CYSTIC FIBROSIS IN SOUTH AFRICA

Spectrum of disease, diagnosis, and determinants of outcome

Marco Zampoli

ZMPMAR001

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Supervisors

Prof. Brenda M. Morrow

Prof. Heather J. Zar

Declaration

I, Marco Zampoli, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions by those acknowledged.

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i. Zampoli M, Kassanjee R, Verstraete J, Westwood A, Zar HJ, Morrow BM. Trends in cystic fibrosis survival over 40 years in South Africa: An observational cohort study. *Pediatric Pulmonology*. 2022 Apr;57(4):908-18.
ii. Marco Zampoli, Janine Verstraete, Marlize Frauendorf, Reshma Kassanjee, Lesley Workman, Brenda M. Morrow, and Heather J. Zar. Cystic fibrosis in South Africa: spectrum of disease and determinants of outcome. *ERJ Open Res* 2021; 7: 00856-2020.
iii. Marco Zampoli, Jenna Sykes, Janine Verstraete, Stephanie Y Cheng, Brenda Morrow, Michael S. Pepper, Cheryl Stewart, Heather J. Zar and Anne L. Stephenson. Global disparities in cystic fibrosis outcomes prior to *CFTR* modulators: a CF registries cohort study in South Africa and Canada; *Journal of Cystic Fibrosis 2023; published online 12 September 2023* .
iv. Zampoli M, Verstraete J, Nguyen-Khoa T, Sermet-Gaudelus I, Zar HJ, Gonska T, Morrow BM. β-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: Do we need new reference ranges? *Pediatric Pulmonology.* 2023 Jan;58(1):187-96.

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M Zampoli

June 2023

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Abstract

Cystic fibrosis (CF) occurs with varying incidence in all populations throughout the world but much less is known about the epidemiology and outcomes of CF in low-or-middle income countries (LMIC) compared with high income settings. Continued improvement in CF-related outcomes and survival witnessed in the past decades is attributed to multiple factors yet sub-optimal quality of CF care, and limited CF diagnosis capacity continue to exist in most LMIC, including South Africa (SA). CF treatment and prognosis have been transformed by the recent introduction of highly effective cystic fibrosis transmembrane conductance regulator modulator drugs (CFTRm). However, access to these transformative drugs for those with eligible genotypes is currently limited to high-income countries. Understanding the clinical spectrum of CF in SA and investigating novel techniques to diagnose CF is important to advocate for improved quality of CF care including CFTRm drugs.

The overall aim of this work was to document the spectrum and outcomes of CF in South Africans and to investigate more sensitive measures to diagnose CF and CFTR-related disorders in this population. The studies included in this thesis are divided into two components. This first component comprises observational cohort studies derived from a single-centre CF clinic dataset in Cape Town, SA, and the nation-wide South African CF registry, which was established in 2018 in preparation for this thesis. The CF registry cohort in SA was then compared in a cross-sectional study to a matched Canadian CF registry cohort, and differences in lung function and nutrition outcomes adjusted for known factors. The second component was a prospective study investigating the feasibility and diagnostic utility of the novel β-adrenergic sweat test in a cohort of South Africans with inconclusive CF diagnosis. The chapters of this thesis are presented as published manuscripts, which collectively address the overall aim of this body of work.

The SA CF registry in its first year of inception captured a total of 447 people with CF across both private and public health sectors. Summary demographic descriptions of the cohort include median age of 14.7 years with self-identified White race making up 70% of the CF population, followed by Mixed-race ancestry (19%) and Black Africans (10%). Genotype pattern mirrored ancestry with F508del is the most common variant in Whites and people with Mixed-race ancestry, and 3120+1G>A (class I) the most common variant in Black Africans. Overall, 81% of people with CF (pwCF) in SA have at least one copy of F508del and are, thus, eligible for elexacaftor/tezacaftor/ivacaftor. A key finding of the registry-based studies was that lung function and nutrition outcomes in SA were significantly lower across all ages compared with Canada, attributed to differences in the quality of CF care and social determinants of CF health between the two countries. In SA, poor nutrition was the strongest factor independently associated with severe lung disease and was more prevalent in people living in lower

socioeconomic conditions, including people who were not White. Another key finding was despite significant improvement in overall CF survival at a single centre in Cape Town over the past 40 years, disparities between race groups still exist in SA with increased risk of mortality observed in young children who were not White. People with Black African ancestry, who form the majority of the SA population, are likely to be underrepresented in the SA CF registry, raising concern that CF is being missed or underdiagnosed in the majority of South Africans. Furthermore, the genotype of Black Africans means that none are eligible for CFTRm, which has serious implications for future treatment. These registry-based studies highlight disparities in CF care and outcomes both within SA and compared with a high-income setting – novel findings because SA is one of only a few LMIC with CF registries. Addressing these disparities affecting people with CF in SA will require interventions such as greater awareness of CF in SA, universal newborn screening for CF, focused attention on improving nutrition and overall improvement in the quality of essential CF care, especially as LMIC have disproportionally more pwCF who are ineligible for CFTRm drugs.

The diagnosis of CF using standard approaches may remain inconclusive in a small proportion of individuals, which leads to unnecessary anxiety for families and inappropriate treatment where people do not actually have CF or a CFTR-related disorder. Furthermore, accurate diagnosis of CF is important for research and submission of registry data. The β-adrenergic sweat test was proposed as an easier alternative to other electrophysiological measurements of CFTR function such as nasal potential difference and intestinal current measurements, which are not available in SA. We therefore conducted a study evaluating this hypothesis in adult subject controls, and 32 individuals (mostly children) whose CF diagnosis was inconclusive. Key findings of this study were that the β-adrenergic sweat was superior to sweat chloride test in excluding CF in the majority of subjects and that β-adrenergic sweat secretion in children was lower compared to adults. Implications and novelty of this research are that existing reference ranges for this test may not be applicable in children, and confirmation that the β-adrenergic sweat test is a viable alternative for measuring CFTR function. A number of families benefited from this study by reversal of their incorrect CF diagnosis.

The overriding finding and impact of this work has been to highlight disparities in diagnosis, treatment, and outcomes of CF within SA and in the global context. The current status of CF care in SA mirrors many other LMIC that share similar challenges and barriers to improving CF care, including access to affordable CFTRm drugs. The findings of this thesis have made valuable contributions to local and global advocacy initiatives to improve CF care and access to CFTR drugs for many thousands of pwCF living in LMIC who are being left behind in this new era of CF treatment.

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Establishing the SACFR was a collective monumental effort from the CF community in SA. I would like to specially thank the members of the SA CF Medical and Scientific Advisory Committee for their guidance and enthusiasm, Lesley Workman for designing the data collection instrument and Marlize Frauendorf, who captures registry data in Gauteng and Kwa-Zulu Natal Provinces.

A special word of thanks and sincere appreciation to Janine Verstraete, without whom none of this work could have been accomplished. Janine captures registry data for W. Cape, E. Cape, and Free State Provinces, was overall project manager for all the studies and expertly assisted me in negotiating the complexities of the β-adrenergic sweat test study, SAHPRA regulatory processes and pushing through with participant recruitment in the midst of COVID-19 lockdown conditions!

I would like to thank all my collaborators and co-authors for their contributions and guidance through the individual projects. Special thanks must go to Thao Nguyen Khao and Isabelle Sermet-Gaudelus for hosting me in Paris where I received training on the β-adrenergic sweat test method and Thao ‘s visit to Cape Town for hands-on support through its implementation. Thanks also to Anne Stephenson and Jenna Sykes in Toronto for their expert guidance and support in our combined registries project.

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List of abbreviations

ABPA	allergic bronchopulmonary aspergillosis
BAST	βeta-adrenergic sweat test
CF	cystic fibrosis
CFF	Cystic fibrosis Foundation
CFTR	cystic fibrosis transmembrane conductance regulator
CFTRm	cystic fibrosis transmembrane conductance regulator modulator therapy
CFTR-RD	cystic fibrosis transmembrane conductance regulator- related disorder
CFSPID	cystic fibrosis screen positive, inconclusive diagnosis
DNA	deoxyribose nucleic acid
ETI	elexacaftor, tezacaftor and ivacaftor
FEV1	forced expiratory volume in 1 second
GLI	Global Lung Initiative
HIC	high income country
ICM	intestinal current measurement
IRT	immunoreactive trypsinogen
LCI	lung clearance index
LLN	lower limit of normal
LMIC	low or/and middle-income country(s)
NBS-CF	newborn screening for cystic fibrosis
NGS	next generation sequencing
NHLS	National Health Laboratory Services
NPD	nasal potential difference
NPV	negative predictive value
PPV	positive predictive value
PI/PS	pancreatic insufficiency/pancreatic sufficiency
pwCF	people with cystic fibrosis
QPIT	quantitative pilocarpine iontophoresis
RCWMCH	Red Cross War Memorial Children's Hospital
SA	South Africa(n)
SACFR	South African cystic fibrosis registry
SOP	standard operating procedure
UCT	University of Cape Town

Scope and layout of the thesis

This thesis represents the culmination of my work and research in the field of cystic fibrosis (CF) epidemiology and diagnosis, specifically in South Africa (SA). The thesis layout and structure are as follows:

Chapter 1 Background and aim: This chapter provides a broad updated overview on the diagnosis, clinical spectrum, and treatment of CF, including novel drug therapies. The history and existing knowledge of CF in SA prior to this body of work are summarised, and gaps in knowledge and current challenges encountered with CF diagnosis and care in SA and other low-or-middle income countries (LMIC) are discussed. In addition, the chapter describes the establishment of the SA CF registry (SACFR) in 2018, which laid important groundwork in preparation for the thesis. This chapter will explain the rationale and aim of the thesis.

Chapter 2 / Publication 1: "Trends in cystic fibrosis survival over 40 years in South Africa: An observational cohort study". This first paper provides historical context of CF survival trends over 40 years at a single centre in Cape Town, South Africa, prior to establishing the national CF registry. Although survival in SA has improved in keeping with international experience, the paper highlights prevailing survival disparities in this SA cohort and how it compares to international standards and trends. This paper has been published in *Pediatric Pulmonology* 2022 Apr;57(4):908-18.

Chapter 3 / Publication 2: "Cystic fibrosis in South Africa: spectrum of disease and determinants of outcome". This cross-sectional descriptive study, the first derived from data captured in the newly established SACFR from multiple SA sites, describes the current spectrum of CF in SA, and explores determinants of lung function and nutrition outcomes in 2018 of people captured in the SACFR. This paper has been published in *ERJ Open Res* 2021; 7: 00856-2020.

Chapter 4 / Publication 3: "Global disparities in cystic fibrosis outcomes prior to *CFTR* modulators: a CF registries cohort study in South Africa and Canada". This chapter describes work done in collaboration with researchers in Canada, a high-income country (HIC), to conduct a comparative study of CF outcomes in SA and Canada using CF registries data. This paper identifies and highlights important disparities in CF outcomes between SA, a middle-income country, and Canada even before CFTR modulators were widely available in Canada. CFTR modulators were first licensed in 2019 and have, during the time span of this thesis, rapidly revolutionised the treatment and outlook of CF prognosis, but only in HIC. Addressing global inequity in access to these drugs is an urgent priority for pwCF living in LMIC across the world. This paper was published on-line 12 September 2023, *Journal of Cystic Fibrosis*.

Chapter 5 / Publication 4: "β-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: Do we need new reference ranges?" Establishing the SACFR was an essential and important step in understanding local CF epidemiology and advocating for better care in SA. However, diagnosis of CF and setting criteria for inclusion in the SACFR is at times complex due to mild CF phenotypes or people with inconclusive diagnosis using routine diagnostic tests. This paper describes the results of the new β-adrenergic sweat test technique applied to an SA cohort, mostly children, with inconclusive CF diagnosis, and discusses advantages and limitations of this test. This paper has been published in *Pediatric Pulmonology* 2023 Jan;58(1):187-96.

Chapter 6 Research in context and concluding statements: This chapter summarises the overall findings, broad implications and impact of this work, and relevance to SA and other LMIC, as well as the strengths and limitations. The chapter discusses future directions and recommendations arising from this research.

Chapter 1
Background and aim

Introduction

Cystic fibrosis (CF) is a serious autosomal recessive disorder that occurs with varying frequency in all population groups throughout the world (1). Since CF was first described in the 1930's by Guido Fanconi and Dorothy Andersen, treatment has exponentially advanced leading to improved outcomes and survival (2-4). However, CF remains a life-shortening condition with median predicted survival age now 53.1 years for individuals born between 2017 and 2021 in the United States (US)(5). Mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7, first identified in 1989, result in absent or defective CFTR protein production (6). CFTR protein regulates chloride, bicarbonate, and sodium channel functioning on epithelial cell surfaces in multiple organs and is the basic molecular defect responsible for the spectrum of CF phenotypes. Reduced or absent CFTR expression results in increased sodium and water reabsorption from mucosal surfaces, leading to dehydrated secretions and impaired mucociliary function (7). Although multiple organ systems are affected, the respiratory and digestive tracts are primarily involved. Pulmonary morbidity associated with CF is a consequence of disrupted airway surface liquid, which leads to impaired mucociliary clearance, airway obstruction, chronic airway infection, inflammation, and eventually progressive structural lung damage and pulmonary function decline (8). The usual cause of premature death is respiratory failure. Historically lung transplantation was the only available treatment for such advanced lung disease.

The phenotypic presentation and spectrum of CF disease is wide, ranging from mild CFTR-related disorders (CFTR-RD) to classical CF characterised by pancreatic sufficiency (PS) or insufficiency (PI), progressive bronchiectasis often associated with *Pseudomonas aeruginosa* infection, and variable other organ dysfunction such as hepatobiliary complications. CFTR-RD is defined as a clinical entity associated with CFTR dysfunction that does not fulfil diagnostic criteria of CF and typically manifests as single organ diseases, often diagnosed in adulthood, e.g. diffuse bronchiectasis, recurrent pancreatitis or congenital bilateral absence of the vas deferens (CBAVD) (9). The practice of newborn screening for CF (NBS-CF) has transformed the diagnostic pathway for CF in most high-income countries (HIC) in that the overwhelming majority (93% under 1-year of age in the US; 79% of under-5-year-olds in Europe) of new CF diagnoses are now through NBS-CF (5, 10). An unintended consequence of NBS-CF has been the detection of newborn screen positive infants with inconclusive diagnosis (CFSPID). Infants labelled as CFSPID are mostly asymptomatic, but some will progress over time to develop CF or CFTR-RD (11).

NBS-CF has, thus, increased the recognition and reported incidence of milder CF phenotypes and a wider spectrum of CF disease is now recognised.

The genetics of CF and its relevance for novel therapies

Advances in the molecular understanding of the *CFTR* gene and protein has led to the discovery of distinct classes of *CFTR* variants , each associated with different causes and levels of CFTR dysfunction and phenotypic expression (12). Different classifications exist, but the traditional one describes six classes based on CFTR structure and function (4, 13, 14). Functional classification of *CFTR* variant with examples and the approach to targeted CFTR modulator therapies is summarised in **Figure 1.1**. Over 2000 *CFTR* variants have been described and of these, 401 have been classified as pathogenic or disease-causing (15). A more pragmatic approach to classification of *CFTR* variants is by the level of CFTR protein expression and function associated with the mutation: either minimal function or residual function.

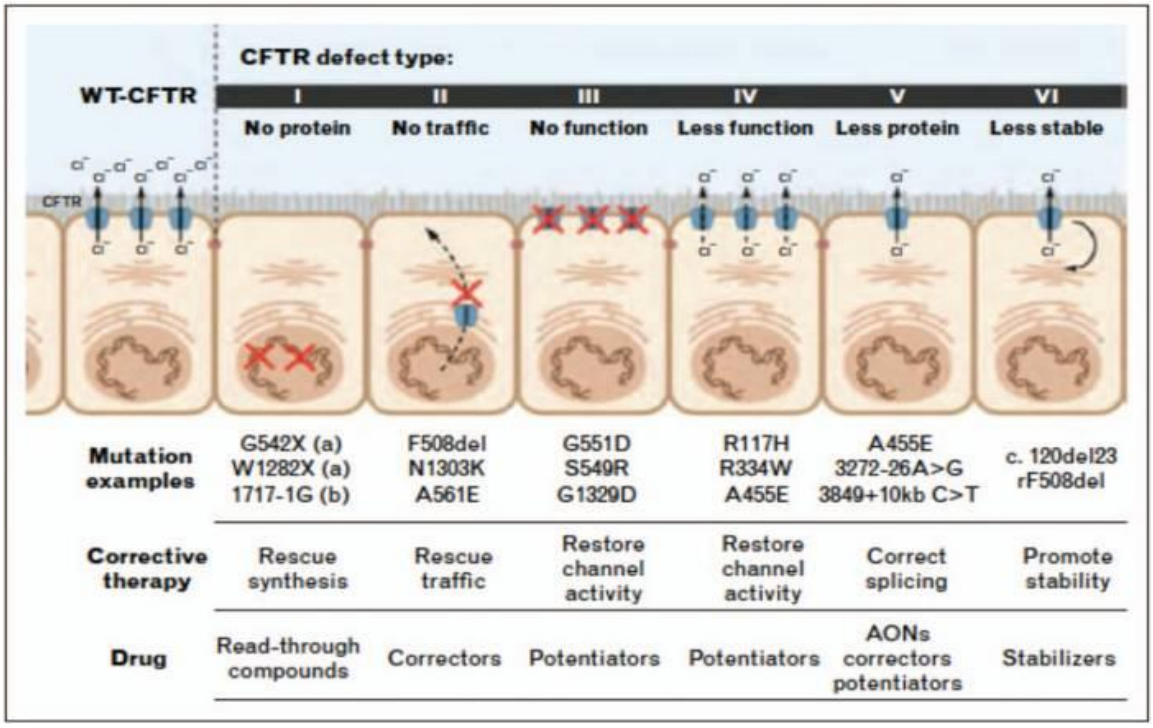

Figure 1.1 Functional classification of *CFTR* variant with examples and approach to targeted CFTR modulator therapies.

Note: Current approved drugs include the potentiator ivacaftor, and the correctors lumacaftor, elexacaftor and tezacaftor. AONs, antisense oligonucleotides; WT, wild type. Reproduced with permission from (22).

The understanding of how each class of *CFTR* variant causes CFTR dysfunction has led to the development of breakthrough novel CFTR modulator therapies (CFTRm) that target the underlying CFTR defect at different molecular levels, thus placing CF at the forefront of precision medicine (12, 14). Potentiator drugs, such as ivacaftor, potentiate the CFTR channel into the open state, allowing improved channel function. Ivacaftor was first approved in 2012 after the first landmark CFTRm clinical trial showing efficacy in people with one copy of the G551D gating (Class III) variant (16). Ivacaftor has subsequently extended its licensing to other class IV and some class V variants (17). Corrector therapies, such as lumacaftor, tezacaftor and elexacaftor, help correct protein misfolding errors, thus improving CFTR protein delivery and function. The next most significant breakthrough in CF treatment was FDA approval in 2019 of the potentiator and corrector triple-combination drug elexacaftor, tezacaftor and ivacaftor (ETI) for people with one or two copies of F508del variant, which followed clinical trials confirming high efficacy of ETI in this population (18-21). Key indicators of efficacy on CFTR function in clinical trials include reduction in sweat chloride by on average 50%, improvements of lung function (FEV1pp) between 13-14%, reduction in pulmonary exacerbations of 63%, improved nutrition and CFQ-R quality of life scores (17, 22).

Beyond clinical trials, real-world data on outcomes in pwCF on ivacaftor, the first CFTRm licensed more than a decade ago, provide compelling evidence over time of improved outcomes in nutrition, pulmonary exacerbations, and lung function. Furthermore, risk for mortality and lung transplantation are also significantly reduced (23-25). Although long-term, real-world data are lacking for ETI, similar outcomes are reported and projected for pwCF receiving ETI, including those with advanced lung disease (23-29). The outlook and long-term prognosis including survival of pwCF has without doubt further improved for those eligible for and with access to CFTRm, especially if initiated at a younger age when end-organ disease is not yet established. Models of pooled clinical trial data project that median survival age will reach 82 years in people homozygous for F508del who commence ETI at age 12-17 years (30). Ivacaftor is currently licensed from age two months and ETI was recently FDA approved from age 2 years.

Conventional CF treatment over the past decades has been symptomatic, with the goal to prevent and delay the inevitable progression of CF and its complications over time. Conventional CF treatment and care guidelines, including the South African Cystic Fibrosis Consensus Guideline, are extensively covered elsewhere and beyond the scope of this thesis (31, 32). CFTR modulators have transformed the long-term outlook and prognosis for the majority (90%) of pwCF in HIC where CFTRm therapy has rapidly become the standard of care for CF treatment. Worldwide, 82% of pwCF who have one or two copies of F508del, and now an expanding number of select variants deemed *in vitro* to be responsive

to ETI, are eligible for ETI (33, 34). Identifying and documenting the complete *CFTR* genotype in each patient and within populations now has important implications that extend beyond simply diagnosis or prognosis but include treatment too. The treatment of CF with CFTRm has rapidly evolved and advanced even within the short time period covered by the work in this thesis. The relevance of CFTRm to my thesis and why it matters for pwCF in SA and other low-or-middle income countries (LMIC) will be expanded on in Chapter 6.

Global CF epidemiology and the contribution of CF registries

Worldwide, 162,428 people are estimated to be living with CF across 94 countries. Of these, an estimated 105,352 (65%) are diagnosed (**Figure 1.2**) whilst approximately 57,076 people live with undiagnosed CF (**Figure 1.3**) (33). Although CF is more common in people with European ancestry, affecting approximately 1 in 3,000 – 1 in 6,000 live births, it is found with varying incidence in all ancestries across the world. In the US, which has a longstanding and robust CF registry, CF incidence per live births is estimated at 1:2500 in Whites, 1:8000 in Hispanic Americans, 1:15,000 African Americans, and 1:35,000 Asian Americans (4, 35). The relative proportion of Hispanic patients diagnosed with CF has increased in the US. California has a large Hispanic population and the highest proportion of Hispanic patients with CF. In Latin American countries such as Brazil, where greater ancestral diversity exists, CF incidence varies from 1:1600 in people with European ancestry to 1:14,000 in people with African ancestry (36). The F508del mutation accounts for approximately 70% of CF alleles across the world (34). Other *CFTR* variants are responsible for the remaining ones, but many are rare and occur with varying frequency in specific regions. Only six variants have a prevalence ≥1% while approximately 50 variants have a prevalence ≥0.1% (34).

In Latin American countries with Spanish ancestry, F508del remains the most common *CFTR* variant with allele prevalence around 50% (37, 38). North Africa, the Middle East and central Asia are other regions where CF incidence and prevalence of *CFTR* variants vary considerably, influenced strongly in some regions by a high rate of consanguinity (4). It is estimated that India and Southeast Asia likely have the highest burden of undiagnosed CF cases in the world. A study from Delhi, India, that screened 955 umbilical cord blood samples for the F508del variant, estimated the incidence of CF in that population to be between 1:40,000 to 1:100,000 live births (39). Although this is lower than people with European ancestry, the absolute number of people born with undiagnosed CF in India is estimated to be substantially higher. In a study by Guo *et al.,* the estimated number of undiagnosed CF cases in India was 37,406, which is similar to the total number in the USA CF registry (33). However, these numbers likely do not represent the real incidence of CF in this region as the unknown prevalence of other rare or unrecognised *CFTR* variants was not considered. Owing to a paucity of

high-quality data, estimates of undiagnosed CF in LMIC are uncertain. According to Guo *et al*., patient registries are available in 45 countries, and used to identify 90% of the estimated diagnosed population throughout the world (33).

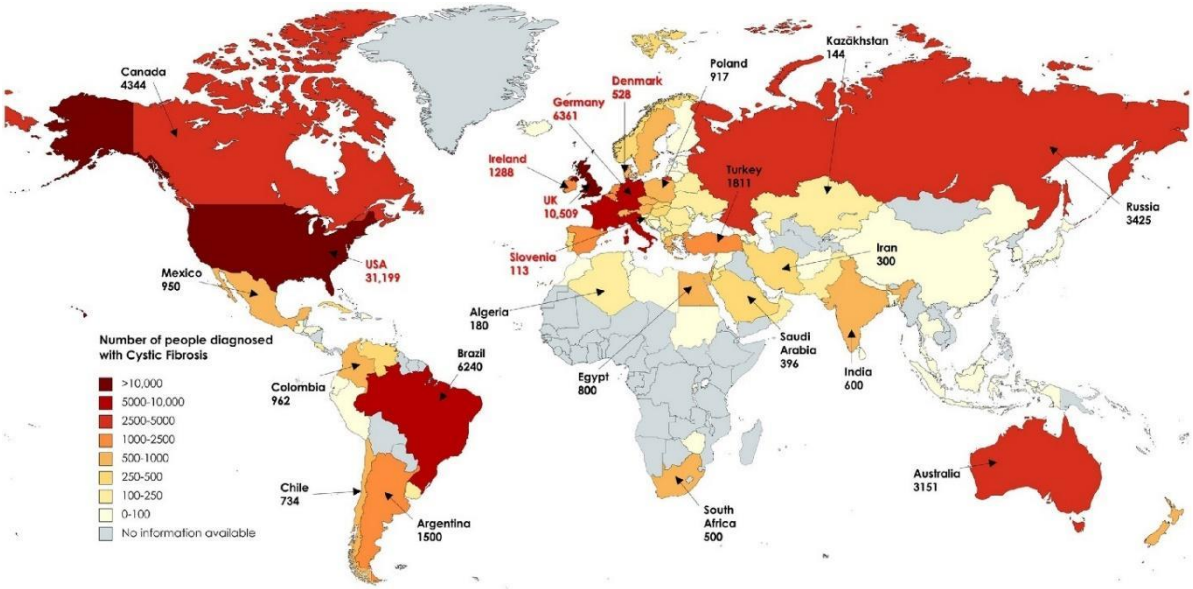

Figure 1.2 World map displaying the estimated *diagnosed* CF patient burden globally [adopted from Guo *et al.* (31)].

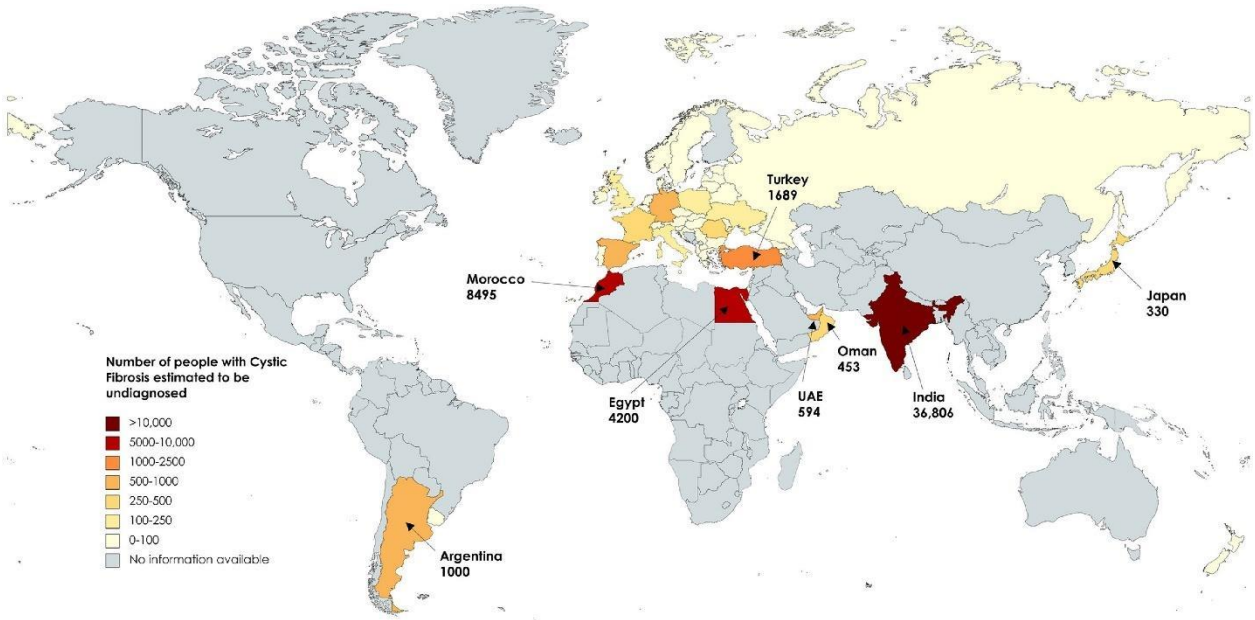

Figure 1.3 World map displaying the estimated number of *undiagnosed* CF patients globally, estimated by epidemiological studies or local CF experts.
[adopted from Guo *et al.* (33)].

Cystic fibrosis in sub-Saharan Africa

The African continent, especially sub-Saharan Africa, is the region of the world with the lowest documented incidence and knowledge of CF (**Figure 1.2** and **Figure 1.3**). Apart from SA and a few case reports from Kenya, Sudan, Rwanda and Ghana, CF is largely invisible in sub-Saharan Africa (40-43), yet *CFTR* variants have been documented in several African population studies and CF is not infrequently diagnosed in children with African ancestry living in countries outside of Africa where diagnostic testing is more available and routine (44). One CF centre in Paris, France, reported the phenotypic presentation and genotype of 17 children with African ancestry diagnosed between 2000 and 2019. The majority (n=29) were diagnosed by NBS-CF, four (23%) had meconium ileus and 14 (83%) were pancreatic insufficient (PI). Importantly, 25% were false negative for *CFTR* variant panel testing in the French NBS-CF programme and the proportion of children with African ancestry diagnosed during this period increased from 2% to 10% (45). A review of all *CFTR* mutations identified across 12 African countries (predominantly Northern Africa and SA) identified 70 *CFTR* variants of which 39 were known disease variants and 29 alleles were unique to Africa (46). A separate study that surveyed published data of causative CF variants in people with African descent living in the Americas (African diaspora) identified 59 *CFTR* variants in the 1,584 tested chromosomes. Of these, 55% remained unidentified, 41 were known disease-causing variants and 17 were of unknown clinical significance (47). Major barriers to advancing knowledge about CF in sub-Saharan Africa include absence of CF awareness and complete absence in most countries of basic CF diagnostic testing, such as sweat test or *CFTR* genotyping (48).

Cystic fibrosis in South Africa

The number of people living with undiagnosed CF in SA is unknown. The true incidence of CF in SA is difficult to establish due to several reasons that include low CF awareness, limited access in remote areas to sweat tests, absence of an integrated national healthcare information system and, until recently, absence of a national CF registry. Furthermore, in the absence of universal NBS in SA, undocumented early CF-related mortality in young infants and children before a diagnosis of CF is even made is highly likely. The prevalence of *CFTR* mutations and incidence of CF vary greatly in the SA population because of the broad diversity of ancestry. Based on carrier frequency rates, estimates in a 2003 study of CF incidence in White, Mixed-ancestry and Black African populations were 1 in 3000 (carrier frequency 1 in 23), 1 in 10,300 (carrier frequency 1 in 55) and 1 in 784 – 13,924 (carrier frequency 1 in 14 to 1 in 59 live births, respectively (49, 50). *CFTR* mutation detection rates using customised panels in 201 clinically diagnosed people with CF from all populations were able to genetically confirm CF with two *CFTR* mutations in 83%, 55% and 21% of White, Mixed-ancestry and Black African individuals, respectively (51). F508del was the most common *CFTR* variant amongst

White individuals (prevalence 76%), whereas 3120+1G>A was the most common variant (prevalence 46%) in Black Africans with an estimated carrier frequency rate of 1 in 90 healthy individuals (50, 51). Both mutations were found with similar frequency in patients with mixed ancestry.

Early reports of CF in Black African children in SA and Kenya date back to 1959 (40, 52). Since then a few small cross-sectional studies from SA have described CF in Black Africans or people with the 3120+1G>A, the so-called "African" variant (53, 54). A case-controlled study in two SA CF centres compared CF diagnosis, nutrition, and lung function outcomes in 35 Black African children with CF to matched controls carrying the F508del variant. Compared with controls, Black African children at diagnosis were more malnourished and fewer presented with meconium ileus. Nutrition and lung function (FEV1 in children ≥6 years) outcomes and changes over time from ages 3-16 years were similar in both groups. In Black Africans, 3120+1G>A was in homozygous and heterozygous state in 65% and 20% of cases, respectively (55). Importantly, a higher number of infant deaths was reported amongst Black Africans compared with controls.

Apart from historical data from one CF centre in the Western Cape province of SA, little is known about the current epidemiology, overall health status and survival of the CF population in South Africa. The first epidemiological description of CF in SA was published in 1978 by Super, who established a register of 299 white patients with CF, of which 179 were alive at the time (56). The fate of this register is unknown but there had been no further attempt to establish a CF registry in SA since that time. In 2006, Westwood described the 33-year follow-up data of 216 children diagnosed with CF between 1974 and 2008 at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town. Median age of diagnosis and survival age over this period were 6 months and 19.8 years, respectively (57). Significantly lower survival age was found in children of mixed ancestry, which was partly attributed to socioeconomic differences along racial lines prevalent in SA society at the time. Although improvement in lung function and nutrition over time has been reported from the same centre (58, 59), survival and other outcomes of the current overall SA CF population are unknown but suspected to be substantially lower than in North America and Europe where estimated median survival age is now over 50 years (60). A small cohort study from one CF centre in Tshwane Province, SA, reported an average FEV1 decline of 5.3% per annum, substantially greater than international norms (61).

Establishment of the South African Cystic Fibrosis Registry (SACFR)

The origins and contributions made by CF registries to advancing our understanding of the epidemiology of CF and improved survival are highlighted in two previous comprehensive reviews (62, 63). Registries of 'big data' for a rare disease like CF have been instrumental in harnessing data for research and monitoring trends of disease outcomes that reflect standard practice. Epidemiological

and longitudinal data are currently recorded in CF registries in the USA, Canada, Europe, Australasia, and Brazil, with coverage rates reported to be as high as 90% of the CF population (63). Cystic fibrosis registries have contributed to many areas of CF research including documenting changing demographics and improving survival; identification of novel and rare *CFTR* mutations; detecting interventions that improve nutrition; and identifying microbiological and other determinants of lung function decline and survival (62). In addition, registries have been used to estimate direct healthcare utilisation costs related to CF care, important for planning and policy determination for all sectors of healthcare provision (64). Furthermore, comparing different cohorts to benchmark outcomes is a powerful tool to influence change in practice that improves quality and standards of care (63). For example, as analysis of the proportion of children compared to adults and age of death are useful and key indicators of quality of CF care in a country, i.e. the younger the cohort is, the lower the quality of CF care **(Table 1.1).** Similarly, international comparisons of CF populations through registries have led to important research to understand the driving factors contributing to disparate CF outcomes (60, 65). As more countries establish CF registries, harmonisation of registry data will be important for meaningful sharing and comparison of data.

Table 1.1 South African CF registry summary data 2018–2022

	2018	2019	2020	2021	2022
People living with CF	447	514	525	523	520
New diagnoses	28	30	11	10	6
Deaths	3	5	5	17	11
Lung Transplants	3	2	2	1	3
CFTR Modulator Therapy*	0	0	4	7	30
In 2021, 6 patients emigrated **8 patients lost to follow-up (not seen for 3 consecutive years)** **8 patients had their diagnoses reversed - all in the Western Cape** ***** Generic formulation privately sourced in Argentina, out-of-pocket					

No national CF patient registry existed in SA prior to 2018, which was a major barrier for advancing knowledge and care of people with CF in SA. A joint initiative led by Marco Zampoli, in partnership with the SA CF Medical and Scientific Advisory Committee (MSAC) and the SA CF Association (SACFA), launched the SACFR registry in 2018. Recruitment of pwCF was initiated through formation of the SACFR steering committee, which includes representatives of all known CF clinics in both public and

private health sectors across SA. The SACFR is owned by SACFA and the SACFR steering committee. Ethics approval from all public health sector CF clinics affiliated to other University Hospitals was obtained from the relevant institutions and the private health sector clinics. The SACFR uses RedCap® as its database platform, with strict control over who has access and editing rights, and administered by Marco Zampoli and two data collectors. Signed informed consent and assent (from 12 years of age) is a requirement for pwCF to be included in the registry.

Data extraction from medical records and data entry into the SACFR is performed by two qualified data managers who visit each participating clinic or private practice once annually to update the registry. Demographic, CF diagnosis and genotype information is captured for all new cases. Strict CF diagnostic criteria must be met to be included in the SACFR with a confirmed CF diagnosis **(Table 1.2).** Unconfirmed or inconclusive diagnosis cases are captured in the SACFR as either "CFTR-RD" if meeting these criteria, or "Diagnosis to be confirmed".

Further details and variable definitions of the SACFR are provided in **Appendix A**. The SACFR adopted similar data collection methods as the European CF Society Registry, including single, best annual measurements for lung function and nutrition. Additional variables added to the SACFR, which are unique to SA circumstances, include: composite measures of socioeconomic status (SES) such as public or private health care, reliance on public transport; household amenities and receipt of social welfare grants; human immunodeficiency virus (HIV) status and infection with *Mycobacterium tuberculosis.* Since 2018, a few revisions to data variables were made for COVID-19 infection and complications, and more recently CFTRm drug therapy. SACFR reports have been published and are freely available on the SACFA website (66). Updated summary information from the SACFR is presented in **Figure 1.4** and **Table 1.3.**

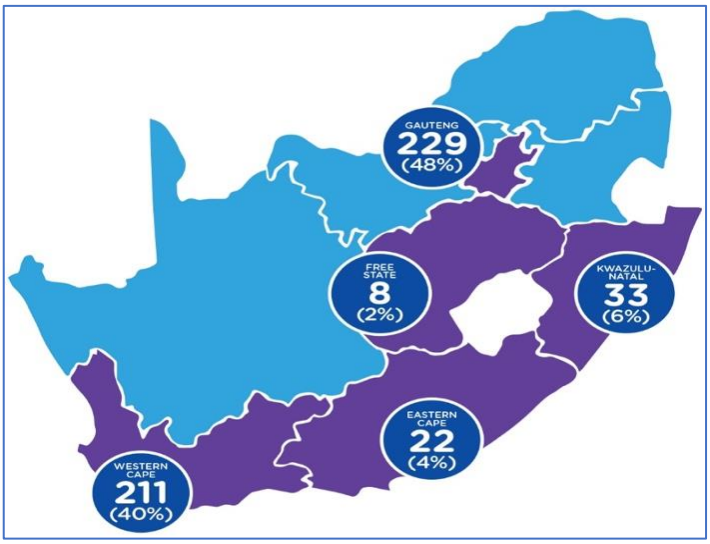

Figure 1.4 Distribution by province of numbers of pwCF in SA

When the diagnosis of CF is unclear

Accurate diagnosis or exclusion of CF is essential for providing appropriate treatment, genetic counselling, and data collection in CF registries. Diagnosis of CF is supported by demonstration of abnormal CFTR function and confirmed by identification of two disease-causing *CFTR* variants on DNA analysis. The sweat chloride test (SCT), first developed 64 years ago, remains the gold standard physiological CFTR function test recommended in clinical practice to diagnose CF. Standards and guidelines for the SCT are published (71-73). Although reliable and accurate in most cases, sweat chloride concentration levels become difficult to interpret in the intermediate range (30-59 mmol/L), because this may be 'normal', falsely elevated for any reason, or abnormal due to CF or CFTR-RD, indicating some residual but decreased expression of functioning CFTR protein (71). The lower limit of intermediate sweat chloride reference range was revised for all ages from 40 mmol/L to 30 mmol/L by a Cystic Fibrosis Foundation (CFF) Expert Consensus panel (71). This revision of reference range was guided by the recognition that a small but significant number of infants identified in CF registries, with two disease-causing *CFTR* variants, had sweat chloride levels between 30 and 40 mmol/L.

The SCT has several limitations in practice. Sweat collection and chloride analysis methods are operator-dependent, time consuming (especially the Gibson and Cooke method) and exhibit a high variability in results, especially in inexperienced hands (74). False positive and false-negative results may arise from technical errors, or other rare underlying medical conditions (75). Another challenge with the SCT in many LMIC where CF diagnosis is largely still symptom-based is a higher-than-expected rate of false positive sweat tests or falsely elevated chloride values due to underlying malnutrition, acute illness and dehydration – more common in hotter climates and in conditions of poverty (76, 77). An audit of 288 sweat tests performed in 2017 at the reference laboratory at RCWMCH in Cape Town, South Africa, found approximately 20% of SCTs had chloride levels in the intermediate range (30-59 mmol/L) and 10% ≥ 60 mmol/L. Yet CF was confirmed genetically in only 6 cases (2%) (personal communication). Substantial within- and between-test variability further adds to difficulty with interpretation of sweat chloride levels in the intermediate or borderline zones (78, 79). Sweat conductivity testing is a cheaper, simpler, and more widely available alternative to SCT in many LMIC and has been validated as a suitable screening test for CF where a NaCl equivalent value ≥ 80 or 90 mmol/L is considered positive (80-82). Sweat conductivity is often the only type of sweat test available in LMIC and in Brazil's NBS-CF programme it performs as well as SCT (83). According to the SACFR report of 2020, 75% of pwCF had at least one sweat test recorded of which a third was only sweat conductivity (66). No validated reference ranges for intermediate sweat conductivity exist. Interpretation of increased sweat conductivity – but less than 90 NaCl equivalent mmol/L – is therefore, difficult, especially if CF or CFTR-RD must be excluded.

Table 1.2 Current status and comparison of CF registry population characteristics around the world (2020)

Country/Region	CF incidence (per live births)	Number of individuals	Newborn screening	Proportion (%) children <18 yrs	Median age CF population (yrs)	Median FEV1% predicted adults ≥18 yrs	Median BMI (kg/m2)	Median estimated survival age (yrs)	Median CF age (yrs) at death	Lung transplant	Proportion (%) with at least one copy F508del	CFTR modulator therapy
Canada (67)	1/3,600	4,332	Yes	38%	23.8	64.7%	22.6	55.4	42.0	Yes	90%	Yes
USA (68)	1/4,500	31,411	Yes	43%	23.8	77.1%	23.1	50.0	34.1	Yes	86%	Yes
Australia (69)	1/3,700	3,538	Yes	45%	20.2	74%	23.3	54.3	30.7	Yes	90%	Yes
European CF Registry: includes Israel, Russia, Turkey, and Eastern European countries (10)	-	52,246	Yes, but not all countries	47%	19.4	70.2%	21.9	51.7	32.0	Yes	81%	Most countries in Western Europe, some Eastern Europe
South Africa (66)	1/3,000 – 1/14,000	525	No	55%	16.7	58.5%	21.6	-	25.8	Yes	79%	No
Brazil (70)	1/7,500 – 1/15,000	6112	Yes	74%	10.4	Not reported	20.8	-	17.4	Yes	53%	Only ivacaftor

Table 1.3 Modified SACFR CF diagnosis inclusion criteria

1. Two sweat chloride tests ≥ 60 mmol/L or sweat conductivity ≥ 90 NaCl equivalent mmol/L *and* clinical features compatible with CF, *or*
2. DNA analysis/genotyping identifying two disease-causing *CFTR* variants as reported at the time in CFTR2 database (15), *or*
3. Sweat chloride < 60 mmol/L and both of the following criteria are met:
    i. DNA analysis/genotyping identified two disease-causing *CFTR* variants; *and*
    ii. Clinical phenotype consistent with CF.
4. CFTR-RD diagnosis if all the following conditions are met:
    i. sweat chloride 30-59 mmol/L
    ii. one or more *CFTR* variants of unknown or variable clinical significance
    iii. clinical phenotype consistent with CFTR-RD

There is, therefore, a growing need, especially in SA and other LMIC, for alternative measures of CFTR function to overcome these diagnostic challenges. Several other CFTR function measurement techniques have been developed and include nasal potential difference (NPD), intestinal current measurement (ICM) and β-adrenergic sweat gland stimulation (77, 84, 85). Of these, NPD and ICM have been more commonly described, mostly in research settings, to exclude CF or to confirm CF and CFTR-RD. NPD and ICM, however, are invasive, expensive and require highly specialised technical skill to perform. βeta-adrenergic sweat test stimulation, a novel test that measures the rate of sweat production through evaporimetry, has shown greater correlation in adults with underlying CFTR expression than sweat chloride (84). Furthermore, β-adrenergic sweat testing is easier to perform and less invasive than NPD and ICM. Although safe and well tolerated in a pilot study, this method was unable to discriminate CF from CFTR-RD in children 4-6 years of age (86). All these techniques, however, require considerable technical expertise and are therefore performed in only a few specialised centres across the world. Diagnostic pathways and guidelines for CF, especially in settings where NBS-CF creates cohorts of children with CFSPID, include alternative measures of CFTR function such as NPD or ICM in their recommendations in situations where the SCT and genotyping are inconclusive (87).

Rationale and aim

Overall outcomes and survival of pwCF have significantly improved in the past decades. The estimated median predicted survival age in the US in 2021 was 53 years with a steady decline in mortality rate to less than 1 death per 100 individuals in the CF registry (5). Similar improvements in survival age are reported in CF patient registries from other HIC including Canada, Europe, and Australia (10, 67, 69). These improvements in CF outcomes were achieved by interventions such as CF-NBS programmes, multidisciplinary CF care, early identification, and treatment of pulmonary infections such as *P. aeruginosa*, aggressive nutritional interventions, lung transplantation and, more recently, CFTR modulators (4). However, these remarkable improvements in CF outcomes are documented mostly in HIC. Poorer socioeconomic conditions, inferior-quality healthcare and malnutrition are strong determinants of poorer CF outcomes and survival throughout the world (4, 88-90). These factors, often linked to disparities by ancestry, are more prevalent in many LMIC where CF outcomes are less favourable than in HIC. Documenting determinants of CF outcomes in SA and comparing these to international standards is important to advocate for improving care and access to new treatments.

Diagnosing CF correctly, including mild phenotypes and CFTR-RD is important for accuracy of cohort descriptions, appropriate medical care, and genetic counselling. Capacity for advanced CFTR function tests, such as NPD or ICM, did not exist in SA. Furthermore, genetic testing for *CFTR* mutations and

next generation sequencing (NGS) was not widely available in SA due to high cost. Consequently the diagnosis of CF remains inconclusive in some people, more so in people with Mixed and Black African ancestry because commercially available CFTR panel testing kits in SA are more suited for White people with European ancestry.

Overall aim

To describe the spectrum of CF disease in South Africans and to investigate more sensitive measures to diagnose CF and CFTR-RD.

Specific objectives

Objective 1: To describe historical trends in CF survival in SA and factors associated with increased risk of mortality.

Objective 2: To describe the current spectrum of CF in SA and investigate determinants of poor lung function and nutrition outcomes.

Objective 3: To compare lung function and nutrition outcomes of the current SACFR population to a matched CF registry cohort from a high-income country.

Objective 4: To investigate the diagnostic utility of the β-adrenergic sweat test in SA as an alternate measure of CFTR function in people with an inconclusive CF diagnosis.

Hypotheses

1. Cystic fibrosis survival has improved in SA and mortality risk is associated with ancestry and early lung function in childhood.
2. Nutrition and lung function outcomes in SA are worse in SA compared to HIC, and determinants of poorer outcomes in SA are multifactorial.
3. The β-adrenergic sweat test performs better than SCT when investigating people with inconclusive CF diagnoses and is a feasible alternative for advanced measurement of CFTR function.

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Chapter 2

Trends in Cystic Fibrosis survival over 40 years in South Africa: An observational cohort study

Authors: Marco Zampoli (1,2), Reshma Kassanjee (3), Janine Verstraete (1), Anthony Westwood A (1), Heather J Zar (1,2) and Brenda M Morrow (1).

Affiliations:

1. Department of Paediatrics and Child Health, University of Cape Town, South Africa
2. MRC Unit for Child and Adolescent Health, University of Cape Town, South Africa
3. Centre for Infectious Disease Epidemiology and Research (CIDER), School of Public Health and Family Medicine, University of Cape Town, South Africa

Corresponding author: M Zampoli
Address: 4th floor ICH Building
Red Cross War Memorial Children's Hospital
Klipfontein Rd
Rondebosch, 7700
Telephone: 021 658 5309
Email address: m.zampoli@uct.ac.za

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Data availability statement: Deidentified participant data are available upon reasonable request from the corresponding author. Data analysis plans are provided in **On-line supplement A**.

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Take home message: CF survival has significantly improved in South Africa after year 2000 but mostly in people older than 10 years. Increased risk of death persists in non-white children less than 10 years and children with poor lung function at age 5-8 years.

Abstract

Introduction: Temporal trends in CF survival from low-middle-income settings are poorly reported. We describe changes in CF survival after diagnosis over 40 years from a South African (SA) CF center.

Methods: An observational cohort study of people diagnosed with CF from 1974 to 2019. Changes in age-specific mortality rates from the year 2000 (versus before 2000) were estimated using multivariable Poisson regression. Data were stratified by current age < or ≥ 10 years and models controlled for diagnosis age, sex, ethnicity, genotype, and *P. aeruginosa* (*PA*) infection. A second analysis explored association of mortality with weight and FEV1z-scores at age 5-8 years.

Results: 288 people (52% male; 57% Caucasian; 44% p.Phe508del homozygous) were included (median diagnosis age 0.5 years: Q1,Q3: 0.2, 2.5); 100 (35%) died and 30 (10%) lost to follow-up. Among age >10 years, age-specific mortality from year 2000 was significantly lower (adjusted hazard ratio aHR: 0.14; 95% CI: 0.06,0.29; p<0.001), but not among age <10 years (aHR: 0.67; 95% CI: 0.28,1.64; p=0.383). In children <10 years, Caucasian ethnicity was associated with lower mortality (aHR 0.17; 95% CI 0.05,0.63), and longer times since first *PA* infection with higher mortality (aHR 1.31; 95% CI 1.01,1.68). Mortality was 7-fold higher if FEV1z was < -2.0 at age 5-8 years (aHR 7.64; 95% CI 2.58,22.59).

Conclusion: Overall, CF survival has significantly improved in SA from year 2000 in people older than 10 years. However, increased risk of mortality persists in young non-Caucasian children, and with FEV1z<-2.0 at age 5-8 years.

Keywords: Cystic fibrosis, South Africa; survival; low-middle income

Introduction

Cystic fibrosis (CF) is a genetic disease that occurs with varying frequency in all population groups throughout the world. Although significant strides have been achieved with improving survival in the past decades, it remains a serious life-shortening condition with median survival age now reported in North America to be approximately 40-50 years (1-3).

