open pilot study was designed to investigate the potential correlation between sleep disorders and carbon dioxide by performing overnight TOSCA and blood gases in patients with CF and different levels of serum bicarbonate.

12.3.2.1 Subjects

Inclusions criteria for this study are as follows:

- Age of 18 or more;
- Confirmed diagnosis of cystic fibrosis (abnormal sweat test and/or presence of two CF-causing mutations).

Exclusion criteria for the study are::

- Colonisation with BCC;
- Use of NIV or CPAP at night;
- Inability to provide consent;
- BMI >30.

12.3.2.2 Data collection

All subjects meeting the inclusion and exclusion criteria should be approached by a member of the research team during a hospital stay. Those agreeing to take part in the study are to undergo an assessment of their oxygen level with overnight oximetry. Treatment including oxygen therapy or NIV is initiated, if required, following the results of this test.

In addition, subjects are asked to complete the Epworth Sleepiness Scale, and to undergo a transcutaneous monitoring of CO_2, a respiratory polygraphy to monitor airflow and respiratory effort during sleep and to rule out any SDB, and a morning blood gas.

Demographic data, microbiology status, medications, and lung function results are collected from the medical notes.

12.3.2.3 Statistical analysis

No sample size calculation was performed for this pilot study. I planned for a full dataset on 40 subjects (10 per each subgroup).

Normal distribution of measured variables will be assessed by visual inspection and using the Shapiro-Wilks test. Results will be expressed as number and percentage, mean and standard deviation (SD) when normally distributed or medians and interquartile range when not normally distributed.

Estimates of differences will be provided with 95% confidence intervals. Inferential statistics might be used for strengthening preliminary results, however this is not intended to be part of the outcomes of this pilot trial. Paired t-test or non-parametric alternative will be used as appropriate for comparison.

Magnitude of outcomes, confidence intervals and other measures of statistical variability will be used to power future prospective larger studies, if appropriated.

12.3.2.4 Ethics and funding

ResMed provided equipment for the study to be carried out, but had no involvement in study design. The research protocol received HRA approval via the Yorkshire and Humber REC (19/YH/0305) and local CSU and R&I approval.

12.3.3 Conclusion

Ethical approval and equipment (NOX) for the study were obtained just before the first wave of the COVID-19 pandemic hit the United Kingdom in February 2020. As such, the study was temporarily halted in line with HRA, PHE and NHS England guidance as this was not a COVID- or drug trial.

Subjects that would have been eligible to take part in the study were included in the Clinically Extremely Vulnerable group for COVID-19, as such reducing their attendance to hospital and minimising their contact in hospital and at home with other individuals was of the utmost importance during the pandemic.

Therefore, even when resumption of non-COVID priority studies was allowed, I decided to keep this pilot study on hold, with the aim of starting it later on when the pandemic is better controlled. Amendments will also be required to improve feasibility in view of the change of circumstances, secondary to the pandemic itself and the introduction of Kaftrio. These amendment might include the enrolment of subjects at home, with home visit to set-up and collect the equipment, and having a comparison group, including those patients who are not receiving Kaftrio due to their genotype.

12.4 Conclusion

Serum bicarbonate correlates and appears to be a good predictor of hypoventilation in obese patients who are referred to the sleep clinic with possible sleep-disordered breathing.

Further studies to assess if serum bicarbonate level rises in other conditions, including CF, are required. In light of this, I have HRA approval to conduct a study in people with CF which I am planning to commence after a temporary stop caused by COVID-19. Results of this study in CF would also help in understanding whether nocturnal respiratory support might be indicated earlier on in the course of the disease.

Part 3

General discussion and conclusions

Chapter 13
General discussion

The aim of this research project was to characterise acid-base disturbances in people with CF, and to understand the clinical relevance and aetiology of elevated serum bicarbonate in this complex multisystem disease.

Five core research questions were identified as described in detail in Chapter 4. These were:

1. Is the concentration of serum bicarbonate elevated in people with CF, even in absence of metabolic alkalosis?
2. Is the raised serum bicarbonate concentration clinically relevant?
3. What is the distribution of acid-base disturbances in patients with CF, and is it different from that of the general population?
4. What is the cause of raised serum bicarbonate in patients with CF?
5. If there is a ventilatory component leading to increased concentration of serum bicarbonate and, if so, can it be modified by non-invasive ventilatory support?

A series of eight research studies were designed to address these overarching questions, and both their methodology and results are discussed in each respective chapter.

In this general discussion, I provide a holistic overview of the outcomes of the studies and how the overall results help answer the five core research questions described above.

13.1 Is serum bicarbonate raised in CF?

At the start of this project, I hypothesised that the concentration of serum bicarbonate was raised in people with CF compared to individuals with non CF respiratory disorders and the general population. I postulated that CF-specific comorbidities, such as pancreatic insufficiency, osteoporosis, recurrent courses of antibiotics, certain genotypes and diabetes, could be independently associated with increased serum bicarbonate and acid-base disturbances.

In this thesis, I have shown that the average concentration of serum bicarbonate in individuals with CF is at the upper limit of the normal range (ULN), with a sizeable fraction (>20%) of measurements above the ULN. Levels are also higher in the CF population when compared to those reported for the general population, and non-CF respiratory conditions.

In Chapter 5, I analysed a large dataset of 2,800 regular annual assessments and showed that serum bicarbonate concentration in clinical stable CF patients is on average

27 mmol/L (Table 5.1), with over 20% of measurements being >28 mmol/L. These levels are significantly higher than those reported in healthy adults (the NHANES III cohort) where mean serum bicarbonate of 24.9 mmol/L were observed in the general population [360,361].

In Chapter 8, I further characterised the impact of acute pulmonary exacerbations on serum bicarbonate and showed that pulmonary exacerbations in individuals with CF are associated with higher serum bicarbonate levels when compared to patients with other respiratory disorders (28 vs 24 mmol/L) (Table 8.3, Figure 8.1).

I identified a high degree of variability in serum bicarbonate levels in people with CF, with levels varying according to age and sex. A correlation was also seen between serum levels, disease severity and several CF related comorbidities.

Serum bicarbonate levels were elevated in individuals in the 12 months before death, increasing significantly the closer the patient was to death (Chapter 6). These findings suggest that respiratory failure is likely to play an important role in elevating serum bicarbonate and that levels could potentially be used as a biomarker of disease severity.