International CF registries have been invaluable in our understanding of CF epidemiology and trends in CF survival estimates (4). Improving median survival age is attributed to many factors including newborn screening (NBS), improved nutritional interventions, active surveillance, prevention and treatment of infections, new therapies, and organ transplantation (5, 6). In addition, novel CFTR modulator drug therapy is anticipated to significantly further improve CF survival (7). Factors associated with increased risk of earlier death include, minimal function *CFTR* mutation genotypes, lower lung function (FEV1% predicted), chronic infection with *Pseudomonas aeruginosa, Burkholderia cepacia complex* and *methicillin*

resistant staphylococcus aureus, female gender, increased pulmonary exacerbations and poor nutrition (4, 5, 8).

Little is known about the epidemiology and survival of CF in low and middle-income countries (LMIC). Biological, environmental, and socioeconomic factors that potentially impact CF survival differ in LMIC compared to high income countries (9). Sub-optimal CF care and restricted access to CF drugs, including novel CFTR modulator therapies, is anticipated to further widen the gap in CF survival between high and low-income countries (9). A 33-year follow-up study of children diagnosed with CF between 1974 and 2008 in Cape Town, South Africa (SA), reported median survival age over this period of 19.8 years (10). Significantly lower survival age was found in children of mixed-race ethnicity, which is partly attributed to the Apartheid government enforced racial and socioeconomic differences prevalent in SA society over the study period. Prior to the first democratic elections in SA in 1994, non-Caucasians under Apartheid ideology were subjected to segregated and inferior health care and education, and denied equal employment opportunities compared to Caucasians (11). Similarly, in the United States (US), lower CF survival age was observed in Hispanics compared to non-Hispanics (12). Encouragingly, a more recent report from two paediatric CF centres in SA comparing Black African children to children with homozygous or heterozygous Phe508del genotype, who were all either Caucasian or mixed race, found no significant difference in outcomes (13). Although improvement in lung function (LF) and nutrition has been reported in the Cape Town cohort (14, 15), survival estimates in the same population are unknown but suspected to be lower than high income countries. Recent data published from SA and the US reported lower FEV1 at age 6-8 years was associated with increased risk of mortality in childhood (16, 17).

The CF clinic at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, SA, has been recording demographic and survival data of its cohort since 1974. We explored temporal change and factors affecting CF survival after diagnosis in this cohort over the past four decades, during which important socio-political changes and interventions in CF care have occurred.

Methods

Study design and population

This observational cohort study included all children diagnosed and managed with CF at RCWMCH, born between October 1974 and December 2019. A diagnosis of CF was confirmed by either: 1) two sweat chloride tests > 60 mmol/L or sweat conductivity > 90 mmol/L *and* clinical features compatible with CF in the absence of two pathogenic *CFTR* mutations, *or* 2) two pathogenic *CFTR* mutations identified.

The multidisciplinary CF clinic at RCWMCH follows-up children to 18 years age. Thereafter patients are transitioned to adult services in either the private or public health sectors. Follow-up survival status in December 2019 was established from different sources: 1) the RCWMCH CF clinic database which is continuously updated; 2) cross-referencing the SA CF registry established in 2018 that prospectively

collects data from 2018 onwards and 3) personal communication with the SA CF Association which retains a strong network in the SA CF community (18).

Standards of CF care over the study period

Standards of CF care in the study cohort have evolved over the study period. Important milestones to highlight include: improved access to tertiary healthcare for indigent and non-Caucasian children after SA's first democratic elections in 1994; establishment of a multidisciplinary CF clinic at RCWMCH in 1996; introduction of routine practices to monitor lung disease such as sputum microbiological surveillance, spirometry and *P. aeruginosa* eradication protocols in 2000; alignment of CF care with international standards from 2000 and publication of SA CF Consensus guidelines, last updated in 2017 (19). Essential CF care and CF medications including pancreatic enzyme replacement therapy, hypertonic saline, azithromycin, and antimicrobials are feely available in the public sector, but expensive CF therapies such inhaled tobramycin solution, recombinant DNase and organ transplantation are restricted or not available in the public sector. Newborn screening (NBS) for CF is not widely performed in SA and CFTR modulator therapies are not available in SA. Lung transplantation was until 2017 only available for adults with private health insurance. A second adult lung transplant centre in the public health sector was established in 2017.

Survival data variables

The following information was captured in the CF clinic database: date of birth; ethnicity; sex; age of CF diagnosis; sweat test results; *CFTR* mutations (categorised as p.Phe508del homozygous, p.Phe508del heterozygous or other); and age of first documented *P. aeruginosa* infection. Survival status on 31 December 2019 was categorised as alive, died or lost to follow-up (LTFU) in cases where follow-up data were missing or unknown. Date of death or date last seen if LTFU were recorded.

Nutrition and LF were not captured in the CF clinic database. For this study, we retrospectively collected nutrition data (weight and length) from medical records for each participant at CF diagnosis (within 3 months) and first stable measurement at age 5-8 years. Weight, height and Body Mass Index (BMI-for age z-scores) were calculated using WHO anthropometric reference equations (20). Where available, first stable pre-bronchodilator spirometry volumes recorded age 5-8 years and corresponding weight/height measurements on the day were selected. Forced expiratory volume in one second reported as z-scores (FEV1z) were calculated with the Global Lung Initiative (GLI) ethnic-specific reference equations (21).

Statistical analysis

Descriptive analyses. Demographic, clinical and outcome variables were described by frequencies of categories (categorical variables) and measures of centrality and spread (numeric variables), comparing characteristics by year of diagnosis (cohort A, diagnosis before year 2000 and cohort B, diagnosis from year 2000) using chi-squared tests. Mortality was described by (i) Kaplan-Meier survival functions

stratified by date of diagnosis (cohort A and cohort B), and (ii) age-specific mortality rates as fitted by a Poisson regression, stratified in two consecutive time periods: period 1 (1975-1999) versus period 2 (2000-2019).

Survival analysis. The formal analysis employed Poisson models and focused on the association between age-related mortality rates and calendar period, comparing mortality in period 1 and period 2. To simplify model forms and allow proportional hazards assumptions to hold (assessed by Schoenfeld residuals), mortality after diagnosis was analysed separately in children younger than versus at least 10 years of age and adjusted for age at diagnosis, sex, ethnicity (Caucasian or other), genotype (p.Phe508del homozygous, p.Phe508del heterozygous or other), any previous *P. aeruginosa* infection, time since first *P. aeruginosa* infection and current age. In a second analysis, which considered mortality after recorded LF and nutrition measurements at age 5-8 years, LF impairment (FEV1z < versus ≥ -2) and weight (WAZ ≤ versus > 2) were also included. In all models, mortality varied continuously with age, using natural cubic splines (see **On-line supplement A** for further details). Unadjusted and adjusted hazard ratios (aHRs) and 95% CIs are reported.

Ethical considerations

Approval to conduct this study was obtained from the University of Cape Town's Human Research Ethics Committee (HREC 032/2019) (Appendix D).

Results

Cohort characteristics (**Table 2.1**)

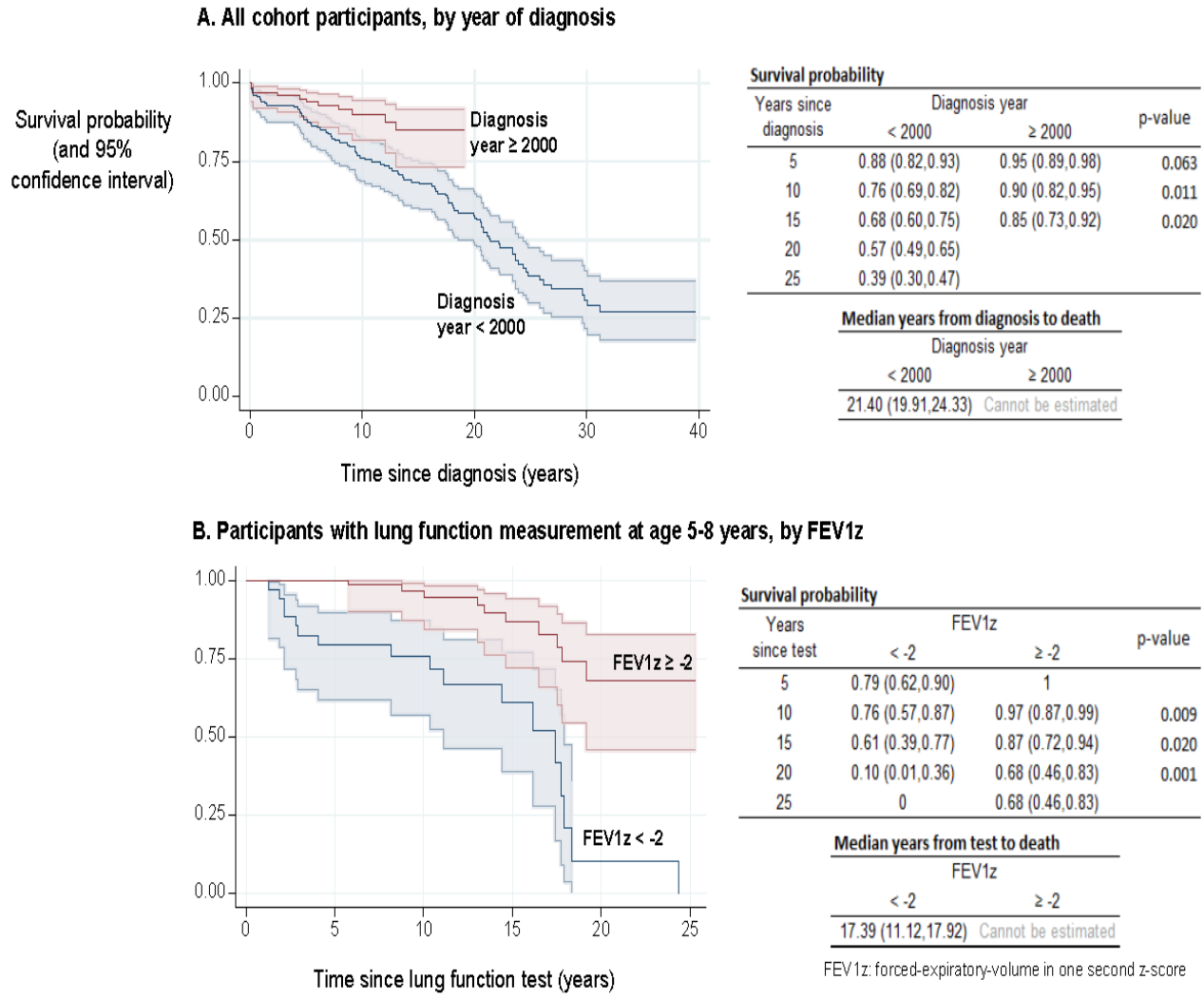
Two hundred and eighty-eight children (52% male; 57% Caucasian; 44% p.Phe508del homozygous were diagnosed with CF, 156 in cohort A (diagnosis year < 2000) and 132 in cohort B (diagnosis year ≥ 2000). Median age of diagnosis was 0.5 years (Q1, Q3: 0.2, 2.50); 100 (35%) died and 30 (10%) were lost to follow-up. Four adults received a lung transplant and were alive in December 2019. Cohort A and B were comparable for age of diagnosis, ethnicity, genotype, sex, nutritional status and LF at age 5-8 years (**Table 2.1**). Median follow-up period for cohort A was 18.2 years (range: 0.1, 39.6) and 8.1 years (range: 0.1, 19.1) in cohort B.

Descriptive analysis of survival mortality rates (**Figure 2.1**, **Figure 2.2** and **Figure 2.3**)

Overall, probability of surviving 10 years after CF diagnosis in cohort A and cohort B increased from 76% to 90% (p=0.01), respectively (**Figure 2.1**). The probability of surviving 10 years after LF measurement at age 5-8 years was 21% higher among children with FEV1z ≥ -2.0 compared to those with FEV1z <-2.0 (97% vs 76%, p=0.009) (**Figure 2.1**). Median age of death increased significantly from 5.5 years (Q1, Q3: 0.5-7.6) before 1990, to 22.5 years (Q1,Q3: 19.7-25.2) after 2010 (**Figure 2.2A**), corresponding with the aging cohort of median age 3 years and 12 years for these two periods, respectively (**Figure 2.2B**). The

proportion of recorded deaths (total *n*=100) under 18 years of age decreased from 84% before year 2000, to 42% after year 2000, to 21% after year 2010.

Temporal trends in age-related mortality rates before 2000 (period 1) and from year 2000 (period 2) are presented in **Figure 2.3**. After an initial peak in early childhood (< 2 years age), estimated mortality rates declined until adolescence when mortality steadily increased with age, more so in period 1. Observed average mortality by age 2-years, over the full study period, was significantly lower in Caucasians compared to non-Caucasians (1.2 deaths per 100 person-years vs. 10.3 deaths per 100 person-years; unadjusted HR 0.12; 95% CI 0.01, 0.53; *p*=0.001).



Survival probability

Years since diagnosis	Diagnosis year < 2000	Diagnosis year ≥ 2000	p-value
5	0.88 (0.82,0.93)	0.95 (0.89,0.98)	0.063
10	0.76 (0.69,0.82)	0.90 (0.82,0.95)	0.011
15	0.68 (0.60,0.75)	0.85 (0.73,0.92)	0.020
20	0.57 (0.49,0.65)		
25	0.39 (0.30,0.47)		

Median years from diagnosis to death

Diagnosis year < 2000	Diagnosis year ≥ 2000
21.40 (19.91,24.33)	Cannot be estimated

Survival probability

Years since test	FEV1z < -2	FEV1z ≥ -2	p-value
5	0.79 (0.62,0.90)	1	
10	0.76 (0.57,0.87)	0.97 (0.87,0.99)	0.009
15	0.61 (0.39,0.77)	0.87 (0.72,0.94)	0.020
20	0.10 (0.01,0.36)	0.68 (0.46,0.83)	0.001
25	0	0.68 (0.46,0.83)	

Median years from test to death

FEV1z < -2	FEV1z ≥ -2
17.39 (11.12,17.92)	Cannot be estimated

FEV1z: forced-expiratory-volume in one second z-score

Figure 2.1 Kaplan-Meier survival functions for (A) over time since CF diagnosis, stratified by year of diagnosis (cohort A: 1974-1999 versus cohort B: 2000-2019); and (B) lung function at age 5-8 years (FEV1z score less than versus at least -2).

Analysis of age-specific mortality by time period (**Table 2.2**)

In the adjusted analysis of children < 10 years age, 233 children were included with an average of 7.0 years of time at risk each and 22 recorded deaths. There was no evidence of temporal changes in age-specific mortality rates (aHR: 0.67, 95% CI: 0.28, 1.64, *p*=0.383). Caucasian ethnicity was associated with 83% lower mortality (aHR 0.17; 95% CI 0.05, 0.63; *p*=0.008) compared to non-Caucasian ethnicity. Furthermore, each additional year since first *P. aeruginosa* infection, in the first five years after infection, was associated with a 31% higher mortality (aHR 1.31; 95% CI 1.02,1.68; *p*=0.036). No statistical evidence of an association of mortality with sex, age of diagnosis, any *P. aeruginosa* infection and genotype were found.

In the adjusted analysis of children ≥ 10 years age, 173 children were included with an average of 9.6 years of time at risk each and 64 recorded deaths. No factors other than time period were found to be associated with mortality. The age-specific mortality rate in period 2 (from year 2000) was 86% lower than period 1 (before year 2000: aHR 0.14, 95% CI: 0.06,0.29, *p*< 0.001). Interpretation is limited to individuals less than 20 years old due to insufficient data for older people.

Analysis of age-specific mortality after lung function measured age 5-8 years (**Table 2.3**)

In the analysis of 189 children (62 deaths, average time at risk per person of 16.4 years) who had documented LF at age 5-8 years, age-specific mortality after age 5-8 years was 91% lower in period 2 (from year 2000) compared to period 1 (before year 2000; aHR 0.09; 95% CI 0.02, 0.51; *p*=0.007); and 7.6-fold higher if FEV1z was < -2.0 compared to ≥ -2.0 (aHR 7.64; 95% CI 2.58, 22.59; *p* < 0.001). There was no evidence of associations of mortality with sex, age of diagnosis, *P. aeruginosa* infection (status or years since first infection) or genotype. The mortality-age relationships were allowed to be distinct by ethnicity, and therefore the impact of ethnicity is not explicitly noted as it varies by age – see **On-line supplement A** for further details.

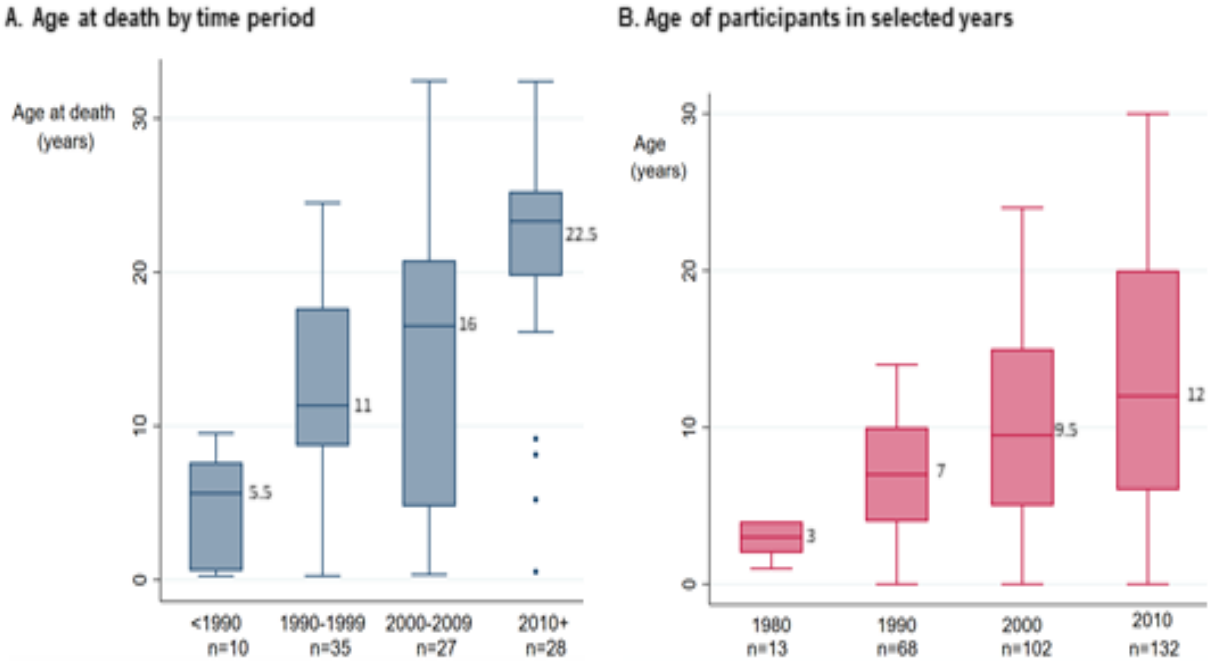


Figure 2.2 Ages of participants in the cohort

A) Ages at times of death, by time period; B) Ages of those in the study at the start of selected years. Boxes and dividing lines indicate quartiles, whiskers span the remaining ages up to 1.5 times the interquartile range, bullets indicate the remaining outlying ages.

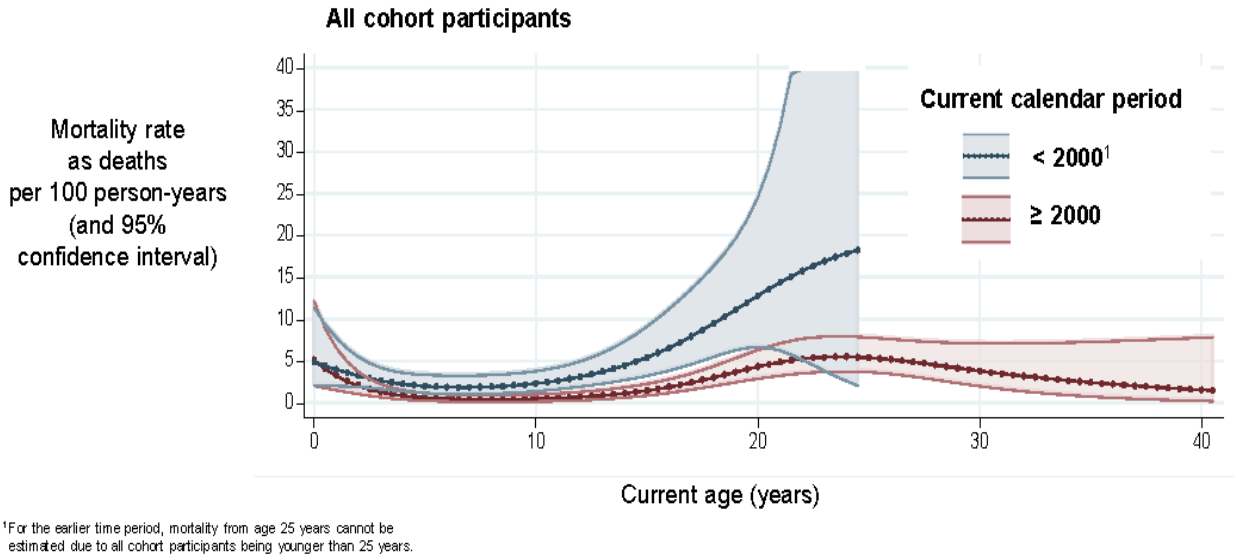


Figure 2.3 Age-specific mortality rates for two consecutive calendar year periods (Period 1: 1974-1999 versus period 2: 2000-2019) for all cohort participants as obtained by a descriptive Poisson regression model.

Legend: For the earlier time period, mortality from age 25 years cannot be estimated due to all cohort participants being younger than 25 years.

Discussion

This study documented improved CF-survival over four decades at a SA CF centre in keeping with trends reported from high income settings, despite limited access to lung transplantation and more recently, CFTR modulator drugs (1-3, 9). Significantly improved survival, however, was noted only in people older than 10 years and no factor other than time period from year 2000 was independently associated with improved survival. Although substantially improved survival was found among children less than age 10 years, it was not statistically significant, although the precision of the analysis is limited by the small number of children and deaths. Non-Caucasian ethnicity and greater time since first *P. aeruginosa* infection were risk factors associated with increased mortality in this age group. In addition, severe LF impairment at age 5-8 years in children who survived to this age, was associated with a 7-fold increased risk of mortality compared to children without severe LF impairment. Overall, our study findings suggest early life factors have a significant impact on CF-survival in SA, which has implications for interventions that aim to improve CF outcomes in SA and other similar LMIC.

Direct comparison of mortality rates and median ages of death to other countries is limited due to different follow-up periods, study designs and statistical methods that we applied to accommodate follow-up time from diagnosis (instead of birth), and insufficient accumulated follow-up time that could not estimate median survival age in more recent periods. However, consistent with worldwide observations, we documented clear reduction in mortality risk in more recent years. Although median survival age in those diagnosed in cohort A (before year 2000) was 21 years (**Figure 2.1**), median survival age is projected to be significantly higher in those diagnosed in cohort B (after year 2000), but less than reported in Canada and Europe where median survival age now has reached 51 years (2, 22). Further evidence from the SA CF registry for lower survival age in SA compared to high income countries today is the higher proportion of children (56%) with CF compared to adults (18).

Annual mortality rate in the US (1987-2019) has steadily declined from over 2 per 100 person-years to less than 1.5 per 100 person-years. In contrast, as depicted in **Figure 2.3**, in period 2 (from year 2000) estimated mortality rates in the SA cohort were highest in the under-2 years age group and then peaked again in mid-20's (approximately 5 deaths per 100 person-years). As reported from studies looking at European CF Patient Registry data, the proportion of CF deaths in childhood reflects the availability of resources to diagnose and treat CF in different countries, with significantly higher mortality reported in middle-low-income European countries compared to high income European countries (23, 24). For years 2008-2013, the proportion of recorded deaths occurring in under 18-year-olds in Europe was 11.9% (23). In contrast, in our study the proportion of recorded deaths occurring under age 18-years after year 2010 was 21%. Several factors in Europe were independently associated with increased childhood CF mortality including female sex, FEV1 below 40% predicted, BMI below 2 standard deviation scores and need for

domiciliary oxygen (23). The 7-fold increased mortality risk in the SA cohort with severe LF impairment (FEV1z < -2.0) at age 5-8 years highlights an important need in SA for early life interventions such as NBS for CF. NBS for CF has improved long-term CF survival in Italy (25). The feasibility of NBS for CF in SA and other LMIC must be explored as early diagnosis and nutritional interventions are key to improving LF and CF survival. The proportion of children in this study with severe malnutrition at diagnosis and poor LF age 5-8 years was not significantly different if diagnosed in cohort A and cohort B. Although our data could be underpowered, it points to unchanging early-life factors impacting CF-related health outcomes in childhood, which may partly explain why survival did not significantly improve in children under 10 years of age observed in this study.

Worldwide, disparity in CF-survival between ethnicity is linked to socioeconomic status (SES) and quality of health care (12, 26). In a recent study from Mexico, low SES was associated with a 4-fold increased risk of mortality with significantly lower median survival age of 15.1 years in the low SES group compared to 29.4 years in the high SES group (27). Although SES was not recorded in the SA cohort, ethnicity is a proxy for SES in SA where although being in the minority, average household income in Caucasians is significantly higher than non-Caucasians (28). The higher mortality in non-Caucasian children under 10 years of age observed in this study is a surrogate for SES disparities between ancestries that still exist in SA today as a legacy of Apartheid.

Although our study expanded a period of over 40 years, our data and analyses were limited by relatively small numbers of individuals and recorded deaths followed up at one CF center in SA. This limits the generalisability of our findings to SA and the statistical power of our analyses to identify and accurately estimate associations or temporal changes in the younger age group. However, the clinical spectrum and profile of CF at our center is similar to that documented in the recently established SA CF registry, suggesting our findings may be informative of the rest of SA (18). As demonstrated in simulations by Sykes *et al.*, more than 15% of individuals lost to follow-up may lead to underestimation of median survival age and censuring people receiving a lung transplant or missing deaths, an overestimation of median survival age (29). A strength of our data is thus a relatively low number of people lost to follow-up (10%), inclusion of lung transplant recipients and accurate recording of deaths throughout the study period. Furthermore, reasons for lost to follow-up data are diverse and outcomes are assumed to be similar to those not lost to follow-up. Our data therefore minimises potential bias of survival estimates. Another limitation of our data was absence of detailed CF-related clinical information such as LF, nutrition and microbiology captured in the clinic database. We retrospectively retrieved from old medical files key LF, nutrition and *P. aeruginosa* infection data where this information was available but the large amount of missingness (e.g., length/height measurements) for these variables restricted our analyses to smaller numbers.

In summary, this study has documented improving CF survival in SA over four decades, despite socioeconomic disparities and limited access to life-extending interventions such as lung transplantation and CFTR modulator drugs. Newborn screening for CF and CFTR modulator drugs are interventions needed in SA and other LMIC to further reduce childhood CF mortality and improve long-term CF survival on par with high income countries.

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Table 2.1 Cohort characteristics, stratified by year of diagnosis (1975-1999 versus 2000-2019)

	Total (n = 288)	**Diagnosis year < 2000, cohort A (n = 156)**	**Diagnosis year ≥ 2000, cohort B (n = 132)**	***p*-value (cohort A versus B)†**
Year of diagnosis (year): median (Q1,Q3)	1998 (1988,2009)	1989 (1983,1995)	2010 (2005,2015)	-
Age at diagnosis (months): n (%)	288	156	132	0.622
< 3	97 (33.7)	49 (31.4)	48 (36.4)	
3-23	106 (36.8)	58 (37.2)	48 (36.4)	
≥ 24	85 (29.5)	49 (31.4)	36 (27.3)	
Age at diagnosis (years): median (Q1,Q3)	0.5 (0.2,2.5)	0.6 (0.2,2.7)	0.4 (0.2,2.2)	
Ancestry: n (%)	288	156	132	0.684
Caucasian	163 (56.6)	90 (57.7)	73 (55.3)	
Non-Caucasian	125 (43.4)	66 (42.3)	59 (44.7)	
Sex: n (%)	288	156	132	0.679
Female	138 (47.9)	73 (46.8)	65 (49.2)	
Male	150 (52.1)	83 (53.2)	67 (50.8)	
Genotype: n (%)	264	134	130	0.118
p.Phe508del homozygous	116 (43.9)	66 (49.2)	50 (38.46)	
p.Phe508del heterozygous	100 (37.9)	49 (36.6)	51 (39.2)	
Other	48 (18.2)	19 (14.2)	29 (22.3)	
WAZ at diagnosis (z-score): n (%)	205	86	119	0.837
< -2	72 (35.1)	32 (37.2)	40 (33.6)	
≥ -2 and < -1	45 (22.0)	19 (22.1)	26 (21.9)	
≥ -1	88 (42.9)	35 (40.7)	53 (44.5)	
WAZ at diagnosis (z score): median (Q1,Q3)	-1.3 (-2.9,-0.1)	-1.4 (-3.0,-0.1)	-1.2 (-3.0,-0.0)	
HAZ at diagnosis: n (%)	141	34	107	0.960
< -2	41 (29.1)	10 (29.4)	31 (29.0)	
≥ -2 and < -1	31 (22.0)	8 (23.5)	23 (21.5)	
≥ -1	69 (48.9)	6 (47.1)	53 (49.5)	
HAZ at diagnosis (z score): median (Q1,Q3)	-1.1 (-2.5,0.3)	-1.1 (-2.6,0.3)	-1.0 (-2.5,0.3)	
***P. aeruginosa* infection occurrence:** n (%)	263	137	126	†
Yes	201 (76.4)	122 (89.1)	79 (62.7)	
No	62 (23.6)	15 (11.0)	47 (37.3)	
Age of first *P. aeruginosa* infection (years): median (Q1-Q3)	3.4 (1.0,7.6)	4.1 (0.9,8.0)	2.6 (1.1,6.5)	†
***P. aeruginosa* infection occurrence by age 5, among those still alive at age 5:** n (%)	244	142	102	†
Yes	105 (43.0)	61 (43.0)	44 (43.1)	
No	139 (57.0)	81 (57.0)	58 (56.9)	
FEV1z at age 5-8 years (z-score): n (%)	139	59	80	0.188
< -2	39 (28.1)	20 (33.9)	19 (23.8)	
≥ -2	100 (71.9)	39 (66.1)	61 (76.3)	
FEV1z at age 5-8 years (z-score): median (Q1-Q3)	-1.0 (-2.3,-0.1)	-1.1 (-2.7,-0.1)	-1.0 (-1.9,0.0)	
FEV1 percent predicted at age 5-8 years: n (%)	139	58	80	0.516

≤ 40%	7 (5.1)	4 (6.9)	3 (3.8)	
41-79%	39 (28.3)	18 (31.0)	21 (26.3)	
≥ 80%	92 (66.7)	36 (62.1)	56 (70.0)	
FEV1 percent predicted at age 5-8 years (%): median (Q1-Q3)	87.8 (67.6,99.2)	86.0 (65.1,98.4)	88.1 (74.9,100.0)	
WAZ at 5-8 years (z-score): n (%)	192	112	80	0.468
< -2	25 (13.0)	15 (13.4)	10 (12.5)	
≥ -2 and < -1	54 (28.1)	35 (31.3)	19 (23.8)	
≥ -1	113 (58.9)	62 (55.4)	51 (63.8)	
WAZ at 5-8 years (z score): median (Q1,Q3)	-0.7 (-1.5,-0.1)	-0.8 (-1.6,-0.3)	-0.5 (-1.4,0.3)	
HAZ at 5-8 years (z-score): n (%)	168	88	80	0.435
< -2	22 (13.1)	11 (12.5)	11 (13.8)	
≥ -2 and < -1	50 (29.8)	30 (34.1)	20 (25.0)	
≥ -1	96 (57.1)	47 (53.4)	49 (61.3)	
HAZ at 5-8 years (z score): median (Q1,Q3)	-0.9 (-1.5,-0.1)	-0.9 (-1.5,-0.3)	-0.8 (-1.7, 0.0)	
BMI at 5-8 years (z-score): n (%)	168	88	80	0.620
< -2	10 (6.0)	6 (6.8)	4 (5.0)	
≥ -2 and < -1	27 (16.1)	16 (18.2)	11 (13.8)	
≥ -1	131 (78.0)	66 (75.0)	65 (81.3)	
BMI at 5-8 years (z score): median (Q1,Q3)	-0.2 (-0.9,0.4)	-0.4 (-1.1,0.2)	0.0 (-0.7,0.6)	
Status on 31 December 2019: n (%)	288	156	132	†
Alive	158 (54.9)	44 (28.2)	114 (86.4)	
Died	100 (34.7)	88 (56.4)	12 (9.1)	
Lost-to-follow-up	30 (10.4)	24 (15.4)	6 (4.6)	
Duration of follow-up per person (years): median (Q1-Q3)	12.0 (5.7,20.1)	18.2 (9.1,23.8)	8.1 (3.6,12.8)	‡

HAZ: Height-for-age z score; WAZ: Weight-for-age z-score; FEV1z: forced-expiratory-volume in one second z-score; Q1: Quartile 1; Q3: Quartile 3

† Based on a chi-squared test, using the categorical form of each variable. Tests for difference were not performed for occurrence and age of first *P. aeruginosa* infection and status on 31 December 2019 because the different follow-up periods for individuals in Cohorts A and B imply these statistics are expected to be different and cannot be meaningfully directly compared.

Table 2.2 Unadjusted and adjusted mortality hazard ratios for the full CF cohort, describing the change in mortality by time period (period 1 1974-1999 vs period 2 2000-2019) while controlling for patient and disease characteristics as obtained by Poisson regression models separately fitted to two age groups (less than versus at least 10 years) and always allowing for age-specific mortality rates

	Current age < 10 years					**Current age ≥ 10 years**				
		Unadjusted hazard ratio		**Adjusted hazard ratio (aHR) (*p*-value‡: <0.001) d/t = 22/1633**			**Unadjusted hazard ratio**		**Adjusted hazard ratio (aHR) (*p*-value‡: 0.201) d/t¶ = 51/1662**	
	d/t¶	**Estimate (95% CI)**	***p*-value**	**Estimate (95% CI)**	***p*-value**	**d/t¶**	**Estimate (95% CI)**	***p*-value**	**Estimate (95% CI)**	***p*-value**
Time period (ref: before 2000)	36/1856					64/1957				
Year 2000 onwards		0.55 (0.28,1.08)	0.081	0.67 (0.28,1.64)	0.383		0.25 (0.14,0.46)	<0.001	0.14 (0.06,0.29)	<0.001
Sex (ref: female)	36/1856					64/1957				
Male		0.99 (0.52,1.91)	0.987	0.61 (0.25,1.51)	0.286		0.93 (0.57,1.52)	0.775	0.77 (0.41,1.42)	0.395
***P. aeruginosa* infection (ref: never)**	29/1750					55/1777				
Yes (current or previous)		1.68 (0.37,7.63)	0.501	1.37 (0.27,6.96)	0.705		3.33 (0.88,12.63)	0.077	3.42 (0.85,13.71)	0.083
Time since *P. aeruginosa* infection (years#)	29/1750	1.28 (1.06,1.55)	0.010	1.31 (1.02,1.68)	0.036	55/1777	1.00 (0.92,1.08)	0.944	0.99 (0.90,1.08)	0.760
Ethnicity (ref: not Caucasian)	36/1856					64/1957				
Caucasian		0.24 (0.11,0.51)	<0.001	0.17 (0.05,0.63)	0.008		0.96 (0.58,1.60)	0.877	0.56 (0.29,1.08)	0.082
Genotype (ref: p.Phe508del homozygous)	27/1714					58/1818				
p.Phe508del heterozygous		3.17 (1.22,8.26)	0.018	1.56 (0.55,4.43)	0.408		0.75 (0.43,1.31)	0.314	0.72 (0.38,1.35)	0.303
Other		3.69 (1.24,10.97)	0.019	1.95 (0.58,6.59)	0.284		0.59 (0.25,1.42)	0.239	0.36 (0.13,1.02)	0.054
Age at diagnosis (ref: < 3 months)	36/1856					64/1957				
3-23 months		0.97 (0.48,1.99)	0.941	1.01 (0.40,2.54)	0.977		0.81 (0.43,1.52)	0.505	1.43 (0.68,3.01)	0.346
≥ 24 months		0.67 (0.21,2.13)	0.496	0.78 (0.15,3.91)	0.760		0.95 (0.52,1.72)	0.861	1.65 (0.78,3.49)	0.186

Note: Knots for age natural cubic spline are at 2, 4, 6, and 8 years (<10 years) and 15, 20, 25 and 30 years (≥ 10 years). See **On-line supplement A** for fitted splines.

‡ *p*-value from testing whether the inclusion of factors adds value to a model with only the time period. For the age ≥ 10 years group, there is therefore no evidence that any factor is related to mortality in the adjusted model other than time period.

Years since first infection for the first 5 years (1-5), with a dampening effect and less granular treatment of years thereafter - values of 7.5 and 12.5 are used to capture 5-10 years and > 10 years after first infection. Included as an interaction term to only apply to those who have had an infection.

¶ d/t: number of deaths over aggregate person-years in the data used for the analysis.

Table 2.3 Unadjusted and adjusted mortality hazard ratios for the cohort with lung function measurements at age 5-8 years, describing the change in subsequent mortality by time period (period 1: 1974-1999 vs period 2: 2000-2019) and lung function impairment (FEV1z less than versus at least -2) while controlling for patient and disease characteristics; as obtained by Poisson regression models separately, always allowing for ethnicity-and-age-specific mortality rates.

		Unadjusted hazard ratio		**Adjusted hazard ratio (aHR) (*p*-value‡: 0.7162) d/t¶ = 24/1965**	
	d/t¶	**Estimate (95% CI)**	***p*-value**	**Estimate (95% CI)**	***p*-value**
Time period (ref: before 2000)	62/3107				
Year 2000 onwards		0.21 (0.11,0.40)	<0.001	0.09 (0.02,0.51)	0.007
Lung function impairment at 5-8 years (ref: Fev1z ≥ -2)	27/2069				
Fev1z < -2		6.80 (3.02,15.34)	<0.001	7.64 (2.58,22.59)	<0.001
Weight-for-age at 5-8 years (WAZ ≤ -2)	62/3107				
WAZ > 2		1.95 (0.99,3.83)	0.054	1.92 (0.34,10.64)	0.457
Sex (ref: female)	62/3107				
Male		1.02 (0.61,1.70)	0.939	1.15 (0.39,3.34)	0.803
***P. aeruginosa* infection (ref: never)**	56/2975				
Yes (current or previous)		3.25 (0.89,11.84)	0.074	4.92 (0.38,63.63)	0.223
Time since *P. aeruginosa* infection (scale 1-7)	56/2975	0.99 (0.91,1.08)	0.847	0.93 (0.77,1.11)	0.408
Genotype (ref: p.Phe508del homozygous)	53/2889				
p.Phe508del heterozygous		0.89 (0.47,1.67)	0.709	2.59 (0.83,8.08)	0.102
Other		0.64 (0.26,1.55)	0.325	0.95 (0.21,4.32)	0.949
Age at diagnosis (ref: < 3 months)	62/3107				
3-23 months		1.04 (0.56,1.92)	0.913	1.13 (0.37,3.47)	0.835
≥ 24 months		0.93 (0.49,1.77)	0.836	1.28 (0.40,4.13)	0.679

Note: Knots for age natural cubic spline are at 15, 20, 25 and 30 years. See **On-line supplement A** for fitted splines.

‡ *P*-value from testing whether the inclusion of factors adds value to a model with only the time period and lung function impairment variable. There is therefore no evidence supporting the inclusion of additional factors.

Years since first infection for the first 5 years (1-5), with a dampening effect and less granular treatment of years thereafter - values of 7.5 and 12.5 are used to capture 5-10 years and > 10 years after first infection. Included as an interaction term to only apply to those who have had an infection.

¶ d/t: number of deaths over aggregate person-years in the data used for the analysis.

FEV1z: forced-expiratory-volume in one second z-score.

On-line supplement A (Chapter 2/Publication 1)

This appendix provided supplementary content to the article:

Trends in Cystic Fibrosis survival over 40 years in South Africa by Zampoli M, Kassanjee R, Verstraete J, Westwood A, Zar HJ and Morrow BM.

A. Mortality rates by age for multivariable models

The age-mortality relationships fitted in the multivariable models are shown below.

Analysis was performed in Stata (version 15)[1].

Missing data. Observations with missing values were excluded from the corresponding analyses, assuming outcomes were missing at random given model terms, and all sample sizes are reported. Weight-for-age at diagnosis and BMI at 5-8 years (29% and 13% missing, respectively) were excluded from models to preserve sample sizes.

Age (continuous, in years) was included in all models using natural cubic splines. The Stata function mkspline was used to generate the spline basis functions for age. Including these basis functions in the model allowed for a flexible relationship between age and mortality, **thus allowing for the estimation of the 'effect' of factors of interest on *age-specific* mortality**, as presented in the main article.

As described in Methods of the article, likelihood ratio (LR) tests (using a strict significance threshold of 0.05) were used to decide whether the mortality-age relationship should be distinct by ethnicity (Caucasian or other) or sex (male or female).

A limitation of the analyses presented in this work is the small sample (of individuals and deaths) that was used to fit the multivariable models, restricting our ability to obtain precise age-mortality relationships.

[1] StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.

Model 1: Age less than 10 years

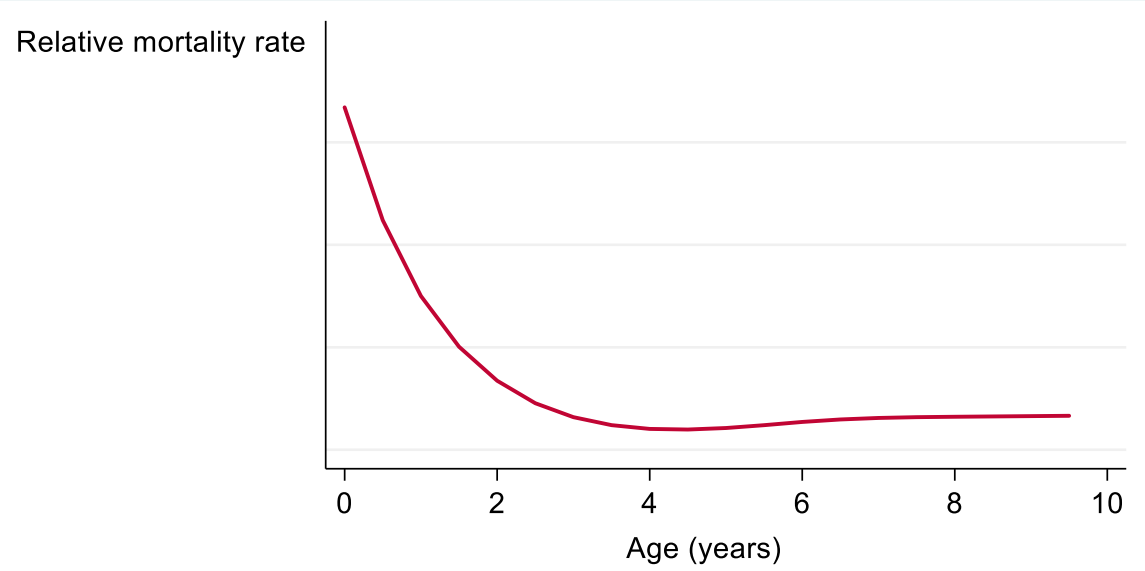
Knots are at 2, 4, 6, and 8 years.

A likelihood ratio test did not justify allowing for the age terms to vary by ethnicity (p = 0.104). It did suggest that the age terms should vary by sex (p = 0.006). However, when allowed to do so, we found unreasonably large variation in mortality by age, probably due to overfitting to our small dataset, and we thus did not allow for modifications by sex.

The estimated coefficients for the age spline basis functions are:

	Coefficient (95% CI)	***p*-value**
Basis function 1	-0.80 (-1.38,-0.22)	0.007
Basis function 2	1.82 (-0.74,4.38)	0.163
Basis function 3	-4.23 (-11.78,3.32)	0.272

At a point estimate level, this implies the following variation of mortality rates by age (no absolute numbers are provided on the y-axis as the exact mortality rate would depend on the value of all other terms included in the model – for example, the person's sex or the infection genotype):



Model 2: Age 10 years and older

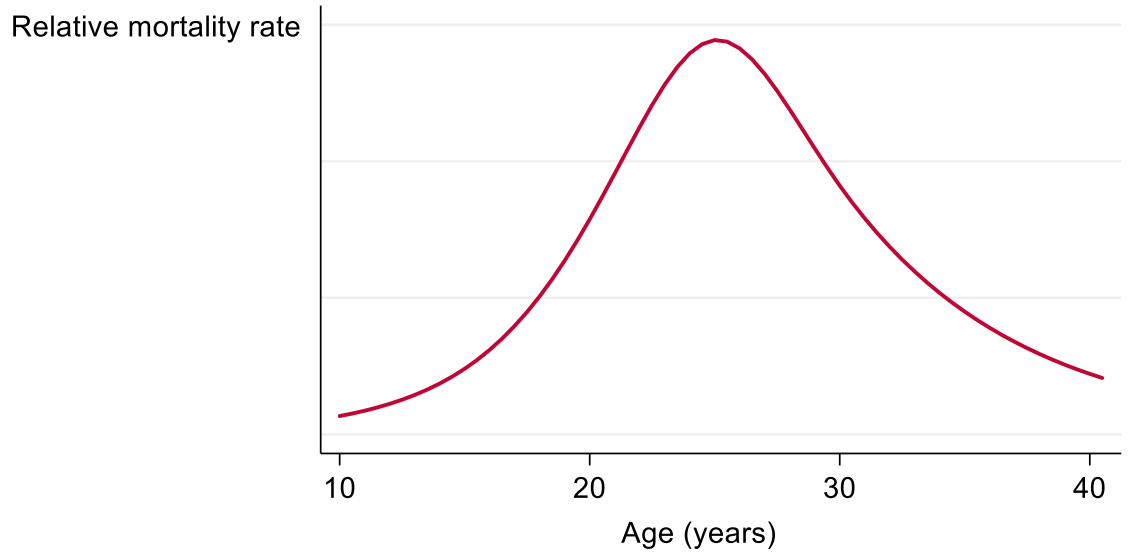
Knots are at 15, 20, 25, and 30 years.

Likelihood ratio tests did not justify allowing for the age terms to vary by ethnicity (p = 0.749) or sex (0.052) – though it is worth nothing that the p-value for sex is just above the threshold used.

The estimated coefficients for the age spline basis functions are:

	Coefficient (95% CI)	***p*-value**
Basis function 1	0.25 (0.10,0.40)	0.001
Basis function 2	-0.15 (-0.73,0.43)	0.611
Basis function 3	-0.14 (-1.95,1.66)	0.878

At a point estimate level, this implies the following variation of mortality rates by age:



Model 3: After lung function measurement at 5-8 years

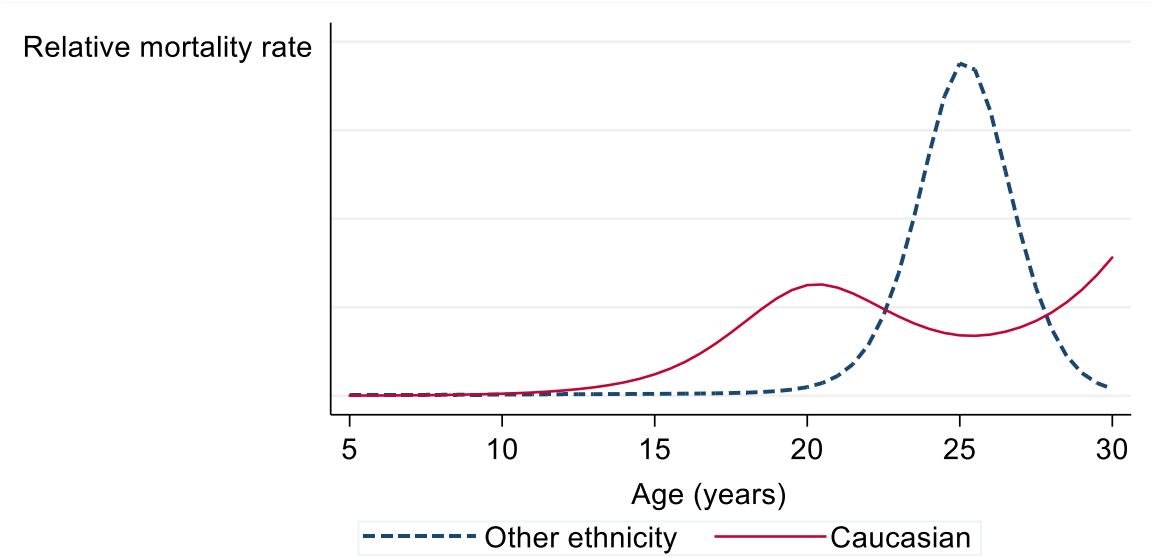
Knots are at 15, 20, 25, and 30 years.

A likelihood ratio test did not justify allowing for the age terms to vary by sex (p = 0.310). It did suggest that the age terms should vary by ethnicity (p = 0.006) – and therefore the age-splines are ethnicity specific.

The estimated coefficients for the age spline basis functions are:

	Other ethnicity		Caucasian	
	Coefficient (95% CI)	***p*-value**	**Coefficient (95% CI)**	***p*-value**
Basis function 1	0.08 (-0.16,0.33)	0.501	0.47 (0.22,0.72)	<0.001
Basis function 2	1.93 (0.23,3.63)	0.026	-1.25 (-2.42,-0.08)	0.037
Basis function 3	-7.73 (-15.22,-0.24)	0.043	3.46 (-0.48,7.40)	0.086

At a point estimate level, the fitted shapes for mortality rates by age and ethnicity follow:



These patterns should not be over-interpreted as data were particularly limited for this analysis, with only 22 deaths observed in the analysis dataset.

Author contribution statements and approval for inclusion of publication in PhD dissertation by A/Prof. Marco Zampoli [student no. ZAMMAR001].

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We confirm and approve of including the above publication in his PhD dissertation, for which we are listed as co-authors. We further confirm that we are not currently registered as a postgraduate student nor intend to use this publication in our own or other postgraduate dissertations.

We agree that his role in the publication was: to conceptualise and design the study protocol; oversee data collection and analysis; data interpretation; drafting and critically revising the manuscript as first author; submitting and approving the final revised manuscript for publication.

Our roles in the publication were the following:

Author name	Contribution	Signature
Reshma Kassanjee	To conceptualise and design the study protocol; perform data analysis and advise with data interpretation; draft and critically revise the manuscript, including tables and illustrations; approving the final revised manuscript for publication.	
Janine Verstraete	To conceptualise and design the study protocol; perform data collection and analysis; critically review and revise the manuscript, including tables; approving the final revised manuscript for publication.	
Anthony Westwood	To perform historical data collection and approving the final revised manuscript for publication.	
Heather J Zar	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and co-author; and approving the final revised manuscript for publication.	
Brenda M Morrow	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and senior author; and approving the final revised manuscript for publication.	

Chapter 3

Cystic Fibrosis in South Africa: spectrum of disease and determinants of outcome

Authors: Zampoli M (1,2), Verstraete J (1), Frauendorf M (3), Kassanjee R (4), Workman L (1), Morrow B M (1) and Zar H J (1,2).

Affiliations

1. Department of Paediatrics and Child Health, University of Cape Town
2. South African MRC Unit for Child and Adolescent Health, University of Cape Town
3. Milpark Netcare Hospital, Johannesburg, South Africa
4. Centre for Infectious Disease Epidemiology and Research (CIDER), School of Public Health and Family Medicine, University of Cape Town

Corresponding author:

A/Prof M Zampoli

5th floor ICH Building

Red Cross War Memorial Children's Hospital

Klipfontein Rd

Rondebosch, 7700

Telephone: +27 21 658 5309

E-mail address: m.zampoli@uct.ac.za

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Take home message

Analysis of the recently established South African (SA) CF registry showed that MRSA and undernutrition was associated with severe CF-lung disease. Highly effective CFTR modulator therapy would benefit the majority of people with CF in SA.

Abstract

Introduction: Little is known about cystic fibrosis (CF) in low-middle income settings. This study aimed to describe the spectrum and outcomes of CF in South Africa (SA) from the recently established SA CF registry (SACFR).

Methods: Demographic, diagnosis and clinical data was extracted from the SACFR. Cross-sectional univariable and multivariable regression analysis of best forced expiratory volume in one second (FEV_1; age ≥ 6 years) and nutrition (all ages) in 2018 was conducted to investigate factors associated with severe lung disease (SLD; FEV_1 < -3.0 z-score) and undernutrition.

Results: By December 2018, ancestry of 447 individuals included in the SACFR was Caucasian (315; 70%), mixed (87; 19%) and Black African (41; 9%). Median diagnosis age was 7.6 months (IQR 2.7,37.1). Genotype was p.Phe508del homozygous (220; 49%); p.Phe508del heterozygous (144; 32%) and neither p.Phe508del or unknown Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR)* variant in 83 (19%); the second most frequent *CFTR* variant was 3120+1G>A, common in Black Africans. Median age of patients in 2018 was 14.7 years (IQR 7.4,24.4). SLD was independently associated with chronic *methicillin resistant S. aureus* (MRSA) (aOR 16.75; 95%CI 1.74-161.50), undernutrition (aOR 5.20; 95%CI 2.23-12.13) and age (aOR 2.23 per 10-years; 95%CI 1.50-3.31). Undernutrition was associated in univariable analysis with low weight at diagnosis, non-Caucasian ancestry, chronic *P. aeruginosa* infection and lower socioeconomic status.

Conclusion: Interventions targeting MRSA infection and nutrition are needed to improve CF outcomes in SA. Most people with CF in SA are eligible for highly effective CFTR modulator therapy.

Introduction

Cystic fibrosis (CF) occurs with varying frequency in all population groups throughout the world. Although CF survival has improved over the past two decades, it remains a life-shortening condition with median survival age for a person with CF in 2018 in the United States (US) approximately 47 years (1). Nutrition, lung function (LF) and rate of LF decline are important predictors of CF-related morbidity and mortality (2, 3). Preserving LF and slowing the rate of LF decline is key to improving CF survival.

CF registries from high income countries have contributed significantly to our understanding of CF epidemiology and survival (4, 5). Epidemiological and longitudinal data of over 72000 people living with CF today are currently recorded in CF registries in the US, Canada, Europe, Australasia and Brazil, with coverage rates reported to be as high as 90% of the CF population (5). Several known modifiable and non-modifiable factors in high income countries have been associated with lower LF and accelerated LF decline in CF (6-8). However, it is unclear if similar or other determinants of CF lung disease exist in low- or middle-income countries (LMIC) such as South Africa (SA). Socioeconomic factors such as poverty, limited access to appropriate healthcare, CF medications and social complexity are more prevalent in LMIC and important factors associated with poor CF-related outcomes such as LF and survival (9-11). Lower LF and survival has been documented in Hispanic CF populations in the US compared to non-Hispanics (12, 13). Ancestry and socio-economic status (SES) are expected to be significant determinants of CF-related outcomes in SA, which is reported to be one of the most unequal societies in the world (14).

South Africa launched its CF registry in 2018 and adopted similar data collection methods as the 2017 European CF registry (15). South Africa is categorised by the Word Bank as a high-middle income country and has a population of nearly 60 million (16). Healthcare infrastructure and services are provided to most of the population through a resource-constrained public health system and a smaller but well-resourced private healthcare system. The prevalence of Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR)* mutations in the SA population and incidence of CF also varies greatly. P.Phe508del is the most common mutation amongst Caucasians (prevalence 76%), whereas 3120+1G>A the most common mutation (prevalence 46%) in Black Africans (17), with an estimated carrier frequency rate of 1 in 90 healthy individuals (18). A review of *CFTR* mutations identified across 12 African countries (predominantly Northern Africa and South Africa) identified 70 *CFTR* mutations of which 39 were known disease-causing mutations and five novel mutations (19). There is, therefore, an urgent need to investigate the spectrum and determinants of CF disease in the SA population, especially in non-Caucasian people. The aim of this study was to describe the spectrum of CF in SA and explore LF and nutrition outcomes captured in the SA CF registry (SACFR).

Methods

Study design and population:

A descriptive cross-sectional study was conducted using anonymised data extracted from the SACFR, a multi-centre public-private collaboration designed to collect similar data and variable definitions as per the 2017 European CF patient Registry report (20) and SACFR 2018 patient registry report (21). The SACFR was established in 2018 and enrols consenting adults and children receiving CF care in SA.

Recruitment of CF registry participants was initiated through formation of the SACFR steering committee which represents all known CF care clinics in public and private health sectors. In addition, the SA CF Association, the local CF advocacy organisation actively promotes participation in the SACFR through its press and social media networks. Data extraction from medical records and data entry into the SACFR is performed by two qualified data managers who visit each participating site on an annual basis. Demographic, CF diagnosis and genotype information were extracted and described for all individuals diagnosed in SA up to the end of December 2018. Annual review data for period 1 January to 31 December 2018 were extracted, including outcome variables: 1) best documented pre-bronchodilator forced expiratory volume in one second (FEV_1) and; 2) accompanying weight/height (age six years and older), or best weight/height if no LF was recorded. In the event of death, the best recorded measurements in 2018 prior to dying were included in analyses. FEV_1 was reported as z-scores calculated with the Global Lung Initiative (GLI) ethnic-specific reference equations (22). Severe lung disease (SLD) was defined as FEV_1z score $\leq$ -3 (23). Undernutrition for the purpose of this study was defined according to age group: World Health Organization (WHO) nutritional reference equation weight-for-height z-score (WHZ) < -1 SD in children < 2 years age; Body Mass Index z-score (BMI kg/m^2) < -1.0 in children age 2-17 years; and BMI < 18.5 kg/m^2 in adults ≥18 years age.

Modified SACFR CF diagnosis inclusion criteria and SACFR variables

People with a confirmed diagnosis of CF were captured in the SACFR if they met the following modified SA CF diagnostic criteria:

1) Two sweat chloride tests > 60 mmol/L or sweat conductivity > 90 mmol/L and clinical features compatible with CF *or*

2) DNA analysis/genotyping identified two disease-causing *CFTR* mutations as reported at the time in CFTR2 (24) database *or*

3) Sweat chloride ≤ 60 mmol/L chloride and both of the following criteria are met: a) DNA analysis/genotyping identified two disease-causing *CFTR* mutations; *and* b) clinical presentation consistent with typical or atypical CF or a CF-related disorder (CFRD) (25). People diagnosed with a CFRD were included in the SACFR.

Additional variables added to the SACFR included: composite measures of SES (e.g. public or private health care, reliance on public transport; household amenities and receipt of social welfare grants); other infections including *Haemophilus influenzae*, *methicillin resistant staphylococcus aureus (MRSA), aspergillus fumigatus*, other fungus/mould species, human immunodeficiency virus (HIV) and *mycobacterium tuberculosis*. Chronic pulmonary infection with CF pathogens was defined by Modified

Leeds criteria (26). Chronic pulmonary infection status was classified as unknown if less than four respiratory samples were submitted for culture during the year or the infection status of each pathogen could not be established from past medical records.

Standard of CF care in South Africa

Multidisciplinary CF care in the public sector is provided at six CF centres located in tertiary hospitals. Individual practitioners with CF expertise provide CF care in the private sector. Essential CF care and CF medications including pancreatic enzyme replacement therapy (PERT), hypertonic saline, azithromycin and antimicrobials are feely available in the public sector, but expensive CF therapies such as inhaled tobramycin solution, recombinant DNase and organ transplantation are restricted or not available in the public sector. Private health insurance schemes in SA vary considerably in what they reimburse for CF care, ranging from basic care equivalent to the public health sector, to comprehensive care that includes reimbursement for inhaled tobramycin solution, recombinant DNase and unlimited CF investigations (e.g. sputum cultures), however, often with additional out of pocket co-payments by the members. Off-label prescription of inhaled intravenous antibiotic formulations (e.g. gentamycin, amikacin and colimycin) for treating *P. aeruginosa* infection is widely practised due to lack of affordable alternatives. Reliable sweat chloride testing or sweat conductivity testing in SA is available only in the main cities, and limited *CFTR* panel testing is available. Newborn screening for CF is not widely performed in SA and CFTR modulator therapy is not available in SA. South African Consensus CF guidelines were published in 2017 with recommendations for appropriate standards of CF care for the SA setting (27).

Statistical analysis

Data preparation and analyses were conducted in R (v3.3.3), using the glm function to fit the regression models. Descriptive statistical tests were reported for the spectrum of clinical features and therapies, using data captured in the SACFR. Reported measures of centrality and spread were guided by whether distributions are approximately normal. Groups of individuals (by ancestry or age) were compared using chi-squared or Fisher's exact (categorical variables), Kruskal-Wallis (medians of continuous variables) or ANOVA (means of continuous variables) tests. Differences in pulmonary therapies by whether SLD occurred were assessed using chi-squared tests; ANOVA was used to compare FEV1 percentage predicted (pp) means by age category; and Pearson correlation coefficients were used to relate BMI scores to FEV1z scores.

The primary outcome measure in people aged six years and older was LF; and nutrition for all ages the secondary outcome. The frequencies of these binary outcomes were analysed using univariable and

multivariable logistic regression models, producing unadjusted and then adjusted Odds Ratios (aORs) for known or suspected demographic, genotype, socioeconomic, nutritional, microbiological and co-morbidity/complication risk/protective factors. To reduce the limitations posed by multiple factors considered in testing, we produced adjusted *p*-values for the unadjusted ORs, using Holm's method. All factors in univariable analysis with unadjusted *p*-values < 0.2 are presented in tables. Variables with Holms-adjusted *p*-values < 0.2 in univariable analyses were included in the multivariable models, as well as the confounder age. A *p*-value of < 0.05 was considered statistically significant.

This study aligns with the Declaration of Helsinki 2013 and was approved by the Faculty of Health Sciences Human Research Ethics Committee, UCT (HREC R007/2018) and the SACFR Steering Committee.

Results

Demographic and clinical characteristics at time of CF diagnosis, December 2018 **(Table 3.1)**

By 31 December 2018, 447 individuals (235, 52.6% female) with confirmed CF diagnoses were captured in the SACFR. Twelve individuals were excluded from the study as diagnostic criteria of the SACFR were not met and one patient declined registry consent. Median age of diagnosis was 7.6 months (IQR 2.7,37.1), with 253 (56.6%) diagnosed under one year of age. Sixty-eight (15.2%) presented with neonatal bowel obstruction (any form of meconium ileus presentation diagnosed clinically and managed operatively or non-operatively), the rest were diagnosed based on symptoms or an affected sibling. Only one child whose family immigrated to SA was diagnosed in the US through newborn screening. Weight and height measurement at diagnosis was missing for 131 (31.3%) and 183 (43.7%), respectively, of 419 children diagnosed < 18 years age. The median WAZ of children at diagnosis was -2.2 (IQR -3.8,-0.9) of which 37.2% were severely underweight for age (WAZ < -3.0). Median WAZ differed significantly by ancestry (p<0.001) and was lower in non-Caucasian groups (-4.2 to -2.8) than Caucasians (-1.5). One hundred and eighteen (26%) patients had one sweat chloride test and 94 (23%) two; 68 (15%) had one sweat conductivity test and 34 (8%) two. At least one sweat chloride test and/or one sweat conductivity test was documented in 212 (47%) and 102 (24%) people, respectively. One hundred and fifty-two (34%) people did not have any sweat tests documented. Reasons for missing sweat test were not documented in the SACFR but include either not done or results missing from medical records.

Complete genotype diagnosis was available in 398 (89%): p.Phe508del was the most common *CFTR* variant identified in homozygous (49.2%) or heterozygous (32.2%) state. 3120+1GA was the second most common *CFTR* variant: 23 (5.1%) in homozygous and 39 (8.7%) in heterozygous state, each

mostly in non-Caucasians (p<0.001); with 56.1% Black Africans homozygous for 3120+1GA. Incomplete genotyping (one or two unknown *CFTR* variants) differed significantly by ancestry (p<0.001) with greater prevalence among those of Mixed ancestry (21.8%) and Black Africans (29.3%) compared to Caucasians (5.7%) (**Table 3.1**).

General description of SACFR cohort in 2018 (n=413) **(Table 3.2)**

Twenty people not seen in 2018 for follow-up, 10 lung transplant (three in 2018) and two liver transplant recipients were excluded from analysis. The median age of the SACFR cohort in 2018 was 14.7 years (IQR 7.4,24.4), with 242/413 (58.6%) children younger than 18 years of age. There were more non-Caucasian children (n=99, 40,9%) compared to adults (n=25, 14,6%) (p<0.001). Except for one, all Black Africans (n= 39) were less than 18 years old. One hundred and seventy-one (41.4%) people received care exclusively in the public health sector and 242 (58.6%) received care partially or exclusively in the private health sector. Human Immunodeficiency virus (HIV) testing was documented in 202 (45.2%) of which one adolescent was HIV-infected and three children were HIV-exposed, uninfected. There were three reported deaths in 2018, all in adults. Demographic, socioeconomic, nutritional, microbiological and co-morbidity/complication details are presented in **Table 3.2**.

Lung function and correlates of severe lung disease in 2018 **(Table 3.2, Figure 3.1, Figure 3.2, Table 3.3)**

Lung function measurements in 2018 were available in 292 individuals (140 children ≥6 years and 152 adults; no LF documented n=41) (**Table 3.2**). The distribution of LF across age categories is shown in **Figure 3.1** with significant differences by age (p<0.001) and a clear trend of decreasing FEV_1pp with increasing age, and largest observed decline in median FEV_1pp between 6–11-year (95.9pp) and 12-17 year (75.7pp) age groups.

As expected, there were significant differences between the age groups 0-6 years, 6-17 years and ≥18 years for the majority of microbiology cultures and pulmonary therapies (**Table 3.2**). Comparison between children aged 6-17 years and adults ≥18 years showed that ever had (p<0.001) and chronic *P. aeruginosa* infection (p<0.001) and isolation of fungus or mould species (p=0.015) was more prevalent in adults. Isolation of any *non-tuberculous mycobacteria* (NTM, 1.2% and chronic MRSA infection (6.3%) was uncommon in all ages; one child had confirmed *M. tuberculosis* infection. Classification of 'chronic infection' status for multiple pathogens was not possible in approximately *one third* of individuals due to insufficient number of sputum samples collected in the year of follow-up. Most individuals ≥6 years were receiving low-dose azithromycin; 280 (84.1%) inhaled antibiotics; 191 (57.4%) inhaled hypertonic saline 164 (49.2%) and recombinant DNase 111 (33.3%).

BMI was associated with LF, shown in **Figure 3.2**: There were significantly positive correlations between BMI z-scores and FEV_1 z-scores in children aged 6-17 years (Pearson correlation co-efficient 0.39; 95% CI: 0.24,0.52; p<0.001) and between BMI (kg/m^2) and FEV_1 z-scores in adults aged ≥ 18 years (0.28; 95% CI: 0.13,0.42; p<0.001).

Twenty-six (18.6%) children and 70 adults (46.1%) had study defined SLD, of which two children (2.1%) and 23 adults (15.1%) had FEV_1 less than 40pp. No evidence of association was observed with SLD and receiving inhaled hypertonic saline (p=0.256) or recombinant DNase (p=0.742). Conversely, low dose azithromycin (98% vs. 79%; p<0.01) and inhaled antibiotic therapies (75.5% vs. 51.4%; p<0.01) were prescribed more frequently in individuals with SLD than without SLD.

Univariable (p<0.2) and adjusted multivariable associations with SLD are presented in **Table 3.3**. Older age (aOR 2.23 per 10-year units: 95% CI 1.50,3.31) and undernutrition (aOR 5.20; 95% CI 2.23, 12.13) were independently associated with SLD, as was chronic MRSA infection (aOR 16.75; 95% CI 1.74-161.50) although there is substantial uncertainty in magnitude of the association. Chronic *P. aeruginosa* infection was associated with SLD, but the effect was not significant (aOR 1.98; 95% CI 0.90, 4.34) after adjusting for other variables, as shown in **Table 3.3**.

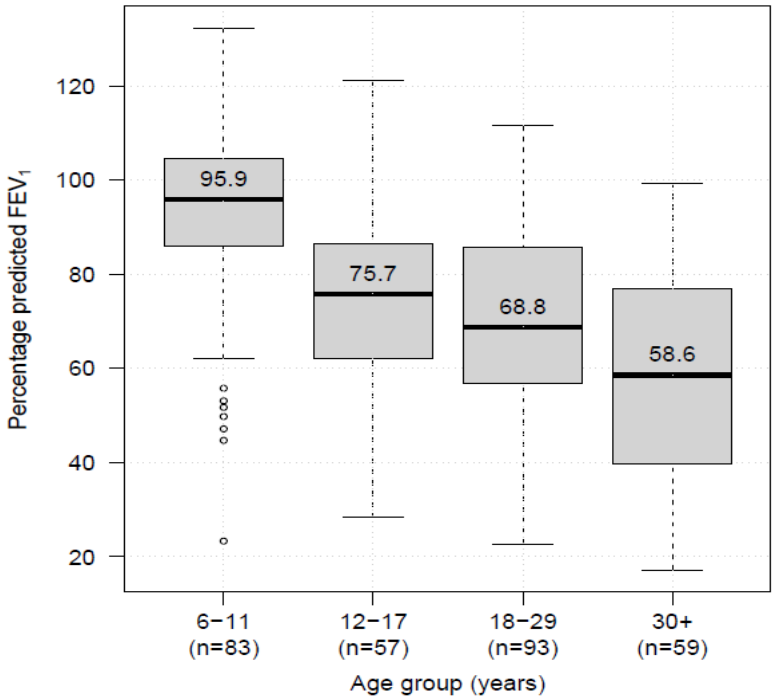

Figure 3.1 FEV_1 percentage predicted by age category in adults and children ≥ 6 years in SA CF registry, 2018.

Note: Boxes indicate first to third quartiles, the dividing line the median, whiskers the remaining points up to length 1.5 times the IQR, and markers any remaining outliers.

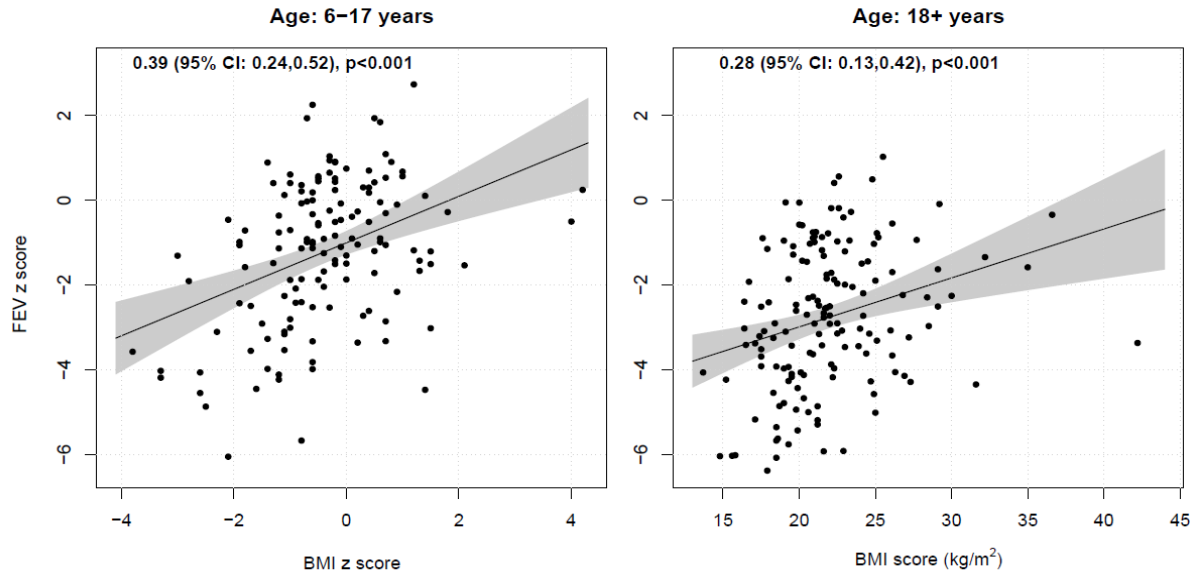

Figure 3.2 Scatter plot of FEV_1 z-scores versus BMI z-scores (children aged < 18 years) or BMI measurements (adults aged ≥ 18 years) in SA CF registry, 2018, with Pearson correlation coefficients indicated

Nutrition in 2018 **(Table 3.4**)

Nutritional measurements in 2018 were available in 237 children and 161 adults, of which the majority (354, 88.9%) were pancreatic insufficient. Overall, study-defined undernutrition was present in 63 (26.6%) children and 28 (17.4%) adults.

On univariable analysis, undernutrition was associated with low WAZ at diagnosis, non-Caucasian ethnicity, 3120+1G>A genotype, chronic *P. aeruginosa infecti*on and indicators of low-SES: receiving public healthcare and a social welfare grant (**Table 3.4**). While receiving a social welfare grant tended towards statistical significance (aOR 1.81; 95% CI 0.92-3.57), multivariable analysis did not identify any independent associations with undernutrition.

Discussion

This first comprehensive description of CF in SA highlights the importance and value of a CF registry in understanding the unique spectrum and epidemiology of CF in LMIC such as SA. Our findings highlight several important aspects which have diagnostic and therapeutic implications for future CF care in SA and other LMICs. These include ethnic-specific genotypes, limitations in areas of CF diagnosis and care, and correlating nutrition and LF outcomes. Furthermore, the higher proportion of children compared to adults, similarly observed in other LMIC such as Brazil may be the effect of lower survival age in SA compared to high income countries where adults outnumber children (11). The newly established

SACFR is, therefore, a useful tool in the long-term to prioritise and guide interventions that could improve CF outcomes in SA where median survival age in 2008 at a single centre was below 20 years (28).

The true number of people living with CF in SA is unknown. Based on *CFTR* carrier frequency rates of studies dating back to 1999, estimates of CF incidence in Caucasian, Mixed-race and Black African populations are 1 in 3000 (carrier frequency 1 in 23), 1 in 10300 (carrier frequency 1 in 55) and 1 in 784-13924 (carrier frequency 1 in 14 to 1 in 59 live births), respectively (18, 29). A national survey in 2016 reported nearly 56 million people in SA, of which the majority (45 million) were Black Africans and 4.5 million Caucasian (30). By extrapolation, the estimated number of children born with CF in the same generation would have been 3214 Black Africans and 1500 Caucasians. There appears to be a significant discrepancy in population estimates and documented number of people with CF in all ancestries, especially Black Africans. Early reports of CF in Black Africans in SA and Kenya date back to 1959 (31, 32). Since then, more studies from SA, Rwanda and Sudan have described CF in children of African ancestry with the 3120+1G>A mutation (18, 33-37). It is notable that all, but one, Black Africans with CF in the SACFR are children. This may be explained, in our opinion, by increased awareness and diagnosis of CF in non-Caucasian people in the last decade or increased mortality in non-Caucasians before CF is diagnosed, and represents an area of future research that stems from the SACFR. Cystic fibrosis expertise and diagnosis capacity are located only in a few cities across SA. It is likely that there are some people receiving care outside recognised CF care centres or practices that have not been captured in the SACFR. Furthermore, fragmented health care systems and lack of diagnostic capacity in under resourced rural provinces are factors in our experience that contribute to delayed or missed CF diagnosis in children in SA. Increased CF-related infant mortality and malnutrition in children from lower socioeconomic groups has been previously documented in SA (28, 37). In the absence of newborn screening, we suspect high numbers of undocumented CF-related infant deaths may be occurring in SA and incorrectly attributed to malnutrition or infectious disease, which is common in poor and rural communities. The high proportion (37%) of children in our study with severe malnutrition at the time of CF diagnosis is supportive of this hypothesis.

As expected, the genotype profile of CF in SA is closely linked to ancestry (**Table 3.1** and Supplementary Table 1). p.Phe508del is the most common mutation in Caucasians and people with mixed ancestry. Approximately 80% of people with CF in SA have at least one copy of p.Phe508del mutation and are therefore eligible for triple combination (elexacaftor/ivacaftor/tezacaftor) CFTR modulator therapy. In contrast, 3120+1G>A, a class 1 minimal function mutation, was the second most common mutation in people with mixed ancestry and the most common mutation in Black Africans.

Importantly, incomplete or unknown genotyping was present in 11% of people, with higher prevalence observed in people with mixed ancestry and Black Africans. Similar genotype profiles and increased prevalence of rare or unknown *CFTR* mutations have been reported in Brazil, an LMIC which shares demographic and socioeconomic characteristics with SA (38). These findings highlight the limitation of commercial *CFTR* testing kits, which are more suited for people of European descent, and the need for LMIC to develop and adopt genotyping strategies that are more appropriate for local populations. Rare, unknown and 3120+1G>A collectively comprise nearly a third of all alleles in the SACFR population This has implications for diagnostic strategies including newborn screening, and highlights the importance of the sweat test to confirm CF diagnosis in LMIC where availability of full *CFTR* genotyping and next generation sequencing is often limited or absent and may lead to a diagnosis of CF being unconfirmed or missed. Of concern, only half of registry entries had at least one sweat chloride/sweat conductivity test documented. Furthermore, sweat conductivity was sometimes the only sweat test reported. Although sweat conductivity has been validated to diagnose CF associated with minimal function *CFTR* mutations, interpretation of intermediate conductivity reference ranges is problematic and the utility of sweat conductivity in diagnosing atypical CF or CF-related disorders is unknown (39). The high number of missing sweat test results in the SACFR in our opinion is explained by either lack of access to sweat testing outside the main cities and missing results from medical records, especially in older patients. Improving documentation and accessibility to sweat chloride testing in SA is highlighted through this study as an important priority. Another implication of our findings is recognition that most Black Africans, owing to the high prevalence of the 3120+1G>A mutation, will not benefit from currently licensed CFTR modulator therapies. Advocacy to include African people in global *CFTR* modulator drug development initiatives is another priority.

Poor LF and nutrition are important co-dependent predictors of survival in CF (8). Identifying modifiable factors that preserve LF decline and improve nutrition is key to improving CF survival. After adjusting for age and factors associated with poor LF that were identified in univariable analyses, undernutrition was the strongest independent modifiable factor associated with SLD. These findings mirror differences observed with LF, nutrition and survival outcomes in Canada compared to the US. Better outcomes in Canada have been attributed to differences in childhood nutrition, access to universal free CF care and access to lung transplantation. However, LF and nutrition outcomes in the US are improving, which is attributed to introduction in the US of high-fat, high-calorie diets, newborn screening, and improved access to CF health care (8, 40). Interventions that improve CF nutrition, particularly amongst poor communities in public health care services in SA where undernutrition is most prevalent, are therefore key to improving CF survival in SA. Newborn screening for hereditary conditions including CF is available only on request in the private sector and for a fee to the public,

and therefore rarely performed in South Africa. This presents a significant barrier to improving CF outcomes in SA, especially because severe malnutrition, as reported in this study, is common in SA at the time of diagnosis. Chronic *P. aeruginosa* and MRSA infections are additional modifiable factors associated with lower LF in SA, which is consistent with international observations (6). Inhaled antibiotic therapies (e.g. tobramycin, aztreonam) and dry powder antibiotic formulations are either not available or very expensive relative to household income in South Africa. The average disposable household income per annum in SA is approximately 2300 USD (41). The cost of one month of rDNAse and tobramycin inhalation solution is approximately three- and 10-fold the average monthly household income, respectively. Active surveillance to detect early infections and aggressive eradication protocols using effective low-cost alternate approaches such as inhalation of gentamycin intravenous solution for *P. aeruginosa* eradication can be more widely adopted throughout SA (42).

Our LF data must, however, be interpreted with caution due to the small number of individuals with chronic MRSA and up to a third of people with missing chronic lung infection status data because of insufficient sputum samples collected during the year of review or infrequent clinic visits. This highlights another important deficit in SA CF care needing attention. The SACFR adopted European CF registry chronic infection status definitions in line with international CF registry harmonisation guidelines (20). In our experience, factors preventing frequent sputum sampling practises in SA include fragmented health services, limited access to multidisciplinary CF care and financial constraints in people with limited or no private health insurance who need to pay out of pocket for surveillance or routine laboratory investigations.

Interpretation of the SACFR data is limited by the absence of longitudinal or retrospective data which has excluded CF-related outcomes and deaths prior to 2018, children who may have died of CF without being diagnosed or people in whom informed consent for inclusion in the registry was not yet obtained. Longitudinal cohort data captured in future by the SACFR will be helpful to establish early life determinants of LF and CF survival in South Africa. Although we estimate most people alive and diagnosed with CF are captured in the SACFR, we suspect there are still a small number of CF patients receiving care outside the recognised participating SACFR clinics and practices. In addition, several patients were excluded from the LF and nutrition analyses due to missing data, which is an inherent challenge with registry data collection and analysis. Missing SACFR data is a major limitation of this study as data entry into the registry relies on relevant measurements and investigations being accurately recorded in the medical records by treating clinicians. Routine CF care and frequency of CF visits is unregulated and not standardised in SA. Private sector care is strongly influenced by individual patient financial resources and variable levels of reimbursement by private health insurers. Public

sector care, which serves predominantly uninsured poorer patients, is inconsistent at different clinics and frequency of routine attendance at CF clinics is dependent on access to reliable transport and other socioeconomic factors. People with CF living in remote or rural areas infrequently attend participating CF clinics, which could be another factor contributing to missing data. Missing data or incomplete information relating to CF diagnosis in older children and adults was an additional limitation.

In summary, this first comprehensive overview of CF in SA has identified important epidemiological data, which is useful to guide strategies and interventions to improve CF diagnosis capacity and CF-related outcomes. Based on genotype, most people with CF in SA are eligible for highly effective CFTR modulator therapy which is currently not available in SA. Accelerating affordable access to CFTR modulator therapy and improving nutrition and treatment of MRSA and *P. aeruginosa* infections will lead to better LF outcomes in SA.

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Name	Affiliation
Dr Fiona Kritzinger	The Chest and Allergy Centre, Netcare Christian Barnard Memorial Hospital, Cape Town, SA
Dr Taryn Gray	The Chest and Allergy Centre, Netcare Christian Barnard Memorial Hospital, Cape Town, SA
Dr Julie Morrison	Department of Paediatrics and Child Health, Stellenbosch University, Tygerberg Hospital, Cape Town, SA
Prof Pierre Goussard	Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, SA
Prof Greg Calligaro	Centre for Lung Infection and Immunity, Division of Pulmonology, Department of Medicine and UCT Lung Institute and South African MRC/UCT Centre for the Study of Antimicrobial Resistance, University of Cape Town, Cape Town, SA
Prof Paul Wilcox	UCT Private Academic Hospital, Cape Town, SA
Dr Tony Biebuyck	Panorama Medi-Clinic Hospital, Cape Town, SA
Dr Susan Klugman	Department of Paediatrics and University of Witwatersrand, Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, SA
Dr Cathy Baird	Netcare Milpark Hospital, Johannesburg, SA
Dr Siza Sezani	Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Pretoria, Pretoria, SA; Steve Biko Academic Hospital
Prof Robin Green	Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Pretoria, Pretoria, SA; Steve Biko Academic Hospital
Dr Carla Els	Netcare Linksfield Hospital, Johannesburg, SA
Dr Dave Richard	Sandton Medi-Clinic, Johannesburg, SA
Prof Refiloe Masekela	Department of Paediatrics and Child Health, Nelson R Mandela School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, SA
Dr Reratilwe Mphahlele	Department of Paediatrics and Child Health, Nelson R Mandela School of Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, SA
Dr Graham Lawrence	Busamed Hillcrest Private Hospital, Durban, SA
Dr Jonathan Egner	Netcare St Augustine's, Durban, SA
Dr Paul Gebers	Netcare Greenacres Hospital and Life St. George's Hospital; Port Elizabeth, SA

Table 3.1 Demographic and clinical information, at time of diagnosis, among the CF population in South Africa, December 2018, stratified by ancestry

	Caucasian n = 315	**Mixed n = 87**	**Black African n = 41**	**Indian n = 4**	**Total n = 447**
Sex[¤]: n (%)	**n = 315**	**n = 87**	**n = 41**	**n = 4**	**n = 447**
Female	179 (56.8)	40 (46.0)	15 (36.6)	1 (25.0)	235 (52.6)
Diagnosis age	**n = 309**	**n = 87**	**n = 39**	**n = 4**	**n = 439**
Diagnosis age in months, median (IQR)	8.9 (2.0,40.0)	6.9 (2.3,26.6)	6.4 (3.5,8.9)	54.9 (4.5,130.2)	7.6 (2.7,37.1)
Diagnosis age in years: n (%)					
< 1	167 (54.0)	53 (60.9)	31 (79.5)	2 (50.0)	253 (57.6)
1-3	57 (18.4)	15 (17.2)	2 (5.1)	0 (0)	74 (16.9)
3-10	46 (14.9)	14 (16.1)	5 (12.8)	1 (25.0)	66 (15.0)
10-17	21 (6.8)	3 (3.4)	1 (2.6)	1 (25.0)	26 (5.9)
≥ 18	18 (5.8)	2 (2.3)	0 (0)	0 (0)	20 (4.6)
Nutritional status					
WAZ at diagnosis (age 0-17 years)	**n = 183**	**n = 68**	**n = 34**	**n = 3**	**n = 288**
Median (IQR) [¤¤]	-1.5 (-3.2,-0.5)	-2.8 (-4.1,1.6)	-4.2 (-5.3,-3.1)	-4.0 (-4.5,-4.0)	-2.2 (-3.8, -0.9)
WAZ < -1.0: n (%)	118 (64.5)	53 (77.9)	34 (100)	3 (100)	208 (71.9)
WAZ < -3.0: n (%)	50 (27.3)	29 (42.6)	26 (76.5)	2 (66.7)	107 (37.2)
HAZ at diagnosis (age 0-17 years)	**n=157**	**n=45**	**n=32**	**n=2**	**n=236**
Median (IQR) [¤¤]	-1.6 (-3.2,-0.4)	-1.8 (-3.9,-0.6)	-2.3 (-3.9,-0.6)	-1.0 (-4.0,-1.0)	-1.8 (-3.3,-0.5)
HAZ < -1.0: n (%)	102 (65.0)	31 (68.9)	24 (75.0)	1 (50.0)	158 (66.9)
HAZ < -3.0: n (%)	40 (25.5)	15 (33.3)	12 (37.5)	1 (50.0)	68 (28.8)
BMI at diagnosis in kg/m^2 (age ≥ 18 years)	**n = 6**	**n = 0**	**n = 0**	**n = 0**	**n = 6**
Median (IQR)	22.8 (18.6, 24.8)	-	-	-	22.8 (18.6, 24.8)
BMI < 18.5: n (%)	1 (16.7)	-	-	-	1 (16.7)
Neonatal bowel obstruction: n (%)	**n = 315**	**n = 87**	**n = 41**	**n = 4**	**n = 447**
Yes	57 (18.1)	8 (9.2)	3 (7.3)	0 (0)	68 (15.2)
Unknown	16 (5.1)	1 (1.1)	2 (4.9)	0 (0)	19 (4.3)
Sweat Testing					
Sweat chloride (mmol/L)	**n = 134**	**n = 62**	**n = 15**	**n = 1**	**n = 212**
Mean (SD)	105 (18)	107 (17)	115 (24)	109 (-)	106 (18)
Sweat conductivity in (mmol/L)	**n = 63**	**n = 21**	**n = 18**	**n = 0**	**n = 102**
Mean (SD)	104 (16)	110 (23)	105 (20)	-	106 (19)
Genotype					
p.Phe508del[¤¤]: n(%)	**n = 315**	**n = 87**	**n = 41**	**n = 4**	**n = 447**
Homozygous	183 (58.1)	36 (29.9)	0 (0)	1 (25.0)	220 (49.2)
Heterozygous	102 (32.4)	40 (46.0)	1 (2.4)	1 (25.0)	144 (32.2)

3120+1G>A; c.2988+1G>A[¤¤] : n(%)	**n = 315**	**n = 87**	**n = 41**	**n = 4**	**n = 447**
Homozygous	0 (0)	0 (0)	23 (56.1)	0 (0)	23 (5.1)
Heterozygous	8 (2.5)	19 (21.8)	12 (29.3)	0 (0)	39 (8.7)
Incomplete genotyping (one or two unknown *CFTR* variants) [¤¤]: n (%)	18 (5.7)	19 (21.8)	12 (29.3)	0 (0)	49 (11.0)
Most common *CFTR* mutation allele frequencies[#]: alleles, n (%)	**n = 630**	**n = 174**	**n = 82**	**n = 8**	**n = 894**
F508del; c.1521_1523delCTT/ p.Phe508del	468 (74.3)	92 (52.9)	1 (1.2)	3 (37.5)	564 (63.1)
3120+1G>A2,3; c.2988+1G>A	8 (1.3)	19 (10.9)	58 (70.7)	0 (0)	85 (9.5)
Other# (< 1% allele frequency)	46 (7.3)	22 (12.6)	8 (9.8)	5 (62.5)	81 (9.1)
Unknown	24 (3.8)	24 (13.8)	15 (18.3)	0 (0)	63 (7.0)
3272-26A>G1; c.3140-26A>G	15 (2.4)	8 (4.6)	0 (0)	0 (0)	23 (2.6)
394delTT1; c.262_263delTT /p.Leu881IlefsX22	18 (2.9)	0 (0)	0 (0)	0 (0)	18 (2.0)
A455E; c.1364C>A / p.Ala455Glu	11 (1.7)	5 (1.7)	0 (0)	0 (0)	16 (1.8)
N1303K1; c.3909C>G / p.Asn1303Lys	10 (1.6)	0 (0)	0 (0)	0 (0)	10 (1.1)
R553X; c.1657C>T / p.Arg553X	7 (1.1)	3 (1.7)	0 (0)	0 (0)	10 (1.1)
G542X1; c.1624G>T / p.Gly542X	9 (1.4)	0 (0)	0 (0)	0 (0)	9 (1.0)
G551D; c.1652G>A / p.Gly551Asp	8 (1.3)	1 (0.6)	0 (0)	0 (0)	9 (1.0)

Notes: excludes 12 people for whom diagnostic criteria of the SACFR were not met; column percentages calculated with recorded number of entries as denominator value

[¤] Indicates significance of differences in characteristic by ancestry, $p<0.05$ and

[¤¤] $p<0.001$

[#] Other *CFTR* mutations, see **Online Supplement** (Table 1).

Table 3.2 Clinical, lung function and nutritional characteristics of children and adults in the SA CF registry captured in 2018, stratified by age

	0-6 years	**6-17 years**	**≥ 18 years**	**Total**
Sex: n (%)	**n=80**	**n=162**	**n=171**	**n=413**
Female	40 (50.0)	87 (53.7)	94 (55.0)	221 (53.5)
Age in years	**n=80**	**n=162**	**n=171**	**n=413**
Median (IQR)	3.4 (2.1,5.0)	11.3 (8.8,14.6)	26.9 (21.6,34.3)	14.7 (7.4,24.4)
Ancestry¤¤: n (%)	**n=80**	**n=162**	**n=171**	**n=413**
Caucasian	41 (51.3)	102 (63.0)	146 (85.4)	289 (70.0)
Mixed	19 (23.7)	38 (23.4)	24 (14.0)	81 (19.6)
Black African	20 (25.0)	19 (11.7)	1 (0.6)	40 (9.7)
Indian	0 (0)	3 (1.9)	0 (0)	3 (0.7)
Pancreatic insufficient: n (%)	**n=80**	**n=162**	**n=171**	**n=413**
Insufficient	75 (93.8)	144 (88.9)	146 (85.4)	365 (88.4)
Socioeconomic factors				
Household cigarette smoke¤¤: n	**n=80**	**n=162**	**n=171**	**n=413**
Yes	13 (16.3)	39 (24.1)	8 (4.7)	60 (14.5)
Receiving social welfare grant:	**n=80**	**n=162**	**n=171**	**n=413**
Yes	15 (18.8)	31 (19.1)	19 (11.1)	65 (15.7)
Private health insurance¤¤: n (%)	**n=80**	**n=162**	**n=171**	**n=413**
Yes	40 (50.0)	86 (53.1)	116 (67.8)	242 (58.6)
Microbiology: n (%)	**n=80**	**n=162**	**n=171**	**n=413**
Age in years of 1st *P. aeruginosa*, median (IQR)	1 (0.0, 2.0)	4 (1.0, 8.0)	5 (1.0, 17.0)	3 (1.0, 9.0)
Ever had *P. aeruginosa*¤¤: n (%)	27 (33.8)	58 (35.8)	102 (59.6)	187 (45.3)
Chronic *P. aeruginosa*¤¤: n (%)				
Yes	8 (10.0)	28 (17.3)	80 (46.8)	116 (28.1)
Unknown*	39 (48.8)	53 (32.7)	56 (32.7)	148 (35.8)
Chronic MSSA¤¤: n (%)				
Yes	6 (7.5)	40 (24.7)	26 (15.2)	72 (17.4)
Unknown*	39 (48.8)	53 (32.7)	58 (33.9)	150 (36.3)
Ever had MRSA: n (%)	2 (2.5)	15 (9.3)	9 (5.3)	26 (6.3)
Chronic MRSA¤: n (%)				
Yes	0 (0)	7 (4.3)	7 (4.1)	14 (3.4)
Unknown*	38 (47.5)	52 (32.1)	59 (34.5)	149 (36.1)
Chronic *B. cepacia*¤ : n (%)				
Yes	0 (0)	5 (3.1)	8 (4.7)	13 (3.1)
Unknown*	38 (47.5)	52 (32.1)	61 (35.7)	151 (36.6)
Chronic *aspergillus spp*¤: n (%)				
Yes	0 (0)	15 (9.3)	14 (8.2)	29 (7.0)
Unknown*	37 (46.3)	52 (32.1)	58 (33.9)	147 (35.6)
Chronic *H. Influenzae*¤: n (%)				
Yes	1 (1.3)	4 (2.5)	1 (0.6)	6 (1.5)
Unknown*	39 (48.8)	54 (33.3)	60 (35.1)	153 (37.0)
Another fungus/mould¤: n (%)	12 (15.0)	26 (16.0)	45 (26.3)	83 (20.1)
Any NTM isolate: n (%)	0 (0)	2 (1.2)	3 (1.8)	5 (1.2)

Pulmonary therapies: n (%) (> 3 months continuous)	**n=80**	**n=162**	**n=171**	**n=413**
Inhaled hypertonic saline	45 (56.3)	85 (52.5)	79 (46.2)	209 (50.6)
Recombinant DNase ¤¤	9 (11.3)	40 (24.7)	71 (41.5)	120 (29.1)
Inhaled antibiotics¤¤	31 (38.8)	71 (43.8)	120 (70.2)	222 (53.8)
Low-dose azithromycin¤¤	47 (58.8)	127 (78.4)	153 (89.5)	327 (79.2)
Complications/comorbidity): n	**n=80**	**n=162**	**n=171**	**n=413**
ABPA	0 (0)	8 (4.9)	10 (5.8)	18 (4.4)
CF-related diabetes¤¤	0 (0)	9 (5.6)	53 (31.0)	62 (15.0)
CF-related liver disease¤				
With cirrhosis ‡	2 (2.5)	11 (6.8)	10 (5.8)	23 (5.6)
Without cirrhosis	5 (6.3)	29 (17.9)	29 (17.0)	63 (15.3)
Pneumothorax	0 (0)	1 (0.6)	1 (0.6)	2 (0.5)
Haemoptysis major (>250 ml)	1 (1.3)	5 (3.1)	1 (0.6)	7 (1.7)
Occurrence of malignancy	0 (0)	0 (0)	2 (1.2)	2 (0.5)
Nutritional status				
WHZ (current age 0-2 years)	**n=16**	-	-	**n=16**
Median (IQR)	-0.6 (-1.4,1.2)	-	-	-0.6 (-1.4,1.2)
WHZ <-1	7 (43.8)	-	-	7 (43.8)
BMIZ (current age 2-17 years)	**n=60**	**n=161**	-	**n=221**
Median (IQR)	0.4 (-0.4,1.1)	-0.5 (-1.1,0.4)	-	-0.3 (-1.0,0.6)
BMIZ <-1	6 (10.0)	50 (31.1)	-	56 (25.3)
BMI in kg/m^2 (age ≥ 18 years)			**n=161**	**n=161**
Median (IQR)			21.2 (19.2, 23.8)	21.2 (19.2,23.8)
Undernutrition#¤: n (%)	**n=76**	**n=161**	**n=161**	**n=398**
Yes	13 (17.1)	50 (31.1)	28 (17.4)	91 (22.9)
Lung function	-	**n=140**	**n=152**	**n=292**
FEV_1pp ¤¤: n (%)				
> 70	-	107 (76.4)	62 (40.8)	169 (57.9)
40-70	-	30 (21.4)	67 (44.1)	97 (33.2)
<40	-	3 (2.1)	23 (15.1)	26 (8.9)
FEV_1pp¤¤: Median (IQR)	-	87.2 (71.3,102.0)	64.6 (50.1,83.1)	77.4 (58.1 91.8)
FEV_1z: n (%)	-			
≤ -1.0 ; > -2.0	-	30 (21.4)	25 (16.4)	55 (18.8)
≤ -2.0 ; > -3.0	-	15 (10.7)	28 (18.4)	43 (14.7)
≤ -3.0	-	26 (18.6)	70 (46.1)	96 (32.9)
FEV_1z ¤¤: Median (IQR)	-	-1.0 (-2.4,0.1)	-2.8 (-4.0, -1.4)	-1.9 (-3.4,-0.7)

Note: Excludes 20 not seen in 2018, 2 with previous liver transplants and 12 with previous lung transplants

BMIZ: body mass index z-score

WHZ: weight-for-height z-score

NTM: non-tuberculous mycobacterium

FEV_1z: Forced expiratory volume in one second z-score

FEV_1pp: Forced expiratory volume in one second percent predicted

ABPA: allergic bronchopulmonary aspergillosis

MSSA: methicillin sensitive *S. aureus*

MRSA: methicillin resistant *S. aureus*

[¤] Indicates significance of differences in characteristic by age group, $p<0.05$ and

[¤¤] $p<0.001$

[#] Undernutrition definition: World Health Organization (WHO) nutritional reference equation WHZ < -1 SD in children < 2 years age; BMI z-score (BMI kg/m2) < -1.0 children aged 2-17 years; and BMI < 18.5 kg/m2 in adults ≥ 18 years age.

[‡] includes liver cirrhosis with portal hypertension

*Unknown: Chronic pulmonary infection status was classified as unknown if less than four respiratory samples were submitted for culture during the year or the infection status of each pathogen could not be established from past medical records

Table 3.3 Unadjusted and adjusted associations with severe lung disease in children ≥ 6 years and adults in the SA CF registry, 2018

	Severe lung disease of $FEV_1z \leq -3.0$ (n=292)				
	Univariable analysis[#]			Multivariable analysis (n=190)	
	n	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Neonatal bowel obstruction (ref: no)	275	0.58 (0.28,1.19)	0.126	NS	
Age diagnosis (units: 10-years)	285	1.26 (0.90,1.76)	0.180	NS	
Current age (units: 10-years)	292	1.99 (1.55,2.54)	<0.001*	2.23 (1.50,3.31)	<0.001
p.Phe508del (ref: neither record)					
Homozygous	292	0.77 (0.37,1.57)	0.069	NS	
Heterozygous	292	1.45 (0.70,3.01)			
Caucasian (ref: other ancestry)	292	1.50 (0.83,2.72)	0.171	NS	
Ever had *P. aeruginosa* (ref: no)	279	4.36 (1.65,11.49)	0.001*	1.66 (0.34, 8.12)	0.529
Time since first *P. aeruginosa* isolate (per 10-year interval)†	133	2.12 (1.38,3.25)	<0.001*	1.25 (0.85, 1.84)	0.259
Chronic *P. aeruginosa* (ref: no)	201	3.81 (2.08,6.95)	<0.001*	1.98 (0.90, 4.34)	0.088
Chronic MSSA (ref: no)	198	0.57 (0.30,1.10)	0.089	NS	
Ever had MRSA (ref: no)	261	2.46 (0.98,6.18)	0.055	NS	
Chronic MRSA (ref: no)	198	8.88 (1.89,41.73)	0.001*	16.75 (1.74, 161.50)	0.015
Other fungus or mould (ref: no)	292	2.19 (1.22,3.93)	< 0.009*	1.31 (0.58, 2.93)	0.518
ABPA (ref: no)	261	0.29 (0.06,1.30)	0.064	NS	
Household cigarette smoke exposure/smoker (ref: no)	292	0.62 (0.30,1.28)	0.181	NS	
CF-related diabetes (ref: no)	286	3.79 (2.10,6.84)	<0.001*	1.03 (0.46, 2.33)	0.939
CF-liver disease with cirrhosis (ref: no)	282	2.87 (1.15,7.14)	0.073	NS	
Undernutrition[‡] (ref: no)	292	3.16 (1.72,5.83)	<0.001*	5.20 (2.23,12.13)	<0.001

[#] All variables with unadjusted *p*-values <0.2 in the univariable analyses are tabulated. Refer to **Online Supplement (Table 3)** for full set of univariable results.