Thanks to the large-scale data sets available, a number of factors independently associated with changes in serum bicarbonate in patients with CF were identified.

13.1.1 Age and sex

Serum bicarbonate increases more rapidly with age in people with CF compared to the general population. On average, serum bicarbonate increased at a rate of 1.56 mmol/L every 10 years in patients with CF (Chapter 5), compared to the 0.24 mmol/L in healthy adults [360,361].

The age related increase in bicarbonate was present in both female and male individuals with CF, in line with what has been previously reported in the general population. Serum bicarbonate levels were more elevated in males compared to females with CF, with a difference in the medians of 0.9 mmol/L (Chapter 5, Figure **5.3**)

Bicarbonate levels, as previously described, were higher in the 12 months before death, the rate increasing to 1.27 mmol/L per month in the last six months of life. By restricting the analysis to the last 12 months of life, males with CF no longer had higher serum bicarbonate levels compared to females (**Figure 6.5**). However, the rate of increase when approaching death was steeper in female patients.

A “gender gap” in CF is well established, whereby female patients have a faster decline and more severe illness [450–454]. In conditions of advanced and severe disease, serum bicarbonate tends to increase in a more pronounced way in female patients, who are known to be more at risk of decline [450].

13.1.2 CF-related comorbidities

To understand how much age and sex are genuine independent factors associated with different levels of bicarbonate, and how much serum bicarbonate concentration is a proxy of disease severity, each CF-related comorbidity was modelled independently, and in a full-variable model (Chapter 5, Section 5.4.2.4)

Due to the progressive life-limiting nature of CF, older patients are bound to have more complications and worsening overall health. A link between age and decline in lung function is well established in the literature [102,364,366,450,455,456].

CF liver disease, and especially cirrhosis, was associated with increased concentration of serum bicarbonate. Similar findings have been reported in the general population and may reflect a role of the liver in the regulation of acid-base balance [367].

Similarly, bone metabolism may be linked to acid-base balance, with metabolic acidosis and low serum concentration of bicarbonate being linked to osteoporosis in the general population. These changes in acid-base balance appear to promote bone reabsorption and prevent bone formation [368,457]. This contrasts with the results of the study in Chapter 5, which demonstrated higher, rather than lower, serum bicarbonate concentration in individual with CF related osteoporosis. This may be partially explained by the relationship between low bone mineral density, reduced lung function and increased disease severity [458–460], and further supports the fact that serum bicarbonate may be a useful biomarker of disease severity.

In the general population, diabetes and impaired glucose tolerance, irrespective of chronic renal disease, are associated with lower concentrations of serum bicarbonate [360,369]. In contrast, I found the opposite, with CFRD being linked to increased bicarbonate levels. As discussed in Section 1.5.3.2, diabetes occurs in a large proportion of adults with CF, and is a marker of poor prognosis, especially when uncontrolled. While post-hoc analysis on the basis of glycaemic control, as assessed by HbA1C, was not performed, it is conceivable that people with abnormal glucose tolerance may have more severe disease than those with normal glucose tolerance.

When controlling for all CF-related comorbidities, the independent impact of age was reduced to 0.03 mmol/L per year (0.30 mmol/L every 10 years), in line with the general population.

13.1.3 Genotype and CFTR modulators

CFTR is primarily an anion channel responsible for the transport of chloride and bicarbonate, and it also acts as a regulator for other anion exchangers and cation channels (Chapter 1, Section 1.1.1). While the genotype/phenotype correlation in CF is

influenced by gene modifiers and environmental factors, the expression and activity of CFTR vary depending on how each specific mutation affects the transcription of the protein (Section 1.1.2). This led to the hypothesis that the specific genotype could affect the concentration of serum bicarbonate in people with CF (Chapter 4, Section 4.1.1).

However, no difference in serum bicarbonate concentration was observed between the different genotypes (Figure **5.4**). As part of this analysis, the population was arbitrarily subdivided into three cohorts: F508del homozygous, F508del heterozygous, and all others. These categories have been previously reported in the literature and were chosen for simplicity. However this classification of highly heterogeneous mutations may have missed subtle differences. For example, individuals in the "heterozygous" and "others" groups can present genotypes that result in widely different expressions of CFTR. Some might have mutations that result in reduced expression of WT-CFTR or decreased channel activity, such as gating or residual function mutations, whereas others might have genotype which cause complete absence of CFTR, as class I mutations. Unfortunately, a more granular classification was not possible due to small sample sizes in certain groups.

To further assess the role of genotype on serum bicarbonate levels, I looked at the effects of treatment with CFTR modulators. If serum bicarbonate concentration were dependent on CFTR dysfunction, then treatment should theoretically reduce bicarbonate levels.

Treatment with small molecule therapy was associated with a higher concentration of bicarbonate, but this is likely a reflection of disease severity rather than alteration in CFTR function (Figure **5.5**, Chapter 5). For most of the study period (2007-2020), the majority of individuals treated with CFTR modulators were receiving it via the compassionate use scheme, with the entry criteria requiring the presence of severe lung disease. Therefore, most of the patients on CFTR modulators had advanced lung disease, oxygen or respiratory support requirement, and were under consideration for lung transplantation. When looking at the cohort of people with CF being treated with CFTR treatment, it is likely that this simply reflect a proxy of disease severity. In addition, in most cases the CFTR modulators prescribed during the study period resulted in a relatively modest improvement in lung function and reduction in sweat test. This contrasts with the recent introduction of ELX/TEZ/IVA which has proved much more efficacious both *in vitro* and *in vivo,* and theoretically is more likely to correct CFTR function in the kidney.

This was confirmed by the full factor model presented in Chapter 5 Section 5.4.2.4, showing that, once correcting for all comorbidities, age, sex, and other prognostic factors, the role of CFTR modulators on serum bicarbonate was statistically significant, but clinically and physiologically negligible.

In the prospective HAST study, which included a small but more homogeneous cohort of patients (Chapter 9), a third of individuals were on CFTR modulators. Treatment, at this time, was available through clinical trials or as part of standard of care and patients were therefore more clinically stable and had less comorbidities compared to the retrospective historical analysis.

In this group, concentrations of serum bicarbonate were lower compared to the historic cohort. This may in part due to the improved overall health achieved with treatment with CFTR modulators, but may also reflect the normalisation of renal bicarbonate handling following the partial systemic correction of CFTR function [242].