* Unadjusted *p*-values are shown; indicated variables had adjusted *p*-values < 0.2 using Holm's method and were thus included in the multivariable regression model.

† Included as an interaction term, to apply only to those who have had ever had *P. aeruginosa*

[‡] Undernutrition includes: WHZ<-1.0, <2years age; BMIz<-1.0, 2-17 years; or BMI<18.5 kg/m^2, ≥18 yrs

ABPA: allergic bronchopulmonary aspergillosis

MSSA: methicillin sensitive S. aureus

MRSA: methicillin resistant S. aureus

Severe lung disease: $FEV_1z < -3$ in children ≥ 6 years

Table 3.4 Unadjusted and adjusted associations with undernutrition in children and adults in the SA CF registry, 2018

	Undernutrition‡ (n=398)				
	Univariable analysis#			Multivariable analysis (n=190)	
	n	Unadjusted OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
WAZ < -1.0 at diagnosis (ref: ≥ -1)	270	2.18 (1.07,4.44)	0.024*	excluded to preserve n due to large % missing	
Current age (unit: 10-years)	398	0.82 (0.66,1.01)	0.062	0.96 (0.76,1.21)	0.698
P.Phe508del (ref neither record)					
Homozygous	398	0.50 (0.27,0.92)	0.043	NS	
Heterozygous	398	0.85 (0.46,1.58)			
3120+1G>A hetero/homozygous	398	2.55 (1.42,4.58)	0.002*	1.32 (0.66,2.68)	0.438
Caucasian (ref: other ancestry)	398	0.35 (0.22,0.57)	<0.001 *	0.56 (0.28,1.12)	0.100
Receiving social welfare grant (ref: no)	398	2.99 (1.70,5.25)	<0.001 *	1.81 (0.92,3.57)	0.088
Private health insurance (ref: no)	398	0.46 (0.28,0.73)	<0.001 *	0.87 (0.46, 1.63)	0.661
Ever had MRSA (ref: no)	351	1.86 (0.80, 4.35)	0.165	NS	
Chronic MRSA (ref: no)	255	2.31 (0.77, 6.94)	0.145	NS	
Chronic *P. aeruginosa* (ref: no)	256	1.76 (1.00,3.12)	0.050	NS	

All variables with unadjusted *p*-values <0.2 in the univariable analyses are tabulated. Refer to **Online Supplement (Table 4)** for full set of univariable results.

* Unadjusted *p*-values are shown; indicated variables had adjusted *p*-values < 0.2 using Holm's method and were thus included in the multivariable regression model.

‡ Undernutrition includes: WHZ < -1.0, < 2 years age; BMIz < -1.0, 2-17 years; or BMI < 18.5 kg/m^2, ≥ 18 years

MRSA: methicillin resistant S. aureus

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We confirm and approve of including the above publication in his PhD dissertation, for which we are listed as co-authors. We further confirm that we are not currently registered as a postgraduate student nor intend to use this publication in our own or other postgraduate dissertations.

We agree that his role in the publication was: to conceptualise and design the study protocol; oversee data collection and analysis; data interpretation; drafting and critically revising the manuscript as first author; submitting and approving the final revised manuscript for publication. Our roles in the publication were the following.

Author name	Contribution	Signature
Reshma Kassanjee	To conceptualise and design the study protocol; perform data analysis and advise with data interpretation; draft and critically revise the manuscript, including tables and illustrations; approving the final revised manuscript for publication.	
Janine Verstraete	To conceptualise and design the study protocol; perform data collection and analysis; critically review and revise the manuscript, including tables; approving the final revised manuscript for publication.	
Marlize Frauendorf	To perform data collection, critically review the manuscript and approve the final revised manuscript for publication.	
Lesley Workman	To design data collection tool, data cleaning and analysis; critically review the manuscript and approving the final revised manuscript for publication.	
Heather J Zar	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and senior author; and approving the final revised manuscript for publication.	
Brenda M Morrow	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and co-author; and approve the final manuscript for publication.	

Chapter 4

Global disparities in cystic fibrosis outcomes prior to CFTR modulators: a CF registries cohort study in South Africa and Canada

Authors: Marco Zampoli (1, 2), Jenna Sykes (3), Janine Verstraete (1), Stephanie Y Cheng (4), Brenda Morrow (1), Michael S. Pepper (5), Cheryl Stewart (6), Heather J. Zar (1, 2) and Anne L. Stephenson (3)

Affiliations

1. Department of Paediatrics and Child Health, University of Cape Town, South Africa
2. South African MRC Unit for Child and Adolescent Health, University of Cape Town, South Africa
3. Department of Respirology, Adult Cystic Fibrosis Program, St. Michael's Hospital, University of Toronto, Toronto, Canada
4. Cystic Fibrosis Canada, Toronto, Canada
5. Institute for Cellular and Molecular Medicine, SAMRC Extramural Unit for Stem Cell Research and Therapy, Department of Immunology, University of Pretoria, South Africa
6. Department of Basic Medical Sciences, The University of the West Indies, Mona, Jamaica

Corresponding author:

Marco Zampoli, MD
Department of Paediatrics and Child Health
5th floor ICH Building
Red Cross War Memorial Children's Hospital
Klipfontein Rd, Rondebosch
7700
Cape Town, South Africa
Tel: +27 216585309
Email: m.zampoli@uct.ac.za

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Abstract

Background: Outcomes of cystic fibrosis (CF) differ between low-middle income and high-income countries, but comparative data are lacking. We compared South African (SA) and Canadian CF outcomes to explore what disparities existed prior to access of CFTR modulators in Canada.

Methods: A cross-sectional study of SA and Canadian CF registries data for period 1 January to 31 December 2018. CF registry data were harmonised between countries to compare lung function and nutrition outcomes. Poor nutrition was defined as BMI z-score < -1 in children and < 18.5 kg/m^2 in adults. Standardised mean difference (SMD) >10 was considered significant.

Results: After excluding Canadians on CFTR modulators and lung transplant recipients, data on 4049 Canadian and 446 SA people were analysed. Compared to Canada, people in SA were younger (median age 15.8 years vs. 24.1 years: SMD 52) with fewer males (47.8% vs 54.2%; SMD 12.5) and White (70.9% vs. 93.3%; SMD 61.3). Class I-III *CFTR* mutation frequency was similar in SA (n= 384, 86.1%) and Canada (n=3426, 84.9%). After adjusting for age, gender, diagnosis age, genotype, *P. aeruginosa* infection and pulmonary treatments, FEV1pp was 8% lower (95% CI 5.4% to 10.7%) and poor nutrition 1.5-fold more common (OR 1.56; 95% CI 1.12-2.16) in SA compared to Canada.

Conclusion: Lung function and nutrition was significantly lower in SA compared to Canada. Global disparities in CF outcomes between high and low-middle income countries are likely to widen as CFTR modulators are rapidly scaled up in only high-income countries.

Key words: Outcome disparities; cystic fibrosis; South Africa; Canada

Introduction

International cystic fibrosis (CF) registries from high income countries (HIC) have documented improving CF survival estimates over past decades (1). Improving survival age is attributed to many factors including newborn screening (NBS), improved nutritional interventions, active surveillance, prevention and treatment of infections and organ transplantation (2). In addition, novel cystic fibrosis transmembrane conductance regulator (CFTR) modulator drug therapy is anticipated to significantly further improve CF outcomes and survival (3, 4). In contrast to HIC, less is known about the epidemiology and outcomes of CF populations in low-and middle-income countries (LMIC) where conditions of poverty, poor healthcare infrastructure and limited resources to diagnose CF prevail. Furthermore, ethnicity and CF genotype differ significantly in LMIC compared to HIC with populations of predominantly European descent (5, 6). Studies reporting *CFTR* mutation prevalence in Brazil and

Africa have described higher rates of uncommon or novel mutations than HIC, which may be an important determinant of CF outcomes in these settings (6, 7). However, factors such as socioeconomic status (SES), living conditions, delayed CF diagnosis, nutrition, pulmonary infections, and limited access to expensive CF therapies may be more important determinates of CF outcomes in LMIC compared to HIC.

South Africa (SA) is a middle income country with a population of nearly 60 million with diverse ancestry and conditions of extreme socioeconomic disparity (8). Healthcare infrastructure and services are provided through a resource-constrained public health system and well-resourced private healthcare. Newborn screening for CF is not available. Essential CF care and CF medications including pancreatic enzyme replacement therapy (PERT) are available, but expensive CF therapies such as inhaled tobramycin solution, recombinant DNase are scarce and CFTR modulator therapies are not available. Poor nutrition, methicillin resistant staphylococcus aureus (MRSA) infection and non-White ancestry have all been associated with poorer CF outcomes in SA (9, 10). In contrast, Canada is an HIC with a population of almost 40 million. Universal healthcare including NBS for CF is provided for free, which allows Canadians to access most CF medical care without additional personal financial costs. The majority of essential CF medications and lung transplants are paid for by government programs including the early CFTR modulator drugs (ivacaftor), prior to 2018 for a small number of people with CF (pwCF) who were eligible.

Examining differences in health outcomes between CF care centres has spawned a body of evidence focused on quality improvement initiatives in an effort to explain such differences and improve outcomes (1, 11). Similarly, international comparisons of the CF population through registries have led to important research to understand the driving factors contributing to disparate CF outcomes (12, 13). Comparing current CF health outcomes in SA and Canada, and factors that potentially explain any difference in outcomes, may be helpful for planning targeted interventions and advocating for improved access to novel therapies where outcomes are less favourable. We, therefore, harmonised data in the SA and Canadian CF registries to compare demographic, clinical and health outcomes characteristics between pwCF receiving care in SA and Canada at a time prior to when CFTR modulator therapy was more widely available in Canada.

Methods

Study design and populations

This is a cross-sectional cohort study of CF populations captured in the SA and Canadian CF registries for the calendar period 1 January to 31 December 2018. The Canadian CF Registry (CCFR) was

developed in the 1970s and is managed by Cystic Fibrosis Canada. Consented data are entered into the CCFR by the 42 CF centres across Canada. Universal access to health care and incentivised data entry programs for participating CF centres within Canada ensure that the majority of pwCF are captured in the CCFR. The SA CF Registry (SACFR) is a public-private collaboration that was launched in 2018 and includes CF care centers at six university hospitals and several private practice clinics across SA with expertise in CF care. Consented annual review registry data are collected by independent data capturers covering different regions of SA, who extract and capture registry data from medical records at each participating clinic or practice. By 31 December 2018, it was estimated that over 80% of the known SA CF population in care was captured in the SA CF registry (9).

Harmonization of CF registry data

A combined data dictionary outlining variable definitions was systematically created using a mapping strategy in order to make data directly comparable (see **On-line Supplement B**). Demographic, CF diagnosis and genotype information were collected. Genotype was classified as homozygous F508del, heterozygous F508del, not F508del and missing. *CFTR* mutations were further categorised as class I-III and class IV-VI. Clinical information in 2018 on sputum microbiology, CF-related complications, CF therapies including number of courses of intravenous antibiotics, were recorded from both countries. Due to inability to harmonize most sputum microbiology data captured in the registries as a result of differing data collection definitions, sputum microbiology reporting was restricted to any isolate of *P. aeruginosa,* methicillin resistant *staphylococcus aureus* (MRSA) *and* non-tuberculous *mycobacterium species* (NTM).

Outcomes

Lung function (LF) was measured by forced expiratory volume in one second (FEV_1) expressed as a percentage of the predicted values (FEV_1pp) for healthy age, race and sex-matched controls using Global Lung Initiative (GLI) reference equations (14). Non-White people in SA were entered as "Other" ethnicity in GLI equations in line with published guidelines (15). In order to harmonize LF measurements, the highest recorded stable pre-bronchodilator FEV_1pp in 2018 for subjects six years and older were selected from the SA and Canadian registries as this is how FEV1pp is captured in the SA CF registry. Lung function impairment was classified as severe (FEV_1pp ≤ 40), moderate (FEV_1pp 41-69), mild (FEV_1pp 70-89) and normal (FEV_1pp ≥ 90). In the event of death, lung transplantation or starting CFTR modulator therapy in 2018, patients were included but the FEV_1pp measurements in 2018 prior to these events were included in analyses. People who started CFTR modulators, died or were transplanted prior to 2018 were excluded from formal analyses (**Figure 4.1**).

Body mass index (BMI) measurements were selected from the same day as FEV_1 measurements. BMI z-scores (BMIz) were calculated for children 0-18 years using World Health Organization reference equations (16). For individuals 18 years of age and older, BMI (kg/m^2) was calculated and classified according to World Health Organization guidelines. Poor nutrition was defined as BMIz < -1 for age < 18 years or BMI < 18.5 kg/m^2 for age 18 years and older; overweight was defined as BMIz > 2 for age <18 years or BMI >24.9 kg/m^2 for age 18 years and older.

Statistical methods

Demographic and clinical variables were summarized by country, with categorical variables expressed as frequency and proportion, and continuous variables as means with standard deviation (SD) if parametric and medians with range if non-parametric. Differences between countries were compared using the Mann-Whitney test for continuous variables and the Chi-square test for categorical variables. Standardised mean difference (SMD) was calculated to identify statistically and clinically meaningful differences between the two countries, with an SMD of greater than 10 as the threshold (17). The magnitude of difference in LF and poor nutrition between the two countries was estimated by linear regression and logistic regression, respectively. Confounders were chosen *a priori* based on clinical knowledge and prior literature and included: age, sex, genotype, diagnosis age, any *P. aeruginosa* infection, poor nutrition, and the pulmonary treatments recombinant human DNAse (rhDNAse) and macrolides. BMI was dichotomized as poor nutrition against the combined categories of adequate weight and overweight. Statistical analysis was conducted using R version 4.0.3. All *p*-values are two-sided and assessed at $p<0.05$.

Ethical considerations

Approval has been obtained for the University of Cape Town Research Ethics Committee (HREC 032/2019) SA, and St. Michael's Hospital (REB 20-310), Toronto. Informed consent and assents were collected in SA and Canada in accordance with local and institutional and registry requirements.

Results

Demographic and diagnosis information

Data on 4049 Canadian and 446 SA pwCF in 2018 were analysed (**Table 4.1** and **Figure 4.1**). Compared to Canada, the CF population in SA was younger (median age 15.8 years vs. 24.1 years: SMD 52) with a lower proportion of males (47.8% vs 54.2%; SMD 12.5) and White people (70.9% vs. 93.3%; SMD 61.3). Age of CF diagnosis was slightly younger in Canada and more children were diagnosed by NBS in Canada compared to SA (n=550, 13.6% vs n=1, 0.2%, SMD 54.6); the one case in SA was diagnosed by

NBS in California, USA, and the family relocated to SA. Poor nutrition at time of diagnosis was present significantly more in SA compared to Canada (n=131, 53.5% vs n= 1117, 35.5%: SMD 36.8).

Sixty-one (1.3%) and 12 (2.7%) pwCF in Canada and SA, respectively, had incomplete genotyping recorded in the CF registries (**Table 4.1**). F508del was the most common *CFTR* mutation in both countries with similar proportions of people who were homozygous for F508del (SA 47.8% vs. Canada 42.4%). In SA, 3120+1G>A was the second most prevalent mutation (allele frequency 9.9%) and in Canada, 621+1G->T the second most prevalent mutation (allele frequency 2.0%). Overall, *CFTR* mutations class I-III were present with similar frequency in SA (n= 384, 86.1%) and Canada (n=3426, 84.9%).

Microbiology

P. aeruginosa was isolated in 2018 more frequently in SA than Canada (SA n= 192, 43.0% vs Canada n= 1458, 36.0%; SMD 26.3). Infection rates of MRSA and NTM were similar in both countries. Comparison of other infections or chronic infection status between countries could not be reliably determined due to different definitions and data capturing approaches.

Complications and CF therapies

The frequency of allergic bronchopulmonary aspergillosis (ABPA) and CF-related liver disease was similar in both countries (**Table 4.2**). CF-related diabetes (Canada 22.2% vs SA 15.9%; SMD 12.7) and pancreatic sufficiency (Canada 18% vs SA 10%; SMD 22) was more common in Canada than SA. Azithromycin and hypertonic saline inhalations were prescribed more frequently in SA and rhDNAse more frequently used in Canada (**Table 4.2**). More pwCF in SA than Canada received one or more courses of intravenous antibiotics (SA n= 202, 45.3% vs Canada n= 969, 23.9%; SMD 46.4). Three hundred and seventy-six (9.3%) pwCF in Canada had received a lung transplant in 2018 or earlier compared to 11 (2.5%) in SA.

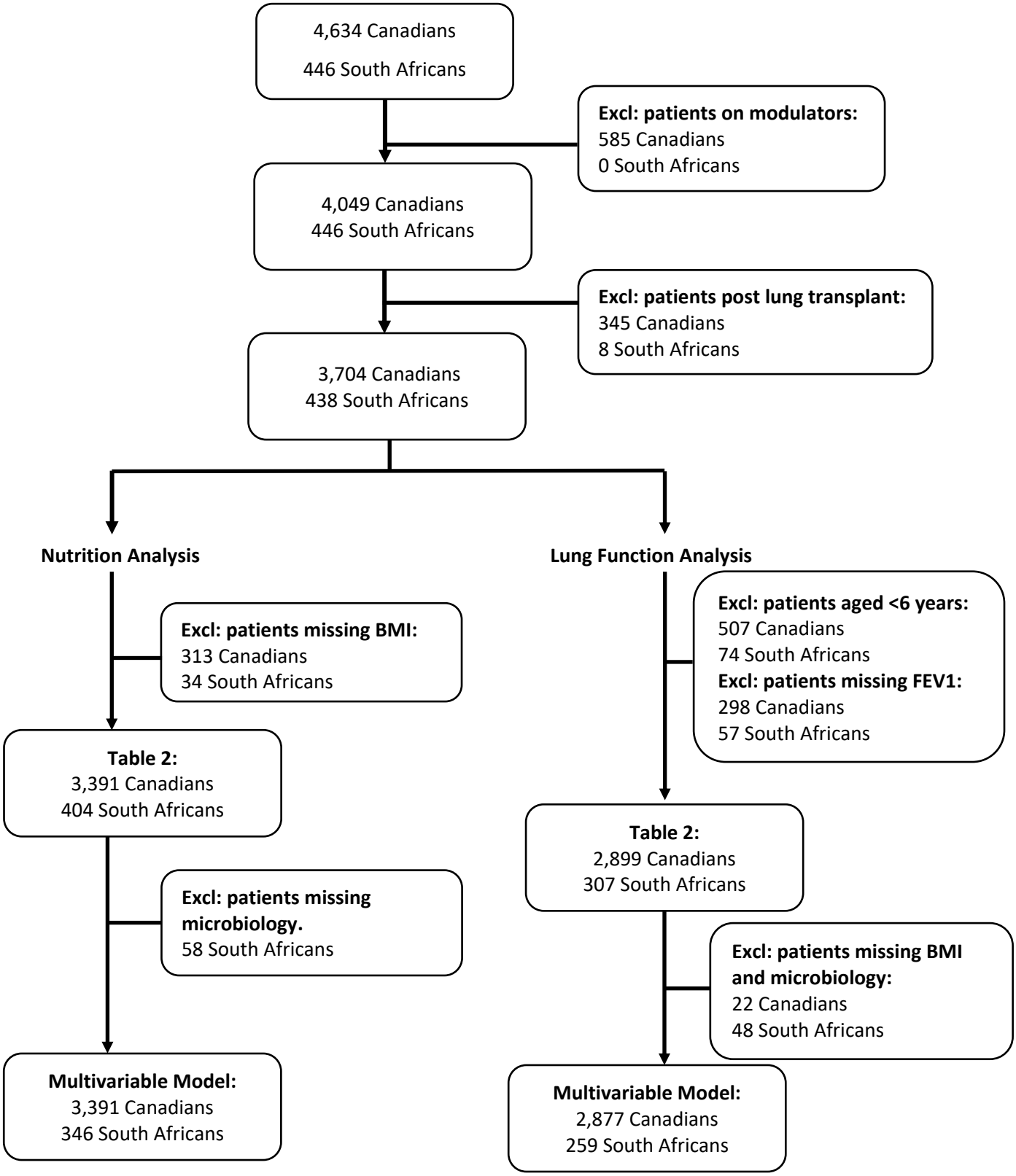

Figure 4.1 Cohort flow diagram for Canadian and South African CF registry participant selection – 1 January to 31 December 2018

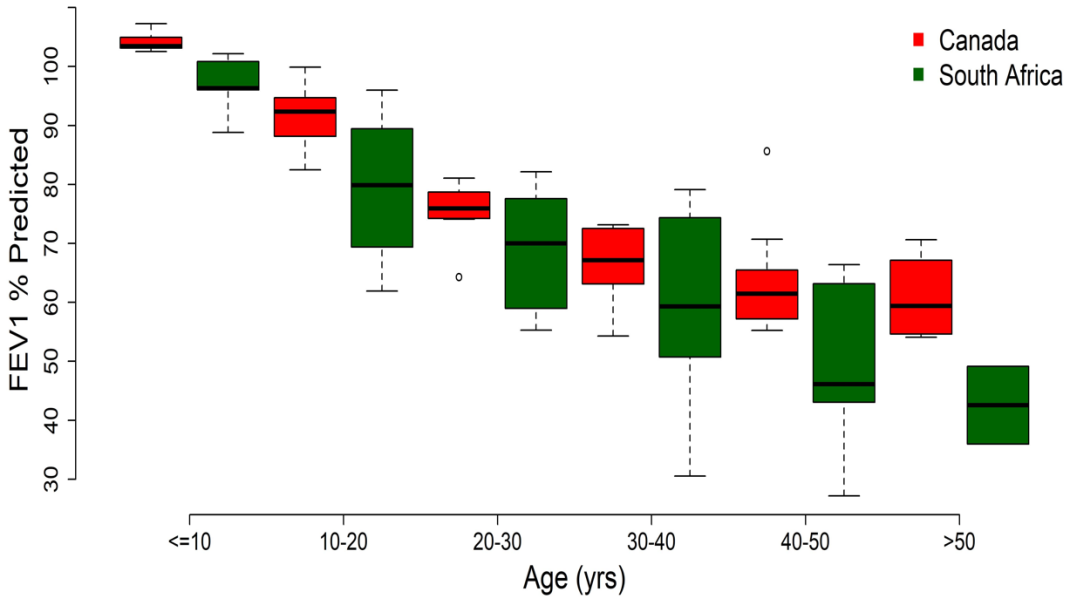

Figure 4.2 Comparison of lung function (FEV1pp) by age categories (6 years and older) between the South African and Canadian CF Registry cohorts in 2018.

Notes: Horizontal bar represents median value and boxes 25-75% IQR; maximum and minimum range end indicated by thin horizontal bars

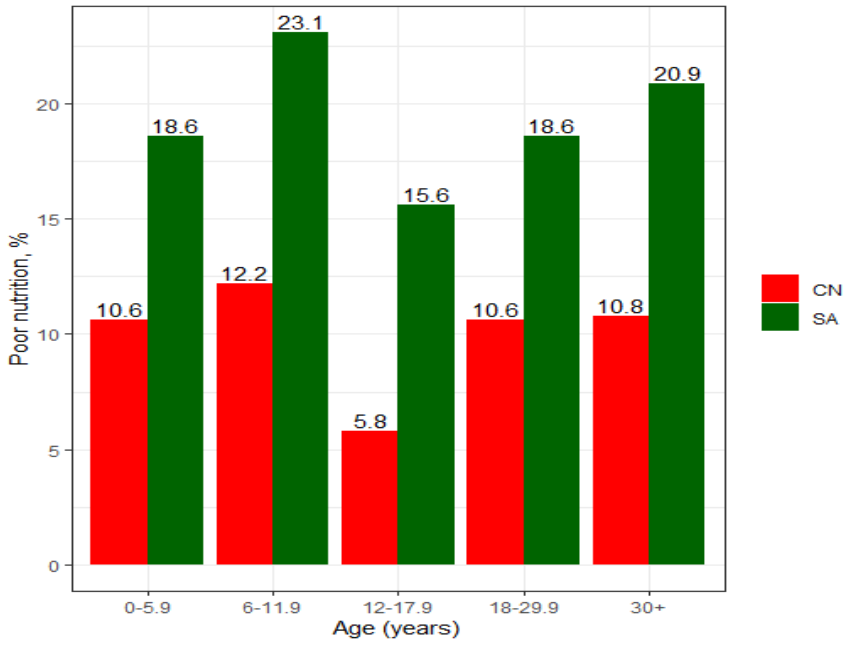

Figure 4.3 Proportion (as percentage) of people with CF in the South African and Canadian CF Registry cohorts with poor nutrition in 2018, by age categories.

Notes: Poor nutrition was defined as BMI z-score < -1 for age < 18 years or BMI < 18.5 kg/m2 for age 18 years and older; differences between countries in all categories are statistically significant (SMD ≥ 10).

Outcomes

Forty-two (1.0%) and three (0.7%) adults in Canada and SA died in 2018, respectively (**Table 4.2**). There were no deaths in children less than 18 years in either country in 2018. Details of the number of LF and nutrition measurements in 2018 in pooled analysis are presented in **Figure 4.1**.

Lung function

Lung function was significantly lower across all ages in SA compared to Canada, including children 6-11.9 years age (**Figure 4.2**). After adjusting for age, sex, genotype, diagnosis age, any *P. aeruginosa* infection, poor nutrition, and pulmonary treatments, FEV_1pp was 8% lower in SA compared to Canada (95% CI 5.4% to 10.7%; *p*-value <0.0001) (**Figure 4.3**). The impact of nutrition on LF did not differ significantly by country.

Nutrition

Poor nutrition was significantly more prevalent across all age groups in SA compared to Canada (SA 91, 22.5% vs Canada 373, 11.0%: SMD 31.2) including children 0-5.9 years (**Table 4.2** and **Figure 4.3**). After adjusting for age, sex, genotype, diagnosis age and any *P. aeruginosa* infection, poor nutrition was 1.5-fold more common in SA compared to Canada (OR 1.56; 95% CI 1.12-2.16). Other factors associated with poor nutrition in both countries were age of diagnosis 2-18 years, *P. aeruginosa* and pulmonary treatments (**Table 4.3**).

Discussion

This registry-based study comparing health outcomes between SA and Canada has identified significant health disparities, specifically less favourable LF and nutrition outcomes in SA compared to Canada that are attributable to factors over and above age, *CFTR* mutation class, sex, diagnosis age, *P. aeruginosa* infection, lung transplantation and CFTR modulator therapy. A further observation is that these differences were already present in early childhood suggesting early life health factors may be important determinants for poorer outcomes observed in SA. This study was not specifically designed to identify which additional factors were associated with poorer outcomes in SA. However, it is reasonable to assume that these may include lower SES, inferior standards of CF care, limited access to publicly funded health care and expensive therapies such as rhDNase and inhaled tobramycin. Socioeconomic conditions and quality of healthcare delivery are important determinants of CF outcomes, even in high income settings (18, 19). We could not include robust measures of SES as these were not captured by either registry. However, the gross domestic product per capita of Canada is 7-fold higher than SA which indirectly reflects the difference in healthcare expenditure between the

countries (20). Although lung transplant recipients and pwCF on CFTR modulators were excluded from the formal analyses, inclusion of these factors would likely contribute to a wider difference in observed health outcomes between SA and Canada. Documenting and monitoring differences in CF-related outcomes is important in advocacy for improved quality of CF care and equitable global access to CFTR modulators (21).

A strength of this study is it illustrates the value of CF registries and how they can contribute to our understanding of CF epidemiology and outcomes in different global and socioeconomic settings. However, harmonization of registry variables as required in our study is key to meaningful comparisons between different registry populations (22). Our study is the first attempt to directly compare CF outcomes between a LMIC and HIC and highlights important disparities after adjusting for known factors and differences in genotype and ancestry characteristics between countries. South Africa shares similarities in CF epidemiology and unequal or limited health care resources with other LMIC. The disparity findings of our study are therefore relevant to other LMIC settings. Poor nutrition across all ages was more prevalent in SA compared to Canada and was the strongest factor negatively impacting LF in the adjusted multivariable analysis. Poor nutrition is an important modifiable aspect of CF care which needs greater attention in SA as similarly demonstrated in previous studies comparing nutrition outcomes between countries (22). In addition, improved sputum surveillance and more aggressive *P. aeruginosa* treatment is another aspect of CF care which could be improved in SA.

People in LMIC represent 10% of an estimated 105,352 known diagnosed pwCF in 45 countries across the world of which only 12% are receiving triple combination CFTR modulator therapy (23). However, the hidden global burden of undiagnosed CF disease is uncertain but estimated to be substantially greater than currently known and compounded by a paucity of high-quality data and limited capacity to diagnose CF in many LMIC (23). Highlighting existing global disparities is especially important at a time when CFTR modulator therapy is rapidly becoming the standard of care in most HIC. Simulated projections of the Canadian CF Registry population have estimated that universal introduction of triple combination CFTR modulator therapy (tezacaftor/ivacaftor/elexacaftor) in 2021, would by 2030, reduce the number of CF-related deaths by 15% (4). The delay and unlikely availability of CFTR modulator drugs in LMIC for a long time due to prohibitive pricing and patent protection laws, will undoubtedly lead to further widening of global disparities in CF outcomes between LMIC and HIC (24, 25). Ironically, earlier access to CFTR modulator therapy in LMIC will likely lessen the burden and complexity of CF care in LMIC due to less severe disease and fewer CF-related complications.

Another disparity highlighted by our study is the absence of NBS for CF in SA and the impact this likely has on explaining the lower nutritional status seen in the SA cohort. Although only 13% of Canadians

with CF in 2018 were diagnosed by NBS and median diagnosis age was slightly younger in Canada compared to SA, evidence that NBS improves long-term CF outcomes and survival is unequivocal (26). Poorer nutrition and LF outcomes were present already in young SA children compared to children in Canada and supports the need to explore the feasibility of NBS for CF in SA. Although challenging and seldom prioritised by governments in LMIC, NBS for CF has been successfully implemented in several LMIC (26-29).

Our study has several limitations which were taken into consideration with interpretation of the findings. First, missing registry data significantly diminished the sample size of the formal analyses which reduced the precision of our estimates. Second, the SA registry data is unlikely to be an exhaustive representation of the CF population in SA as the true incidence, including undiagnosed cases of CF in SA, especially in the non-White population, is unknown. There is insufficient population data to accurately predict the expected incidence of CF in the majority, Black African SA population, but it is significantly lower than in White and mixed-race populations (30). Data of the 446 SA patients in this study were extracted from the SA CF registry after its first year of inception in 2018 and is, in our view, the best representation of the available background diagnosed CF population in SA at the time. Follow-up and retention in the SA registry is high and it is unlikely that there are significant numbers of pwCF receiving care outside the designated CF clinics in SA, nor is it likely that CF-related deaths in SA registry patients were missed. Third, cross-sectional analysis of registry cohorts limits the interpretation of observed age-trends as they cannot be interpreted as average trends of individuals over time. Longitudinal analysis of individual trends over time between SA and Canada will enhance the interpretability of the observed differences observed in this study. Based on established knowledge, we selected variables *a priori* to include in the multivariable models for LF and nutrition outcomes. It is possible that inclusion of more variables such as a wider range of pulmonary infections might alter the overall differences in outcomes between countries due to unmeasured interactions. However, harmonization of all pulmonary infection definitions between the two registries was not possible, thus limiting the inclusion of other infections in the models. A slightly greater proportion of pancreatic sufficient pwCF in Canada compared to SA may be a factor contributing to better nutrition and LF outcomes in Canada. However, the proportional difference of people with pancreatic sufficiency is small and unlikely to be statistically significant. In addition, adjusting for class IV-VI *CFTR* mutations and older age of diagnosis would have accounted for the differences in proportions of pwCF with milder CF phenotypes with pancreatic sufficiency between the two countries.

In conclusion, this study has demonstrated the value of CF registry-based research and has highlighted important disparities in CF outcomes between a HIC and LMIC settings, which has global relevance for

countries with similar socioeconomic conditions and challenges impacting on CF care. In addition to improving the quality of established CF care, affordable and equitable global access to CFTR modulators in LMIC must be prioritised in order to reduce the inevitable widening CF outcome disparities between HIC and LMIC that will result from CFTR modulators becoming standard of care in HIC across the world.

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Name	Affiliation
Dr Paul Gebers	Netcare Greenacres Hospital and Life St George's Hospital, Port Elizabeth
Dr Susan Klugman Dr Cathy Baird	Charlotte Maxeke Academic Hospital, University of the Witwatersrand, Johannesburg
Dr Tumi Pitso Prof Robin Green	Steve Biko Academic Hospital, University of Pretoria
Dr Cathy Baird	Netcare Milpark Hospital, Johannesburg
Dr Carla Els	Netcare Linksfield Hospital, Johannesburg
Dr Dave Richard	Sandton Medi-Clinic, Johannesburg
Prof Refiloe Masekela Dr Reratilwe Mphahlele	Inkosi Albert Luthuli Central Hospital, University of KwaZulul Natal, Durban
Dr Graham Lawrence	Busamed Hillcrest Private Hospital, Durban
Dr Jonathan Egner	Netcare St Augustine's Hospital, Durban
Dr Fiona Kritzinger Dr Taryn Gray	The Chest and Allergy Centre, Christian Barnard Memorial Hospital, Cape Town
Dr Julie Morrison Prof Pierre Goussard	Tygerberg Hospital, University of Stellenbosch, Cape Town
Prof Greg Calligaro	Groote Schuur Hospital, University of Cape Town
Prof Paul Wilcox	UCT Private Academic Hospital, Cape Town
Dr Tony Biebuyck	Panorama Medi-Clinic Hospital, Cape Town

Table 4.1 Demographic and diagnosis information among the CF population in Canada and South Africa, December 2018, excluding people on CFTR modulatory therapy

	Canada (N=4,049)	**South Africa (N=446)**	**SMD**
Sex: n (%)			
Female	1856 (45.8)	233 (52.2)	12.8
Male	2193 (54.2)	213 (47.8)	
Age in years, median (range)	24 (0.1-82.5)	15.8 (0-53.6)	52
Age in years: n (%)			
0-5.9 years	507 (12.5)	74 (16.6)	11.6
6-11.9 years	514 (12.7)	96 (21.5)	23.6
12-17.9 years	505 (12.5)	77 (17.3)	13.5
18-29.9 years	1061 (26.2)	121 (27.1)	2.1
≥ 30 years	1462 (36.1)	78 (17.5)	43
Race: n (%)			
White	3765 (93)	316 (70.9)	60
Mixed	28 (0.7)	85 (19.1)	64.7
Black African	34 (0.8)	41 (9.2)	39
Other/Unknown	222 (5.5)	4 (0.9)	26.3
Diagnosis age in years, median (range)	0.5 (0-75.8)	0.6 (0-47.8)	17.6
Diagnosis age in years: n (%)			
0-2	2696 (66.6)	307 (68.8)	4.8
2-18	1008 (24.9)	119 (26.7)	4.1
≥ 18	345 (8.5)	20 (4.5)	16.4
Newborn screening diagnosis: n (%)	550 (13.6)	1 (0.2)	54.6
Meconium ileus: n (%)	519 (12.8)	68 (15.2)	7.0
Nutritional status at diagnosis	n=3146	n=245	
Normal weight	1863 (59.2)	107 (43.7)	31.5
Poor nutrition	1117 (35.5)	131 (53.5)	36.8
Overweight	166 (5.3)	7 (2.9)	12.3
Sweat test at diagnosis, mean (SD)			
sweat chloride test (mmol/L)	92.8 (23.8)	107.4 (21.9)	63.5
sweat conductivity (mmol/L)	N/A	105.8 (19.5)	N/A
no sweat test documented: n (%)	1879 (46.4)	152 (34.1)	25.3
Genotype: n (%)			
F508del:			
Homozygous	1715 (42.4)	213 (47.8)	10.9
Heterozygous	1774 (43.8)	145 (32.5)	23.4
Other	499 (12.3)	76 (17)	13.4
Missing/Incomplete genotyping (one or two unknown *CFTR* variants)	61 (1.5)	12 (2.7)	8.3
Mutation class: n (%)			
I - III	3426 (84.6)	384 (86.1)	7.8
IV - VI	502 (12.4)	44 (9.9)	
Missing/unclassified	121 (3)	18 (4)	5.7
Most common *CFTR* mutation allele frequencies: alleles, n (%)			
F508del	3,436 (84.9)	358 (80.3)	N/A

3120+1G>A	0	44 (9.9)	
621+1G->T	80 (2.0)	0	
Unknown	65 (1.6)	12 (2.7)	
3272-26A>G	0	7 (1.6)	
Other	0	7 (1.6)	
G551D	0	3 (0.7)	
M1101K	41 (1.0)	0	
G542X	35 (0.9)	0	
711+1G->T	25 (0.6)	0	
A455E	21 (0.5)	5 (1.1)	
N1303K	21 (0.5)	5 (1.1)	
L206W	19 (0.5)	-	
3849+10kbC>T	0	1 (0.2)	
R1162X	0	1 (0.2)	
W1282X	15 (0.4)		

SMD: Standardised mean difference, values > 10 indicate clinically important differences

Table 4.2 Clinical, lung function and nutritional characteristics of CF registry populations in South Africa and Canada in 2018, excluding people on CFTR modulatory therapy

	Canada (N=3,391)	**South Africa (N=404)**	**SMD**
Microbiology: n (%)			
Any *P. aeruginosa*	1458 (36)	192 (43)	26.3
Missing	157 (3.9)	65 (14.6)	37.6
Any MRSA	243 (6)	28 (6.3)	5.5
Missing	157 (3.9)	80 (17.9)	46.3
Any NTM isolate	197 (4.9)	5 (1.1)	21.2
Missing	157 (3.9)	77 (17.3)	44.6
Pulmonary therapies: n (%)			
Hypertonic saline	1523 (37.6)	211 (50.6)	26.4
Recombinant human DNase	1904 (47)	122 (29.2)	37.4
Inhaled antibiotics	2250 (55.6)	224 (53.8)	3.5
Azithromycin	1527 (37.7)	335 (80.3)	96.2
Intravenous antibiotic courses in 2018			
0	3080 (76.1)	244 (54.7)	46.1
1	579 (14.3)	101 (22.6)	21.6
2	224 (5.5)	52 (11.7)	22
≥ 3	166 (4.1)	49 (11)	26.3
Lung transplant recipients (2018 or earlier)	376 (9.3)	11 (2.5)	28.9
Liver transplant recipients (2018 or earlier)	28 (0.7)	0 (0)	N/A
Complications/comorbidity: n (%)			
ABPA	70 (1.8)	20 (5.2)	18.7
CF-related diabetes	900 (22.2)	71 (17.2)	12.7
CF-related liver disease with cirrhosis	135 (3.3)	22 (5.4)	10
Pancreatic insufficient	3310 (82.1)	377 (89.8)	22.1
Vital status in 2018			
Alive on 31 December 2018	4007 (99)	443 (99.3)	4
Died in 2018	42 (1)	3 (0.7)	4
Age at death (years): median (range)	33.7 (13.6-82.4)	36 (32-38)	7.4
Alive, but not seen in 2018	156 (3.9)	21 (4.7)	4.2
Nutritional status: n (%)			

Normal weight	2448 (72.2)	280 (69.3)	6.3
Overweight	570 (16.8)	33 (8.2)	26.4
Poor nutrition	373 (11.0)	91 (22.5)	31.2
Proportion with poor nutrition: n (%)			
F508del homozygous	186/1429 (13%)	38/194 (19.6%)	17.9
F508del heterozygous	137/1494 (9.2%)	32/133 (24.1%)	40.8
Class I-III mutation	328/2882 (11.4%)	79/353 (22.4%)	29.7
Class IV-V mutation	30/411 (7.3%)	7/35 (20%)	37.6
Lung function (age ≥ 6 years)	**n=2899**	**n=307**	
FEV_1pp: n (%)			
≥ 70	1962 (67.7)	171 (55.7)	24.8
40-69	705 (24.3)	101 (32.9)	19.1
< 40	232 (8)	35 (11.4)	11.5
FEV_1pp: Median (range)			
F508del homozygous	82.9 (61-98.7); N=1296	78 (60.8-92.9); N=180	15.6
F508del heterozygous	86.3 (61.3-100.2); N=466	78 (53.1-92.7); N=64	29.4
Other/missing genotype	84.9 (64.1-100.2); N=1435	64.1 (51.7-85.8); N=120	56.8
Class I-III mutation	83 (61.7-99.2); N=2650	75.9 (56.2-90.1); N=310	27.7
Class IV-V mutation	93 (71.1-103.2); N=438	61.6 (51.4-85.1); N=40	74.4

NTM: non-tuberculous mycobacterium

FEV_1z: Forced expiratory volume in one second z-score

FEV_1pp: Forced expiratory volume in one second percent predicted

ABPA: allergic bronchopulmonary aspergillosis

MRSA: methicillin resistant *S. aureus*

SMD: Standardised mean difference, values >10 indicate clinically important differences

Table 4.3 Adjusted multivariable analyses comparing lung function (age 6 years and older) and nutrition outcomes between South African (SA) and Canadian (CN) CF registry cohorts 2018

	Lung function (N=2877 CN, 255 SA) #		**Poor nutrition (N= 3391 CN, 346 SA) # †**	
Variable	**Coefficient (95% CI)**	***P*-value**	**Odds Ratio (95% CI)**	***P*-value**
Intercept	90.19 (88.49 – 91.9)	<0.0001	-	-
Country (SA vs CN)	-8.12 (-10.78 – -5.46)	<0.0001	1.56 (1.12 – 2.16)	0.0078
Sex (Male vs female)	-1.04 (-2.41 – 0.32)	0.14	0.88 (0.72 – 1.08)	0.23
Mutation class				
I-III	Ref		Ref	
IV-V	5.07 (2.82 – 7.32)	<0.0001	0.92 (0.63 – 1.35)	0.68
Missing	-2.35 (-6.26 – 1.55)	0.24	1.72 (1.01 – 2.91)	0.045
Age (centered at 18 years)			0.97 (0.96 – 0.98)	<0.0001
* Age-18 – term 1	-679.79 (-727.86 – -631.72)	<0.0001		
*Age-18 – term 2	221.8 (181.58 – 262.02)	<0.0001		
Age at diagnosis				
<2 years	Ref		Ref	
2-18 years	1.3 (-0.26 – 2.85)	0.10	-	
≥ 18 years	11.07 (8.12 – 14.02)	<0.0001	-	
Any *P. aeruginosa*	-2.89 (-4.38 – -1.4)	0.00015	1.2 (0.96 – 1.51)	0.11
Azithromycin	-9.5 (-11.11 – -7.88)	<0.0001	1.45 (1.12 – 1.88)	0.0045
rhDNase	-7.18 (-8.63 – -5.73)	<0.0001	1.37 (1.1 – 1.71)	0.0055
Poor nutrition	-16.01 (-18.12 – -13.9)	<0.0001	-	

*Age was modeled as a quadratic relationship between age and lung function

Excluding lung transplant recipients, people started on CFTR modulator therapy before 2018 and children less than 6 years (no lung function recorded)

†Lung function excluded from the nutrition modelling to not exclude children less than 6 years without LF measurements

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On-line Supplement B (Chapter 4/Publication 3)

Cystic fibrosis registries diagnosis inclusion criteria

Patients captured on the Canadian Cystic Fibrosis Registry (CCFR) and the South African CF Registry (SACFR) for the calendar year 2018 were included according to the diagnostic criteria below. For both countries: 1) patients consented to have their data collected by the registries, and 2) were alive in the year 2018.

Diagnostic Inclusion Criteria:

Canadian Registry:

For a diagnosis of CF, the patient must meet the standard guidelines for having CF (based on clinical symptoms, sweat testing, genotyping; J Pediatr 2008).

SACFR:

Two sweat tests value > 60 mmol/L chloride: CF diagnosis accepted.

One sweat test value > 60 mmol/L chloride or sweat conductivity > 90 mmol/L and DNA Analysis/Genotyping – two identified disease-causing CF mutations as reported in CFTR2 database: CF diagnosis accepted; or

Sweat value less than or equal to 60 mmol/L chloride then both these should be fulfilled:

a. DNA Analysis/Genotyping – two identified disease-causing CF mutations; and

b. Clinical Presentation consistent with typical or atypical CF.

Sweat conductivity without DNA confirmation of two disease causing *CFTR* mutations as reported in CFTR2 database will be accepted for SA registry only if both following criteria are met:

a. clinical presentation consistent with typical CF

b. at least two separate sweat conductivity measurements ≥ 90 mmol/l

Table 4.4 Explanation of data variables extracted from the Canadian and South African Cystic Fibrosis Registries

Variable	Description	
Demographic Data		
Age	Calculated as age in years from Date of Birth (DOB) to 31 December 2018. DOB was set to the 15th of the month in both registries to protect confidentiality.	
Sex	Male or Female	
Ancestry/Ethnicity	*South Africa* Caucasian Black African Other: Mixed race, Asian, Indian Unknown	*Canada* Caucasian Black Other: Asian, South Asian, First Nations People, Hispanic, two or more races Unknown
Diagnosis		
Diagnosed by newborn screening	Yes, No Not available in SA	
Meconium Ileus	Yes, No or Unknown	
Age of diagnosis	Calculated from DOB to Date of Diagnosis in months	
Genotype categorised	Categorised as: p.Phe508del homozygous (Yes/No) p.Phe508del heterozygous (Yes/No) Other/Not p.Phe508del Unknown	
CFTR mutation class	Categorised as: Class I-III (minimal function) Class IV-VI (residual function)	
Mutation allele frequency	Frequency of 10 most frequently occurring Allele mutations	

	Includes category for unknown mutation
Sweat test	Chloride value in mmol/L If two chloride values are recorded the first sweat test value should be calculated for that patient for analysis.
Sweat conductivity (SACFR only)	Conductivity value in mmol/L If two conductivity values are recorded the first value should be calculated for that patient for analysis.
Lung Function	
FEV_1 Litres	The best pre-bronchodilator FEV_1 value (litres) for the 2018 calendar year is selected. Includes children 6 years and older at date of lung function Excludes lung function measurements taken after lung transplant Date of this measurement collected for nutrition and GLI calculations
FEV_1 percentage predicted value (%)	Calculated according to Global Lung Index (GLI) using the best pre-bronchodilator FEV_1 value (litres) for the 2018 calendar year.
Nutrition	
Pancreatic status in 2018	Determined by fecal elastase measurements and/or prescription of pancreatic enzyme replacement therapy. Pancreatic Sufficient Pancreatic Insufficient Unknown
Weight (Kg)	Weight at the time of best pre-bronchodilator FEV_1 value for 2018 is selected. If no FEV_1 recorded for the year, the last weight measurement of the year is selected. Date of this measurement collected for nutrition calculations

Length/height (cm)	Height at the time of best pre-bronchodilator FEV_1 value for 2018 is selected. If no FEV_1 recorded for the year, the last height measurement of the year is selected. Date of this measurement collected for nutrition calculations.
Nutrition classification	Weight in kg Length in cm BMI – kg/m2 for patients >18 years BMI z-score (BMIz) parameters calculated according to World Health Organisation references equation children 0-19 years.
Microbiology	
Any Isolates in 2018	Any *Pseudomonas* isolated in 2018 Any MRSA isolated in 2018 Any NTM isolates in 2018 Recorded as: Yes, No or Unknown *Other infections and chronic infection status of specific infections could not be sufficiently harmonized between countries to permit meaningful comparisons.
Complications	
Cystic fibrosis related Diabetes in 2018	Yes, No or Unknown Diagnosis of CF related diabetes requiring insulin. This includes people who have impaired glucose tolerance in Canada but not South Africa. If someone has Type 1 diabetes or juvenile diabetes, this can be included under the category of 'CF-related' diabetes.
Liver cirrhosis/portal hypertension	Yes, No, or Unknown Includes clinical (hepatosplenomegaly), radiologic (U/S, CT scan that shows enlarged spleen or nodular liver), pathologic (liver

	biopsy) evidence of cirrhosis and splenomegaly which implies portal hypertension or if there is endoscopic evidence of oesophageal or gastric varices in the reporting year. If a patient only has elevated liver enzyme tests with no evidence of cirrhosis or portal hypertension, do NOT check this complication. In Canada cirrhosis was recorded between 2015-2018 ensuring the most recent estimate.
ABPA	Yes, No or Unknown in 2018. ABPA is defined as anyone diagnosed with ABPA by a physician based on clinical judgment, biochemistry, or other laboratory testing in the reporting year.
Therapies	
Pulmonary	
hypertonic saline	Yes, No or Unknown minimum of 3 months of therapy in the reporting year
rhDNase	Yes, No or Unknown minimum of 3 months of therapy in the reporting year
macrolides	Yes, No or Unknown minimum of 3 months of therapy in the reporting year
Inhaled antibiotics	Yes, No or Unknown minimum of 3 months of therapy in the reporting year Yes, includes any inhaled treatment preparation from the SA and Canadian registries including gentamycin, amikacin, aztreonam, tobramycin, colistin, levofloxacin, vancomycin, or any other inhaled antibiotic
Number of IVI courses per year	Yes, No or Unknown

	If Yes – 1/2/3/4/5 or more Canadian Registry: Combining variable for pulmonary admission and home IV events
GIT	
Pancreatic Enzyme Replacement Therapy	Yes, No or Unknown
Transplant in 2018	
Received liver transplant in 2018	Yes, No
Received liver transplant prior to 2018	Yes, No
Received lung transplant in 2018	Yes, No
Received lung transplant prior to 2018	Yes, No
Modulators	
CFTR modulators (any)	Yes, No
Deaths	
Number of deaths in 2018	
Age of death	Calculated in years from DOB to Date of Death

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We confirm and approve of including the above publication in his PhD dissertation, for which we are listed as co-authors. We further confirm that we are not currently registered as a postgraduate student nor intend to use this publication in our own or other postgraduate dissertations.

We agree that his role in the publication was: to conceptualise and design the study protocol; oversee data collection and analysis; data interpretation; drafting and critically revising the manuscript as first author; submitting and approving the final revised manuscript for publication.

Our roles in the publication were the following:

Author name	Contribution	Signature
Jenna Sykes	To conceptualise and design the study protocol; perform data and statistical analysis and advise with data interpretation; draft and critically revise the manuscript, including tables and illustrations; approving the final revised manuscript for publication.	
Stephanie Y Cheng	To conceptualise and design the study protocol; perform data extraction and cleaning from the Canadian CF Registry, critically review the manuscript, approving the final revised manuscript for publication.	
Janine Verstraete	To conceptualise and design the study protocol; perform data extraction and cleaning from the SA CF registry; critically review and revise the manuscript, including tables; approving the final revised manuscript for publication.	
Michael S. Pepper	To conceptualise and design the study protocol, contribute genotype data to the SA CF registry, critically review and approve the manuscript for publication.	
Cheryl Stewart	To conceptualise and design the study protocol, contribute genotype data to SA CF registry, critically	

	review and approve the manuscript for publication.	
Heather J Zar	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and co-author; and approve the final revised manuscript for publication.	
Brenda M Morrow	To conceptualise and design the study protocol; critically review and revise the data analysis and manuscript as co-supervisor and co-author; and approve the final revised manuscript for publication.	
Anne L. Stephenson	To conceptualise and design the study protocol; oversee and advise on analysis and data interpretation; critically review all drafts and revisions of the manuscript including tables and illustrations; approve the final revised manuscript for publication as senior author.	

Chapter 5

Beta-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: do we need new reference ranges?

Authors: Marco Zampoli MD (1,2), Janine Verstraete PhD (1), Thao Nguyen-Khoa PhD (3,4,5), Isabelle Sermet-Gaudelus PhD (4,5,6), Heather J Zar PhD (1,2), Tanja Gonska PhD (7), and Brenda M Morrow PhD (1).

Affiliations:

1. Department of Pediatrics and Child Health, University of Cape Town, South Africa
2. MRC Unit for Child and Adolescent Health, University of Cape Town, South Africa
3. Laboratories of Biochemistry and Newborn screening, Necker-Enfants Malades hospital Assistance Publique-Hôpitaux de Paris, France
4. Centre de Référence Maladies Rares Mucoviscidose et Maladies apparentées, Necker-Enfants Malades Hospital, Assistance Publique – Hôpitaux de Paris, Paris Descartes University, France
5. Institut national de la santé et de la recherche médicale U1151, Institut Necker Enfants Malades
6. European Respiratory Network for Rare Diseases of the Lung
7. Division of Pediatric Gastroenterology, Hepatology and Nutrition, University of Toronto, and the Program of Translational Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada.

Corresponding author: M Zampoli

Address	5th floor ICH Building Red Cross War Memorial Children's Hospital Klipfontein Rd Rondebosch, 7700
Telephone	021 658 5309
Email address	m.zampoli@uct.ac.za

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Key words: Cystic fibrosis; β-adrenergic sweat test; evaporimetry; children.

Abstract

Background: Investigating inconclusive cystic fibrosis (CF) diagnosis in children is difficult without advanced cystic fibrosis transmembrane conductance regulator (*CFTR*) function tests. This study investigated the utility of beta (β)-adrenergic sweat test to exclude CF in participants with inconclusive diagnosis (CF suspects) in South Africa.

Methods: β-adrenergic sweat test and sweat chloride tests (SCT) were performed simultaneously in CF suspects and adult controls (healthy, *CFTR* heterozygotes and CF). Cholinergic and β-adrenergic induced sweat rate was measured by evaporimetry (transepithelial water loss, TEWL: gm $H_2O/m^2/hr$) following intradermal injections. Next generation sequencing of *CFTR* was performed in CF suspects. CF diagnosis was defined by genotype.

Results: Thirty-seven controls (10 healthy, 14 CF, 13 *CFTR* heterozygotes) and 32 CF suspects (26 children; 6 adults) were enrolled. Six were excluded from formal analyses due to β-adrenergic sweat test failure. In adults, evaporimetry was superior to SCT for diagnosis of CF with β-adrenergic: cholinergic ratio TEWL ≤ 0.05 achieving 100% sensitivity and specificity. Twenty-two CF suspect children (age range 3.4-15.6 years) completed β-adrenergic sweat testing of which none had CF confirmed by genotyping: β-adrenergic:cholinergic ratio >0.05 successfully excluded CF in all but one child who was *CFTR* heterozygous. Median peak β-adrenergic TEWL and β-adrenergic:cholinergic ratio in *CFTR* negative and *CFTR* heterozygous children was significantly lower than adult controls.

Conclusion: β-adrenergic sweat test is more accurate than SCT for excluding CF in children with inconclusive diagnosis. Established reference ranges for β-adrenergic sweat test may not be suitable for children due to lower β-adrenergic sweat secretion compared to adults.

Introduction

The diagnosis of cystic fibrosis (CF) is confirmed by demonstration of reduced or absent cystic fibrosis transmembrane conductance regulator (*CFTR*) function and/or identification of two pathogenic *CFTR* variants (1). Reduced or absent *CFTR* activity in sweat glands results in reduced chloride reabsorption by epithelial cells and increased sweat chloride concentration (≥ 60 mmol/L), which is the hallmark of CF diagnosis. Quantitative pilocarpine iontophoresis cholinergic sweat gland stimulation is the gold standard in clinical practice to diagnose CF (1). However, access to sweat chloride test (SCT) is scarce and increasingly unaffordable in low-middle income countries (LMIC) where clinical presentation of CF in children is poorly distinguished from more common conditions such as non-CF bronchiectasis,

malnutrition, tuberculosis and human immunodeficiency virus infection (2). Although the SCT in conjunction with supportive genotyping is accurate in most cases of CF, SCT in the intermediate range (30-59 mmol/L) without supportive genotyping may not always distinguish CF from CFTR-related disorders (CFTR-RD) associated with residual *CFTR* activity, from subjects without CF (1). Furthermore, genotyping alone is unable to confirm or exclude CF in the presence of *CFTR* variants of variable or unknown clinical significance. In such cases, adjunctive measurement of *CFTR* function can aid the diagnosis or exclusion of CF or CFTR-RD.

The SCT has several limitations with respect to interpretation of borderline sweat chloride results associated with inconclusive *CFTR* genotyping. False positive and false-negative results may be the result of insufficient sweat volume or erroneous collection techniques, or other rare underlying medical conditions (3). Furthermore, age-related variation of sweat electrolytes due to sweat gland maturation; and within and between test variability makes interpretation of sweat chloride levels in the intermediate or borderline zones difficult (4, 5). Several techniques to measure *CFTR* activity have been developed and include the electrophysiological tests nasal potential difference (NPD) and intestinal current measurement (ICM), and the β-adrenergic sweat gland stimulation test (3, 6, 7). Of these, NPD and ICM are most widely described in research settings and clinical practice. Both however, are expensive and require highly specialised technical skill and expertise which are not available in LMIC, including South Africa.

The novel *CFTR*-dependent β-adrenergic sweat test (BAST) measures rate of sweat production by evaporimetry or sweat droplet imaging (6, 8, 10). BAST is easier to perform and less invasive than NPD and ICM and demonstrates a greater ability than SCT to discriminate between CF, CFTR-RD, *CFTR* heterozygotes and subjects without *CFTR* variants (6, 11). The BAST is safe, validated and well tolerated in adults but the diagnostic utility, feasibility and safety in children and populations with diverse ethnicity is not well established (12). We therefore conducted a pilot study of BAST in a South African cohort of predominantly children with inconclusive CF diagnosis to evaluate the safety, feasibility, and utility of BAST to exclude CF or CFTR-RD in an LMIC setting where newborn screening for CF, NPD and ICM are not available.

Methods

Study design and setting

We prospectively recruited a series of consenting participants fitting the following groups at Red Cross War Memorial Children's Hospital, Cape Town, South Africa, between February 2020 and April 2021:

Healthy adults ≥ 18 years (healthy controls)

a. Adults with confirmed CF diagnosis and two pathogenic CFTR variants (CF).

b. Adults who were a parent of a child with confirmed CF diagnosis and two pathogenic CFTR variants (CFTR heterozygous).

c. Children (≥ 3 years age) and adults ≥ 18 years with suspected CF based on clinical symptoms and inconclusive SCT and/or previous *CFTR* genotype (CF suspects).

Study procedures

Study participants each underwent simultaneous SCT on the right forearm and BAST on the left forearm (**Figure 5.3** and **Figure 5.4**) according to standardised protocols (see online supplement material). SCT was conducted with the Macroduct® sweat stimulation and collection system (ELITechGroup, USA) and sweat chloride analysis with Sherwood® Mk II Chloride Analyser 9265 (Sherwood Scientific Limited, Cambridge, UK).

Sweat rate was measured using two evaporimetry probes (CyberDerm RG-1; Dasylab, Broomall, PA, USA®) placed onto the forearm skin, using a previously described technique (6, 12). The reference probe was positioned medially and the measuring probe laterally (**Figure 5.3**, Supplementary material). Evaporimetry was expressed as transepithelial water loss (TEWL, grams of water loss/m^2/hour). The β-adrenergic sweat secretion test was performed as described by Quinton *et al.* (2012) by intradermal injection of atropine sulphate (0.2 mL, 44μg/mL) below the reference probe, followed by sequential intradermal injection of carbachol (0.1 mL, 0.1 μg/mL), atropine sulphate (0.2 mL, 44μg/mL), and finally 0.2 mL β-adrenergic cocktail containing atropine sulphate (8.0 μg), isoproterenol (4.4 μg) and aminophylline (0.84 mg) below the measuring probe (6). Stock solutions were prepared by a research pharmacist at least 48 hours prior to participant testing and stored at 4 °C to ensure drug stability (13).

Peak/Δ cholinergic, peak/Δ β-adrenergic TEWL and β-adrenergic:cholinergic ratio were recorded and calculated in each participant. A previously described 'modified' BAST protocol and analysis measuring only peak/Δ β-adrenergic TEWL was applied in children < 6 years age where the full protocol could not be followed, or if cholinergic secretion in response to carbachol was not detected for any reason (12). Details of the BAST technique, 'modified' BAST protocol or analysis, standard operating procedures and output measurements are provided in the online data supplement material.

CFTR variant analysis

All CF suspects had whole blood samples collected for next generation sequencing of *CFTR* by Invitae Laboratories (1400 16th Street, San Francisco, CA 94103, USA) (14). *CFTR* variants in parents of children were determined by their child's genotype. Healthy adult volunteers were not tested for *CFTR* variants. Participants were stratified for analysis into three final *CFTR* genotype categories: A) CF or CFTR-RD; B) *CFTR* heterozygous and C) *CFTR* negative/healthy control (no *CFTR* variant identified or healthy adult control). CF and CFTR-RD were grouped together based on previous reporting that BAST was unable to differentiate CF from CFTR-RD in children (12). The Wong and Baker faces pain rating scale was used to measure self-reported pain before and two minutes after the BAST for all participants (15). Participants were monitored throughout and for at least 5 minutes after BAST and SCT for any adverse events.

Clinical data collection in CF suspects

Clinical information of CF suspects, including age, ethnicity, sex, and symptoms, were documented by participant questionnaire and from the medical records. Physical examination of CF suspects including weight and height measurements was performed at the study visit and analysed with Body Mass Index (BMI) or BMI-z scores according to the World Health Organisation (WHO) reference equations. Routine CF-related investigations including chest radiograph, Computed Tomography (CT) chest scan, microbiology of respiratory samples, fecal elastase and spirometry were extracted from the medical records where available. Pre-bronchodilator spirometry values were calculated according to Global Lung Initiative reference equations (16). Previous SCT results collected by Gibson and Cooke method were documented and the mean sweat chloride value (mmol/L) for each participant calculated.

Statistical analysis

Reported measures of centrality and spread were guided by whether distributions were approximately normal. Groups of individuals (by recruitment, diagnosis, ethnicity, sex or age) were compared using chi-squared or Fisher's exact (categorical variables), Kruskal-Wallis (medians of continuous variables) or ANOVA (means of continuous variables) tests. Pearson correlation coefficients described associations of age and sex between SCT, BAST results. Data in adults and children were analysed and presented separately due to absence of BAST reference data in children. Receiver operating characteristic (ROC) curves were plotted to describe and compare the diagnostic accuracy of BAST and SCT in adults ≥ 18 years to exclude CF/CFTR-RD, and to select the optimal sensitivity and specificity cut-offs for the diagnosis of CF/CFTR-RD. For analysis and comparisons, final *CFTR* genotype diagnosis category was used as the diagnostic standard due to absence of BAST reference data in the local study

population. All data analyses were conducted using SPSS Windows 27.0 (IBM SPSS Inc., Chicago, IL, USA) and Statistica Windows Version 13.0 (TIBCO Software Inc., Palo Alto, CA, USA).

Ethical considerations

The study was approved by the University of Cape Town's Human Research Ethics Committee (HREC 032/2019) and the South African Health Products Regulatory Authority (SAHPRA trial ref. 20190602). Signed informed consent and assent (children aged 7-17 years) was obtained from all participants. Ethical approval to recruit healthy control children was not obtained.

Results

Study population characteristics

Ten healthy adults, 14 adults with CF (10 pancreatic insufficient; 4 pancreatic sufficient), 13 adult *CFTR* heterozygotes and 32 CF suspects (26 children and six adults) were recruited between February 2020 and April 2021. Detailed clinical and CF diagnostic investigations including genotype and evaporimetry results of all participants are presented in **Table 5.1**.

Median age of CF suspects was 10.9 years (range 3.5-36) with similar distribution of sexes (47% male). The majority were of mixed ethnicity (69%) with fewer Black Africans (28%) and Caucasians (3%).

The majority of CF suspects (29/32, 90%) had chronic respiratory symptoms with abnormal chest radiographs (13/31, 48%) or CT chest scans (12/13, 92%). Forced expiratory volume in first second percent predicted (FEV_1pp) was < 80 in 20/30 (67%) and a recognised CF-associated bacterium was isolated from sputum in 7/31 (26%) participants. Low fecal elastase was recorded in 4/21 (19%), and 14/32 (44%) had a BMIz < -1.0 or BMI <18.5. After extensive *CFTR* testing of CF suspects, CF was diagnosed genetically in one adult, CFTR-RD in one child and four children identified as *CFTR* heterozygotes (**Table 5.1**).

No serious adverse events were recorded in any participants who completed the BAST. Minor adverse events occurred in five participants: one healthy adult had anxiety, one child had inconsolable crying, and three participants (one adult and two children) had redness at the injection site, which resolved without intervention.

BAST in adults

Forty-three adults underwent BAST of which 41 were included in analyses and grouped into the following final *CFTR* genotype categories: 14 CF; 12 *CFTR* heterozygotes; and 15 *CFTR* negative /healthy controls. Receiver operator curve analysis in adults is presented in **Table 5.2**. Evaporimetry performed better than SCT ≥ 60 mmol/L for diagnosis and exclusion of CF. Δ β-adrenergic ≤ 4.5 TEWL

and β-adrenergic:cholinergic ratio ≤ 0.05 achieved 100% sensitivity and specificity for genetic CF diagnosis, thus confirming the 0.05 cut-off to be optimal for confirmation and exclusion of CF/CFTR-RD in this adult cohort.

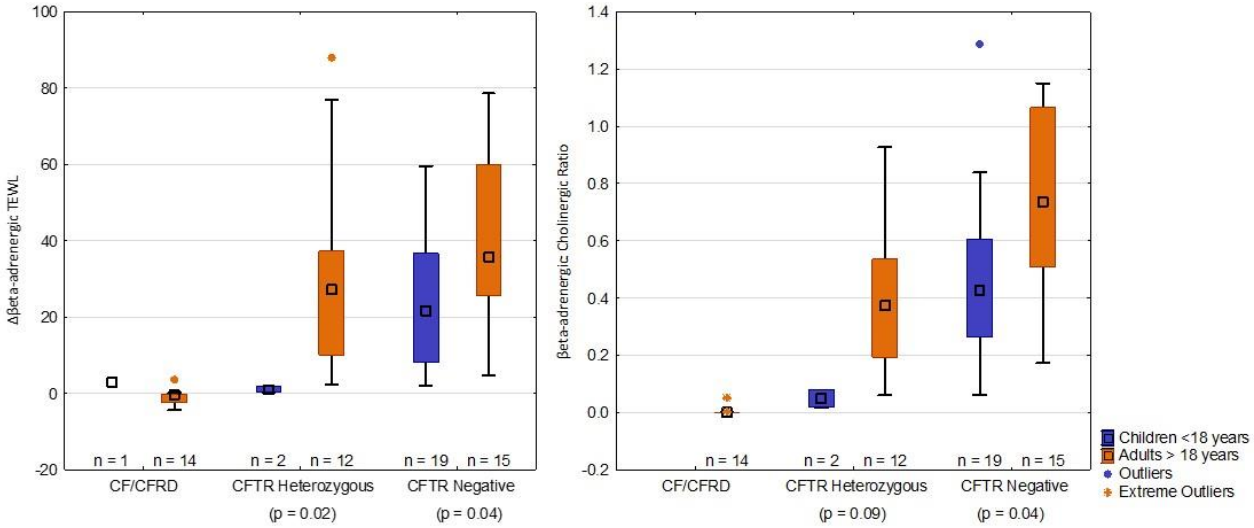

Figure 5.1 Peak β-adrenergic and β-adrenergic:cholinergic ratio indices plotted by *CFTR* diagnosis category for adults and children.

Notes: Boxes indicate first to third quartiles, the dividing line the median, whiskers the non-outlier range and markers the outliers. P-values indicate differences between adults and children with Mann-Whitney U test.

Two participants' (one CF and one *CFTR* heterozygote) BAST results were excluded due to technical failure, and the modified BAST analysis was applied in three females due to failed cholinergic secretion; two of these three females had insufficient sweat volume collected with the simultaneous SCT. BAST correctly diagnosed one CF suspect adult (participant no. 32) with intermediate SCT and p.Phe508del/ 3849+10kbC->T variants. False positive SCT was observed in three adults who were *CFTR* negative, including one male (participant no. 31) who was human immunodeficiency virus (HIV) infected. Median Δ β-adrenergic TEWL and median β-adrenergic:cholinergic ratio in CF, *CFTR* heterozygotes and *CFTR* negative/healthy control groups is presented in **Figure 5.1**. Median β-adrenergic:cholinergic ratio in *CFTR* heterozygotes was half that of *CFTR* negative adults but the difference did not reach statistical significance.

BAST in children

Twenty-six CF suspect children underwent BAST of which 22 (one CFTR-RD; two *CFTR* heterozygotes and 19 *CFTR* negative) were included in BAST analyses. Four children (two *CFTR* negative and two *CFTR* heterozygotes) were excluded due to BAST failure: three due to movement artefact rendering

evaporimetry tracing unreadable, and one child refused testing all together. The modified BAST protocol or analysis was applied in two children. No children were diagnosed with CF based on *CFTR* testing and one child (participant no. 7) was diagnosed with CFTR-RD based on intermediate SCT and two *CFTR* variants (3272-26A>G1 and 12789+2insA). False positive SCT was observed in three children and intermediate SCT observed in 12 children of which one had CFTR-RD and 11 were *CFTR* negative. Of the 22 children tested by BAST, Δ cholinergic TEWL was < 30 in nine (41%), Δ β-adrenergic TEWL < 4.5 in six (27%) and β-adrenergic:cholinergic ratio <0.05 in one. Of the six children with Δ β-adrenergic TEWL < 4.5, one had CFTR-RD (participant no. 7), two were *CFTR* heterozygotes and three were *CFTR* negative (i.e., 5/6 were false positive results). However, the β-adrenergic:cholinergic ratio in children was > 0.05 in all but one child who was heterozygous with c.1210-34TG[11]T[5] variant (participant no.4).

Effect of age and sex on BAST in *CFTR* negative/healthy adult subjects

Median Δ cholinergic TEWL was significantly higher in males compared to females but similar in children and adults. Median Δ β-adrenergic TEWL was significantly higher in males compared to females and significantly higher in adults compared to children. Median β-adrenergic:cholinergic ratio was similar between sexes but significantly lower in children compared to adults (**Table 5.3**, on-line supplement). Scatterplots of BAST responses and age demonstrate no relationship with Δ cholinergic TEWL, and a trend toward positive correlation with Δ β-adrenergic TEWL and β-adrenergic:cholinergic ratio (**Figure 5.2**).

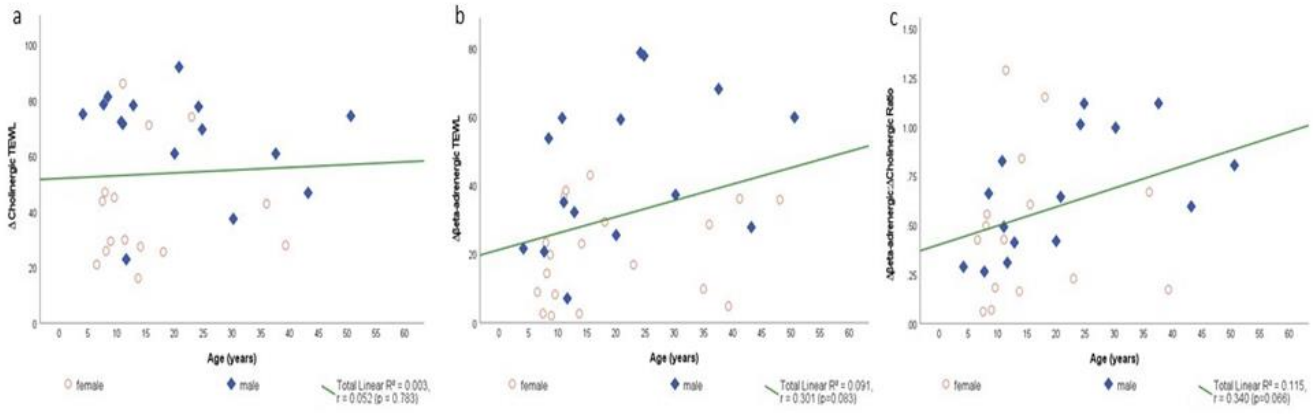

Figure 5.2 Scatterplots of Δ cholinergic TEWL (2a), Δ β-adrenergic TEWL (gmsH_2O/m^2/hr) (2b), β-adrenergic:cholinergic ratio (gmsH_2O/m^2/hr) (2c) and age for all *CFTR* Negative participants, fitted with linear $r^{2.}$

Discussion

This pilot study in a South African cohort validates previous studies demonstrating the accuracy and safety of BAST in the diagnosis and exclusion of CF or CFTR-RD where first tier routine investigations are inconclusive. BAST may therefore also be a suitable and more accurate alternative to SCT where SCT is not available or unaffordable as in many LMIC. We further demonstrated that BAST in young children can be successful, but that technical difficulty, interpretation and acceptability of the test are challenges in this age group. Our study findings suggest there is a need to establish reference ranges for healthy children due to lower β-adrenergic secretion in children without *CFTR* variants compared to adult controls. BAST is a suitable and simpler alternative to NPD or other established advanced CFTR activity tests in clinical or research settings where *CFTR* genotyping is non-diagnostic or not available. Furthermore, in our experience, technical aspects of BAST with evaporimetry were relatively easy to learn and implement, which is an advantage over other established CFTR activity tests.

Eccrine sweat glands are stimulated independently by cholinergic and β-adrenergic conductive pathways but cholinergic responses after maximal stimulation are greater than β-adrenergic responses (17). Cholinergic stimulation is *CFTR*-independent and mediated by calcium and potassium-dependent conductance. In contrast, β-adrenergic stimulation is mediated only by cAMP-dependent chloride conductance and is thus *CFTR*-dependent. Recognition of *CFTR*-dependent β-adrenergic pathways in sweat electrophysiology and early observations of reduced sweat secretory responses in *CFTR* carriers and absent responses in people with CF led to the development of the BAST as a diagnostic instrument (18, 19). The original validation study using evaporimetry in adults by Quinton *et al.* reported 100% sensitivity and specificity for CF, CF heterozygotes and healthy subjects, with a TEWL cut-off at 4.5 grams $H_2O/m^2/hr$, whereas TEWL in subjects with CFTR-RD was variably low or absent (6). Other studies with adult subjects employing bubble imaging-based ratiometric sweat rate assays after β-adrenergic stimulation report similar accuracy in distinguishing CF, *CFTR* heterozygotes and healthy controls with β-adrenergic:cholinergic ratio cut-off values of 0.0055 or less (8, 9). Sweat bubble imaging techniques are technically difficult to operate but are more sensitive than evaporimetry at detecting small but physiologically important differences that distinguish CF from CFTR-RD or measuring the effects of CFTR modulating drugs on CFTR activity (8, 10, 13, 20). In our study the clinical objective was to exclude CF or CFTR-RD in people presenting with clinically suspected CF for which evaporimetry performed well with optimal cut-off values in adults of Δ β-adrenergic 4.5 TEWL and β-adrenergic:cholinergic ratio 0.05. We found a high number of false positive SCT in this cohort, in which BAST with evaporimetry successfully excluded CF/CFTR-RD in all cases, and correctly diagnosed two adults with CF who had intermediate sweat chloride levels and residual function mutations known to be associated with lower or normal sweat chloride levels (21). Reasons for falsely elevated sweat

chloride levels in the intermediate and positive range in this population warrants further investigation and may include several known biological factors including malnutrition, environmental deprivation, acquired CFTR dysfunction from exposure to cigarette smoke or mineralocorticoid deficiency. Significant intra-individual variability of SCT over time may also explain discrepant SCT results observed in our cohort (22-25). One adult male in this study with repeatedly high SCT values (90 mmol/L) was newly diagnosed with HIV-infection and had an ichthyosis-like skin disorder. False positive SCT in HIV infection has been reported in a child but the mechanism is unknown (26).

A novel finding of our study was an age-dependent effect on β-adrenergic secretion, with lower β-adrenergic secretion and β-adrenergic:cholinergic ratios in *CFTR* negative children compared to adults who were *CFTR* negative or healthy controls. Lower cholinergic secretion sweat volume and β-adrenergic secretion rate in females compared to males have been previously reported (6, 12, 27). We were unable to extensively investigate or report BAST in children with CF or were *CFTR* heterozygotes due to small numbers and study protocol restrictions to only adult control subjects. Most CF suspect participants in this cohort were children with CF-like symptoms who did not have genetically diagnosed CF/CFTR-RD. Our findings in these participants thus provides valuable 'non-CF' control data for BAST in children, which has not been previously reported. Our data suggests that due to lower β-adrenergic secretion driven likely by difference in sweat gland maturation, peak β-adrenergic secretion using a cut-off of 4.5 TEWL as per adult references does not accurately discriminate between CF, CFTR-RD, *CFTR* heterozygous or *CFTR* negative status in young children. However, the β-adrenergic:cholinergic ratio, which is less influenced by sex, may be more useful than peak β-adrenergic secretion in young children as supported by our data of children with Δ β-adrenergic < 4.5 TEWL, where β-adrenergic:cholinergic ratio > 0.05 excluded CF/CFTR-RD in all but one child. However, further studies are needed to establish optimal age and sex-dependent references ranges of the BAST in children as conflicting data in older children has been published suggesting established reference ranges are valid (11).

There are several limitations to this study that we considered when interpreting our results. Firstly, we did not do extensive genetic testing in healthy adult controls due to budgetary constraints, thus it is possible that some were asymptomatic *CFTR* heterozygotes, which could have lowered their β-adrenergic secretion. However, the study objective was to confirm or exclude CF/CFTR-RD, which would not have influenced related analyses. Second, several participants were excluded from analysis due to failure of BAST for different reasons including accidental needle re-puncture of the skin during injection of BAST drugs or technical factors due to movement artefact. Absent or poor cholinergic secretion sweat rate has been previously reported and could be caused by inadvertent subcutaneous

injection of carbachol, which is too deep from dermal sweat glands (6). Our protocol and regulatory requirements did not allow recruitment of healthy children controls and permitted only one repeat carbachol injection if it failed and repeating the full BAST protocol in study participants was not permitted. Third, we applied at our discretion a 'modified' protocol in young children where the full protocol was not possible and similarly calculated Δ β-adrenergic TEWL in participants where β-adrenergic secretion was recorded but cholinergic secretion failed. Although Δ β-adrenergic secretion calculations with this approach differ slightly, they are unlikely to change substantially as the reference TEWL measurement is within a 5 gmsH_2O/m^2/hr margin of error. Furthermore, documenting Δ β-adrenergic secretion above the CF range remains useful to exclude CF, even in the absence of recording cholinergic secretion. Cholinergic pre-stimulation with pilocarpine is shown to potentiate β-adrenergic secretion, therefore omitting or failure of this step could have altered β-adrenergic secretion calculations which must be taken into consideration when β-adrenergic secretion is within CF range (8, 28). Increasingly iontophoresis as drug delivery method in place of intradermal injection is being explored as a less invasive approach, which is desirable in children (8, 29, 30). Although iontophoresis of pilocarpine and atropine is successful, iontophoresis of a β-adrenergic cocktail solution has had variable success (8, 30).

In summary, BAST accurately excluded CF or CFTR-RD in the majority of children with inconclusive CF diagnosis in this ethnically diverse South African cohort. BAST is feasible and safe in children but interpretation of BAST in younger children needs further investigation due to lower β-adrenergic secretion observed in children. β-adrenergic:cholinergic ratio performed better than peak β-adrenergic TEWL for excluding CF in children.

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Table 5.1 Demographic characteristics and investigations of recruited subjects with inconclusive CF diagnosis (CF suspects) ranked by age

Study no.	Age	Sex	Ethnicity	Sweat chloride tests		FEV1pp	Sputum microbiology	Faecal Elastase	*CFTR* variants	*CFTR* genotype category	Evaporimetry		
	(yrs)			Mean previous Cl (mmol/L)	study visit Cl (mmol/L)			mean (µg/g)			Δ Cholinergic	Δ βeta-adren	Ratio β-adren:Chol
1	3.4	M	Mixed	65	14			500	p.Val520Ile[§]/_	CFTR Hetero	23.39	1.84	0.079
2	4.1	M	Mixed	65	44			393	_/_	CFTR negative	75.02	21.6	0.288
3*	5.7	M	Mixed	43		112			3272-26A>G1/ _	CFTR Hetero			
4	6.0	F	Mixed	68	35	56	PA	321	c.1210-34TG[11]T[5] /_	CFTR Hetero	12.21	0.22	0.018
5*	6.3	M	Mixed	70	32	91		500	p.Ala280Ser[§]/ _	CFTR Hetero			
6	6.5	F	Black African	48	20	75			_/_	CFTR negative	20.98	8.93	0.426
7†	6.6	F	Caucasian	50	48	94		438	3272-26A>G1 / 12789+2insA[§]	CFTR-RD		2.94	
8	7.5	F	Mixed	75	66	70		251	_/_	CFTR negative	43.74	2.64	0.060
9	7.7	M	Black African	83	31	53		133	_/_	CFTR negative	78.53	20.71	0.264
10	7.9	F	Black African	65	15	109			_/_	CFTR negative	46.98	23.32	0.496
11	8.1	F	Mixed	53	45	106		437	_/_	CFTR negative	25.87	14.35	0.555
12	8.4	M	Mixed	63	36	78		476	_/_	CFTR negative	81.28	53.7	0.661
13‡	8.7	F	Black African	60	50	56		135	_/_	CFTR negative		19.8	
14	8.9	F	Mixed	56	12	100		257	_/_	CFTR negative	29.34	2.03	0.069
15	9.5	F	Black African	43	37	60		138	_/_	CFTR negative	45.08	8.2	0.182
16	10.7	M	Mixed	45	50	61	H.Influenzae		_/_	CFTR negative	72.23	59.62	0.825
17	11.0	M	Black African	74	12	73		425	_/_	CFTR negative	85.98	36.71	0.427
18	11.0	F	Mixed	51	60	67		500	_/_	CFTR negative	71.52	35.1	0.491
19	11.4	F	Mixed	67	35	85		386	_/_	CFTR negative	29.9	38.49	1.287
20	11.6	M	Mixed	84	61	80		30	_/_	CFTR negative	22.85	7.06	0.309
21*	12.3	F	Mixed	71	47	76	MSSA, H.Influenzae	369	_/_	CFTR negative			
22	12.8	M	Mixed	60	14	93		462	_/_	CFTR negative	78.19	32.19	0.412

23	13.7	F	Black African	20	22	23	PA, Aspergillus fumigatus		_/_	CFTR negative	16.08	2.63	0.164
24*	14.0	F	Mixed	74	22	47		141	_/_	CFTR negative			
25	14.1	F	Mixed	59	62	87	MSSA	500	_/_	CFTR negative	27.41	22.98	0.838
26	15.6	F	Mixed	50	27	73		420	_/_	CFTR negative	71.06	42.99	0.605
27	18.1	F	Mixed	69	63	71		470	_/_	CFTR negative	25.54	29.38	1.150
28	20.0	M	Mixed	87	67	74		368	_/_	CFTR negative	60.88	25.51	0.419
29	20.8	M	Mixed	44	26	60			_/_	CFTR negative	91.92	59.17	0.644
30	24.2	M	Black African	51	44	56		233	_/_	CFTR negative	77.67	78.63	1.012
31	30.2	M	Black African	93	90	61	MSSA	222	_/_	CFTR negative	37.41	37.24	0.995
32	36.0	M	Mixed	47	47	58	PA		p.Phe508del / 3849+10kbC->T	CF	64.62	-0.6	0.000

Footnotes: PA: *Pseudomonas aeruginosa*; MSSA: methicillin sensitive staphylococcus aureus; FEV_1pp: forced expiratory volume in first second percent predicted; CF/CFTR-RD: Cystic fibrosis/Cystic fibrosis-related disorder; CFTR: Cystic fibrosis transmembrane regulator protein; Hetero: heterozygous

*Participants were excluded from analysis due to technical failure with the BAST
† A modified BAST protocol/analysis was used
‡ Failed cholinergic response
§ CFTR variant of uncertain significance

Table 5.2 Receiver Operator Curve analysis for sweat chloride test and β-adrenergic sweat test for CF/CFRD diagnosis in adults

	Sensitivity	Specificity	PPV	NPV
SCT ≥ 60 mmol/L	85.7%	88.0%	80.0%	91.7%
Δ β-adrenergic ≤ 4.5 TEWL	100.0%	96.3%	93.3%	100.0%
β-adrenergic: cholinergic ratio ≤ 0.00	85.7%	100.0%	100.0%	92.3%
β-adrenergic: cholinergic ratio ≤ 0.002	92.9%	100.0%	100.0%	96.0%
β-adrenergic: cholinergic ratio ≤ 0.050	100.0%	100.0%	100.0%	100.0%

PPV: positive predictive value

NPV: negative predictive value

SVCT: sweat chloride test.

SCT AUC=0.969 (95% CI 0.923,1.000) $p<0.001$; Δ β-adrenergic AUC=0.997 (95% CI 0.987, 1.00) $p<0.001$; β-adrenergic: Cholinergic Ratio AUC =1.00 (95% CI 1.00,1.00) $p<0.001$

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On-line supplement C (Chapter 5/Publication 4)

β-adrenergic sweat test in South Africans with inconclusive cystic fibrosis diagnosis

Authors: Marco Zampoli, Janine Verstraete, Thao Nguyen-Khoa, Isabelle Sermet-Gaudelus, Heather J Zar, Tanja Gonska and Brenda M Morrow

Study procedures

Evaporimetry

Sweat production and rate was measured by two evaporimetry probes (CyberDerm RG-1; Dasylab, Broomall, PA, USA®) placed against the forearm skin using a previously described technique (1, 2, 6, 12). The probes were cleaned with an alcohol swab and air-dried before a diagnostic test was run to ensure optimal room temperature (20–22^{o}C) and humidification (10-50 Percentage Relative Humidity). With the participant in a comfortable seated position with both forearms stretched-out on the table, the skin was cleaned with distilled water and dried. Thereafter, reference probe A was positioned medially and measuring probe B laterally and secured to ensure perpendicular orientation to the skin (see **Figure 5.3**). Evaporimetry was expressed as transepithelial water loss (TEWL, grams of water loss/m^2/hour) and the one-minute mean was recorded manually for analysis. The baseline TEWL reading was taken for a minimum of 2 minutes to ensure that it was stable with a reading between 5-15 units and a reading of within 5 units between the measurement and reference probes.

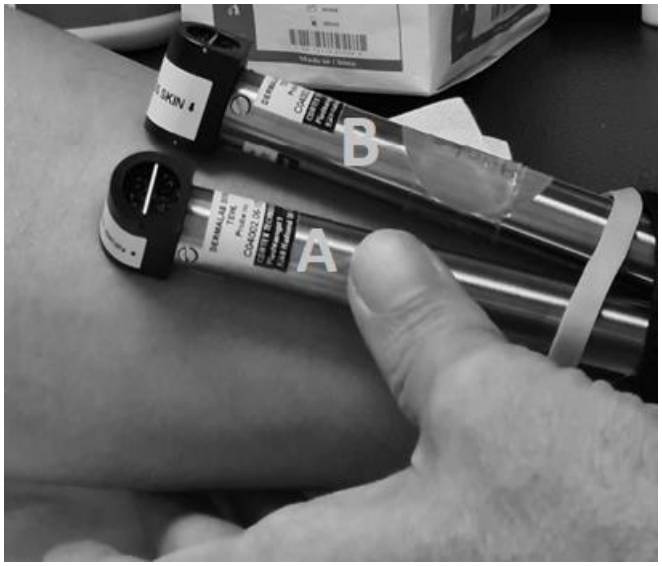

Figure 5.3 Evaporimetry probes measuring transepithelial water loss.

Notes: The reference probe A was positioned medially and the measuring probe B laterally.

Intradermal injections

β-adrenergic sweat test was performed as described by Quinton *et al.* (2012) (6). Stock solutions of the drugs were prepared by a research pharmacist at least 48 hours prior to participant testing and stored

at 4 °C. The skin on the forearm was cleaned with an alcohol swab before each intradermal injection. All drugs were given as an intradermal injection with a 30 G ½ hypodermic needle and injected in the centre of the test area to form a visible wheal of <1cm diameter. The same puncture site was used for repeated injections below the measuring probe. The injected sites were wiped with saturated mineral oil and excess oil was blotted with soft gauze before the probes were replaced in such a way that the needle puncture site was outside the rim of the probe measuring area. The time of each injection and the minute mean probe reading was recorded manually for analysis.

Reference probe site: Cholinergic inhibition as control was achieved by injection of atropine sulphate (0.2 mL, 44µg/mL) below the reference probe: blue-line readout (**Figure 5.5**).

Measuring probe site: Carbachol (0.1 mL, 0.1 µg/mL) was injected below the measuring probe. TEWL readings (red-line readout, **Figure 5.5**) were recorded until the carbachol response plateaued. Thereafter, atropine sulphate (0.2 mL, 44µg/mL) was injected into the same puncture site to reverse the effects of the carbachol until TEWL returned to within 5 units of the reference probe. Finally, 0.2 mL β-adrenergic cocktail containing atropine sulphate (8.0µg), isoproterenol (4.4µg) and aminophylline (0.84mg) was injected. TEWL data were recorded until a steady plateau was reached for at least two minutes. The maximum reading was recorded at least 5 minutes after the β-adrenergic cocktail injection to avoid falsely elevated readings due excessive fluid in the skin after the third injection. If no response was observed, recording continued for a minimum of 8 minutes.

A 'modified' protocol using a single injection of β-adrenergic cocktail under the measurement probe was applied in children < 6 years age or where the full protocol could not be followed, or cholinergic secretion in response to carbachol failed for any reason (12). Children were offered audio-visual entertainment on a tablet during the procedure to make them feel as comfortable as possible (**Figure 5.4**).

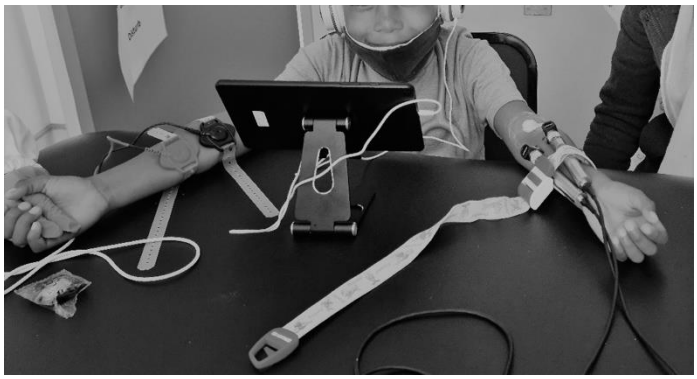

Figure 5.4 Research participant with SCT testing on the right forearm and BAST testing on the left forearm, and audio-visual entertainment on the tablet in the centre.

Calculations and quality control

Peak cholinergic, Δ cholinergic, peak β-adrenergic, Δ β-adrenergic TEWL and β-adrenergic:cholinergic ratio was calculated in each participant as shown in **Figure 5.5**. For those who followed a modified protocol, the Δ β-adrenergic TEWL was calculated as the peak β-adrenergic response less the reading on the reference probe (blue line) at the same time point. All BAST tests were assessed for technical quality with stable tracing at both the reference and measuring probe. Where tracing stability could not be established the BAST was excluded from analysis. The cholinergic response was deemed to have failed in those without a visible response to carbachol injection or if the peak cholinergic response was < 30 TEWL and the β-adrenergic:cholinergic ratio was ≥1.0. Only Δ β-adrenergic calculation was included for analysis in participants with modified protocol or where cholinergic response failed. Other technical exclusions included puncturing of the skin with administration of the BAST drugs. Examples of the TEWL readouts are illustrated in **Figure 5.6**.

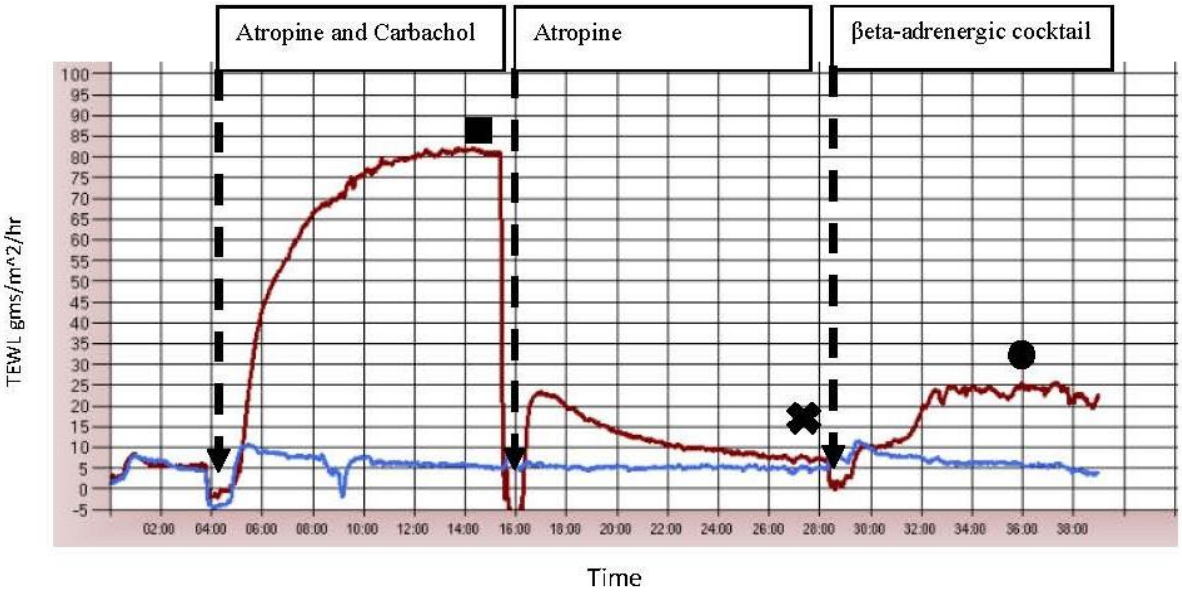

Figure 5.5 A TEWL read-out in CFTR negative participant to indicate readings after injection of drugs and for data analysis.

Notes: The red line indicates the evaporimetry response on the measuring probe and the blue line indicates the evaporimeter response on the reference probe. The points of injection are indicated; □ indicates maximal carbachol reading; **X** indicates lowest atropine reading; ● Indicates maximal β-adrenergic reading; Δ cholinergic = □**-X;** Δ β-adrenergic = ●-X.

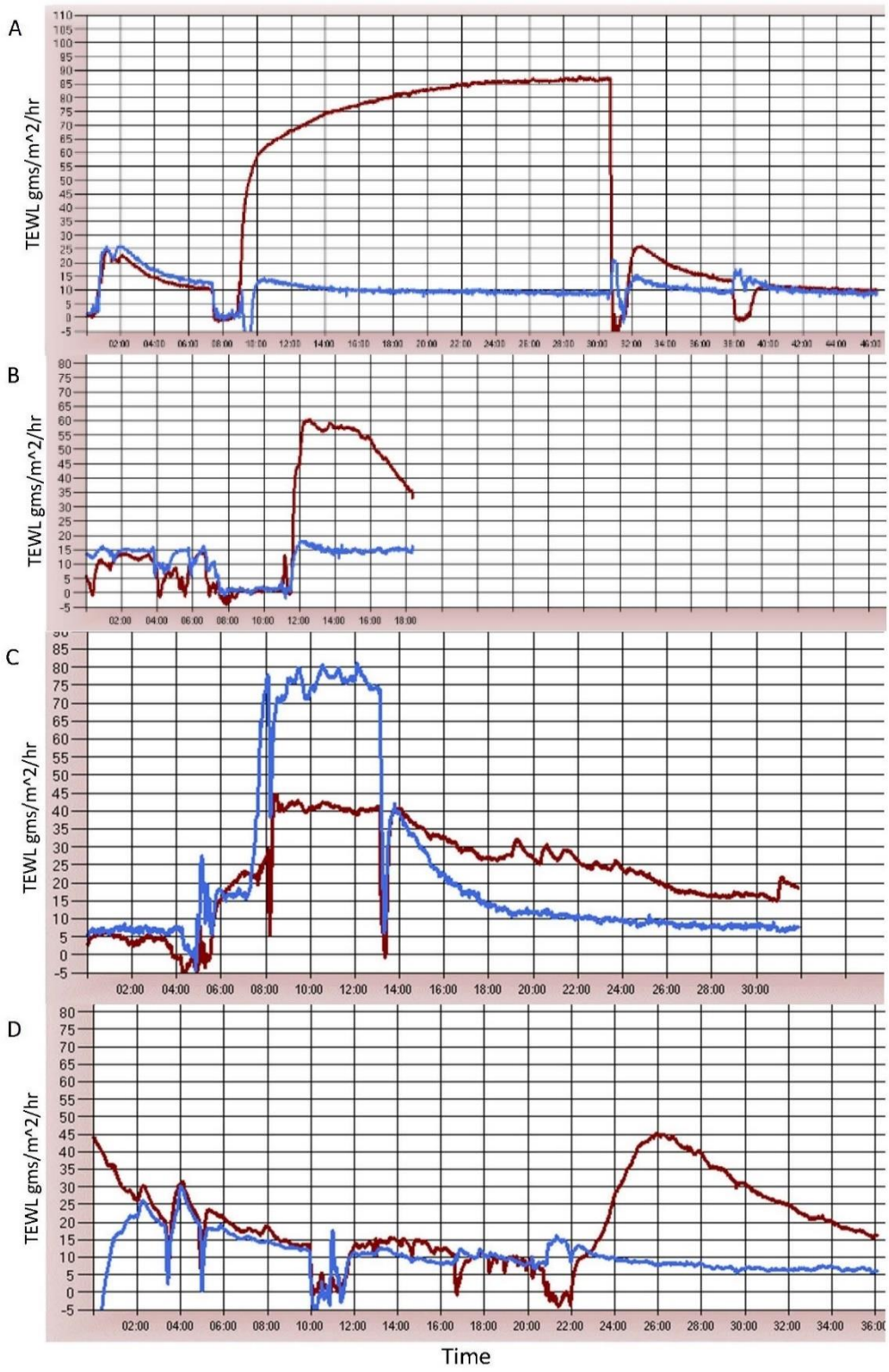

Figure 5.6 A: TEWL read-out of the full study protocol in an individual who has CF. B: TEWL read-out of the modified paediatric protocol in an individual who is *CFTR* negative. C: TEWL read-out of a BAST which was excluded due to technical failure. D: TEWL read-out of a BAST in which the cholinergic response failed.

Notes: The red line indicates the evaporimetry response on the measuring probe and the blue line indicates the evaporimetry response on the reference probe.