Further studies to explore the impact of CFTR modulators on serum bicarbonate concentration before and after the initiation of treatment are needed. These should be performed taking into consideration the other confounding factors which have described above.

13.1.4 Lung function

Lung disease is one of the main clinical manifestation of CF and is characterised by mucus plugging, progressive lung damage and chronic bacterial infection, interspersed with clinical decline following acute pulmonary exacerbations (Section 1.4). CF-related lung disease and end stage respiratory failure remain the main cause of morbidity and mortality in this population.

In routine clinical practice, lung function is used to monitor disease progression. Traditionally, FEV_1 has been considered a primary marker of disease severity and prognosis in CF, although normal results do not equate to an absence of underlying parenchymal lung disease [102,103]. In the early 1990s, it was shown that patients with advanced lung disease and FEV_1 <30% had a 50% mortality rate at 2 years [372].

Currently, thanks to radical improvements in the multidisciplinary care of people with CF, those with FEV_1 <30% have an improved average survival time which is now greater than 5 years [103,370,371], and the accuracy of FEV_1 <30% as a predictor of death is much more debatable, with rate of decline being probably more relevant. This notwithstanding, a significant reduction in lung function remains an important negative prognostic marker, with the cut-off of FEV_1 <40% being routinely used to define advanced lung disease [105], and FEV_1<30% remaining an important criterium for referral to lung transplant services [375].

In Chapter 5, serum bicarbonate was shown to correlate with lung function, mainly due to the strong negative correlation in patients with an $FEV_1 \leq 40\%$ (Figure **5.10**). When the analysis is controlled for the presence of age and CF related comorbidities, the general independent correlation of FEV_1 and bicarbonate, although statistically significant, is clinically negligible (0.15 mmol/L for every 10% points of FEV_1 reduction).

Crucially, when assessing the rate of change in bicarbonate and known prognostic factors in the 12 months prior to death, a significant and visible increase in serum bicarbonate was observed despite only a minimal worsening in lung function (Chapter 6).

This highlights the poor prognostic value of FEV_1<30%, and supports the observation that elevation in serum bicarbonate is associated with severe disease, and that an upward trend in bicarbonate levels may provide a more accurate biomarker of progression to end-stage disease.

13.1.5 Apparent inconsistencies in serum bicarbonate collected at HASTs

The historical analysis demonstrated that the concentration of serum bicarbonate is higher in people with CF compared to the general healthy population. Higher bicarbonate levels are also associated with known prognostic markers of disease, increased with age and disease severity, and concentration further spikes in the six months preceding death.

However, I had conflicting result in one study which found similar serum bicarbonate levels in individuals with CF and other chronic respiratory conditions (26.5 vs 27 mmol/L, p=0.420) (Chapter 9). In order to interpret this discrepancy, one has to look at the inclusion criteria for the study population.

Data was collected on individuals with CF or other respiratory diseases seeking to be cleared to fly without supplemental oxygen. As a result, these patients are not only clinically stable, but also deemed fit to fly (>60% negative HAST outcomes). All patients with CF attending the Leeds Unit, are in general referred for a flight test, irrespective of lung function. Most patients would have been deemed fit enough to fly and were most likely fitter than the control group. In contrast, the historic cohort included people with severe CF, advanced lung disease and serious comorbidities.

Therefore, I attribute the lower average level of serum bicarbonate seen in Chapter 9 to the clinical stability of this cohort of patients, further confirming my interpretation of serum bicarbonate as a prognostic predictor.

13.2 Is the raised concentration of serum bicarbonate clinically significant?

As discussed in Section 13.1, serum bicarbonate concentration in individuals with CF is independently associated with several CF-specific comorbidities, sex, age and advanced lung disease, confirming the hypothesis that serum bicarbonate can be a marker of severity of the CF disease. This is further supported by data from the studies summarised in Chapter 6 and Chapter 8.

In the cohort discussed in Chapter 8, serum bicarbonate concentrations were higher during CF exacerbations compared to patients with other lung diseases who were admitted with a primary acute respiratory conditions [28 (25.5-28.5) vs 24 (23-24.75), p=0.004] (

Figure 8.1).

Serum bicarbonate levels were also significantly higher in those needing lung transplantation (Figure **5.8**). As advanced lung disease and respiratory failure are the main criteria for referral for lung transplantation [374], elevation in bicarbonate may be the result, in part, of the compensation for hypercapnia.

The sole study available in the literature looking at a potential prognostic role of serum bicarbonate in people with CF requiring lung transplantation, failed to identify serum bicarbonate as a predictor of outcome or clinical deterioration [381]. In this study, patients referred for lung transplantation had variable serum bicarbonate levels between 24 and 40 mmol/L, and elevated values of serum bicarbonate appeared to be equally distributed in individuals with a survival greater than two years, or lower than one year. In this study, sample collection in the last years before a lung transplantation was punctual and sporadic in terms of chronology and the methodology did not equate to monitoring the trend in serum levels over time.

Contrary to the study by Doershuk and Stern [381], the historical analysis on the cohort presented in Chapter 6, was performed in a much larger cohort of patients, all of whom had multiple measurements of serum bicarbonate over a period of 12 months prior to death.

Serum bicarbonate concentration was significantly elevated in individuals with CF close to their death compared to a matched clinically stable cohort [38 (33-41) mmol/L vs 27 (25-29) mmol/L, p<0.001] (Figure **6.2**). This was the results of a progressive and rapid increase in levels observed in the 12 months preceding the death (Figure **6.8**). The mean serum concentration of bicarbonate increased from a mean value of 29.6 mmol/L at 12 months before death, to an average of 37.57 mmol/L in the month preceding death.

Bicarbonate appeared to rise, on average, gradually (0.79 mmol/L per month) in the year prior to death, with the rate of increase being significantly more pronounced in the last six months preceding death (1.27 mmol/L per month), compared to months 7-12 (0.39 mmol/L per month). In contrast, lung function tended to remain stable albeit very low (FEV_1 <30%) throughout the 12 months preceding death of people with CF (Figure 6.6).

These outcomes strongly support the idea that serum bicarbonate and its rate of change over time might be used as a biomarker of clinical severity, as previously observed in other chronic respiratory conditions [274,384–386].

13.3 What is the acid-base balance status in people with CF?

At the start of this research project, I hypothesised that the distribution of acid-base disturbances was different in CF, compared to individuals with other respiratory conditions, both at a time of clinical stability and during pulmonary exacerbations.

The studies which make up this thesis demonstrated an increased prevalence of metabolic alkalosis in CF compared to other respiratory conditions, both in clinical stability (on average 20% of ABGs in CF) and during pulmonary exacerbations (46.7% of ABGs in CF). In this latter case, metabolic alkalosis might also favour secondary hypercapnia, as previously described [223].

In Chapter 7, a large number of capillary blood gases (over 500) performed in clinically stable patients with CF and other respiratory conditions was assessed. The vast majority of blood gases yielded a normal acid-base balance, which was confirmed in a prospective confirmatory trial (Chapter 9). Clinically stable people with CF did however have a higher rate of metabolic alkalosis compared to patients with other respiratory conditions (21.3% vs 14.6%) (Table 7.1).

13.4 What is the cause of raised serum bicarbonate?

At the beginning of this research project, I hypothesised that serum bicarbonate concentration might be elevated in patients with CF in part as a response to hypoventilation, and that it could be a useful marker of respiratory failure, as has been observed in other respiratory conditions [274,384–386].

As discussed in Chapter 2 Section 2.5.2, impaired bicarbonate excretion has been recently observed in murine CF models, as well as in people with CF as a result of the interactions between defective CFTR and pendrin [240,242,243], a result conflicting with antecedent literature but which supports an important mechanism proposed in this thesis.

Thanks to the multiple studies conducted in this thesis, the genesis of raised bicarbonate in patients with CF can be better clarified.

I believe that the combined output these studies demonstrate that the raised concentration of serum bicarbonate in patients with CF is not solely due to advanced lung disease, or to a primary effect of CFTR dysfunction in the kidneys, but rather to a combination of both mechanisms.

13.4.1 Is raised bicarbonate a consequence of ventilatory failure?

Previous studies in patients with obesity hypoventilation syndrome suggest that isolated elevation of bicarbonate is part of the spectrum of ventilatory failure [387,389].

Obese subjects with elevated bicarbonate (and BE) have a response to hypoxic and hypercapnic stimulus similar to the individuals with daytime hypercapnia [387]. In addition, bicarbonate is an independent predictor of respiratory muscle weakness, hypercapnia, and survival in patients with neuromuscular disorders and COPD [274,384–386].

In this research work, I initially confirmed that elevated serum bicarbonate, above the ULN for the biochemistry laboratory at LTHT, appears to be a good predictor of OHS, and could potentially act as a biomarker for ventilation in a similar way to HbA1C in diabetes, at least in this cohort (Chapter 12, Section 12.2). I therefore aimed to explore if the same concept was applicable to people with CF, where bicarbonate could rise in response to hypoventilation.

In Chapter 11, average serum bicarbonate concentration among individuals with CF and severe lung disease was 28.6 mmol/L, despite normal daytime pCO_2 in all but one case. However, subjects in the study had transient hypoventilation and raised CO_2 levels during exercise (Figure **11.3**)**,** despite an increased RR, as previously shown in children [416,434], and in small reports in adults [435,436].

This effect is likely to be a consequence of the inability of people with CF to maintain adequate alveolar ventilation, as a result of excessive dead space ventilation and failure to increase tidal volume appropriately [204,428]. If this pattern repeats throughout the day, it is conceivable that increased bicarbonate reabsorption and reduced secretion in the kidney could be elicited to compensate for the raised pCO_2.

In addition, further supporting data can be drawn from Chapter 7. Individuals with CF who presented with high bicarbonate concentration, but normal CO_2, had a response to the hypoxic stimulus which was intermediate between those with normal blood gases, and people with daytime hypercapnia. In addition, more patients with elevated bicarbonate, with or without elevation of daytime pCO_2, had a clinically significant drop in pO_2 and a positive response to the hypoxic test (Table 7.4). As hypercapnic patients have a blunted response to the hypoxic drive, an elevation of arterial bicarbonate, even in the presence of normal daytime pCO_2, might be on the spectrum of chronic ventilatory failure in a similar fashion to OHS [387].

Finally, while in Chapters 5, 6 and 10, the mechanisms underlying the elevation in serum bicarbonate concentration were not explicitly explored, serum bicarbonate concentration was lower in individuals on treatment with NIV. As NIV was previously shown to stabilise pCO_2 in individuals with CF and severe lung disease [262,330,339], it is conceivable that, at least in part, serum bicarbonate concentration increases to compensate for hypercapnia, and could therefore be an indirect marker of ventilation.

As previous studies have shown that people with CF often present with sleep fragmentation and daytime fatigue and/or sleepiness [189,200,447,448], I planned to investigate the link between these disturbances and transient nocturnal hypoventilation (Chapter 12, Section 12.3). A single-centre, short-term study was designed. This aimed at defining whether individuals with different serum bicarbonate concentration and lung function results had different ventilatory pattern at night, which would elicit an increase in serum bicarbonate as compensatory mechanisms. This would have helped to clarify whether isolated elevated bicarbonate is in the spectrum of ventilatory failure. The study received approvals, but could not be completed due to the COVID pandemic. Once resumed, I believe it will provide very valuable data to further interpret the relation between serum bicarbonate and ventilatory patterns in people with CF.

13.4.2 Is raised bicarbonate caused by defective renal handling?

A renal cause of raised serum bicarbonate and metabolic alkalosis in individuals with CF is often presumed to be due to electrolyte imbalance in the context of Pseudo-Bartter syndrome (Chapter 2, Section 2.5.1). It is only recently that a potential role of defective CFTR in driving this anomaly as a result of lack of regulation of pendrin activity has been hypothesised (Chapter 2, Section 2.5.2).

In Chapter 8, I studied the renal response to acid-balance disturbances and increased serum bicarbonate in individuals with CF. Urinary bicarbonate excretion was similar in individuals with CF and those with other respiratory conditions, despite the higher serum concentration and the increased prevalence of metabolic alkalosis. This was not associated with dyselectrolaemia, suggesting that Pseudo-Bartter syndrome is not responsible for the increased bicarbonate concentration.