Table 5.3 Sweat chloride test (SCT) and evaporimetry results (median value and inter-quartile ranges) of study participants stratified by *CFTR* diagnosis category, age group and sex

	Sex			**Age group**		
	Male (N=30)	**Female (N=31)**	***p*-value**	**Adult ≥18 yrs (N=39)**	**Children < 18 yrs (N=23)**	***p*-value**
SCT volume (uL)						
CF/CFRD	n=10 80.0 (65.0,84.0)	n=5 55.0 (51.0,62.0)	**0.02**	n=14 68.5 (55.0,82.5)	n=1 73.0	0.93
CFTR hetero	n=5 52.0 (51.0,81.0)	n=9 38.0 (37.0,54.0)	0.30	n=12 51.5 (38.0,78.0)	n=2 33.0 (29.0,37.0)	0.13
CFTR negative	n=15 66.0 (30.0,77.0)	n=17 30.0 (22.0,40.0)	**0.05**	n=13 30.5 (22.0,68.5)	n=19 35.0 (20.0,55.0)	0.67
ΔCholinergic						
CF/CFRD	n=10 70.8 (58.0,80.6)	n=4 65.9 (53.7,73.3)	0.84	n=14 70.8 (58.0,74.6)	n=0	
CFTR hetero	n=5 83.1 (74.3,89.0)	n=9 55.3 (50.8,60.5)	0.08	n=12 63.4 (54.7,81.4)	n=2 17.8 (12.2,23.4)	**0.02**
CFTR negative/healthy controls	n=15 72.2 (60.8,78.2)	n=17 29.6 (25.7,46.0)	**<0.002**	n=13 60.8 (37.4,74.0)	n=18 46.0 (27.4,75.0)	0.95
Δβ-adrenergic						
CF/CFRD	n=10 -0.6 (-1.5,-0.2)	n=5 -0.1 (-2.5,0.1)	0.77	n=14 -0.6 (-2.4,-0.2)	n=1 2.9	0.27
CFTR hetero	n=5 29.0 (25.4,76.9)	n=9 13.6 (4.9,30.5)	0.30	n=12 27.2 (10.1,37.3)	n=2 1.0 (0.2,1.8)	0.02

CFTR negative/healthy controls	n=15 37.24 (25.5,59.8)	n=19 19.8 (8.2,33.8)	0.003	n=15 35.8 (25.5,59.8)	n=19 21. (8.2,36.7)	0.04
Ratio β-adren: Cholinergic						
CF/CFRD	n=10 0.00 (0.0,0.00)	n=4 0.00 (0.00,0.01)	0.73	n=14 0.00 (0.00,0.00)		
CFTR hetero	n=5 0.39 (0.29,0.93)	n=9 0.27 (0.74,0.50)	0.21	n=12 0.38 (0.18,0.54)	n=2 0.05 (0.02,0.08)	0.09
CFTR negative/healthy controls	n=15 0.64 (0.41,1.00)	n=16 0.43 (0.17,0.67)	0.17	n=13 0.74 (0.51,1.07)	n=18 0.43 (0.26,0.60)	0.04

CF/CFRD: Cystic fibrosis/Cystic fibrosis-related disorder; CFTR: Cystic fibrosis transmembrane regulator protein; Hetero: heterozygous
Bolded *p*-values indicate significance at $p<0.05$.

Author contribution statements and approval for inclusion of publication in PhD dissertation by Ass/Prof. Marco Zampoli [student no. ZMPMAR001]

PhD dissertation title: Cystic fibrosis in South Africa: spectrum of disease, diagnosis, and outcome.
Publication title: β-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: Do we need new reference ranges? (Chapter 5)
Citation: Zampoli M, Verstraete J, Nguyen-Khoa T, Sermet-Gaudelus I, Zar HJ, Gonska T, Morrow BM. β-adrenergic sweat test in children with inconclusive cystic fibrosis diagnosis: Do we need new reference ranges? Pediatric Pulmonology. 2023 Jan;58(1):187-96.

We confirm and approve of including the above publication in his PhD dissertation, for which we are listed as co-authors. We further confirm that we are not currently registered as a postgraduate student nor intend to use this publication in our own or other postgraduate dissertations.

We agree that his role in the publication was: to conceptualise and design the study protocol; oversee data collection and analysis; data interpretation; drafting and critically revising the manuscript as first author; submitting and approving the final revised manuscript for publication.

Our roles in the publication were the following:

Author name	Contribution	Signature
Thao Nguyen-Khoa	To conceptualise and design the study protocol; provide in-person supervision, training and implementation of research instrument and technology; critically review data analysis and data interpretation; critically review manuscript, including tables and illustrations; approving the final revised manuscript for publication.	
Isabelle Sermet-Gaudelus	To conceptualise and design the study protocol; advise on the research instrument and technology; critically review data analysis and data interpretation; critically review manuscript, including tables and illustrations; approving the final revised manuscript for publication	
Janine Verstraete	To conceptualise and design the study protocol; co-ordinate and manage all SAHPRA regulatory requirements and reporting; data collection and analysis; critically review and revise the manuscript, including tables; approving the final revised manuscript for publication.	
Tanya Gonska	To advise on the research instrument and technology; critically review data analysis and data interpretation; critically review manuscript, including	

	tables and illustrations; approving the final revised manuscript for publication.	
Heather J Zar	To conceptualise and design the study protocol; critically review the manuscript as co-supervisor and co-author; and approving the final revised manuscript for publication.	
Brenda M Morrow	To conceptualise and design the study protocol; critically review the data analysis, interpretation, and manuscript as co-supervisor and senior author; and approving the final revised manuscript for publication.	

Chapter 6
Summary findings and concluding statements

This work has provided the first comprehensive description of CF in SA including insight into key outcomes such as lung function, nutrition, and survival. This was largely made possible by establishing the SACFR, one of only a few national CF registries in the world in an LMIC setting. Although longitudinal data are lacking, cross-sectional analysis of outcome data derived from the SACFR has identified a number of important factors associated with poor outcomes in SA. Furthermore, this study has highlighted differences in CF genotypes by ancestry and disparities in CF outcomes, both in SA and within the global context, which have important implications in the new era of CFTRm treatment.

Spectrum of CF in South Africa (Objective 2 and Objective 3)

Demographic characteristics

There are slightly more females than males with CF in SA which differs to that in other international registries where male sex is associated with survival benefit (1). The reasons for this reverse pattern in SA are unclear and need to be further explored. However, participants in the SA registry are substantially younger than those in HIC. In 2018, the median age was 14.7 years with proportionally more children than adults alive with CF in SA (2). Although more recent SACFR statistics of 2020 indicate the total number and median age of the cohort have increased to 520 and 16.7 years, respectively, the median age in SA is still significantly younger than CF registry populations in HIC where median age ranges between 19.4 in Europe to 24 years in Canada **(Table 4.1** and **Table 4.2)**. Older cohort age in HIC compared to SA and other LMIC is due to a combination of improved survival for multiple reasons and a greater proportion of people with milder CF phenotypes associated with pancreatic sufficiency (PS), who in the absence of NBS-CF are often diagnosed at an older age as reported in our study comparing SA and Canadian CF populations **(Table 4.2**). The lower number of PS CF cases in SA could be explained by either lower frequency of residual function *CFTR* variants in the population, or more likely, less recognition and diagnosis in SA of mild CF phenotypes in older people who may be living with CF but misdiagnosed with other conditions such as asthma, chronic obstructive pulmonary disease (COPD) or non-CF bronchiectasis.

Interestingly, after excluding 12% of Canadian registry individuals diagnosed by NBS, the median diagnosis age in SA (7 months) and Canada (6 months) were similar, with just over two thirds diagnosed under 2 years in both countries **(Table 4.2**). In contrast, 63% of all new diagnoses in the US and 88.5% diagnoses among those less than 6 months old were reported as being detected by NBS in

the US (3). Earlier diagnosis of CF, ideally through NBS, has clear benefits for improved nutritional and other outcomes. Age of diagnosis is unlikely to change in SA without universal NBS for CF (4-6).

Genotype and ancestry

The genotype spectrum of pwCF in SA is strongly determined by ancestry. Not surprisingly, therefore, is the finding that F508del is the most common *CFTR* variant in SA where self-identified White people with European ancestry make up 70% of the CF registry population (2) **(Figure 6.1)**. Almost half of all pwCF in SA were homozygous for F508del, which is similar to other countries with populations of European ancestry. However, unlike the US, Europe, and Australia, in SA White people constitute only 8% of the total population **(Figure 6.2).** A national survey in 2016 reported nearly 56 million people in SA, of which the majority (45 million) were Black Africans and 4.5 million White (7). The second most prevalent *CFTR* variant in SA is 3120+1G>A, a class-1 splicing mutation in intron 16, first described in African Americans and now widely recognised as the most common *CFTR* variant in people with African ancestry (8, 9). One copy of 3120+1G>A was present in at least 85% of Black Africans with CF of whom 56% were homozygous (2). People with Mixed ancestry make up nearly 20% of the CF population and 21% were found to carry the 3120+1GA variant in heterozygous state with either F508del or other variants. The clear differences in genotypes by ancestry have important implications for new drug therapies as 3120+1GA is not responsive to currently licenced CFTRm and therefore almost all Black Africans in SA are not eligible for any approved CFTRm. Furthermore, a greater proportion of the total 11% of pwCF in the SACFR without complete CF genotyping have Black African and Mixed ancestry **(Figure 6.1).** Any existing racial and socioeconomic disparities in pwCF in SA will be further exacerbated by differences in eligibility for CFTRm, which is strongly determined by ancestry.

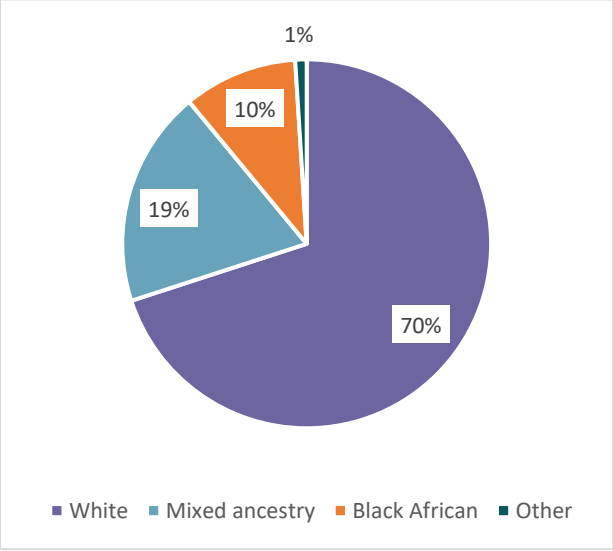

Figure 6.1 Proportions by self-identified ancestry in the SACFR, 2018

(Source: Zampoli *et al*. (2))

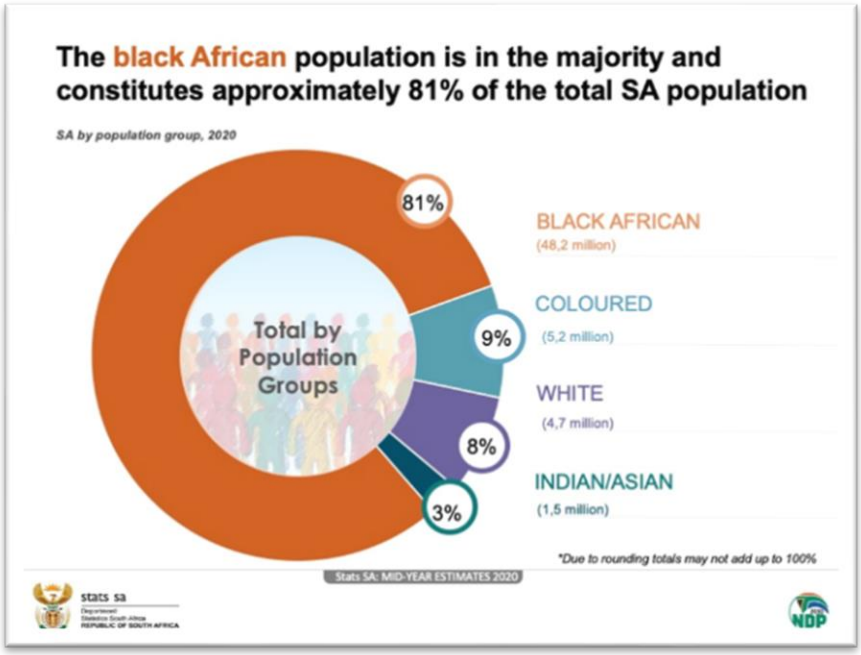

Figure 6.2 Proportions by self-identified ancestry in the general SA population, 2016.

(Source: Statistics SA (7); the term "Coloured" in SA refers to mixed ancestry).

The total number of Black Africans with CF in the SACFR in 2018 was 40 (10%) and 53 (10%) in 2020 (2, 10). As discussed in Chapter 1, the true incidence of CF in Black Africans in SA in unknown but estimated to be in the range between and 1 in 784 to 13 924 live births (11, 12). Although these estimates are imprecise, extrapolation using a conservative incidence of 1:15 000 live births (total live births in SA Black Africans about 1-million in 2020 (13)) would translate to approximately 67 Black African children born in 2020 alone with CF. There appears, therefore, to be a significant discrepancy in population estimates and the number of diagnosed cases of CF in SA, especially in Black Africans. This raises important questions about either the accuracy of these estimates or the possibility that the majority of CF cases in Black Africans are being overlooked, misdiagnosed, or dying in early childhood before a diagnosis of CF is considered. Encouragingly, the proportion of Black Africans diagnosed with CF in SA in 2018 is significantly greater in children aged 0-6 years (25%) compared to ages 6-17 years (11.7%) and adults (< 1%) (2). Reasons for this are speculative but may be due to increased diagnosis rates in young children more recently and/or improved child survival and decreased early childhood mortality of CF in Black Africans.

Outcomes.

Survival (Objective 1)

In line with international trends, improved overall survival, with increasing cohort age and age of death was documented over the 40-year study period (14). The study was not designed to calculate median survival age estimates but significant (86%) reduction in mortality risk was observed after year 2000 in people 10 years and older, and no factor other than time period was associated with survival.

The trend and risk for mortality, however, were different in children < 10 years in whom increased mortality risk persisted after the year 2000. This pattern is concerning and different to what is observed in HIC. Two factors emerged as important determinants for survival: non-White ancestry, and early acquisition of *P. aeruginosa*. The impact of socioeconomic disparities on CF survival are well documented globally but are more striking in SA than anywhere else (15-18). Poor lung function (FEV1z< -2.0) at age 5-8 years at any time during the study period was an additional independent risk factor for increased mortality. This points toward poor health early in life and in school children, and requires targeted interventions to improve survival.

Worldwide, CF survival continues to steadily improve. Estimated median survival age for 2015 to 2019 reached an average 55 years in Canada and Australia, and an even higher 65 years in France (19). Recent projections using person-level simulation models to estimate survival age in the CFTRm era estimate survival will increase to 82.5 years in pwCF homozygous for F508del who initiate ETI at age 12-17 years (20). Current median survival estimates in SA are likely to be the mid-30s (personal observation, SACFR) which is substantially lower than HICs. Without interventions such as NBS-CF, early detection, nutritional support, and treatment of *P. aeruginosa* and reduction in poverty, survival is unlikely to significantly improve in SA, especially in young children with CF. Furthermore, delays in accessing CFTRm therapy and development of new drug therapies for non-responsive *CFTR* variants (e.g., 3120+1G>A) will further widen existing survival disparities both within SA and the rest of the world.

Lung function (Objective 2 and Objective 3)

Lung function is a key measurable outcome in CF which correlates with nutrition, overall health, and survival in all world settings. In this study we found lung function to be significantly lower in SA compared to Canada **(Chapter 4).** Unlike other HIC, Canada in 2018 had not yet implemented universal NBS-CF and CFTRm were not yet widely available, which allowed for better cohort matching than countries where NBS-CF and CFTRm were in place. Factors *other than* NBS-CF, age, sex, age at diagnosis, genotype, nutrition, *P. aeruginosa* infection, pulmonary treatments, lung transplantation and CFTRm must therefore account for observed differences: factors such as inferior quality of CF

care, limited access to expensive therapies and social determinants of health are, therefore, most likely to be important in SA. Importantly, these disparities were already present in early childhood **(Figure 4.2** and **Figure 4.3)**, which suggests that interventions targeting infants and young children are key to improving CF outcomes, including survival in SA.

Within the SA CF registry cohort population, we looked separately at determinants of poor lung function (2). Advancing age, poor nutrition and MRSA infection were independently associated with severe lung disease (SLD, defined as FEV1z < -3.0). Several other known factors associated with poorer lung function outcomes such as *P. aeruginosa* and CF-related diabetes were associated with SLD but not significantly so after adjusted analysis. This cross-sectional study design selected advanced CF lung disease as the primary outcome of interest and, therefore, was unable to explore relationships between less severe lung disease or rate of lung function decline over time. Social determinants of health in SA (e.g. receiving welfare grants, private health insurance), race and genotype (3120+1G >A) did not influence lung function but they were associated with poorer nutrition on univariate analysis.

Nutrition (Objective 2 and Objective 3)

Nutrition is another key CF outcome that correlates closely with LF and survival. Poor nutrition was found to be more common at all ages in SA compared to Canada and in SA was more prevalent in people with lower SES. Malnutrition at the time of CF diagnosis (median age 7 months) was significantly more prevalent in SA (53%) compared to Canada (35%) and in SA, severe malnutrition (WAZ < -3.0) at time of diagnosis was more prevalent in people with mixed ancestry (43%) and Black Africans (77%) compared to Whites (27%) (2) **(Chapter 4).** Although these marked nutritional disparities by ancestry fortunately do not persist with treatment, it does alert us to the possibility that many infants with undiagnosed CF less than 6 months age, especially amongst non-Whites, may be succumbing to severe malnutrition-related complications before CF is diagnosed (21). This is arguably the most compelling reason for why universal NBS-CF must be prioritised in SA and it is the most likely explanation for the underrepresentation of Black Africans diagnosed with CF in SA.

Diagnosis: βeta-adrenergic sweat test (BAST) (Objective 4)

A second part of this study was to pilot the novel β-adrenergic sweat test (BAST) to establish its suitability, especially in children, as an alternative to other established CFTR activity tests in situations where CF diagnosis is inconclusive. Recognition of milder phenotypes and, thus, the full spectrum of CF disease is important in any population for appropriate treatment and counselling.

While the emphasis of previous studies was to describe the spectrum of “typical” CF in SA, they exclude mild phenotypes of CF or CFTR-RD that do not meet strict CF diagnostic criteria. Although the SACFR does include a small number of people with CFTR-RD and unconfirmed CF diagnoses, these

individuals were excluded from the studies. RCWMCH has been following a cohort of children with inconclusive CF diagnosis, i.e. symptoms with positive or borderline sweat chloride levels without genetic confirmation of CF. This led to a pilot and feasibility study using the novel BAST method in combination with NGS of *CFTR* in these individuals to either confirm or exclude CF (22). This study was designed as a pilot study that assumed established reference ranges of the BAST in adults were valid for all ages which was the basis of the study design. Recruiting adult controls subjects thus seemed appropriate but as our results indicated, these assumptions were incorrect.

The main finding was that BAST accurately excluded CF or CFTR-RD in almost all participants (26 children and 6 adults) with inconclusive CF diagnosis. BAST is, therefore, a safe, feasible and simpler alternative to NPD or ICM for measuring CFTR activity and our study found similar diagnostic accuracy of BAST to previous reports in adults. A novel unexpected finding of this study was an age-dependent response of β-adrenergic sweat secretion, with lower β-adrenergic secretion and β-adrenergic:cholinergic ratio in children compared to adults who did not have CF nor carry any *CFTR* variants. This finding has important implications for interpretation of the BAST in children as established reference ranges derived from adults may not be suitable for young children. There is a growing clinical need in countries where NBS-CF takes place to find more discerning diagnostic tests for CF in young children labelled as CFSPID. The BAST has potential to be a suitable candidate in this setting but in its current form has limited application for routine use in young children , e as it requires a series of four intradermal injections , which is technically difficult and not easily accepted for use in infants and young children. Despite the practical and interpretation limitations that we reported, BAST is feasible, safe, and more accurate than SCT for measuring CFTR function in children and adults with inconclusive CF diagnosis, where access to NPD, ICM or other advanced methods to measure CFTR activity are unavailable. As a consequence, eight children had their previous CF diagnosis reversed and were removed from the SACFR.

Strengths and limitations

Studies derived from SACFR data represent the first comprehensive description of pwCF in SA with broad representation from SA's diverse society in terms of socioeconomic, demographic, and healthcare delivery backgrounds. In addition to identifying within-country disparities and factors linked to less favourable outcomes, this work provided benchmarking evidence for disparities in CF outcomes between SA and Canada, thereby also highlighting deficiencies in CF care in SA. South Africa shares many similarities and barriers in CF Care with other LMIC. Knowledge and insight gained by this research in SA may therefore be relevant to other LMIC to support global advocacy efforts for better CF care. A further strength of SACFR data and related studies is that data variables and definitions

were designed to be harmonised with data collection in international CF registries such as the European and Canadian CF registries. Harmonisation of CF registries data is important for robust comparison and CF research in different world settings (23, 24).

A key question and limitation of SACFR data is how well it represents the true CF population in SA, both diagnosed and undiagnosed. SA's complex healthcare delivery infrastructure is fragmented between private and public sectors and there are few incentives for pwCF to attend recognised CF clinics or private practices. It is, therefore, possible that not all diagnosed pwCF in SA are captured in the SACFR, but it is estimated that at least 80% of the known CF population was captured in the first year of establishing the SACFR in 2018. The CF community in SA including CF clinics and specialists, is highly organised and through its network ensures referral to appropriate CF clinics for most pwCF. It is therefore unlikely that large numbers of people with diagnosed CF are missing from the SACFR. Latest numbers of pwCF in the SACFR stand at 523 **(Appendix B)** and follow-up and retention in the SA registry is high, including capturing of key events such as deaths and transplants. The question of how many pwCF are alive in SA but not yet diagnosed, or how many undocumented, undiagnosed CF-related deaths are occurring is challenging without more comprehensive population-based genetic prevalence studies or implementation of universal NBS-CF.

A second limitation of these studies is that longitudinal data were not available due to the SACFR only commencing prospective data collection from 2018. Cross-sectional analysis of registry cohorts limit the interpretation of observed age-trends as they cannot be interpreted as average trends of individuals over time. Longitudinal analysis of registry data of individual trends will with time enhance the interpretability of the observed trends and observed differences between future cohorts. The retrospective survival analysis spanning 40 years at RCWMCH provided valuable survival data for SA, which is generalisable to the rest of SA due to similarities in populations and healthcare infrastructure across SA. Although it was a single-centre study, it highlighted important survival disparities determined by ancestry and socioeconomic status that persist in SA today.

The inclusion of the BAST pilot study was to address a knowledge gap in SA of the existence and prevalence of mild CF phenotypes and CFTR-RD that are likely excluded from the SACFR due to being overlooked and misdiagnosed in SA. Furthermore, as demonstrated by this study, people with incorrect CF diagnosis due to false positive SCTs were identified through this study, which highlights the limitation of SCT. We demonstrated that the BAST is a feasible, safe alternative to NPD or ICM and our experience with acquiring the technology and skills to perform the test supports the position that the BAST is the preferred CFTR function testing modality where SCT is inconclusive. However, limitations of the BAST were also highlighted through our experience, including poor tolerability and

acceptance in very young children; uncertainty of reference standards in children; and difficulty in sourcing and procuring in SA the injectable drugs of the BAST which were not licensed for this indication, thereby creating regulatory barriers for injecting drugs for off-label purposes into children.

Research in context: impact, future research, and recommendations

Establishing the SACFR and the findings of this research have both direct and indirect impact in a number of areas of CF care and research, in SA and internationally.

Advocacy

An important outcome has been to strengthen and support local and international advocacy initiatives addressing inequity in global access to lifesaving CFTRm drugs in LMIC. Re-imbursement agreements for CFTRm drugs have been reached in over 40 countries in the world. However, these are almost exclusively HIC in North America, Europe, Israel, Australia, and New Zealand (25) **(Figure 6.3).** We have demonstrated disparities in CF outcomes at country and international level due to multiple factors including quality of CF care, social determinants of health and ancestry (2, 14) **(Chapter 4**). These studies coincided with the rapid evolution of the CFTRm era, which has revolutionised CF treatment in just a few years since FDA approval of ETI in 2019 (26). Expanded access of CFTRm in only HIC has exposed global disparities in CF care and by all projections, existing disparities in CF treatment and outcomes will widen further if access to CFTRm drugs continues to be determined by wealth, geography and ancestry (20, 25, 27-29). There is no doubt that initiating CFTRm therapies, especially in children, will improve CF care in LMIC. Current barriers to equitable and affordable access to CFTRm in SA and other LMIC have highlighted the recurring problem of how international pharmaceutical patents prevent access to transformative medicines in LMIC. Most countries are signatories to the World Trade Organization agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) and under this agreement, countries are required to offer patents to pharmaceutical companies, which typically are valid for 20 years, thus offering extended period market exclusivity without competition. While protecting intellectual property rights is important, patent regulations and laws are open to abuse. Vertex Pharmaceuticals hold the global monopoly on all CFTRm drugs and have patents in many LMIC that are protected by TRIPS. However, Vertex Pharmaceuticals have not registered, nor do they intend registering any CFTRm in SA, effectively blocking SA from sourcing cheaper generic options such as those which are manufactured in Argentina. The annual estimated minimum production cost of ETI for one person for one year is $5,676, which is over 90% lower than the manufacturers asking price of over $300 000 per year, per person (30). Such prohibitive pricing for a life-long treatment is beyond the reach of governments and health budgets in LMIC. Data derived from the SACFR, and related studies have been used to support a variety of initiatives and campaigns, some of which are highlighted below:

- *Vertex Save Us global campaign*: a global multi-national (60+ countries) activist campaign that calls for equal and affordable global access to CFTRm drugs throughout the world (31).

- *A High Court application* in SA filed by a CF patient requesting the Patent Commissioner to issue a Compulsory License to import a generic ETI and other CFTRm drugs on the grounds that Vertex Pharmaceuticals has abused its patent right by not registering their drugs in SA and blocking the importation and distribution of generic alternatives. This landmark legal challenge, a first for SA, was filed on 07 February 2023 and was widely reported by local and international media outlets, including *the New York Times* **(Appendix C)** (32, 33). The case is being closely followed by international campaigners and other countries for the precedent it may potentially set for not only CFTRm drugs, but also expensive treatments for other rare and orphan diseases. Data from these studies and expert affidavits were filed as supporting documents for the High Court application.
- An investigation by the *Competition Commission* into Vertex Pharmaceuticals for uncompetitive practices in SA relating to CFTRm drugs. Data from these studies and my expert advice has been provided to the Competition Commission in its ongoing investigation into the matter.
- Publication of opinion pieces and letters in support of global efforts and calls for equal and affordable access to CFTRm drugs across the world (25, 34, 35).

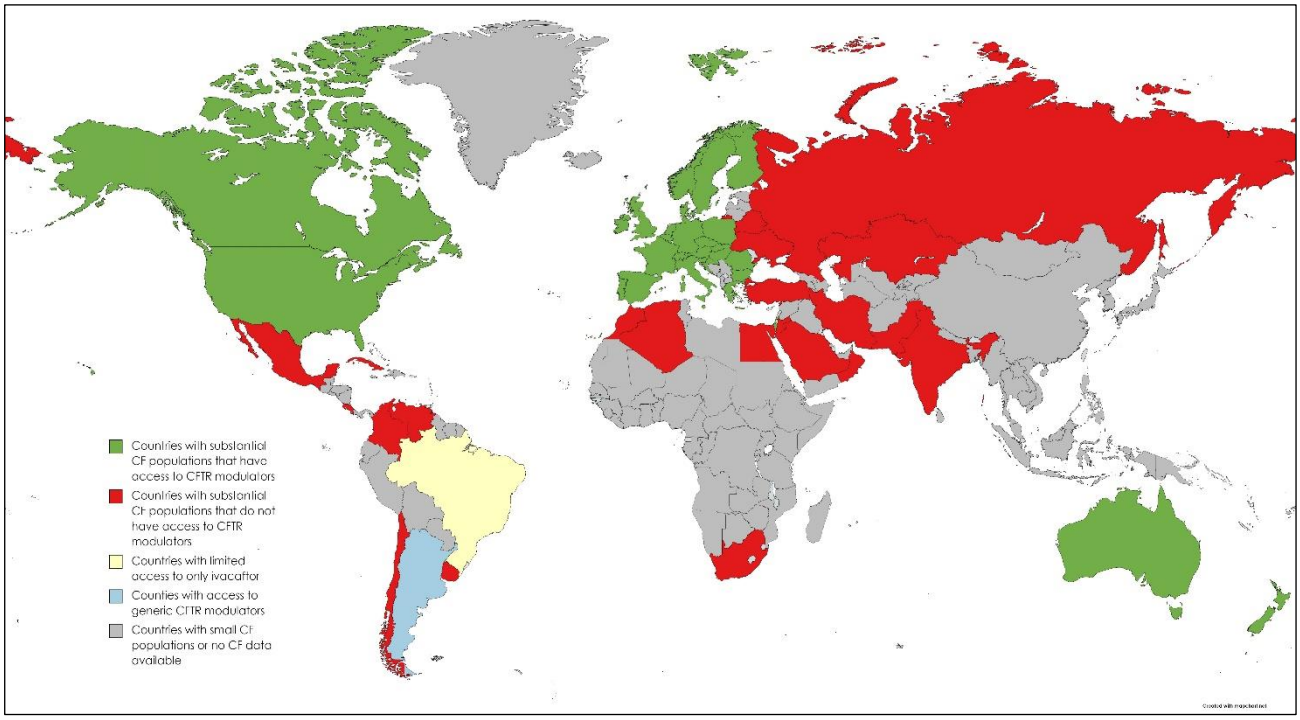

Figure 6.3 World map distribution of countries with access to CFTR modulator drugs or negotiated agreements in place, March 2023

Source: Zampoli *et al*. (25).

International collaborations

A number of international collaborations and research partnerships were created as a result of this work and establishment of the SACFR. The COVID-19 pandemic and concerns of the risk of SARS-CoV-2 infection to pwCF led researchers from numerous countries with CF registries (including SA) to collaborate and pool data on COVID-19 in pwCF. Through this collaboration, we learnt that COVID-19 was not a major threat to all pwCF as expected, especially children. However, solid organ transplant recipients, older age, non-White ancestry, underweight body mass index and CF-related diabetes are associated with increased risk of more disease (36, 37). This multi-national CF Registry Collaboration is still active and has received a competitive research grant to investigate the long-term impact of SARS-CoV-2 infection in pwCF as well as address emerging research priorities for global CF care.

Our work and experience with the BAST in SA have provided new insight and knowledge of this new diagnostic test for CF, especially in children where there was previously sparse data. This work is recognised by international partners in Europe with whom collaboration is ongoing to explore the utility and feasibility of BAST in children with CFSPID.

Future research priorities

1. The true burden of CF in SA and sub-Saharan Africa is unknown. This presents a major barrier for improving care and advocating for better treatment on the continent. Apart from SA, the capacity to diagnose CF is mostly absent in other sub-Saharan African countries. Population-based genomic research to investigate the prevalence of *CFTR* variants in African populations is needed to estimate the expected incidence and hidden burden of CF in sub-Saharan Africa, which will in turn provide evidence to justify improving capacity for diagnosing and managing CF.

2. Universal NBS-CF should be implemented in SA, but which screening approach and strategy is most suited and cost-effective for SA's diverse population and resource-constrained public health sector is unknown. Evidence in these studies suggests that NBS-CF is needed to address racial and socioeconomic disparities in CF outcomes, including early childhood mortality. Specific research questions to address include the feasibility of collecting dry-blood spot samples for immunoreactive trypsinogen (IRT) assay within the first 24 hours after birth; the role of pancreatitis-associated protein (PAP) in an NBS-CF protocol in SA; and if DNA-based testing for common *CFTR* variants should be included in any NBS-CF protocol in SA.

3. The SACFR is providing valuable longitudinal data of CF in SA. Tracking and monitoring trends in CF outcome, including projected survival estimates will be important to measure the impact of any interventions and continued advocacy for improving care.

4. About 90% of the CF registry population in SA and HICs are eligible and stand to benefit from ETI and other CFTRm drugs. There are currently no targeted drug therapies for the remaining 10% of pwCF who have *CFTR* variants not responsive to approved CFTRm drugs, which is proportionally higher in LMIC with populations who have greater ancestral and genotype diversity. Clinical trials of new drug therapies and molecules such as nucleic acid-based therapies should be more inclusive of pwCF living in LMIC. South Africa is ideally positioned to conduct clinical trials for this purpose as well as having the unique position where most pwCF are CFTRm naïve.

Recommendations for policy and practice

1. *Revision of CF treatment guidelines in SA:* Although consensus CF treatment guidelines exist in SA, these were last revised in 2017 and are currently outdated. Strong recommendation for new CFTRm in eligible patients should be included in new guidelines (38).

2. *Improving capacity and resources for CF and CFTR-RD diagnosis*: Expertise and capacity for sweat testing in SA is restricted to the major cities in four of the nine provinces. Less resourced provinces with predominantly rural and poorer populations (e.g. Limpopo, Northern Cape, Mpumalanga, Eastern Cape, and Free State) should develop at least one referral centre that offers sweat testing. The BAST is recommended as a feasible alternative for measuring CFTR function in older children where CF diagnosis is inconclusive. However its wider application outside a research setting is limited due to technical challenges of injecting drugs and limited data on refences ranges in young children. Expert training and procurement of equipment and drugs to perform this test is recommended in at least two specialised CF centres across SA. In addition to sweat testing, NGS of *CFTR* must be offered by molecular genetics diagnostic services in the public sector NHLS. Next generation sequencing is currently only offered by private laboratories in SA.

3. *Implement universal newborn screening for treatable genetic conditions in SA as a public health intervention*: Newborn screening for any condition, including congenital hypothyroidism, is

currently not in place in the public sector in SA. Although there is acknowledgement by the Department of Health that NBS is important, implementation and resources to roll out NBS, including for CF, still have a long way to go. NBS-CF in SA will very likely detect many previously undiagnosed cases, especially in Black Africans, reduce infant mortality and improve overall CF outcomes in SA.

4. *Improved CF care in infants and young children*: Data from these studies have identified a pattern that poor nutrition and lung function outcomes in SA originate in early childhood. Apart from earlier diagnosis through NBS-CF, more intensive nutritional interventions and rehabilitation is required in pwCF, especially young children who are malnourished. Interventions to improve lung health include strengthening of surveillance and treatment of infection with *P. aeruginosa* and MRSA and adopting in routine practice more sensitive lung function techniques such as multiple breath washout to detect early lung disease in pre-school children and children with normal spirometry. Strengthening basic symptomatic CF treatment should not be overlooked in LMIC where proportionally more pwCF are ineligible for CFTRm.

5. *Regulation and advocacy*: Continued advocacy for access to affordable CFTRm drugs, including support for the legal challenge to set aside the patent restrictions is important. Recognition by government and private medical schemes that CF is included as one of the listed conditions that must be re-imbursed in full is needed to address inequalities in CF care that exist in SA.

Conclusion

These studies on CF in SA have demonstrated the value of CF registry-based research and have highlighted important disparities in CF outcomes both within SA and compared to HIC standards. Key findings highlight the importance of strengthening early diagnosis, nutritional support, and lung health. A further finding of this research is that the BAST is a feasible alternative in older children for diagnosing or excluding CF where routine tests are inconclusive. However, the BAST has limited application in very young children in whom validated reference standards have not yet been established. This research has global relevance for other LMIC countries with similar socioeconomic conditions and challenges impacting CF care and outcomes as South Africa. In addition to implementing NBS-CF and improving the quality of established CF care, affordable and equitable global access to CFTRm drugs in LMIC is a priority to prevent the inevitable widening disparity in CF outcomes that will see pwCF in LMIC being left further behind in this new era of CF treatment.

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APPENDICES

APPENDIX A:

SACFR Variables

Appendix A

SACFR Variables

Form	Field Label	Explanation	Notes
Baseline and diagnosis	National Registry Patient Identifier	unique ID autogenerated by Redcap	
	CF Centre Code		User to select code for their centre from drop down list
	CF Centre Patient ID	Hospital folder number (public sector) or SA ID number (private); leave blank if not either	
	Surname		
	First name		
	Date of birth		
	Year of birth		
	Month of birth	1(Jan)-12(Dec)	
	Day of birth	a number 1 up to 31	
	gender	Male or Female	
	Self -identified ethnicity	Caucasian	
		Mixed Race	
		Black African	
		Indian	
		Asian	
		Other	
	Date of CF diagnosis		use 15th day of calendar month if exact date unknown
	Age CF diagnosis in Years	calculated field	
	Diagnosis confirmed?	Yes / No / Diagnosis to be confirmed	Two sweat chloride tests ≥60 mmol/l OR sweat conductivity ≥80mmol/l OR 2 pathogenic CFTR mutations
	Sweat test type 1	Sweat Chloride or Conductivity	In addition to sweat test at diagnosis. Individuals who start CFTR genetic modulator therapy will have sweat
	sweat Chloride level mmol/L 1		

	sweat Chloride level mmol/L 2		test results recorded before and after initiation of therapy
	Sweat test type 2	Sweat Chloride or Conductivity	
	sweat conductivity level 1		
	sweat conductivity level 2		
	meconium ileus	No	
		Yes, operated	
		Yes, not operated	
		Yes, don't know if operated	
	Neonatal screening	Not done in SA	
		Performed, result positive	
		Performed, result negative	
		Performed, result unkown	
	Mutation 1		Drop down list of 50 mutations : select Other if not on this list.; Mutations NOT in CFTR 2 database can only be included if sweat tests consistent with CF diagnosis.
	other mutation 1		
	Mutation 2		
	Other mutation 2		
	Genotype Category	F508del homozygous / F508del heterozygous / Other	
	Weight diagnosis (kg)		
	Length at diagnosis (cm)		
	Faecal elastase at diagnosis (ug/g)		
	Has signed consent been obtained ?	Yes/No	
Status in year of follow-up	Current status	Died during year of follow-up	
		Alive at 31/12 and seen in year of follow-up	
		Alive but not seen in year of follow-up	
		Lost to follow up (not seen for 3rd consecutive year)	

		Emigrated in year of follow up	
		CF diagnosis reversed or excluded in year of follow-up	
	Date of death		use 15th day of calendar month if exact date unknown
	Year of death		
	Month of death		
	Day of death		use 15th day of calendar month if exact date unknown
	Cause of death	Respiratory	
		Liver GI	
		Trauma	
		Suicide	
		Transplant	
		other	
	Date last seen if Lost to Follow-up		use 15th day of calendar month if exact date unknown
	Number of contacts/visits in year of f/up	1-5 or more	
	Health sector point of care	Private / Public / Private and Public	where ambulatory/OPD care is provided
	Lung transplant	Yes/No	
	Year of lung transplant		
	Liver transplant	Yes/No	
	Year of latest liver transplant		
	Ever had Pseudomonas?	Yes/No	
	date first ever pseudomonas		use 15th day of calendar month if exact date unknown
	HIV infection status	Uninfected	
		Exposed, uninfected	
		Infected	
	Education	currently enrolled at school (minor)	
		not attending school (minor)	
		completed high school	

		incomplete high school	
		tertiary education/qualification	
		NA preschool age	
	Employment (adult)	Full-time, part-time, unemployed, student, disabled, retired, NA child	
	Socio-Economic Factors	Active Smoking or household tobacco smoke exposure	
		Informal housing without running water/toilet	
		Informal housing without electricity	
		Receives care dependency or disability grant	
		Relies on public transport	
Growth and lung function	Weight (kg)		
	Height/length (cm)		
	BMI	calculated field	
	BMI z score	calculated field according to WHO	
	Date of Best FEV1 or weight (if no FEV1) recorded for the year	select best FEV1% pred and record the FEV1 Value in Litres	If no lung function test, use day of last height and weight measurement of the year; If neither lung function test nor height and weight measurement carried out, enter -1.00 If only the day is unknown (but the month is known), use 15; If done, but date is unknown, use 1 (for 1/7), or 31 (for 31/12, if the patient was born after 1/7 of the year of f.u; If patient died during the year of follow-up and date of FEV1 is unknown, enter the month of last height and weight measurement of the year;
	Age (years) of best FEV1/weight for the year	Calculated field	
	Month of Best FEV1	1 to 12	
	Day of Month Best FEV1 recorded this year		
	FEV1 (Litres)	Record value in litres of the highest FEV1% predicted of the year according to local reference values. (to 2 decimals; use a point/dot '.' for decimals, not a comma ',')	
	FEV1% pred local ref	record FEV1 % predicted on using local spirometry equations	
	FEV 1 % predicted	calculated field – according to GLI	

	FEV1 z score	calculated field – according to GLI	
	Forced Vital Capacity (Litres)	Record value in litres of the measured with highest FEV1 value of the year according to local reference values. (to 2 decimals; use a point/dot '.' for decimals, not a comma ',') : enter **-1.00** if no FVC measurement	
	Forced Vital Capacity % pred	calculated field	
	Forced Vital Capacity z score	calculated field	
Microbiology	Were respiratory samples collected in year of F/Up?	Yes / No	
	Number of respiratory samples	1 -5 or more	
	Any Pseudomonas infection this year?	Yes / No	
	Chronic pseudomonas infection	Yes / No.	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any haemophilus influeza infection?	Yes / No	
	Chronic haemophilus infection?	Yes / No	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any MSSA infection this year?	Yes / No	
	Chronic MSSA infection this year?	Yes / No	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any Burkholderia cepacia complex infection?	Yes / No	
	Chronic Burkholderia cepacia complex infection?	Yes / No	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any Stenotrophomonas maltophilia infection this year?	Yes / No	
	Any mycobaterial cultures done in the year of F/Up?	Yes / No	

	Any non-tuberculous mycobacterial infection this year?	Yes / No	
	Which NTM infection?	M. Absessus complex	
		M. Avium complex	
		Other	
		Unknown only AFB+	
	Any Mycobacterium tuberculosis infection this year?	Yes / No	
	Any achromobacter spp infection this year?	Yes / No	
	Any MRSA infection this year?	Yes / No	
	Chronic MRSA infection	Yes / No	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any Aspergillus spp infection?	Yes / No	
	Chronic Aspergillus spp infection?	Yes / No	Minimum of 4 sampling episodes per year or other evidence of chronic infection
	Any other fungal or mould infection this year?	Yes / No	
	Which fungus or mould infection?	Candida spp	
		Scedosporium spp	
		Other	Free Text field
	SARS-CoV-2 tested?	Yes / No	
	Was any SARS COV-2-PCR positive or inconclusive?	Yes / No	
	If yes, date of positive test		
	Reasons for SARS COV-2 testing	New Symptoms	
		Household Contact	
		Other Close Contact	
		Pre-spirometry screen	
		Pre-procedure or admission	

		Unclear	
	Other evidence of SARS CoV-2 Illness		Free text field
	SARS-CoV-2 symptoms		Free text field
	SARS-CoV-2 illness severity	asymptomatic	
		treated at home	
		treated at ward	
		treated in ICU	
	SARS-COV-2 management	Free text	
	SARS-COV-2 outcome	Recovered	
		died	
		other complications	
Complications	Pancreatic status	Insufficient/Sufficient	Assume insufficient if on creon
	Faecal elastase done?	Yes/No	
	Faecal elastase level this year	< 200ug/g once	
		< 200ug/g twice	
		≥ 200ug/g once	
		≥ 200ug/g once	
	Faecal elastase level this year (ug/g)		
	Allergic bronchopulmonary aspergillosis this year	Yes/No	Diagnostic criteria: 1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, induced asthma, change in pulmonary function, or increased sputum production) not attributable to another aetiology. 2. Total IgE > 500 IU/ml. 3. Positive skin prick test for Aspergillus antigen (> 3 mm) or positive specific IgE for A. fumigatus. 4. Either: a. precipitins to A. fumigatus or in vitro demonstration of IgG antibody to A. fumigatus; b. or new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (characteristic changes)

			that have not cleared with antibiotics and standard physiotherapy
	Distal intestinal obstruction syndrome	Yes/No	Abdominal pain or constipation needing treatment
	Diabetes : treated with insulin this year	Yes/No	
	Salt depletion	Yes/No	Symptomatic or hyponatraemia
	Pneumothorax requiring chest drain	Yes/No	
	Liver disease this year	No	Liver function will be monitored at each routine visit and after initiation of CFTR genetic modulator therapy. Requires liver function tests and or ultrasound report, Select UNKNOWN if these not done or no evidence of previous liver disease
		Cirrhosis with hypertension / hypersplenism	
		Cirrhosis with hypertension / hypersplenism	
		Cirrhosis hypertension unknown	
		Liver disease without cirrhosis	
	haemoptysis major over 250 ml this year	No/ Yes at least once in year of follow up	
	Port-related complications this year	Yes/No / Does not have a port	
	Occurrence of malignancy this year	Yes/No	
	Other complications co-morbidity this year?	Anxiety or Depression	
		Other Mental health	
		ADHD	
		Nasal Polyps requiring surgery	
		Chronic sinusitis requiring treatment or surgery	
		Systemic hypertension	
		Pulmonary Hypertension	
		Renal failure	

		Other	free text field
Therapies	Inhaled continuous hypertonic saline this year (> 3 months)	Yes/No	
	Inhaled continuous rhDNase this year	Yes/No	
	Inhaled continuous (> 3 months) or alternate month antibiotic this year	Yes/No	
	Inhaled continuous (> 3 months) corticosteroids this year?	Yes/No	
	Use of continuous oral steroids (> 3 months) this year?	Yes/No	
	inhaled continuous (> 3 months) bronchodilator this year?	Yes/No	
	Use of continuous azithromycin (or other macrolide) therapy this year	Yes/No	
	In Oxygen therapy any time this year	No	
		Yes, with exacerbation / acute illness	
		Yes, nocturnally or continuously at home	
	Use of non-invasive ventilation this year?	Yes/No	
	Use or Urso deoxycholic acid this year	Yes/No	
	Use of pancreatic enzymes this year	Yes/No	
	use of proton pump inhibitors this year	Yes/No	
	Gastronomy inserted or used in year of follow-up?	Yes/No	

	Treated with IVI antibiotics this year	Yes/No	
	Number of IVI antibiotic courses this year	1 – 5 or more	
	CFTR modifier therapy	Yes/No	
	If yes, date started		
	CFTR modifier therapy drug name recorded		
	Received SARS CoV 2 vaccine?	Yes/No	
	If yes, date of vaccine recorded		
Fertility	Pregnant in year of follow-up?	Yes/No	
	Pregnancy outcome in year of follow up	Yes/No/ N/A as <16 years	
	Pregnancy outcome other, specify	Carried to term and delivered in year of follow up	
		Preterm delivery	
		Still pregnant at 31/12	
		Spontaneous abortion	
		Medical termination	
		Other	Free text field
	Male patient conceived pregnancy by artificial or natural means in year of follow-up?	Yes/No/ N/A as <16 years	
Missing Data codes			Unknown Not asked Asked but Unknown

SOUTH AFRICAN CYSTIC FIBROSIS REGISTRY

2021 ANNUAL REPORT

CONTENTS

AUTHORS

Zampoli M, Verstraete J., Frauendorf M, and the CF Steering Committee*

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Report can be accessed at: https://sacfa.org.za/

PREFACE

This third edition of the Cystic Fibrosis Registry Annual Report represents a great achievement for the South African Cystic Fibrosis (CF) community, who initiated the registry project in 2018. The relevance and importance of the registry and the information we document through publication of this report cannot be more emphasised in this new era of CF treatment with CFTR modulators. While we do not yet have sufficient longitudinal data to identify patterns or trends of CF care and outcomes in South Africa, we can begin to compare how people with CF in South Africa are doing in comparison to their counterparts in other parts of the world, who are being treated with CFTR modulators. Real-life experience and data on the profound impact of CFTR modulators on people with CF is transforming CF from a serious condition with limited life expectancy to a manageable chronic condition where projected life-expectancy and health is rapidly improving. The South African CF community is living through both an exciting and frustrating time where some families with means are taking the extraordinary step of travelling to Argentina to collect medication for their loved ones. At the same time, South Africa leads the way for other low-and-middle income countries with legal challenges to break the monopoly of CFTR modulator drugs which is protected by international patent agreements. There is much to be optimistic and proud about, but at the same time the stark realities of growing disparities between rich and poor cannot not be ignored. The registry reports will continue to be important ammunition as we move forward with improving CF care for South Africans.

We thank the many contributors, CF families and colleagues who graciously continue to support and share their data toward the South African Cystic Fibrosis Registry.

With thanks

ASSOCIATE PROFESSOR MARCO ZAMPOLI
National Co-ordinator SA CF Registry
Chair: Medical and Scientific Advisory Committee SA

MR ALAN DUNN
Chair: South African
Cystic Fibrosis Association

LIST OF PARTICIPATING CF CARE SITES & STEERING COMMITTEE MEMBERS IN SOUTH AFRICA*

PROVINCE	CENTRE	CONTACT
Eastern Cape	Netcare Greenacres Hospital Life St George's Hospital PE Provincial Hospital	Dr Paul Gebers
Free State	Universitas Hospital	Dr Pieter de Waal Dr Shaun Maasdorp
Gauteng	Charlotte Maxeke Hospital	Paeds: Prof Debbie White Adults: Dr Erica Shaddock
	Steve Biko Academic Hospital	
	Netcare Milpark Hospital	Dr Cathy Baird
	Netcare Linksfield Hospital	Dr Carla Els
	Sandton Medi-Clinic	Dr Dave Richard
KwaZulu-Natal	Inkosi Albert Luthuli Central Hospital	Prof Refiloe Masekela Dr Reratilwe Mphahlele
	Busamed Hillcrest Private Hospital	Dr Graham Lawrence
	Netcare St Augustines	Dr Jonathan Egner
Western Cape	Red Cross War Memorial Children's Hospital	Prof Marco Zampoli
	The Chest and Allergy Centre; Christian Barnard Memorial Hospital	Dr Tarryn Gray Dr Julie Morrison
	Tygerberg Hospital	Dr JLunga Mfingwana Prof Pierre Goussard
	Groote Schuur Hospital	Prof Greg Calligaro
	UCT Private Academic Hospital	Prof Paul Wilcox
	Panorama Medi-Clinic Hospital	Dr Tony Biebuyck

INTRODUCTION

THE SOUTH AFRICAN CYSTIC FIBROSIS REGISTRY

The South African Cystic Fibrosis Association (SACFA) in collaboration with health professionals across South Africa established the South African Cystic Fibrosis Registry (SACFR) in 2018. Medical information obtained through routine medical care is collected and updated on an annual basis. The SACFR has been approved for each participating centre by research ethics committees affiliated to universities throughout SA.

Data is stored in a secure central database. Data is available for scientific purposes on application. All requests are reviewed by the SACFR Steering Committee.

All the data analyses presented in this report have been recalculated to include data that may have been updated or missed in previous years. This ensure that data is compared accurately between different years within this report. However, discrepancies might occur when comparing historical reports with the current one.

At the end of December 2021 there were 523 registered patients who were seen in 2021 across 17 CF Centres in 5 Provinces (this excludes 9 patients whose diagnosis was unconfirmed). This report summarises the diagnostic information and annual review of these patients. The annual review includes record of lung function, nutrition, microbiology, complications, therapies, transplantations and mortality. Due to the retrospective nature of data collection missing/unknown data has been specified.

GLOSSARY & ABBREVIATIONS

EXPLANATION OF TERMS

ABPA:
Allergic Bronchopulmonary Aspergillosis, an allergic reaction to *Aspergillus fumigatus.*

BMI:
Body Mass Index calculated by weight (kg)/[height (m)]^2. This is used as an indicator of nutritional status or to categorise one as underweight or overweight.