If serum bicarbonate was increased as a compensatory mechanism for transient hypoventilation, as discussed above in Section 13.4.1, urinary excretion of bicarbonate and other electrolytes should be similar to that observed in post-hypercapnic status, thereby showing a low urinary chloride (<20 mmol/L) [413]. In this cohort, conversely, I observed a high, rather than low, urinary excretion of chloride, excluding this as the sole cause for hyperbircabonataemia.

These results therefore suggest that a primary role of defective CFTR in the renal handling of bicarbonate, due to dysfunction in the CFTR, pendrin and secretin link, is very possible.

In healthy conditions, urinary bicarbonate concentration should increase in response to increased serum levels via a mechanisms mediated by interaction of CFTR, pendrin and secretin [149]. Conversely, murine models and individuals with CF do not have the expected response of up-regulation of pendrin when exposed to acute blood alkalinization, despite adequate increase in secretin [242,243]. This suggest therefore a

primary role of defective CFTR, as confirmed by preliminary (n=4) data showing increased urinary excretion of bicarbonate following partial correction of CFTR with LUM/IVA [242].

13.4.3 Summary

Taken in isolation, abnormal ventilation and disease severity as discussed in Sections 13.4.1 and 13.4.2 cannot fully explain why serum bicarbonate concentration is elevated in people with CF. Thanks to our additional findings on the renal involvement in serum bicarbonate of people with CF, I have shown the potential presence of a parallel mechanism involved.

Serum bicarbonate increases in people with CF as a response of transient hypoventilation, or as a result of worsening overall health with CF-related comorbidities playing an additional role (Section 13.1). In healthy people, an increase in serum bicarbonate concentration would be compensated in the kidneys via increased bicarbonate excretion in the CCD. Defective CFTR, however, does not allow for an increased expression of pendrin, thereby preventing the normal increase in urinary concentration of bicarbonate.

Further studies addressing ventilation and renal response in people with CF on and off treatment with highly effective CFTR modulators able to restore CFTR function more efficiently than LUM/IVA, would help confirming such conclusion.

13.5 Could ventilatory support affect serum bicarbonate?

In people with CF, serum bicarbonate concentration is raised, at least in part, as a result of compensation for alveolar hypoventilation.

This prompted me to assess whether techniques that provide ventilatory support, such as NIV and NHFT, could reduce serum bicarbonate levels, and in general be effective in people with CF. NIV and NHFT increase alveolar ventilation and reduce work of breathing, affecting thereby gas exchange leading to improved oxygenation and reduction in carbon dioxide levels (Chapter 3).

13.5.1 Non-invasive ventilation

In Chapter 5, mean serum bicarbonate appeared to be lower in individuals who were on treatment with NIV at the time of the measurement being performed. Similarly, in the year before death (Chapter 6), serum bicarbonate was stable, despite being higher than normal, in individuals on treatment with NIV, whereas it increased steeply in subject who did not receive NIV (Figure **6.11**).

This effect was further confirmed in Chapter 10. Before starting treatment with NIV, serum bicarbonate concentration was overall raised in the cohort. However, following

initiation of NIV, the concentration of serum bicarbonate decreased among those individuals had a prolonged treatment of NIV, showing a pre-treatment rate of change +10.8 mmol/L/y compared to –8.39 mmol/L/y after NIV (p=0.046) (Figure 10.8).

As discussed in Section 13.1.4, the correlation between bicarbonate and lung function is strong only when FEV_1 < 40%. While in the cohort on NIV a significant reduction in the rate of decline of lung function was described, this resulted in stabilisation of FEV_1 rather than improvement (Figure 10.9). This suggests that changes in lung function are unlikely to be the cause of reduced bicarbonate levels after NIV. Rather, the rate of change in serum bicarbonate concentration is most likely the consequence of improved alveolar ventilation with stabilisation in pCO_2, which has been previously shown in CF [328,339,341,426].

These results further suggest that serum bicarbonate concentration is, at least in part, driven by ventilatory failure and can be used as a marker of the ventilatory response to NIV treatment. In addition, it supports the hypothesis that bicarbonate concentration is a negative prognostic marker among individuals with CF.

13.5.2 Nasal high-flow therapy

Nasal high-flow therapy has several beneficial effects by virtue of providing high flows (up to 60 L/min) with varying F_IO_2, heated at body temperature and humidified to full saturation [212,258]. Its use in clinical practice in CF is widespread [351–353], albeit the limited evidence in clinical or physiological trials [354].

In view of the positive effects shown by the use of NIV to improve ventilation during exercise in CF [433], and considering the afore mentioned mechanisms of NHFT [212,258] (Chapter 3, Section 3.2.1), I explored its effect during exercise.

In the pilot randomized cross-over trial, presented in Chapter 11, NHFT led to reduced carbon dioxide levels, and respiratory rate in people with CF and advanced lung disease, when undergoing a 6MWT. However, by the nature of the study being short-term, the effects on bicarbonate following this intervention were not explored. It is conceivable that, as hypoventilation appear to be one of the determinants of raised serum bicarbonate levels, improving ventilation and reducing carbon dioxide during exercise could have an effect on bicarbonate concentration as well.

13.6 Strengths of the thesis

The extensive and diverse series of studies conducted as part of this research thesis helps increasing the understanding of both the prevalence and the influencing factors which drive elevated serum bicarbonate in CF. The results also provide key data to help explain the clinical significance and pathophysiological mechanisms underlying these changes.

In addition, the data collected provide the opportunity to characterise the acid-base balance status of patients with CF in conditions of clinical stability, and during pulmonary exacerbations. This new knowledge will allow to further improve care and treatment of people with CF.

The outcomes of the studies included in this thesis address all the key questions identified at the start of the research project. The use of different cohorts and of a combination of retrospective and prospective studies has proven useful in the context of a rare condition as CF, by allowing inclusion of different sub-set of patients in each study,

The retrospective studies focusing on a well characterised historical cohort of patients with CF enabled me to include large numbers of people with CF with a wide spectrum of disease and complications (Chapter 5), and to perform longitudinal assessments both before and in proximity to death (Chapter 6). This latter aspect is of particular importance in the context of CF where, without the use of retrospective data, meaningful longitudinal evaluation of prognostic factors would require prolonged prospective observations that are not compatible with the time frame of a PhD programme.

All prospective studies conducted were successful in either confirming results from retrospective analyses, or providing preliminary data which can be used to assess the viability of larger-scale prospective, potentially multi-centric, follow-up trials.

Overall the studies described in this thesis allowed to explore both the ventilatory and renal aspects of serum bicarbonate and acid-base regulation, and provide a plausible explanation for the disturbances noted in CF.

Further studies are needed to clarify and confirm the newly formulated hypothesis.

13.7 Limitations of the thesis

Specific limitations of each individual study are discussed in each Chapter. In general, the main limitations are related to the nature of the studies.

All studies, both retrospective and prospective, are single-centre, limiting the generalizability of the results. Despite the care provided in the Leeds Regional Adult CF Centre being in line with the recommendations of the ECFS and UK CF Standard of cares [244,245], as in all other centres in the UK, confounding factors intrinsic to the Leeds population and the treatment received cannot be excluded. This is particular relevant for what concerns areas less covered by national and international guidance, such as the use of NIV, and the access to the new highly-effective CFTR modulation via the managed access programme. While the practice in the Leeds CF Centre in these scenarios was similar to what previously described, it is possible that across the world other indications are used, and access to medications might be different.

In addition, while retrospective studies have several benefits as described above, they are limited to the use of data collected for clinical purpose without full standardization. This has resulted in a lack of regular blood gas monitoring (as in Chapter 10), or lack of contemporaneous serum bicarbonate and ABG measures (as in Chapter 5).

Unfortunately, one prospective study planned for this project (Chapter 12, Section 12.3) was not completed as a result of the impact of the COVID-19 pandemic. This would have helped in further clarifying whether transient hypoventilation can occur before patients being symptomatic and be a possible cause of raised bicarbonate.

Finally, a study covering both the aspects of ventilation and renal handling of bicarbonate simultaneously, would have helped confirm my hypothesis that both mechanisms are inextricably linked to the increased in serum bicarbonate levels. As such, I am planning an amendment of the currently approved study to take this into account when investigating hypoventilation during sleep.

13.8 Future work

Future work should explore the role of transient hypoventilation in the daily activity of people with CF, and further characterise the subgroup of patients with high serum bicarbonate despite clinical stability.

A combined approach looking at variable of ventilation, by monitoring transcutaneously and with blood gases the carbon dioxide levels, and of renal compensation should be considered.

The effects of highly effective CFTR modulators should also be studied in the population with advanced and stable lung disease.

13.9 Conclusion

In this thesis, I have shown that serum bicarbonate concentration is higher in individuals with CF than in the general population. This is particularly relevant during period of deterioration and progression of the disease. The rate of increase in bicarbonate concentration appears to be a biomarker of decline and poor prognosis.

While this could be linked to serum bicarbonate being an indicator for chronic and acute on chronic ventilatory failure, I showed that compensation for raised CO_2 is not the only mechanism involved. Serum bicarbonate concentration appears to be linked with chronic or transient hypoventilation, as demonstrated by the stabilisation of bicarbonate on NIV and the observation of raised CO_2 during exercise. However, the role of defective CFTR in the kidney leading to altered bicarbonate excretion was also shown, and could be the consequence of lack of upregulation of pendrin in the CF kidney.

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Appendix A

EMIS Patient information sheet and Consent

St JAMES'S & SEACROFT
REGIONAL ADULT CYSTIC FIBROSIS UNIT
St James's Hospital
Beckett Street, Leeds, LS9 7TF

The Leeds Regional EMIS Clinical Management System and Database

Leeds Adult and Paediatric CF Unit, Leeds teaching Hospital Trust, UK

In 2007, the Leeds Adult and Paediatric CF Units introduced a Cystic Fibrosis Clinical Management System to improve data collection, generate electronic patient record and automate clinic and discharge letters.

The System is fully secure, password protected and any changes to records can be audited.

The benefits include:

- All blood and sputum results can be downloaded electronically to your records so that information is available immediately.
- All letters can be generated automatically at the time of clinic which will avoid delays.
- A summary screen of all your latest results is displayed in one place to help with diagnosis and early recognitions of complications.
- Lung function and weight can be displayed as a simple graph so that you will be able to see how you are doing.
- Drugs can be prescribed electronically and any potential interaction will be displayed on the screen warning the doctor.
- Appointments can be booked and rearranged electronically.
- All correspondence will be available on your electronic record and we will not need to wait for letters to be file.
- The system will speed up time taken to send you the results of your annual assessment.

All your information will be completely confidential and access will be limited to your clinical team. We have always strived to provide a high quality of service and feel that the introduction of EMIS clinical management system will further improve your care. Should you have any questions please do not hesitate to contact one of the consultants who will be happy to discuss any issues.

St JAMES'S & SEACROFT
REGIONAL ADULT CYSTIC FIBROSIS UNIT
St James's Hospital
Beckett Street, Leeds, LS9 7TF

CONSENT FORM (Adult)

Study title: Leeds CF database

		Please Initial
1	I confirm that I have read and understand the Leeds Database fact sheet dated 11th October 2007. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
3	I understand that information about me, collected from my medical notes and other data about me may be looked at by a small number of responsible individuals from the NHS Trust, who are involved in my medical care and maintaining the data contained on EMIS. I give permission for these individuals to have access to my records.	
4.	I understand that information that can identify me personally will **never** be given to anyone or published by the Leeds CF Unit, but that anonymised data that cannot identify me may be shared with researchers both in the UK and in other countries.	
5.	I agree to my clinical data being stored on the Leeds Regional EMIS Clinical Management System and for data to be used for day to day clinical management and for research purposes.	

Name of Patient	Signature	Date
Name of Person Taking Consent	Signature	Date

When completed: 1 for patient; 1 (original) to be kept in medical notes.

Appendix B

Automated reporting tool for blood gas analyses

This research project relied in part on the analysis of large data sets of arterial or arterialised capillary blood gas analysis, which had to be interpreted in terms of acid-base status for the purposes of my analyses.

The manual interpretation of ABG measures is time-consuming and prone to errors. When used in scientific research, it would be recommended, if not required, that these interpretations are repeated by at least two independent clinicians in order to evaluate their consistency and degree of concordance.

Because of the nature of this research work, following this standard approach would have prevented the majority of my quantitative analyses performed on historical datasets, due to the sheer volume of ABG to be interpreted.

As such, in the initial phases on this research, I explored the possibility to develop an automated algorithm for the interpretation of ABG measures. Multiple approaches to the algorithms for the interpretation of blood gas analyses have been developed, as presented in Chapter 4, Section 4.3.2.