Bronchodilator:
Medication that relaxes the muscles in the airways, in the registry this refers to inhaled forms of bronchodilators.

CFRD:
CF related diabetes.

CFTR:
CF transmembrane conductance regulator. This protein which is situated at the cell surface controls the transport of salt and water across the cell. The gene that causes CF gives instructions to the CFTR protein. Everyone has two copies of the gene for CFTR and in people with CF both CFTR genes are affected by a CF-causing mutation.

FEV_1:
Forced expiratory volume in one second, measured during a Lung Function Test or Spirometry.

FEV_1%:
FEV^1 value expressed as a percentage of the average for healthy peers of the same age, height and sex.

Haemoptysis:
Coughing up blood. The complication recorded in the registry is for a major bleed of more than 250ml as many people with CF will often have bleeding in small amounts.

Homozygous:
CF is caused by mutations of the CFTR gene. The gene has two allele, one which is inherited from the mother and one from the father. Both allele need to have a mutation to cause CF. If both mutations on the CFTR gene are the same, the person is said to by homozygous for the mutation.

Heterozygous:
CF is caused by mutations of the CFTR gene. The gene has two allele, one which is inherited from the mother and one from the father. Both alleles need to have a mutation to cause CF. If the CF gene has two different mutations the person is said to by heterozygous.

Max:
Maximum. This refers to the highest value.

Mean:
This is the average value and is calculated by adding up all the values and then dividing it by the number of values that there are.

Meconium Ileus:
This is an obstruction in a baby's small intestine with thick and sticky faeces. In some cases, this obstruction needs to be surgically removed.

Median:
This is the middle number where 50% of the measurements are above this number and 50% of the measurements are below this number.

Min:
Minimum. This refers to the lowest value.

N:
The number of patients in a group for whom the information is not missing.

NaCl:
Sodium chloride, which in the registry refers to inhaled hypertonic saline which is used to loosen thick and sticky secretions in the lungs.

Pancreatic Insufficiency:
When the pancreas does not produce enough enzyme that the body uses to digest food in the small intestine. This will lead to malnutrition if it is not treated with synthetic pancreatic enzyme replacement therapy.

25th Pctl:
25th Percentile which can also be called the first quartile. This value separates the measurements so that 25% of them are below that number and 75% are above it.

75th Pctl:
75th Percentile which can also be called the third quartile. This value separates the measurements so that 75% are below that number and 25% are above it.

Pneumothorax:
A pneumothorax is a collapsed lung and in people with CF this is usually caused by severe lung damage.

rhDNase:
Recombinant human DNase (marketed as Pulmozyme) which is an inhaled solution to assist with loosening of thick and sticky secretions in the lungs.

Z-score:
This is a standardised score and allows us to compare the results from a test to a normal population of the same age and sex. The mean of the normal population is 0 and a standard deviation of 1 indicates how far the value is from the normal or reference population. Negative scores indicate that the value is below that of the mean for the reference population. Positive values mean that the value is above the mean for the reference population.

SUMMARY OF 2021 DATA

OUTCOME		FEMALES	MALES	TOTAL
Patients registered in the SACFR	Number (%)	269 (51.4%)	254 (48.6%)	523
Age (years) at follow-up (on 31/12/2021)	Median (IQR)	17.8 (10.1,29.2)	16.3 (9.8,25.8)	17.2 (9.8,27.3)
Patients ≥18 years (on 31/12/2021)	Number (%)	130 (48.3%)	116 (45.7%)	246 (47.0%)
Age at diagnosis	Mean (SD) (years) Median (IQR) (months)	3.8 (6.6) 8.9 (3.0,42.5)	3.5 (7.3) 7.2 (2.9,37.4)	3.7 (6.9) 7.8 (2.9,39.9)
Patients with at least one F508del allele recorded	Number (%)	217 (80.7%)	195 (76.8%)	412 (78.8%)
Patients living with a lung transplant in 2021	Number (%)	8 (3.0%)	4 (1.6%)	12 (2.3%)
Patients living with a liver transplant in 2021	Number (%)	0	1 (0.4%)	1 (0.2%)
Patients deceased in 2021	Number (%)	8 (3.0%)	9 (3.5%)	17 (3.3%)
Age at death 2021 (years)	Mean (SD) Median (IQR)	27.0 (8.8) 27.5 (22.0,30.5)	29.1 (13.2) 24 (19.0,35.0)	28.1 (11.0) 26 (20.0,32.0)
Patients receiving exclusive Public Health Care	Number (%)	86 (32.0%)	109 (42.9%)	195 (37.3%)
Patients receiving any Private Health Care	Number (%)	166 (61.7%)	132 (52.0%)	298 (57.0%)

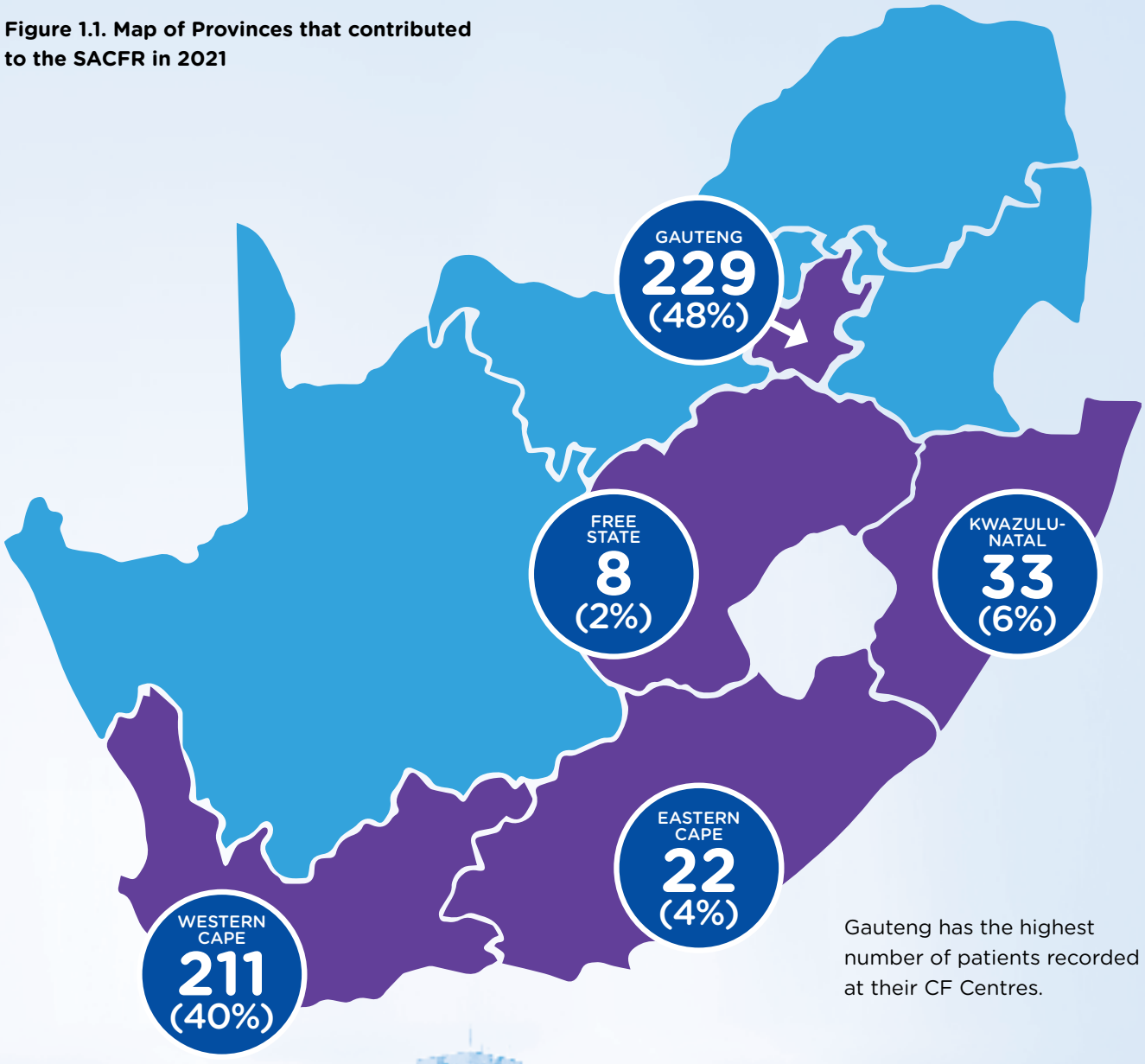
SUMMARY OF 2018-2021 DATA

		2018 N=482	2019 N=515	2020 N=525	2021 N=523
Age (years) on 31 December	Median (IQR)	15.5 (8.3,26.2)	15.7 (8.3,26.1)	16.7 (9.1,27.0)	17.2 (9.8,27.3)
Patients ≥ 18 years	Number (%)	206 (42.7%)	220 (42.7%)	234 (44.6%)	246 (47.0%)
Males	Number (%)	231 (47.9%)	247 (48.0%)	252 (48.0%)	254 (48.6%)
New diagnosis	Number (%)	33 (6.8%)	32 (6.2%)	11 (2.1%)	10 (1.9%)
Age of new diagnosis (months)	Median (IQR)	8.4 (4.0,81.8)	35.8 (5.9,173.8)	7.6 (0.6,162.1)	5.0 (0.7,7.0)
CFTR modulator therapy	Number (%)	1 (0.2%)	2 (0.4%)	4 (0.8%)	7 (1.3%)
Lung transplants	Number (%)	3 (0.6%)	2 (0.4%)	2 (0.4%)	1 (0.2%)
Liver transplants	Number (%)	0	0	0	0
Deaths	Number (%)	3 (0.6%)	5 (1.0%)	5 (1.0%)	17 (3.3%)
CF diagnosis reversed or excluded	Number (%)	0	0	2 (0.4%)	5 (1.0%)
Emigrated	Number (%)	2 (0.4%)	0	7 (1.3%)	6 (1.1%)
Lost to follow up	Number (%)	0	0	1 (0.2%)	3 (0.6%)

DATA REPORT

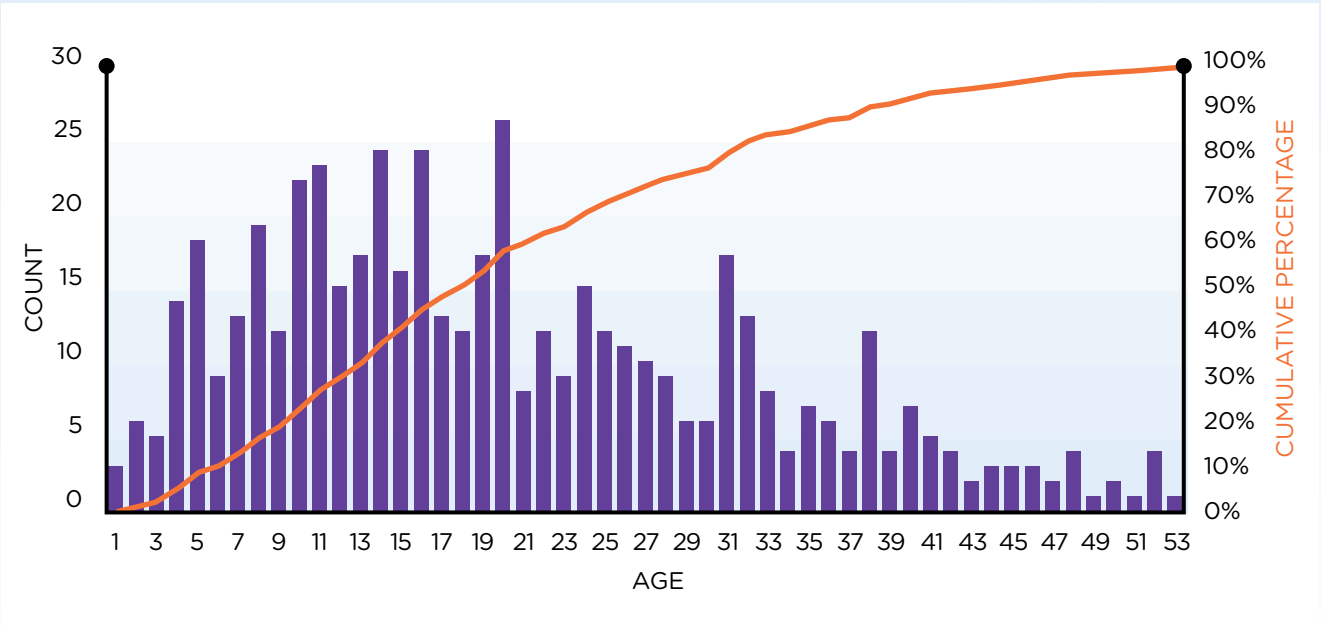
1 DEMOGRAPHICS

Figure 1.1. Map of Provinces that contributed to the SACFR in 2021



Gauteng has the highest number of patients recorded at their CF Centres.

Figure 1.2. Age of people with CF on 31/12/2021



Each purple vertical bar represents the number of patients of that age alive in 2021. The cumulative percentage (orange line) describes how many patients (as a percentage) are below a certain age (e.g., 50% of patients are younger than 17 years of age).

Table 1.1. Socio-demographic information of patients in year 2021, by children, adults and total

SOCIO-DEMOGRAPHIC INFORMATION	CHILDREN <18 YEARS N=277 Number (%)	ADULTS >18 YEARS N=246 Number (%)	TOTAL N=523 Number (%)
GENDER			
Males	138 (49.8%)	116 (47.2%)	254 (48.6%)
Females	139 (51.7%)	130 (48.3%)	269 (51.4%)
NUMBER OF CLINICAL VISITS			
Alive but not seen in the year of follow-up	18 (2.9%)	37 (15.0%)	55 (10.5%)
1	32 (11.6%)	35 (14.2%)	67 (12.8%)
2	30 (10.8%)	33 (13.4%)	63 (12.0%)
3	45 (16.2%)	29 (11.8%)	74 (14.1%)
4	55 (19.9%)	29 (11.8%)	84 (16.1%)
5 or more	85 (30.7%)	69 (28.0%)	154 (29.4%)
SOCIO-ECONOMIC FACTORS			
Active smoking or household tobacco smoke exposure	49 (17.7%)	28 (11.4%)	77 (14.7%)
Informal housing without running water/toilet	8 (2.8%)	0	8 (1.5%)
Informal housing without electricity	5 (1.8%)	0	5 (1.0%)
Receives Care Dependency or Disability Grant	69 (24.9%)	24 (9.8%)	93 (17.8%)
Relies on public transport	61 (22.0%)	9 (3.7%)	70 (13.4%)
None of above	59 (21.3%)	53 (21.5%)	112 (21.4%)
EDUCATION			
Preschool age	68 (24.5%)		68 (13.0%)
Currently enrolled at school (minor)	193 (69.7%)	18 (7.3%)	211 (40.3%)
Not attending school (minor)	2 (0.7%)	2 (0.8%)	4 (0.8%)
Completed High School		55 (22.4%)	55 (10.5%)
Incomplete High School		10 (4.1%)	10 (1.9%)
Tertiary education/ Qualification		67 (27.2%)	67 (12.8%)
EMPLOYMENT			
Employed full-time		91 (37.0%)	91 (17.4%)
Employed part-time		13 (5.3%)	13 (2.5%)
Student		49 (19.9%)	49 (9.4%)
Disabled		6 (2.4%)	6 (1.1%)
Unemployed		25 (10.2%)	25 (4.8%)
Unknown		62 (25.2%)	62 (11.9%)

Multiple Options could be selected for socio-economic factors for each patient.
There were further several patients for which this information was unknown.

2 DIAGNOSIS

People with a confirmed diagnosis of CF were captured in the SACFR if they met the following modified SA CF diagnostic criteria:

1. Two sweat chloride tests > 60 mmol/L or sweat conductivity > 90 mmol/L and clinical features compatible with typical CF or;
2. DNA analysis/genotyping identified two disease-causing CFTR mutations as reported at the time in CFTR2 database or
3. Sweat chloride ≤ 60 mmol/L chloride and both of the following criteria are met:
   - **a.** DNA analysis/genotyping identified two disease-causing CFTR mutations; and
   - **b.** Clinical presentation consistent with typical or atypical CF or a CFTR-related disorder (CFTR-RD).

People diagnosed with a CFTR-RD were included the SACFR.

South Africa does not have routine new-born screening for the diagnosis of Cystic Fibrosis.

Table 2.1. Total new diagnoses for the 2018-2021 and age at diagnosis (in years)

	YEAR OF DIAGNOSIS			
	2018 N=33	2019 N=3	2020 N=11	2021 N=10
Median (half the patients are younger than this age)	0.70	2.99	0.64	0.42
Min (age of youngest patient)	0.20	0.02	0.02	0.00
Max (age of oldest patient)	22.53	30.94	35.83	22.44

Table 2.1 shows the number of new patients diagnosed for each year and the descriptive statistics for age of diagnosis.

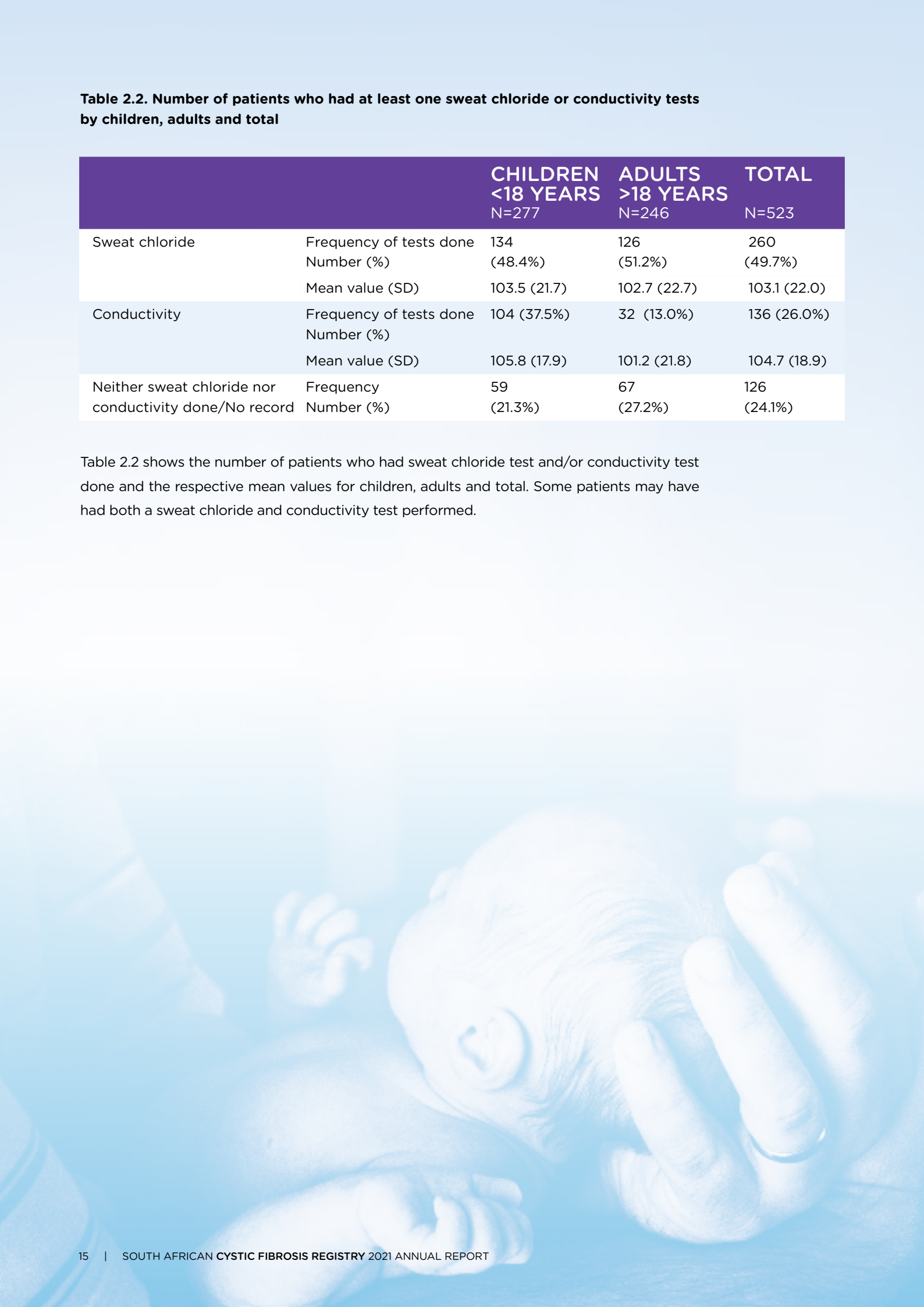
Most patients included in the registry in 2021 were diagnosed younger than 1 year (n=296, 56.6%), with a smaller proportion diagnosed younger than 1 month (n=78, 14.9%) or older than 18 years (n=26, 5.0%). To note those diagnosed 1 month or younger are also included in the sub-group of those diagnosed under one year of life.

For all patients included in the registry in 2021 14.1% had a meconium ileus.

Table 2.2. Number of patients who had at least one sweat chloride or conductivity tests by children, adults and total

		CHILDREN <18 YEARS N=277	ADULTS >18 YEARS N=246	TOTAL N=523
Sweat chloride	Frequency of tests done Number (%)	134 (48.4%)	126 (51.2%)	260 (49.7%)
	Mean value (SD)	103.5 (21.7)	102.7 (22.7)	103.1 (22.0)
Conductivity	Frequency of tests done Number (%)	104 (37.5%)	32 (13.0%)	136 (26.0%)
	Mean value (SD)	105.8 (17.9)	101.2 (21.8)	104.7 (18.9)
Neither sweat chloride nor conductivity done/No record	Frequency Number (%)	59 (21.3%)	67 (27.2%)	126 (24.1%)

Table 2.2 shows the number of patients who had sweat chloride test and/or conductivity test done and the respective mean values for children, adults and total. Some patients may have had both a sweat chloride and conductivity test performed.



3 GENETICS

Cystic Fibrosis is an inherited disease, which means it is passed down in families from parents to the child through the genes. Cystic Fibrosis is caused by mutations of the 'CFTR' gene. Each gene has two alleles: one inherited from the mother and one from the father. People with CF have two mutations or one mutation on each allele. If both mutations are the same, the person is said to be homozygous for that mutation. If the two mutations are different, the person is said to be heterozygous.

If DNA analysis to look for CFTR mutation was carried out but the mutation was not found it was recorded as 'unknown'. There are differences in how DNA testing is carried out in South Africa: some centres test only for the most common mutation (F508del), some use standard kits that test only a limited number of common mutations, and others perform DNA analyses of the whole gene until the mutation is identified.

Table 3.1. Allelic frequencies of the 10 most common mutations in the SACFR database

MUTATION NAME	NUMBER OF ALLELES	PERCENTAGE
ΔF508	653	62%
3120+1G>A	112	11%
3272-26A>G	36	3%
394delTT	23	2%
A455E	16	2%
N1303K	12	1%
G551D	11	1%
R542X	10	1%
3849+10kbC>T	8	1%
R553X	7	1%
Unknown	72	7%

Note: Each patient with CF contributes two allele mutations

This table presents the allele frequency of the 10 most commonly occurring and unknown mutations found in the SACFR database. F508del is the most common CF causing mutation in South Africa, and the world. In all the patients recorded on the SACFR 46% are F508del homozygous (patients who have two F508del mutations) and 33% are F508del heterozygous (patients who have one F508del mutation and one other mutation). 3120+1G>A2,3 is the second most common mutation. 7% of Allele mutations were unknown at 31/12/2021.

4 LUNG FUNCTION

Spirometry is a test done to measure the Forced Vital Capacity in 1 second or FEV_1. This is the amount of air that can be forcefully expired (or blown out) in one second and is an indicator of how well your lungs are functioning. FEV_1 is normally expressed as a percentage of the predicted or expected value (FEV_1%). The predicted value is calculated from a reference population of healthy individuals of the same sex, height, ethnicity, and age.

The Global Lung Function Initiative equations have been used to calculate the percentage predicted FEV_1 scores for this report (See Appendix 1 for more details). A FEV_1% of 100 means that the measurement is equal to the mean or average lung function measurement of people of the same age, sex, ethnicity and height.

Spirometry requires some coordination and practice which is usually only mastered at about six years of age. Therefore, the lung function results only include values for those six years or older. Spirometry values pre-bronchodilator use were recorded.

We further exclude patients from the lung function analyses who have had a lung transplant, as their lung function results are no longer reflective of the severity of their CF lung disease. In patients who had more than one spirometry test done in the year, the test with the best FEV_1% value was recorded.

Table 4.1. FEV1 % predicted descriptive statistics by age group for patients 6 years or older who have never had a lung transplant

AGE AT FEV_1 MEASUREMENT	N*	N MISSING	MEAN	MIN	25th PCTL	MEDIAN	75th PCTL	MAX
6-11	53	24	90.6	37.1	81.2	90.0	101.4	119.8
12-17	68	26	81.0	27.5	67.3	81.9	96.0	141.6
18-29	81	8	65.4	15.4	48.5	64.7	83.8	114.0
30+	66	4	58.8	15.5	41.4	62.2	75.9	112.9

**Excluding 12 lung transplant recipients and children <6 years*

This table shows the FEV1 % predicted value by age group for patients 6 years or older who have not had a lung transplant. The FEV1 % predicted mean and median values decrease as those with CF get older.

Table 4.2 FEV_1% predicted according to severity group by age group and health sector. For patients aged 6 years and older who have never had a lung transplant in 2021

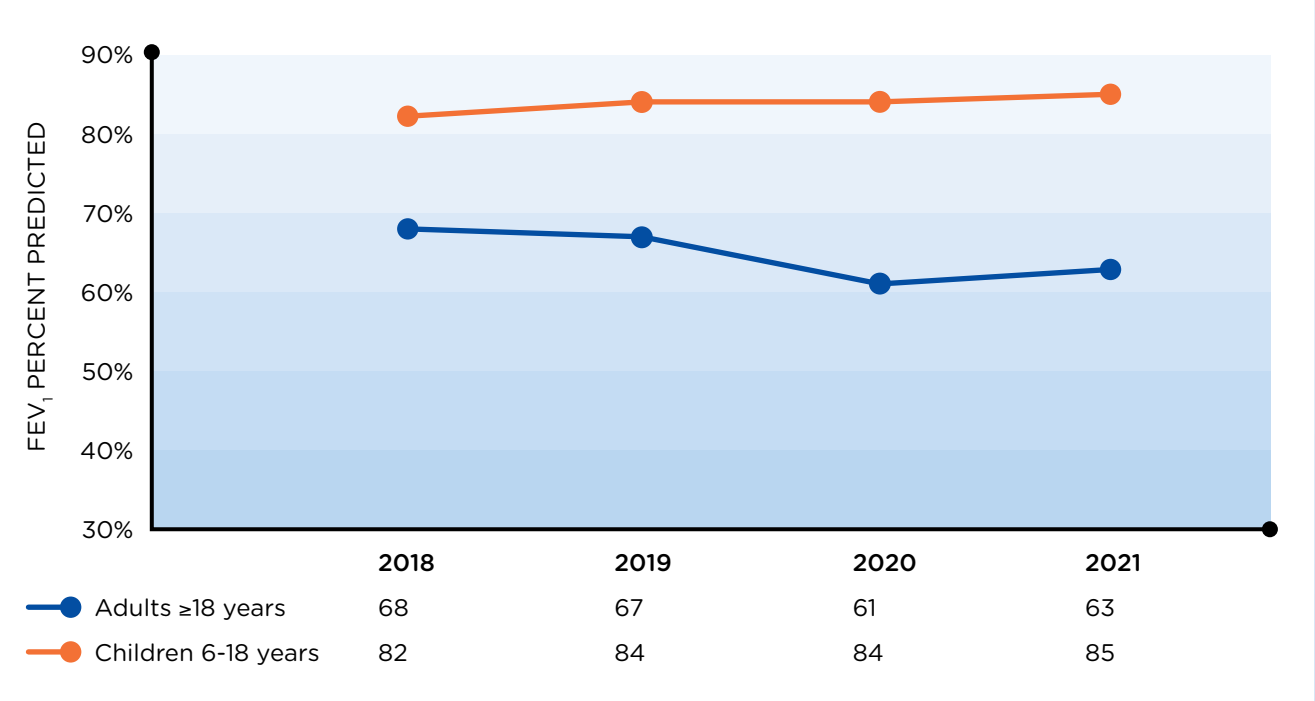
AGE AT FEV_1 MEASUREMENT	N*	MISSING N	FEV_1 <40% Number (%)	FEV_1 40-80% Number (%)	FEV_1 >80% Number (%)
6-11	53	24	1 (1.8%)	11 (20.8%)	41 (77.4%)
12-17	68	26	5 (7.4%)	23 (33.8%)	40 (58.8%)
18-29	81	8	11 (13.6%)	45 (55.5%)	25 (30.9%)
30+	66	4	15 (22.7%)	36 (54.6%)	15 (22.7%)
Total	268	62	32 (11.9%)	115 (42.9%)	121 (45.2%)
HEALTH SECTOR					
Any Private Health Care	195	22	17 (8.7%)	81 (41.5%)	97 (49.8%)
Public Health Care	72	40	15 (20.8%)	34 (47.2%)	23 (32.0%)

**Excluding 12 lung transplant recipients and children <6 years*

The table describes the FEV_1% predicted value by severity according to their age group and overall. Patients with an FEV_1% predicted value higher than 80% are considered to have mild lung disease. Those with and FEV_1% predicted value of 40 – 80% have moderate lung disease and those with lower than 40% have severe lung disease.

The severity of lung disease increases with age in patients with CF.

Figure 4.2. Median FEV_1% predicted for adults and children, included in the registry in 2021, who have never had a lung transplant, 2018-2021



Note: Adults and Children are categorised by the corresponding age each year

The following figure explains how to read a box plot

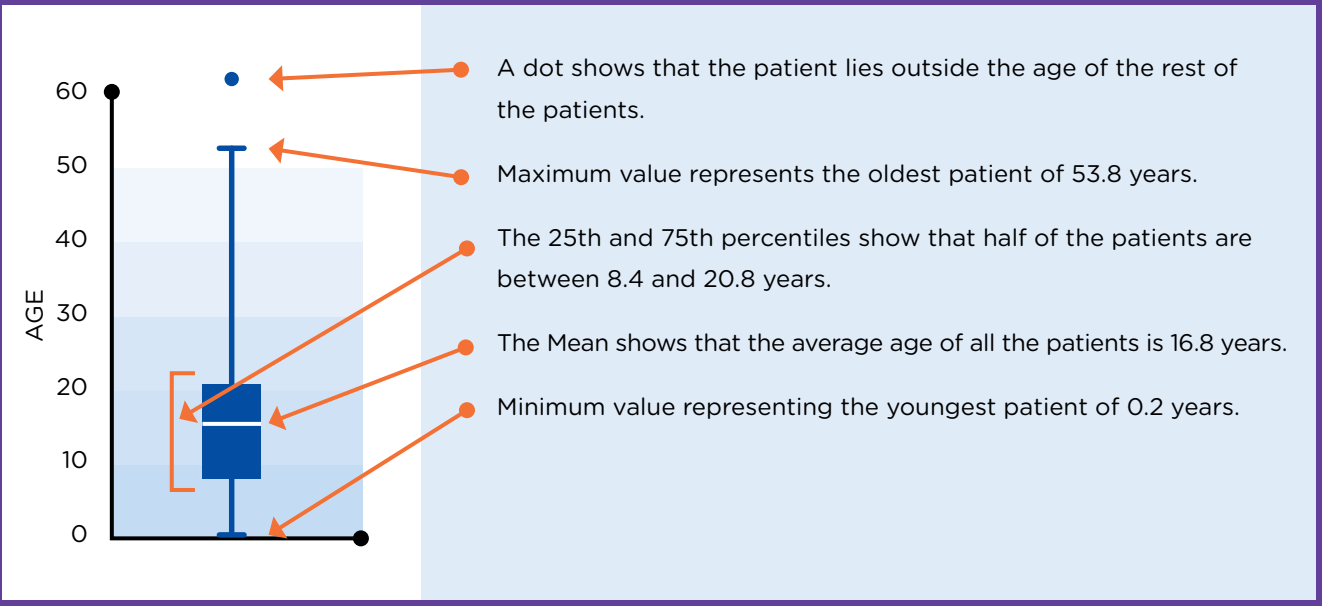
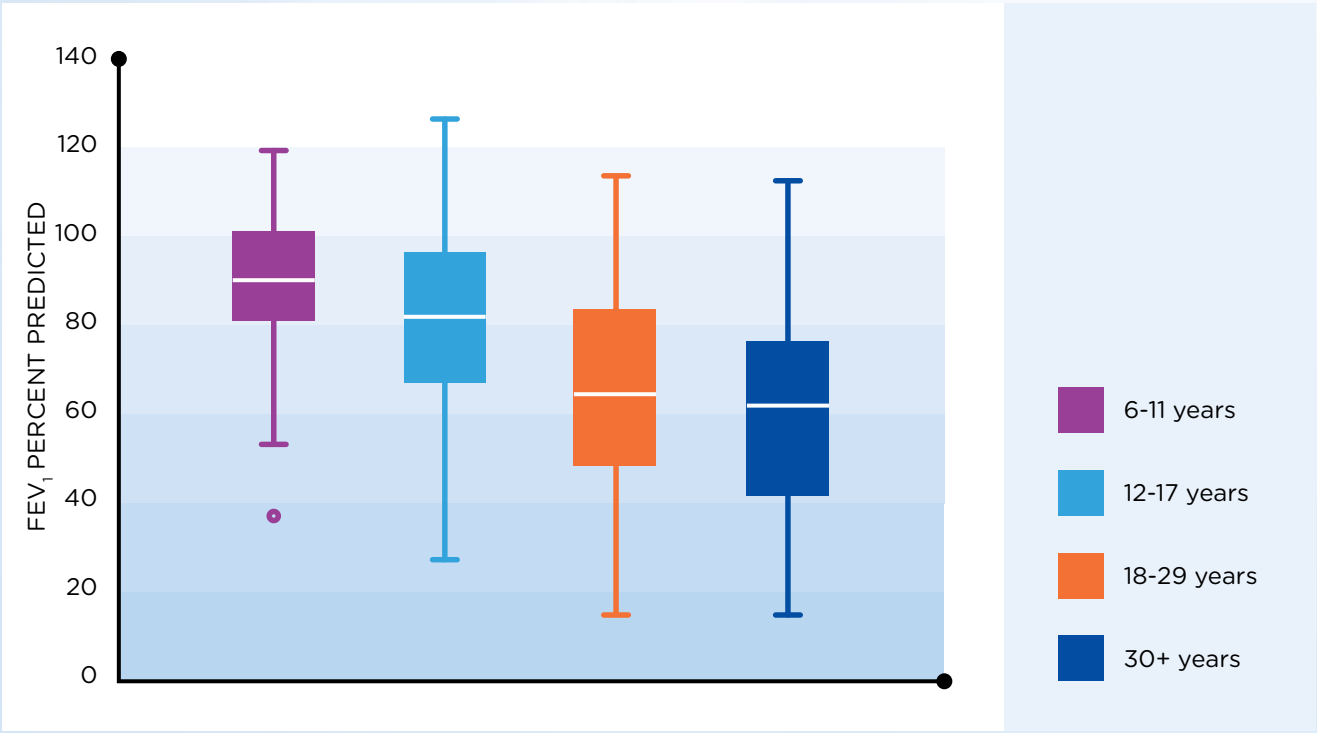


Figure 4.1. FEV1 %predicted box-plot by age group for patients 6 years or older in 2021 who have never had a lung transplant



The box-plot is a graphic representation of the FEV1 by age group, expressed as a % of the predicted value, as detailed in Table 4.1. For each group the middle horizontal line is the median, the x is the mean and the whiskers (vertical lines with end T) are the minimum and maximum. A dot shows that the patient is lies outside the Lung function range of the rest of the patients.

5 MICROBIOLOGY

We collected data on six chronic infections from the Microbiology reports from sputum, cough swabs and/or bronchial alveolar lavage – *Pseudomonas aeruginosa*, *Burkholderia cepacian* complex species, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Haemophilus influenza* and *Aspergillus*.

Chronic infection was determined if the patient fulfilled the criteria in 2021 or had in previous years fulfilled the criteria and the treating doctor had no reason to think that the status had changed:

1. modified Leeds criteria, chronic infection: >50% of respiratory samples collected during 12 calendar months are positive (1/1/2021 - 31/12/2021). At least 4 samples during that period;
2. and/or significantly raised bacteria-specific antibodies according to local laboratories.

If there was no history of chronic infection and the minimum of 4 samples had not been collected during the calendar year the category of missing/unknown was selected for chronic infection. As half of the patients did not meet these criteria the rate of chronic infection has not been reported.

The incidence of chronic *Pseudomonas aeruginosa* infection may be the most accurately captured with the lowest proportion of missing/unknown (44.4%) due to apparent inhaled anti-biotic treatment regime. As such we can report that in 2021 47 (20.7%) children and 88 (52.7%) adults were known to have chronic *Pseudomonas aeruginosa* infection.

Presence of any infection from Microbiology reports from sputum, cough swabs and/or bronchial alveolar lavage was collected for – *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Burkholderia cepacian* species, *Achromobacter* species, *Aspergillus* species, *Stenotrophamonas*, *non-tuberculous mycobacterial* and *mycobacterium tuberculosis*, other fungal and mould infection including *Candida* species and *Scedosporium* species.

Table 5.1. Frequency and number of respiratory samples collected for 2018-2021

MICROBIOLOGY - RESPIRATORY SAMPLES		2018 N=482 Number (%)	2019 N=515 Number (%)	2020 N=525 Number (%)	2021 N=523 Number (%)
Respiratory samples collected	yes	412 (85.5%)	471 (91.5%)	424 (80.8%)	394 (75.3%)
	no	42 (8.7%)	27 (5.2%)	59 (11.2%)	43 (8.2%)
	not seen in year of follow up*	28 (5.8%)	17 (3.3%)	42 (8.0%)	86 (16.4%)
Number of samples	1	54 (11.2%)	60 (11.7%)	96 (18.3%)	91 (17.4%)
	2	51 (10.6%)	62 (12.0%)	82 (15.6%)	87 (16.6%)
	3	58 (12.0%)	68 (13.2%)	71 (13.5%)	70 (13.4%)
	4	66 (13.7%)	80 (15.5%)	53 (10.1%)	57 (10.9%)
	5 or more	156 (32.4%)	195 (37.9%)	120 (22.9%)	88 (16.8%)

**Includes patients not seen but known to be alive.*

Table 5.2. Presence of any infection in children and adults for the year 2021

MICROBIOLOGY RESULTS IN 2021		CHILDREN <18 YEARS N=277 Number (%)	ADULTS >18 YEARS N=167 Number (%)	TOTAL N=394 Number (%)
Any Pseudomonas aeruginosa	No	145 (63.9%)	62 (37.1%)	207 (52.5%)
	Yes	82 (36.1%)	105 (62.9%)	187 (47.5%)
Any Haemophilus influenza	No	199 (87.7%)	156 (93.4%)	355 (90.1%)
	Yes	28 (12.3%)	11 (6.6%)	39 (9.9%)
Any Staphylococcus aureus	No	124 (54.6%)	103 (61.7%)	227 (57.6%)
	Yes	103 (45.4%)	64 (38.3%)	167 (42.4%)
Any Methicillin-resistant Staphylococcus aureus (MRSA)	No	214 (94.3%)	157 (94.0%)	371 (94.2%)
	Yes	13 (5.7%)	10 (6.0%)	23 (5.8%)
Any Burkholderia cepacian complex species	No	221 (97.4%)	151 (90.4%)	372 (94.4%)
	Yes	6 (2.6%)	16 (9.6%)	22 (5.6%)
Any Achromobacter Species Infection	No	223 (98.2%)	158 (94.6%)	381 (96.7%)
	Yes	4 (1.8%)	9 (5.4%)	13 (3.3%)
Any Aspergillus	No	203 (89.4%)	128 (76.6%)	331 (84.0%)
	Yes	24 (10.6%)	39 (17.4%)	63 (16.0%)
Any Stenotrophamonas infection	No	214 (94.3%)	158 (94.6%)	372 (94.4%)
	Yes	13 (5.7%)	9 (5.4%)	22 (5.6%)
Any other fungus or mould	No	180 (79.3%)	132 (79.0%)	312 (79.2%)
Candida species	Yes	39 (17.2%)	26 (15.6%)	65 (16.5%)
		Scedosporium	10 (6.0%)	15 (3.8%)
		Other	2 (3.6%)	5 (1.3%)
		N=277 Number (%)	N=246 Number (%)	N=523 Number (%)
Any non-tuberculous mycobacterial infection	No	192 (69.3%)	155 (63.0%)	347 (66.3%)
	Yes	8 (2.9%)	7 (2.8%)	15 (2.9%)
	Unknown	77 (27.8%)	84 (34.2%)	161 (30.8%)
Any mycobacterium tuberculosis infection	No	198 (71.5%)	155 (63.0%)	353 (67.5%)
	Yes	1 (0.4%)	0 (0%)	1 (0.2%)
	Unknown	77 (27.8%)	84 (34.2%)	161 (30.8%)
SARS-CoV-2	No	56 (20.2%)	83 (33.7%)	139 (26.6%)
	Yes	16 (5.8%)	23 (9.3%)	39 (7.5%)
	Unknown	205 (74.0%)	140 (56.9%)	345 (65.9%)

Table 5.2 shows the frequency of any infection in children, adults and total sample who had respiratory samples in 2021. Non-tuberculous mycobacterial and mycobacterium tuberculosis infection is detected on a separate culture to other infection. Similarly SARS CoV-2 is detected on a separate culture. Unknown refers to individuals who did not have mycobacterial or SARS CoV-2 cultures in 2021 or if culture for mycobacterium or SARS Cov-2 is unknown.

Figure 5.2. Age-specific proportion of any infection in 2021

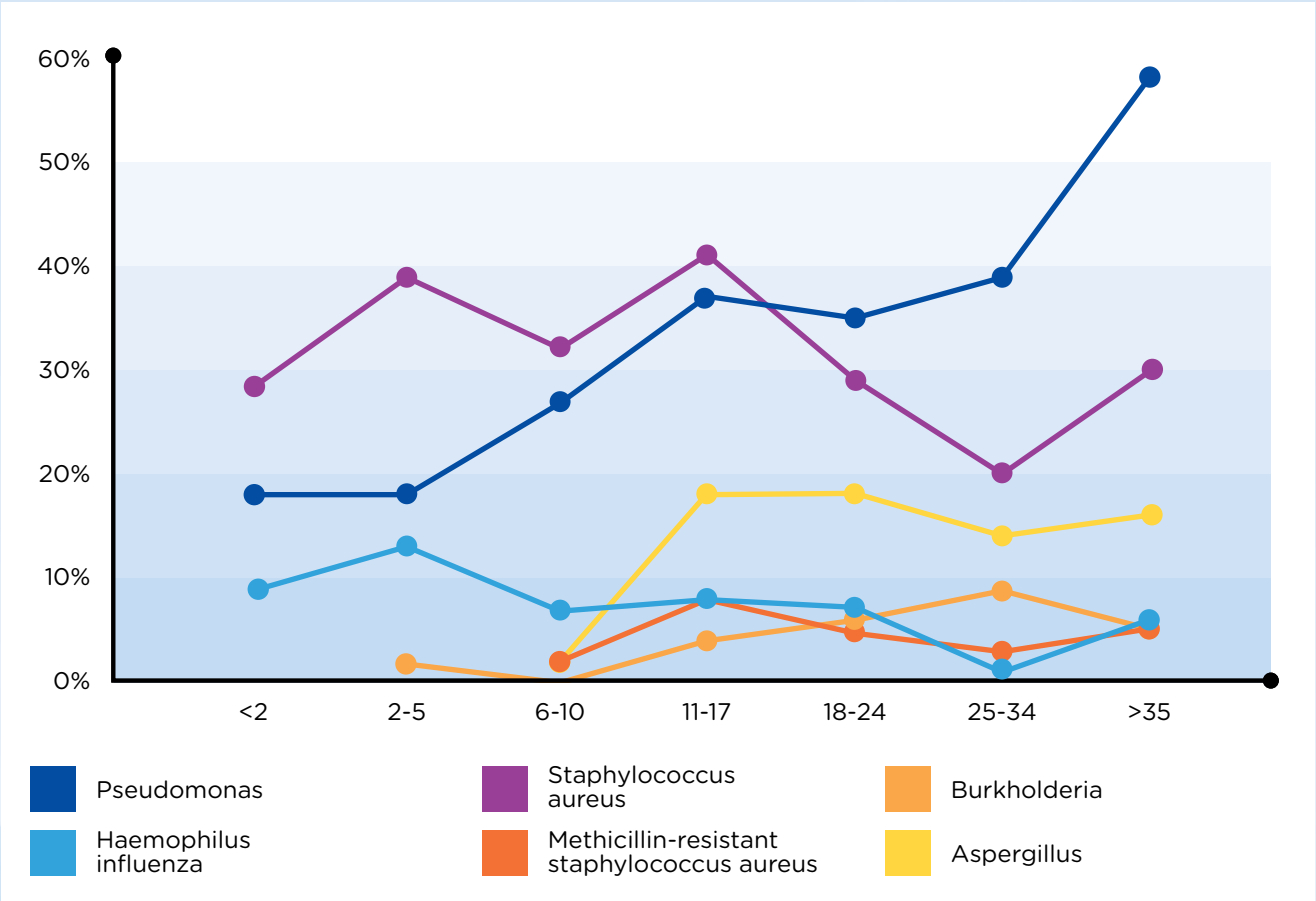


Figure 5.2 shows the age-specific proportion of infections with an incidence of ≥5% in the total SACFR.

6 NUTRITION

Pancreatic insufficiency is the absence of pancreatic enzymes and is diagnosed on the level of faecal elastase in a stool sample. Pancreatic insufficiency was determined by: laboratory confirmation of faecal elastase level or the use of pancreatic enzyme replacement therapy.

The weight and height measured on the date of the best FEV1 value was recorded and, for those who did not perform spirometry, the last measurements of the year were recorded. The weight and height measurements were used to calculated body mass index (BMI). The BMI is a good indicator of nutritional status as it considers the relationship between the weight and the height. For adults, 18 years or older, a BMI of 18.5 to 24.9 kg/m2 is considered normal. Adults with a BMI of less than 18.5 were underweight.

Z-scores were calculated for weight, height, and BMI for children younger than 18 years of age. BMI z-scores are calculated for children aged 2-18 years and a Weight and height for age z-score is calculated for children aged 0-18 years. The scores were calculated using a reference population of healthy individuals of similar age and sex (the WHO reference values were used according to Appendix 1). A z-score of 0 means that the height, weight, or BMI is equal to the mean or average height/weight/BMI of people of the same age and sex. 95% of all individuals in the WHO reference population have a z-score for weight and height between -2 and +2 standard deviations below the mean. A z-score of less than -2 standard deviations in children younger than 18 years is considered underweight.

Table 6.1. Pancreatic status in 2021 for all patients, by age group and overall

PANCREATIC STATUS	CHILDREN <18 YEARS N=277 Number (%)	ADULTS ≥18 YEARS N=246 Number (%)	TOTAL N=523 Number (%)
Insufficient	237 (25.6%)	189 (76.8%)	426 (81.4%)
Sufficient	22 (7.9%)	38 (15.4%)	60 (11.5%)
Unknown	18 (6.5%)	19 (7.7	3 (0.6%)

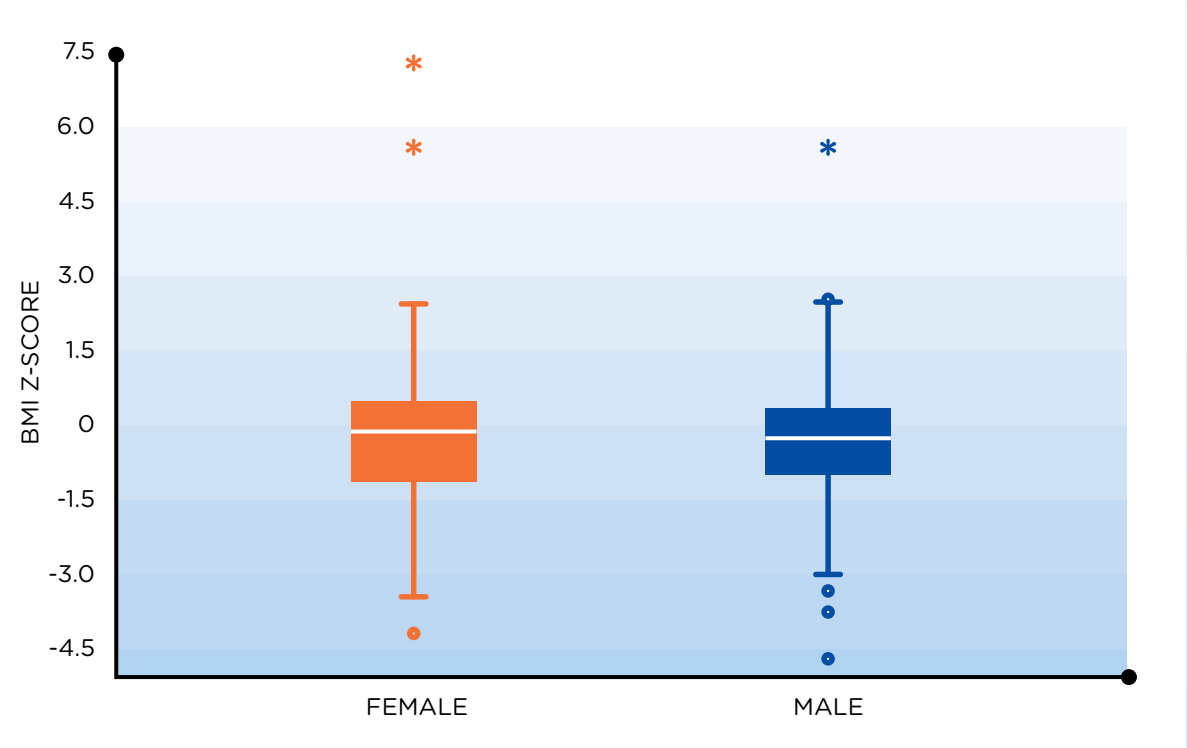
Table 6.1 describes the pancreatic status of those on the SACFR in 2021. The majority of children and adults on the SACFR are pancreatic insufficient and require synthetic pancreatic enzyme replacement therapy. Unknown includes patients not seen in 2021 for consultations or repeat prescriptions.