B.1 Algorithm Development

Among these approaches, the Boston method is the most simple to reliably transpose into a simple static algorithm that can be expanded in a logical calculation. Because of this, an implementation of the Boston method can be easily integrated as a static computation in popular software for data manipulation and analysis, such as Microsoft Excel (as a ‘Formula’), or SPSS (as a ‘Computed Variable’).

The algorithm used is presented below.

```
# ABG interpretation based on the Boston method
#
# ph: pH [adimensional]
# hco3: HCO3- [mmol/L]
# co2: pCO2 [mmHg]

# 0: "Acute Respiratory Acidosis"
# 1: "Compensated Respiratory Alkalosis"
# 2: "Compensated Metabolic Acidosis"
# 3: "Compensated Metabolic Acidosis or Compensated Respiratory Alkalosis"
# 4: "Acute Respiratory Alkalosis"
# 5: "Compensated Metabolic Alkalosis"
# 6: "Compensated Respiratory Acidosis"
# 7: "Compensated Respiratory Acidosis or Compensated Metabolic Alkalosis"
# 8: "Acute Metabolic Acidosis"
# 9: "Partly Compensated Metabolic Acidosis"
#10: "Acute Metabolic Alkalosis"
#11: "Partly Compensated Metabolic Alkalosis"
#12: "Normal ABG"
#13: "Partly Compensated Respiratory Acidosis"
#14: "Partly Compensated Respiratory Alkalosis"
#15: "Unable to determine"

If ((ph < 7.35) && (co2 > 45) && (hco3 >= 22 && hco3 <= 26))
    return 0;
Else If (((co2 - 35) < (hco3 - 22)) && (ph >= 7.35 && ph <= 7.45) && (co2 < 35) && (hco3 < 22))
    return 1;
Else If (((co2 - 35) > (hco3 - 22)) && (ph >= 7.35 && ph <= 7.45) && (co2 < 35) && (hco3 < 22))
    return 2;
Else If (((co2 - 35) == (hco3 - 22)) && (ph >= 7.35 && ph <= 7.45) && (co2 < 35) && (hco3 < 22))
    return 3;
Else If ((ph > 7.45) && (co2 < 35) && (hco3 >= 22 && hco3 <= 26))
    return 4;
Else If (((co2 - 45) < (hco3 - 26)) && (ph >= 7.35 && ph <= 7.45) && (co2 > 45) && (hco3 > 26))
    return 5;
Else If (((co2 - 45) > (hco3 - 26)) && (ph >= 7.35 && ph <= 7.45) && (co2 > 45) && (hco3 > 26))
    return 6;
Else If (((co2 - 45) == (hco3 - 26)) && (ph >= 7.35 && ph <= 7.45) && (co2 > 45) && (hco3 > 26))
    return 7;
Else If ((ph < 7.35) && (co2 >= 35 && co2 <= 45) && (hco3 < 22))
    return 8;
Else If ((ph < 7.35) && !(ph == 0) && (co2 < 35) && (hco3 < 22))
    return 9;
Else If ((ph > 7.45) && (co2 >= 35 && co2 <= 45) && (hco3 > 26))
    return 10;
Else If ((ph > 7.45) && (co2 > 45) && (hco3 > 26))
    return 11;
Else If ((ph >= 7.35 && ph <= 7.45) && (co2 >= 35 && co2 <= 45) && (hco3 >= 22 && hco3 <= 26))
    return 12;
Else If ((ph < 7.35) && (co2 > 45) && (hco3 > 26))
    return 13;
Else If ((ph > 7.45) && (co2 < 35) && (hco3 < 22))
    return 14;
Else
```

Algorithm 1. Static implementation of the Boston method for ABG interpretation.

B.2 Algorithm Validation

The algorithm was validated in a two-step approach. The tool was initially tested for accuracy by myself by reviewing a random sample of 50 blood gas analyses. Further independent validation was performed by asking two respiratory physicians experienced in ABG interpretation (one Consultant and one registrar) to manually interpret a collection 50 blood gases. The manual interpretation was consistent between the two observers and compared to the automated tool.

B.3 Implementations

I developed two implementations of the algorithm. Both implementations can be accessed and used for free at https://abg.giuliaspoletini.com/.

A first implementation of this Algorithm was developed as an HTML5 web application, which can be accessed at https://abg.giuliaspoletini.com/web_app/. This simple web application allows to evaluate a single ABG measure based on the Boston approach, and can be used from any web browser without a download or installation, including from mobile devices.

A second implementation of this Algorithm was developed as an Excel formula. This implementation was used throughout this work to automatically evaluate multiple ABG measures by simply adding this formula to the original database, and linking the correct columns. A template spreadsheet based on this implementation is also available at https://abg.giuliaspoletini.com/.

The Boston approach is not always capable of providing an interpretation of an ABG measure. In such rare cases, a value of “Unable to determine” is returned by the algorithm. This requires a manual interpretation by a clinician. In the context of this thesis this was performed by myself, by applying the Boston Methods and as required introducing the base excess results as an additional aid.

B.4 Source code and licensing

These tools are released as Free and Open-Source Software (FOSS), subject to the MIT Licence (https://en.wikipedia.org/wiki/MIT_License), a permissive software licence allowing virtually any use and modification of the software.

The web application tool is available to the public at https://abg.giuliaspoletini.com/web_app/ subject to the Creative Commons CC BY-NC-SA licence (https://creativecommons.org/licenses/by-nc-sa/2.0/).

Appendix C
The modified Bhalla score

Structural lung changes are a typical feature in CF and can be quite variable in each individual (Section 1.4.3).

Radiological findings can help to differentiate between different phenotypes in CF, and especially to determine the presence of a predominant bronchiectasis pattern or small airways disease with air trapping and mosaic pattern.

Computed tomography is an extremely useful tool to monitor the progression of lung disease in CF, as it allows to identify the extension and severity of the lung involvement in CF.

A CT scoring system allows to describe the findings and abnormalities in a semi-quantitative way. Over the years, multiple scoring systems have been developed, with the most commonly used being the modified Bhalla score [421]. This scoring system allows to identify various abnormalities, including bronchiectasis, peri-bronchial thickening, consolidations, air trapping, ground glass, mosaic pattern, and assess their severity (Fig 1).

CT Abnormalities	Score			
	0	1	2	3
Severity of bronchiectasis	Absent	Lumen slightly greater than adjacent vessel	Lumen 2 to 3 × adjacent vessel	Lumen > 3 × adjacent vessel
Peribronchial thickening	Absent	Airway wall thickness equal to adjacent vessel	Airway wall thickening ≤ 2 × adjacent vessel	Airway wall thickening > 2 × adjacent vessel
Extent of bronchiectasis (BPS)	Absent	1–5	6–9	> 9
Extent of mucous plugging (BPS)	Absent	1–5	6–9	> 9
Sacculations/abscesses (BPS)	Absent	1–5	6–9	> 9
Generations of bronchial divisions	Absent	Up to fourth generation	Up to fifth generation	Up to sixth generation
No. of bullae	Absent	Unilateral	Bilateral	> 4
Emphysema (BPS)	Absent	1–5	> 5	
Collapse/consolidation	Absent	Subsegmental	Segmental/lobar	
Mosaic perfusion*	Absent	1–5	> 5	
Air trapping*	Absent	1–5	> 5	
Acinar nodules*	Absent	Subsegmental/segmental	Lobar	
Thickening of intralobular septae*	Absent	Subsegmental/segmental	Lobar	Diffuse (> 1 lobe)
Ground glass*	Absent	Subsegmental/segmental	Lobar	Diffuse (> 1 lobe)

*Modifications to the original Bhalla score. BPS = bronchopulmonary segments.

Figure A.1 The modified Bhalla score.

Appendix D

TRIPOD Checklist

Section/Topic		Checklist Item	Page
Title	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	n/a
Abstract	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	n/a
Background and objectives	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	119
	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	119 157
Source of data	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	120 158
	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	120 158
Participants	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	120 158
	D;V	Describe eligibility criteria for participants.	120 158
	D;V	Give details of treatments received, if relevant.	n/a
Outcome	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	119 157
	D;V	Report any actions to blind assessment of the outcome to be predicted.	n/a
Predictors	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	122 159
	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	n/a
Sample size	D;V	Explain how the study size was arrived at.	121 159
Missing data	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	121
Statistical analysis methods	D	Describe how predictors were handled in the analyses.	122
	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	122
	V	For validation, describe how the predictions were calculated.	122 159
	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	122
	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	n/a
Risk groups	D;V	Provide details on how risk groups were created, if done.	n/a

Development vs. validation	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	136 165
Participants	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	124 160
	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	133 160
	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	Table 7.6 Table 9.2
Model development	D	Specify the number of participants and outcome events in each analysis.	Table 7.6 Table 9.2
	D	If done, report the unadjusted association between each candidate predictor and outcome.	Table 7.8
Model specification	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Table 7.9
	D	Explain how to the use the prediction model.	Figure 7.7
Model performance	D;V	Report performance measures (with CIs) for the prediction model.	165
Model-updating	V	If done, report the results from any model updating (i.e., model specification, model performance).	n/a
Limitations	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	142 169
Interpretation	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	139 169
	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	139 169
Implications	D;V	Discuss the potential clinical use of the model and implications for future research.	139 169
Supplementary information	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	n/a
Funding	D;V	Give the source of funding and the role of the funders for the present study.	n/a

Appendix E

Borg and Comfort scales

In Chapter 11, participants were asked to rate their dyspnoea and fatigue using the Borg scale (Figure E.1 and Figure E.2), and the comfort they were experiencing (Figure E.3).

Patient instructions

The Borg scale is used to help us understand the intensity or severity of your breathlessness. We will ask you to use this scale to rate the intensity of your breathlessness before, during, and after your exercise.

Please review the scale to see the various levels from which you can choose.

The top of the scale, "0 or nothing at all," means *no breathlessness at all*.

The bottom of the scale, "10 or maximal," means the *most severe breathlessness that you have ever experienced or could imagine experiencing*.

When we ask you to rate the intensity of your breathlessness, please place the tip of your finger on the number that best describes the intensity that you are experiencing at that moment. You may also place a finger between 2 numbers if that better describes the intensity of your breathlessness.

Please let us know if you have any questions before we begin.

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

Figure E.1 Borg scale for Dyspnoea

Scores	Descriptions	
0	Nothing at all	
0.5	Extremely weak fatigue	Just noticeable muscle fatigue.
1	Very weak fatigue	It is like walking slowly and comfortably at your own pace.
2	Weak fatigue	
3	Somewhat fatigue	Muscle fatigue is notable. You want to slow down a bit.
4		
5	Strong fatigue	Tired and hard. It would be nice to take a rest, but you still do not have difficulties in going on.
6		
7	Very strong fatigue	Very tired and heavy. The muscle fatigue is so strong that you wish to stop walking now.
8		
9		
10	Extremely strong fatigue	The muscle fatigue is as strenuous as you have ever experienced before in your life.
*	Absolute maximal level of fatigue	It is the perceived muscle fatigue that is stronger than ever. It is the highest possible level of muscle fatigue.

Figure E.2 Borg Scale for fatigue

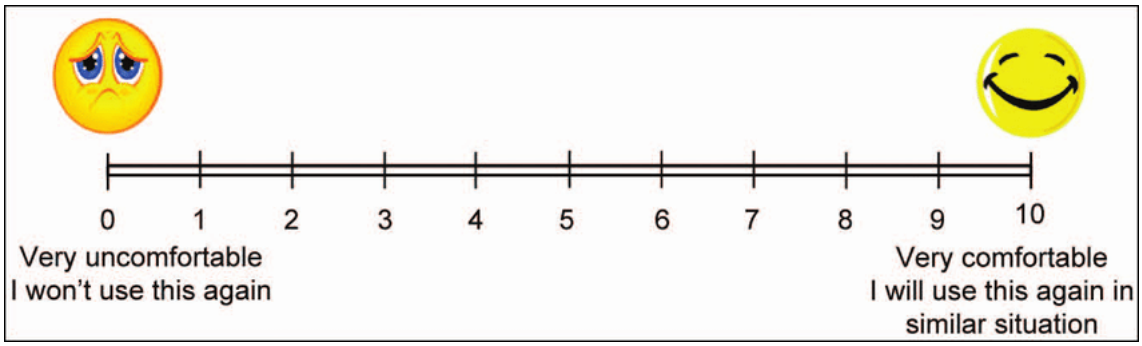

Figure E.3 Comfort visual analogue scale