Table 6.2. BMI Z-scores for patients < 18 years of age by sex and overall in 2021

NUTRITION Z-SCORES 2021	BMI Z-SCORE FEMALE N=114	BMI Z-SCORE MALE N=115	BMI Z-SCORE TOTAL N=229
Mean (average age)	-0.24	-0.26	-0.25
Min	-4.18	-4.71	-4.71
25th pctl (25% of the patients are below this BMI)	-1.07	-1.00	-1.03
Median (half the patients are below this BMI)	-0.09	-0.23	-0.21
75th pctl (75% of the patients are below this BMI)	0.51	0.42	0.49
Max	7.31	5.60	7.31

This table describes the median z-score for height, weight and BMI (the value that separates the highest and lowest half of patients), the mean z-score for height, weight and BMI (the average) and other statistics for children.

Figure 6.1. BMI z-score descriptive statistics for patients younger than 18 years by sex and overall



The box-plot is a graphic representation of the BMI z-scores by sex in children as detailed in Table 6.1. For each group the middle horizontal line is the mean, the coloured box is the 25th and 75th percentile and the whiskers (vertical lines with end T) are the minimum and maximum. A dot shows that the patient is lies outside the range of the rest of the patients and a star shows that the patient lies extremely outside the range of the rest of the patients.

Figure 6.2. Median BMI z-score: for patients by age younger than 18 years

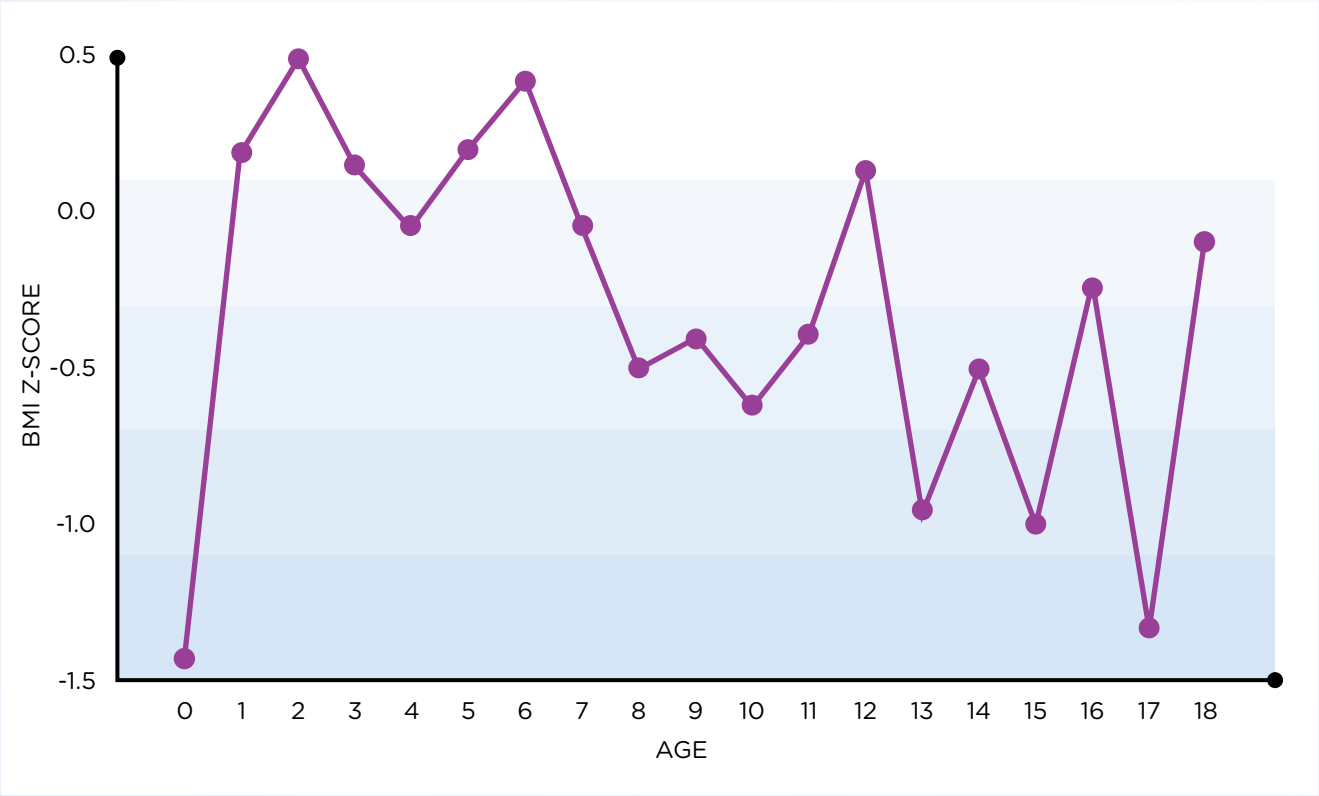


Figure 6.2. shows the median BMI z-score for children across the age range from 0-18 years.

Table 6.3. BMI descriptive statistics for patients 18 years or older by sex and overall in 2021

BMI 2021	BMI FEMALE N=114	BMI MALE N=115	BMI TOTAL N=229
Mean (average age)	21.37	22.00	21.67
Min	14.27	13.59	13.59
25th pctl (25% of the patients are below this BMI)	19.23	18.87	19.09
Median (half the patients are below this BMI)	21.02	21.08	21.05
75th pctl (75% of the patients are below this BMI)	22.85	24.26	23.74
Max	29.71	39.07	39.07

This table describes the median BMI (the value that separates the highest and lowest half of patients), the mean BMI (the average) and other statistics for adults according to sex.

Figure 6.3. BMI descriptive statistics for patients 18 years or older by sex

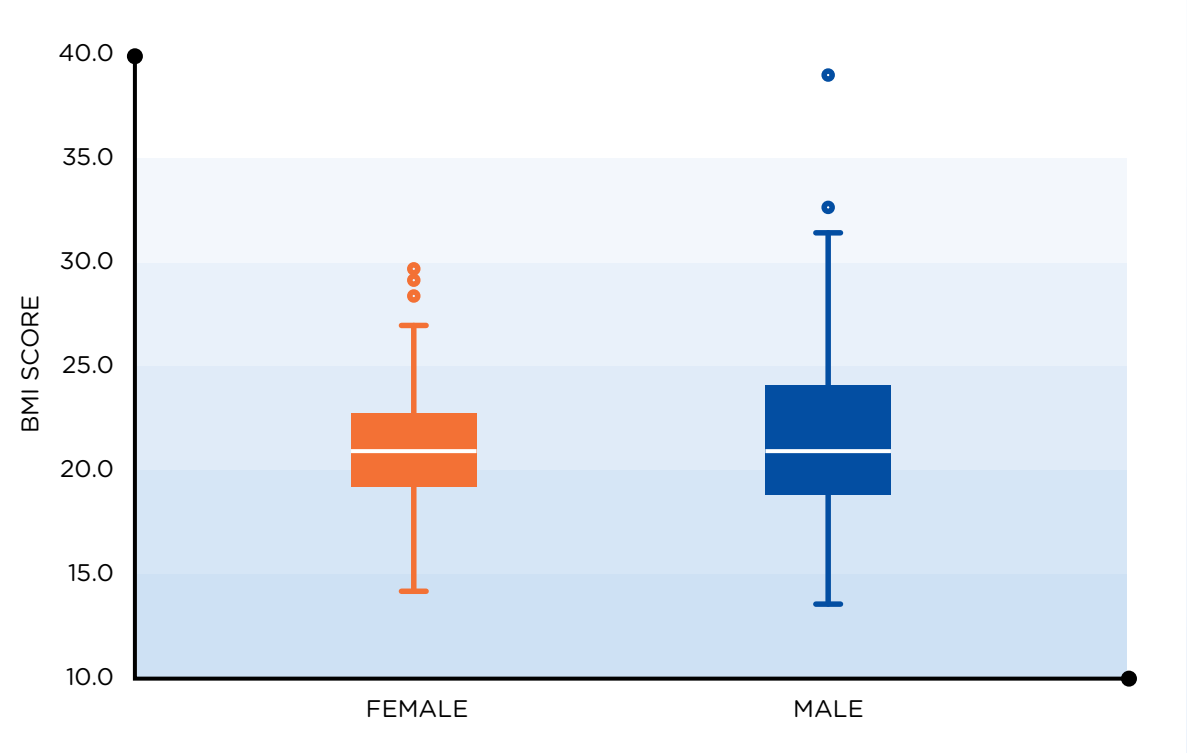


Figure 6.4. Median BMI scores by age for patients older than 18 years

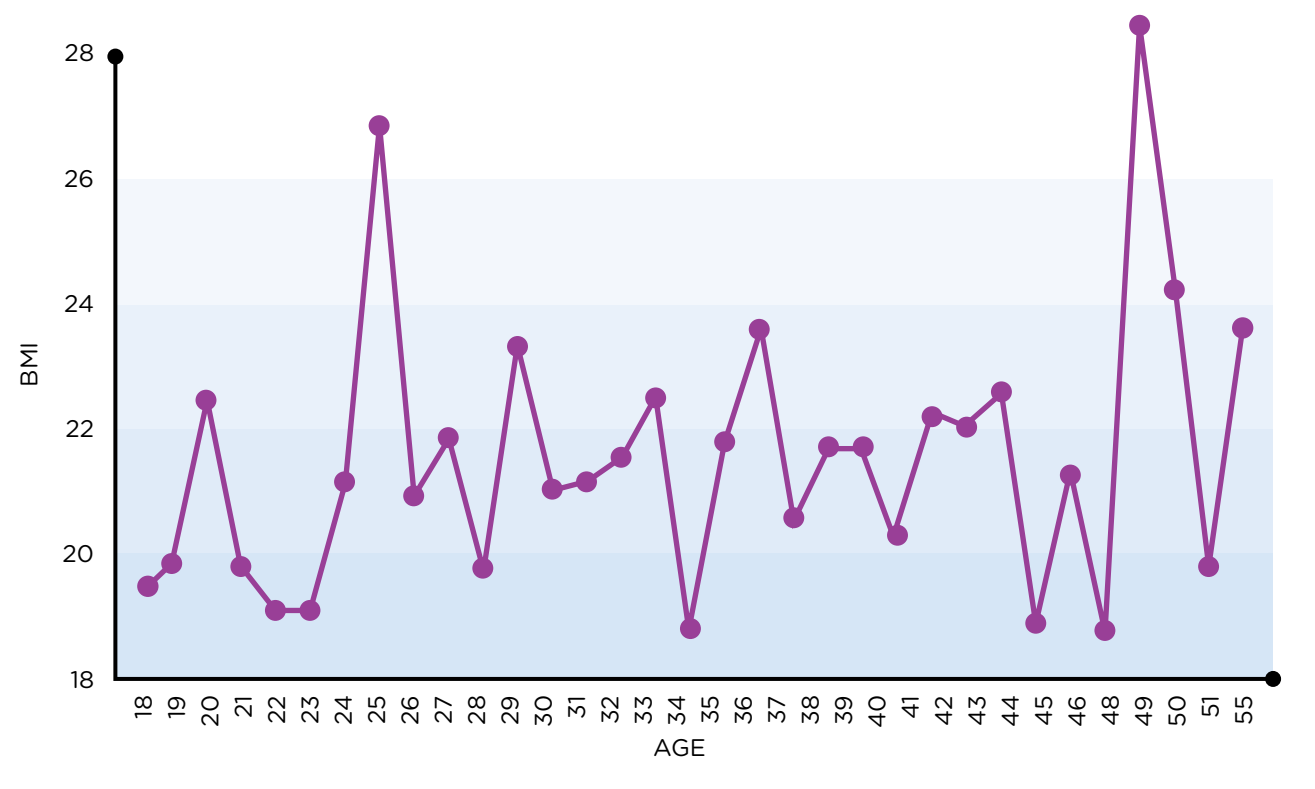


Figure 6.2. shows the median BMI z-score for children across the age range from 0-18 years.

Table 6.4. Nutritional status, by gender and total in 2021

		FEMALE N=114 Number (%)	MALE N=115 Number (%)	TOTAL N=229 Number (%)
BMI z-score for children aged <18 years	<-2	2 (1.8%)	4 (3.4%)	6 (2.6%)
	≥-2 and <-1	30 (26.3%)	24 (20.9%)	54 (23.6%)
	≥-1 and < 0	30 (26.3%)	40 (34.8%)	70 (30.6%)
	≥0 and <1	38 (33.3%)	30 (26.1%)	68 (29.7%)
	≥1	14 (12.3%)	17 (14.8%)	31 (13.5%)
		N=93	N=85	N=178
BMI score adults aged ≥18 years	<18.5	16 (17.2%)	16 (18.8%)	32 (18.0%)
	≥18.5 and <25.0	63 (67.7%)	51 (60.0%)	114 (64.0%)
	≥25.0	14 (15.1%)	18 (21.2%)	32 (17.0%)

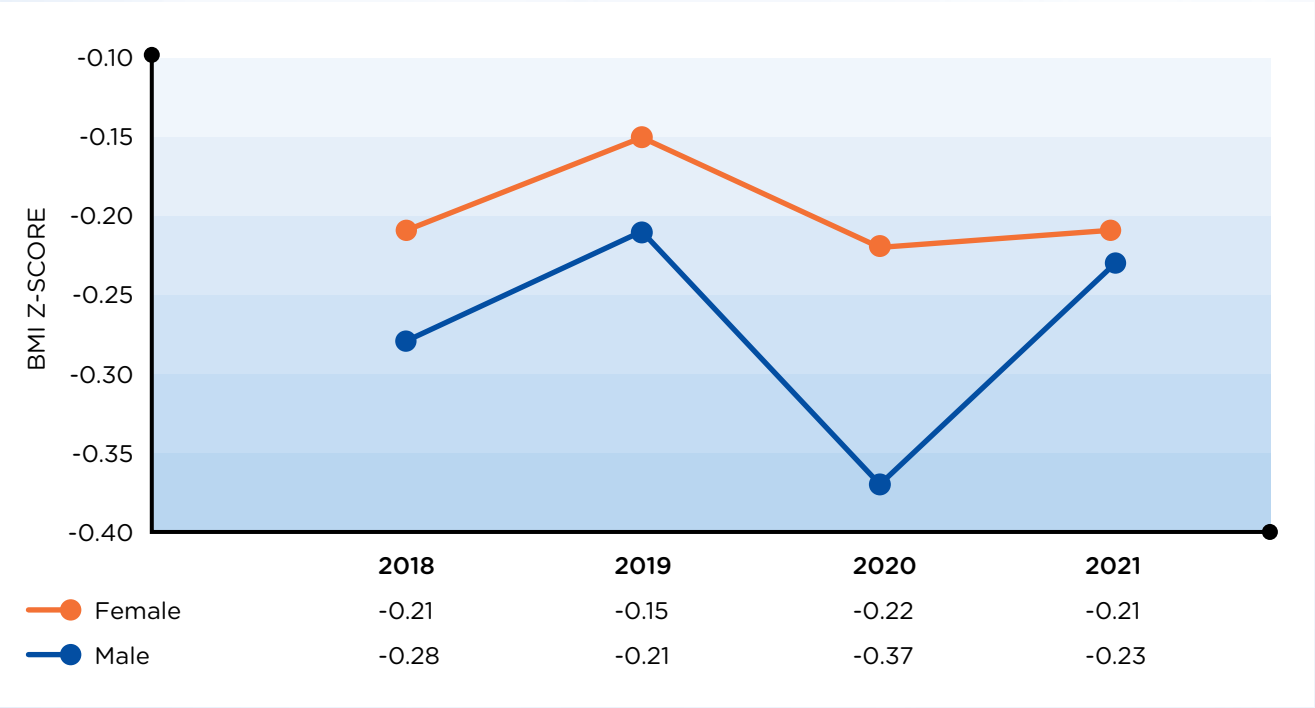
Table 6.4 details the nutritional status according to gender and total. A z-score of less than -1 in children and a BMI or less than 18.5 in adults indicates poor nutrition.

Table 6.5. Nutritional status, by sector of health care and total in 2021

		ANY PRIVATE HEALTH CARE N=103 Number (%)	PUBLIC HEALTH CARE N=126 Number (%)	TOTAL N=229 Number (%)
BMI z-score for children aged 2-18 years	<-2	2 (2.0%)	4 (3.2%)	6 (2.6%)
	≥-2 and <-1	19 (18.4%)	35 (27.8%)	54 (23.6%)
	≥-1 and < 0	35 (34.0%)	35 (27.8%)	70 (30.6%)
	≥0 and <1	30 (29.1%)	38 (30.2%)	68 (29.7%)
	≥1	17 (16.5%)	14 (11.0%)	31 (13.5%)
		N=145	N=31	N=176
BMI score for adults for aged >18 years	<18.5	21 (14.5%)	11 (35.5%)	32 (18.2%)
	≥18.5 and <25.0	95 (65.5%)	18 (58.0%)	113 (64.2%)
	≥25.0	29 (20.0%)	2 (6.5%)	31 (17.6%)

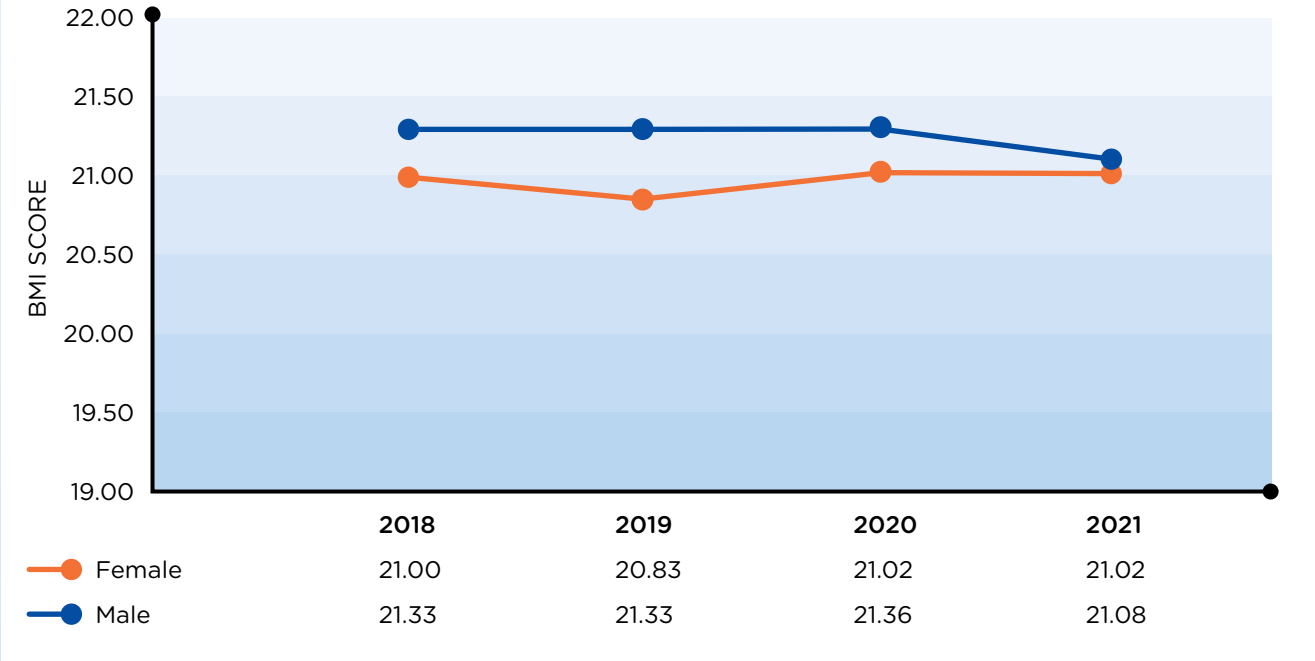
Table 6.5 details the nutritional status according to health sector and total. A z-score of less than -1 in children and a BMI or less than 18.5 in adults indicates poor nutrition.

Figure 6.5. Median BMI z-scores for patients 0-18 years of age, by sex, 2018 to 2021



	2018	2019	2020	2021
Female	-0.21	-0.15	-0.22	-0.21
Male	-0.28	-0.21	-0.37	-0.23

Figure 6.6. Median BMI for patients 18 years or older, by sex, 2018 to 2021



	2018	2019	2020	2021
Female	21.00	20.83	21.02	21.02
Male	21.33	21.33	21.36	21.08

7 COMPLICATIONS & THERAPY

Patients who did not have a port were classified as not applicable (N/A) for port complications. For patients where it was unclear if they had a port or any complications with the port unknown/missing was selected.

We collected information on therapies with consideration to drug class i.e. use of inhaled antibiotics was recorded rather than the generic or brand name of the drug.

Table 7.1. Prevalence of complications in 2021 by age and overall

COMPLICATION IN 2021		CHILDREN <18 YEARS N=277 Number (%)	ADULTS ≥18 YEARS N=246 Number (%)	TOTAL N=523 Number (%)
Allergic Bronchopulmonary Aspergillosis (ABPA)	Missing/unknown	20 (7.2%)	38 (15.4%)	58 (11.1%)
	Yes	7 (2.5%)	17 (6.9%)	24 (4.6%)
Distal Intestinal Obstruction Syndrome (DIOS)	Missing/unknown	19 (6.9%)	33 (13.4%)	52 (9.9%)
	Yes	17 (6.1%)	14 (5.7%)	31 (5.9%)
Salt depletion	Missing/unknown	19 (6.9%)	34 (13.8%)	53 (10.1%)
	Yes	2 (0.7%)	1 (0.4%)	3 (0.6%)
CFRD treated with insulin	Missing/unknown	20 (7.2%)	37 (15.0%)	57 (10.9%)
	Yes	13 (4.7%)	82 (33.3%)	95 (18.2%)
Pneumothorax requiring chest tube	Missing/unknown	19 (6.9%)	33 (13.4%)	52 (9.9%)
	Yes	0	0	0
Haemoptysis major over 250ml	Missing/unknown	19 (6.9%)	33 (13.4%)	52 (9.9%)
	Yes	1 (0.4%)	5 (2.0%)	6 (1.1%)
Malignancy	Missing/unknown	20 (7.2%)	32 (13.0%)	52 (9.9%)
	Yes	0	1 (0.4%)	1 (0.2%)
Port complications	Missing/unknown	19 (6.9%)	26 (10.6%)	45 (8.6%)
	N/A#	235 (84.8%)	175 (71.1%)	410 (78.4%)
	Yes	3 (1.1%)	6 (2.4%)	9 (1.7%)
Drug hypersensitivity	Missing/unknown	19 (6.9%)	22 (8.9%)	41 (7.8%)
	Yes	4 (1.4%)	45 (18.3%)	49 (9.4%)

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COMPLICATION IN 2021		CHILDREN <18 YEARS N=277 Number (%)	ADULTS ≥18 YEARS N=246 Number (%)	TOTAL N=523 Number (%)
Liver disease	Missing/unknown	20 (7.2%)	38 (15.4%)	58 (11.1%)
	No liver disease	226 (81.6%)	148 (60.2%)	374 (71.5%)
	Cirrhosis with portal hypertension/ hypersplenism	6 (2.2%)	9 (3.7%)	15 (2.9%)
	Cirrhosis no portal hypertension/ hypersplenism	3 (1.1%)	2 (0.8%)	5 (1.0%)
	Cirrhosis. Portal hypertension unknown	1 (0.4%)	1 (0.4%)	2 (0.4%)
	Liver disease without cirrhosis	21 (7.6%)	48 (19.5%)	69 (13.2%)
Other complications	None	129 (46.6%)	128 (52.0%)	257 (49.1%)
	Chronic sinusitis requiring treatment or surgery	35 (12.6%)	106 (20.3%)	141 (27.0%)
	Anxiety or depression	4 (1.4%)	55 (22.4%)	59 (11.3%)
	Other mental health	1 (0.4%)	13 (5.3%)	14 (2.7%)
	ADHD	9 (3.2%)	5 (2.0%)	14 (2.7%)
	Pulmonary hypertension	0	12 (4.9%)	12 (2.3%)
	Other	27 (9.7%)	74 (30.1%)	107 (20.5%)

**Patients that were not seen in 2021 are included in the figure of missing/unknown #N/A for port complications refers to those patients who did not have a port in 2021.*

This table shows the frequency of common complications in people with CF in children, adults and overall. One adult had adenocarcinoma of the duodenum.

Table 7.2. Prevalence of therapies used in 2021 by age and overall

THERAPY USED IN 2021		CHILDREN <18 YEARS N=277 Number (%)	ADULTS ≥18 YEARS N=246 Number (%)	TOTAL N=523 Number (%)
Hypertonic saline (NaCl) inhaled >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	168 (60.6%)	106 (43.1%)	274 (52.4%)
rhDNase inhaled >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	52 (18.8%)	97 (39.4%)	149 (28.5%)
Steroids inhaled >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	169 (61.0%)	171 (69.5%)	340 (65.0%)
Inhaled bronchodilator >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	186 (67.1%)	183 (74.4%)	369 (70.6%)
Inhaled antibiotics >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	108 (39.0%)	130 (52.8%)	238 (45.5%)
Oral steroids >3 months	Missing/unknown	21 (7.6%)	38 (15.4%)	29 (11.3%)
	Yes	8 (2.9%)	32 (13.0%)	40 (7.6%)
Proton pump inhibitor	Missing/unknown	22 (7.9%)	40 (16.3%)	62 (11.9%)
	Yes	92 (33.2%)	97 (39.4%)	189 (36.1%)
Gastrostomy inserted or used in 2018	Missing/unknown	24 (8.7%)	39 (15.9%)	63 (12.0%)
	Yes	14 (5.1%)	12 (4.9%)	26 (5.0%)
Marcolides (Azithromycin) >3 months	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	190 (68.6%)	188 (76.4%)	378 (72.3%)
Ursodeoxycholic acid	Missing/unknown	21 (7.6%)	40 (16.3%)	61 (11.7%)
	Yes	34 (12.3%)	40 (16.3%)	74 (14.1%)
Intravenous antibiotics	Missing/unknown	22 (7.9%)	27 (11.0%)	49 (9.4%)
	Yes	66 (23.8%)	101 (41.1%)	167 (31.9%)
Oxygen	Missing/unknown	22 (7.9%)	40 (16.3%)	62 (11.9%)
	Yes, nocturnally or continuously at home	3 (1.1%)	38 (15.4%)	41 (7.8%)
	Yes, with exacerbation	8 (2.9%)	13 (5.3%)	21 (4.0%)
Non-invasive ventilation	Missing/unknown	21 (7.6%)	39 (15.9%)	60 (11.5%)
	Yes	0	2 (0.8%)	2 (0.4%)
CFTR modulator therapy	Missing/unknown	17 (6.1%)	23 (9.3%)	40 (7.6%)
	Yes	1 (0.4%)	6 (2.4%)	7 (1.3%)

**Patients who were not seen or did not get a repeat script in 2021 are included in the figure of missing/unknown.*

This table shows the frequency of common CF therapies used by children, adults and overall in 2021.

Table 7.3. Prevalence of therapies used in 2021 by health sector

THERAPY USED IN 2021		ANY PRIVATE HEALTH CARE N=298 Number (%)	PUBLIC HEALTH CARE N=195 Number (%)	TOTAL N=493 Number (%)
Hypertonic saline (NaCl) inhaled >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	170 (57.0%)	103 (52.8%)	273 (55.4%)
rhDNase inhaled >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	137 (46.0%)	11 (5.6%)	148 (30.0%)
Steroids inhaled >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	219 (73.5%)	119 (61.0%)	338 (68.6%)
Inhaled broncho-dilator >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	244 (81.9%)	123 (63.1%)	367 (74.4%)
Inhaled antibiotics >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	169 (56.7%)	68 (34.9%)	237 (48.1%)
Oral steroids >3 months	Missing/unknown	8 (2.7%)	23 (11.8%)	31 (6.3%)
	Yes	32 (10.7%)	8 (4.1%)	40 (8.1%)
Proton pump inhibitor	Missing/unknown	10 (3.4%)	24 (12.3%)	34 (6.9%)
	Yes	129 (43.3%)	59 (30.3%)	188 (38.1%)
Gastrostomy inserted or used in 2018	Missing/unknown	9 (3.0%)	26 (13.3%)	35 (7.1%)
	Yes	11 (3.7%)	15 (7.7%)	26 (5.3%)
Marcolides (Azithromycin) >3 months	Missing/unknown	10 (3.4%)	23 (11.8%)	33 (6.7%)
	Yes	235 (78.9%)	142 (72.8%)	377 (76.5%)
Ursodeoxycholic acid	Missing/unknown	9 (3.0%)	23 (11.8%)	32 (6.5%)
	Yes	59 (19.8%)	15 (7.7%)	74 (15.0%)
Intravenous antibiotics	Missing/unknown	5 (1.7%)	15 (7.7%)	20 (4.1%)
	Yes	119 (39.9%)	48 (24.6%)	167 (33.9%)
Oxygen	Missing/unknown	9 (3.0%)	24 (12.3%)	33 (6.7%)
	Yes, nocturnally or continuously	33 (11.1%)	8 (4.1%)	41 (8.3%)
	Yes, with exacerbation	12 (4.0%)	9 (4.6%)	21 (4.3%)
Non-invasive ventilation	Missing/unknown	8 (2.7%)	23 (11.8%)	31 (6.3%)
	Yes	1 (0.3%)	1 (0.5%)	2 (0.4%)
CFTR modulator therapy	Missing/unknown	4 (1.3%)	9 (4.6%)	13 (2.6%)
	Yes	7 (2.3%)	0	7 (1.4%)

8 TRANSPLANTS

Table 8.1. Number of patients living with transplanted lungs, by sex at the end of each year

YEAR	FEMALE Number (%)	MALE Number (%)	TOTAL Number (%)	TRANSPLANT PERFORMED IN YEAR OF FOLLOW-UP
2018	7 (3.0%)	3 (1.4%)	10 (2.2%)	3* (0.7%)
2019	6 (2.2%)	5 (2.0%)	12 (2.1%)	2 (0.4%)
2020	8 (3.0%)	4 (1.6%)	12 (2.3%)	2 (0.4%)
2021	8 (3.0%)	4 (1.6%)	12 (2.3%)	1 (0.2%)

**This number excludes 1 Male who died post lung transplant in 2018.*

Table 8.2. Number of patients living with a transplanted liver, by sex at the end of each year

YEAR	FEMALE Number (%)	MALE Number (%)	TOTAL Number (%)	TRANSPLANT PERFORMED IN 2018
2018	0	2 (1.0%)	2 (0.4%)	0
2019	0	2 (0.8%)	2 (0.4%)	0
2020	0	1 (0.4%)	1 (0.2%)	0
2021	0	1 (0.4%)	1 (0.2%)	0

Only transplant recipients who were alive at the commencement of the registry in 2018 were captured. For this reason, the figures may report a lower number of transplanted patients than the true number. There were no new liver transplants performed since the commencement of the registry.

9 MORTALITY

The deaths on the SACFR between 2018-2021 are presented in the tables below

Table 9.1. Number of deaths, by sex and overall for each calendar year

YEAR	FEMALE Number (%)	MALE Number (%)	TOTAL Number (%)
2018	1 (0.4%)	2 (0.8%)	3 (0.6%)
2019	3 (1.1%)	2 (0.8%)	5 (1.0%)
2020	2 (0.7%)	3 (1.2%)	5 (1.0%)
2021	8 (3.0%)	9 (3.5%)	17 (3.3%)

The number of deaths was higher in 2021 compared to the preceding the three-year period.

Table 9.2. Cause of death for each calendar year

YEAR	N	RESPIRATORY	TRANSPLANT	UNKNOWN/ OTHER	CANCER
2018	3	2	1		
2019	5	3	1	1	
2020	5	3	1		1
2021	18	11	2	2	

Most deaths between 2018 and 2021 were due to the respiratory complications.
There was 1 death due to SARS-CoV-2 in 2021 and another due to seizures.

Table 9.2. Age of death per calendar year

AGE AT DEATH	N	MEAN	MIN	25TH PCTL	MEDIAN	75TH PCTL	MAX
2018	3	34.7	32.0	32.0	35.0		37.0
2019	5	24.8	0	9.5	25.0	40.0	51.0
2020	5	27.0	24.0	24.5	25.0	30.5	31.0
2021	17	28.1	13.0	20.0	26.0	32.0	56.0

APPENDIX 1

LIST OF VARIABLES

Adapted from the European Cystic Fibrosis Registry

DEMOGRAPHICS
- CF centre code
- Patient code
- Year of follow-up
- Date of birth
- Gender
- Self-identified ethnicity
- Status of patient
- Cause of death
- Date of death
- Socio-economic status
- Health sector point of care
- HIV infection status

DIAGNOSIS
- Diagnosis confirmed
- Age of diagnosis
- Nutrition at diagnosis (weight and height)
- Type of sweat test
- Chloride value
- Conductivity value
- Meconium ileus
- Neonatal screening
- Faecal elastase
- Genotype – first mutation
- Genotype – second mutation

TRANSPLANT
- Liver transplant
- Year of latest liver transplant
- Lung transplant
- Year of latest lung transplant

LUNG FUNCTION AND NUTRITION THIS YEAR
- Date of best FEV_1 recorded
- Value of best FEV_1 recorded
- Value of best FVC recorded this year
- Height measured at date of best FEV_1 (if no FEV_1 last height of the year)
- Weight measured at date of best FEV_1 (if no FEV_1 last weight of the year)

FERTILITY THIS YEAR
- Pregnant in the year of follow up
- Outcome of pregnancy
- Male patient conceived pregnancy by artificial or natural means in year of follow up

COMPLICATIONS RECORDED THIS YEAR
- Pancreatic status: faecal elastase
- Allergic bronchopulmonary aspergillosis
- Distal Intestinal Obstruction Syndrome
- Diabetes: requiring insulin treated
- Salt depletion
- Pneumothorax requiring chest drain
- Liver disease
- Haemoptysis major over 250ml
- Port-related complications
- Occurrence of malignancy
- Antibiotic hypersensitivities reported

THERAPY THIS YEAR
- Inhaled continuous hypertonic NaCl (>3 months)
- Inhaled continuous rhDNase
- Inhaled continuous (>3 months) or alternate month of antibiotic
- Inhaled continuous (>3 months) corticosteroids
- Use of continuous oral steroids (>3months)
- Inhaled continuous (>3 months) bronchodilators

In oxygen therapy
- Use of non-invasive ventilation
- Use of continuous macrolide
- Use of ursodeoxycholic acid
- Use of pancreatic enzymes
- Use of proton pump inhibitors
- Gastrostomy inserted or used in year of follow up
- Use of IVI antibiotics
- CFTR modifier therapy

MICROBIOLOGY THIS YEAR
- Ever had Pseudomonas aeruginosa and date of 1st infection (excluding sinus or nose swab)
- Any/chronic Pseudomonas aeruginosa infection
- Any/chronic Haemophilus influenza infection
- Any/chronic methicillin-sensitive Staphylococcus aureus infection
- Any/chronic methicillin-resistant Staphylococcus aureus (MRSA) infection
- Any/chronic Burkholderia cepacia complex
- Any/chronic Aspergillus species infection
- Any Stenotrophomonas maltophilia Infection
- Any non-tuberculous mycobacterial infection
- Any mycobacterium tuberculosis infection
- Any achromobacter species infection
- Any other fungal or mould infection

PATIENT INCLUSION CRITERIA

Only patients who fulfil the diagnostic criteria below are included the registry:

1. Two sweat tests value > 60 mmol/L chloride: CF diagnosis accepted

2. One sweat test value > 60 mmol/L chloride or sweat conductivity > 90 mmol/L and DNA Analysis/Genotyping – two identified disease-causing CF mutations as reported in CFTR2 database: CF diagnosis accepted; or

3. Sweat value less than or equal to 60 mmol/L chloride then both of these should be fulfilled:

   a. DNA Analysis/Genotyping – two identified disease-causing CF mutations; and
   b. Clinical presentation consistent with typical or atypical CF

4. Sweat conductivity without DNA confirmation of two disease causing CFTR mutations as reported in CFTR2 database will be accepted for SA registry only if both following criteria are met:

   a. Clinical presentation consistent with typical CF
   b. At least two separate sweat conductivity measurements ≥ 90 mmol/L

DEFINITIONS & EXPLANATIONS FOR SACFR

SWEAT TEST

If a sweat test was not performed on a patient, record "missing/unknown". If a sweat test was done but the result is not known, record "missing/unknown."

Chloride concentration measurement is the preferred analysis in the diagnosis of CF. However, in South Africa due to limit centres that can assess chloride concentration conductivity results are accepted.

The sweat chloride value is reported in millimols per litre (mmol/L). If two chloride values were recorded the mean sweat test value was calculated for that patient for analysis in the report.

The sweat conductivity value is reported in millimols per litre (mmol/L). If two conductivity values were recorded the mean value was calculated for that patient for analysis in the report.

SPIROMETRY

The lung function values recorded in the SACFR are for children over the age of 6 years and exclude those who are lung transplant recipients.

The values reported in the registry should meet the following criteria:

1. Date of birth, gender and height should be recorded for predicted values.
2. Values recorded on the registry are pre-bronchodilator values.
3. The value in litres of the highest available value of FEV_1% predicted (according to local references) of the year should be extracted.
4. Each patient's FVC and FEV_1 measurement must be reported in litres (L), with up to two decimal points.
5. For each reported spirometry value, the date of the test and the patient's height and weight at that date should be reported for calculations of the z-scores.
6. Calculation of the percent of predicted values.

The reference values from the Global Lung Function initiative equations is used, described by Quanjer PH et al. (Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012; 40: 1324–1343). Due to the diverse ethnicity in South Africa race-specific reference values were calculated for the SACFR.

NUTRITION

Weight and height measurements were taken from routine clinical data:

1. Weight is measured after removal of outer clothing and shoes.
2. Height is measured after removal of shoes – stadiometer – top of head in contact with head board, slight pressure.
3. The value of weight and height measurements are recorded on the day of the best FEV1% predicted for that year.

Due to the lack of national reference values for South Africa the z-scores for height, weight and BMI were calculated using the WHO reference values described by Yang and de Onis (Algorithms for converting estimates of child malnutrition based on the NCHS reference into estimates based on the WHO Child Growth Standards. BMC Paediatrics 2008; 8;19 (www.who.int/nutgrowthdb/en).

DEFINITION OF CHRONIC INFECTION IN THE LOWER AIRWAYS

Chronic infection was determined if the patient fulfilled the criteria in 2018 or had in previous years fulfilled the criteria and the treating doctor had no reason to think that the status had changed:

1. Modified Leeds criteria, chronic infection: >50% of respiratory samples collected during 12 calendar months are positive (1/1/2018 -31/12/2018). At least 4 samples during that period;
2. and/or significantly raised bacteria-specific antibodies according to local laboratories.

If there was no history of chronic infection and the minimum of 4 samples had not been collected during the calendar year the category of missing/unknown was selected for chronic infection.

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

Diagnostic criteria:

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, induced asthma, change in pulmonary function, or increased sputum production) not attributable to another etiology.
2. Total IgE > 500 IU/ml.
3. Positive skin prick test for Aspergillus antigen (> 3 mm) or positive specific IgE for A. fumigatus.
4. Either:
    - **a.** precipitins to A. fumigatus or in vitro demonstration of IgG antibody to A. fumigatus;
    - **b.** or new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (characteristic changes) that have not cleared with antibiotics and standard physiotherapy

LIVER DISEASE

Liver Disease is based on ultrasound findings with/without laboratory tests of liver function and/or liver enzymes. If no ultrasound or liver function tests had been done in the patient or the results thereof were unknown the category of missing or unknown was selected. Liver disease was classified as:

1	No liver disease
2	Cirrhosis with portal hypertension/hypersplenism
3	Cirrhosis, no portal hypertension/hypersplenism
4	Cirrhosis. Portal hypertension unknown
5	Liver disease without cirrhosis

PANCREATIC STATUS

Pancreatic status was assessed in the SACFR according to local laboratory values as follows:

a. Pancreatic Insufficiency – Faecal elastase <200 µg/g

b. Pancreatic Sufficiency – Faecal elastase ≥ 200 µg/g

TECHNICAL NOTES

Data manipulation

For pre-natal diagnoses, we set age of diagnosis equal to 0.

Software used for data management and statistical analysis

Data is stored in REDCap LTS 8.10, Vanderbilt University, USA.

Data management and analysis was performed in IBM SPSS Statistics for Windows, version 28, IBM Corp., Armonk, N.Y., USA

PHOTO CREDITS

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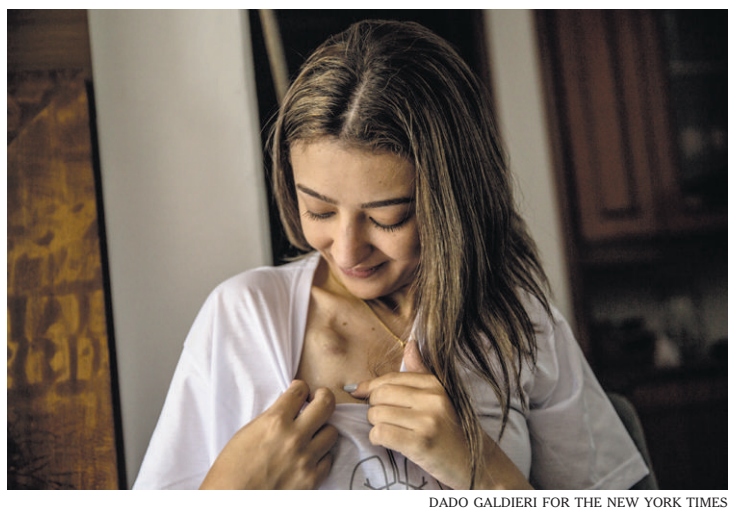
JOÃO SILVA/THE NEW YORK TIMES

Cheri Nel is eligible for Trikafta. She is leading the action to get Vertex to offer the drug in South Africa.

DADO GALDIERI FOR THE NEW YORK TIMES

Rodrigo Rockenbach, center, with his parents in southern Brazil. He started taking Trikafta last month.



DADO GALDIERI FOR THE NEW YORK TIMES

Raphaelle Pereira had been waiting for a lung transplant in Brazil. She improved dramatically on Trikafta.

In Poorer Nations, 'Miracle' Drug Is Kept Out of Reach

Cystic Fibrosis Patients Worldwide Pressure U.S. Manufacturer for Access

By **STEPHANIE NOLEN** and **REBECCA ROBBINS**

When Seshagiri Buddana learned of a powerful new cystic fibrosis drug that was transforming lives in the United States and Europe, he was filled with hope that it could help his son, Hemanth, who had spent much of his childhood in a hospital bed. But the family couldn't get the drug because they live in India.

The drug's maker, Vertex Pharmaceuticals, a large biotech company based in Boston, is not making it available in India or virtually anywhere in the developing world. The company is not trying to sell it, or allowing a local company to make it. Vertex is blocking potential generic competitors by seeking patents in numerous countries.

Hemanth died in December, a day before his ninth birthday and 18 months after he would have been eligible to get the drug, called Trikafta, had he lived in the United States.

Throughout much of Asia, Africa and Latin America, families like Hemanth's are watching Trikafta transform the lives of tens of thousands of cystic fibrosis patients in wealthy countries but say they are blocked by the company at every turn in their efforts to get the drug themselves.

Trikafta, taken as three tablets a day, is the most powerful and widely used of Vertex's four cystic fibrosis medications. With a list price of over $322,000 annually in the United States, it is expected to cost millions of dollars over the course of a patient's lifetime. An analysis led by researchers in Britain found that a year's supply of the drug could be manufactured at an estimated cost of just $5,700.

Vertex has reported more than $17 billion in sales for Trikafta since it was first approved in 2019.

This week, a group of patients and their families in four countries on four continents initiated legal and regulatory steps to try to force their governments to override intellectual property protections and allow a low-cost generic version of Trikafta to be imported or made locally. Under the process, known as compulsory licensing, generic makers would pay Vertex a royalty.

Three of the actions are in India, Ukraine and South Africa — where Vertex has been obstructing efforts to make the drug available, patients and families say. The fourth is in Brazil, where Vertex is trying to win coverage for the drug; the patients and families' concern there is that the brand-name drug will be too expensive.

Cystic fibrosis is a genetic disease that damages the lungs and digestive system. Patients often die in early adulthood, but Trikafta is dramatically extending life expectancy.

"Every patient in the world has access to the internet and wants this drug," Christine Noke, a patient advocate in Turkey, said.

In theory, reaching patients in the developing world would bring in more revenue for a drug company. But some manufacturers resist making their drugs available in poorer countries at lower prices because doing so can erode their ability to charge more in high-income countries.

Vertex, which has a monopoly on transformative cystic fibrosis drugs, said it was pushing to increase access globally.

"Our teams are working every day to expand access to even more patients around the world through a range of routes, including in low-middle-income countries and low-income countries where access barriers are high due to challenging economic conditions and limited health care infrastructure," Heather Nichols, a spokeswoman for Vertex, said.

Ms. Nichols said that Vertex has begun a "product donation program" in low-income countries. She said the company has provided some form of access to at least one of its cystic fibrosis drugs in Brazil, Bulgaria, Estonia, Greece, Latvia, Oman, Poland, Romania, Slovakia and Slovenia. The company declined to specify which lower-resourced countries have access to Trikafta.

The genetic defect that causes cystic fibrosis is most common in people of Northern European ancestry, as are the specific mutations needed for Trikafta to work. The number of cystic fibrosis patients in developing countries who are diagnosed and eligible for the drug is unknown but believed to number in the thousands.

In India, a recent survey counted just 600 diagnosed cystic fibrosis patients. Counting India's tens of thousands of patients who have not been diagnosed, some researchers estimate that India's total cystic fibrosis population is higher than that in Europe.

While a minority of Indians with cystic fibrosis are believed to have mutations that make them eligible for Trikafta, the size of India's population translates into huge numbers of patients who could benefit from Trikafta.

Hemanth Buddana, the Indian boy who died, was given therapies and antibiotics for his frequent lung infections, but there was little available in India to help him breathe or gain weight. Stuck in bed at home in Hyderabad, he taught himself to draw and to speak new languages.

A genetic test confirmed that he would be eligible for Trikafta, which has a U.S. list price 20 times as much as the annual salary Mr. Buddana earns as an operations manager at Google. He joined other parents in pushing the Indian government to find a way to get Trikafta for their children. But there was no progress.

"They say it's a miracle drug, but it's not a miracle if it is not available to everyone who needs it," said Shwetha Sree, who also lives in Hyderabad. Her 5-year-old son, Vihaan, has cystic fibrosis — and the mutation that would make him eligible for the drug when he turns 6, if he were to live in the United States.

Since the fight over access to H.I.V. treatment in sub-Saharan Africa in the early 2000s, some drug companies have agreed to sell their medicines at a profitable but significantly lower price in developing countries. The companies also sometimes work with a drug importer to sell the products in those regions.

There is also compassionate use, through which drug companies supply products to desperate patients in places where they are unauthorized. Vertex said that it has provided its medications free of charge to 6,500 patients worldwide that way. The company declined to say specifically where it has provided the drugs that way and where it is still doing so.

A company can also agree to voluntary licenses, allowing generic manufacturers to make and sell a drug in certain countries, typically in exchange for a royalty.

The Medicines Patent Pool, a United Nations-backed nonprofit that brokers that process by issuing sublicenses to generic manufacturers, said it has had no contact with Vertex.

New drugs typically take longer to reach poorer countries. But frustration with Vertex's failure to provide them with any form of access brought together cystic fibrosis patients online and led to a coordinated campaign for compulsory licensing.

Governments are often reluctant to do compulsory licensing, which capital markets tend to view as an alarming crack in the wall of intellectual property protection. Still, even if governments refuse to issue a compulsory license, the patient actions may pressure Vertex to make Trikafta available in those countries.

Cheri Nel, a 38-year-old investment banker in South Africa who has cystic fibrosis and is eligible for Trikafta, said that she had approached Vertex and suggested several ways the company could increase access and still safeguard its profit and intellectual property. She said she got nowhere and is now leading the action in South Africa.

"There's a balance: You want to keep companies incentivized to investigate and do research and development," she said. "But it does them no financial harm to let us import a generic because they're not even trying to sell it."

Vertex has not registered Trikafta with South Africa's drug regulator, but the company said on Monday that it recently signed an agreement with a distributor there.

In many countries, Vertex has also been seeking patents which deter generic manufacturers from selling the drug there, according to patent filings viewed by The New York Times. The company has a running legal battle with Gador, one of several manufacturers in Argentina making generic versions of Vertex's drugs.

Argentina does not recognize Vertex's intellectual property rights because the company has not joined the global treaty on patent protection. Gador's scientists reverse-engineered the Vertex drugs and began to sell them to Argentine patients. Then patients from foreign countries began to fly to Argentina to buy the drugs, which can cost as little as $18,000 per year using pesos exchanged on the black market.

In the late 2010s, Gador tried to strike a deal with the Turkish government to import its low-cost version of another expensive Vertex cystic fibrosis drug, Orkambi. In 2018, Vertex sued Gador in a Turkish court, arguing that the company was infringing on Vertex's patents. Vertex won, and the government abandoned the Gador deal.

In 2021, after Trikafta had become available in parts of Europe, patients in Turkey began suing their government to try to get the drug.

Today, more than 100 patients who have successfully sued the Turkish social security system are on Vertex medications, mostly Trikafta now. Dr. Bulent Karadag, the head of pediatric pulmonology at Turkey's main cystic fibrosis center, said he had 250 more patients who have been confirmed as eligible for Trikafta but have not been able to get it.

"Some patients say they can't even afford the bus ticket to the hospital, let alone hire a lawyer," he said. The U.S. list price for a year's supply of Trikafta is nearly 60 times as much as the annual salary of a minimum wage earner in Turkey.

Until Vertex's drugs, patients had few options, mainly palliative treatment to help them breathe a bit better, and if they could get one, a lung transplant. Vertex's drugs addressed the underlying cause of the illness, preventing patients' lungs from clogging in sludgy mucus.

Trikafta is stunningly effective at helping patients breathe better, keeping them out of the hospital and extending their lives. Patients and doctors say that the drug's power becomes evident almost immediately.

Raphaelle Pereira, 22, had been waiting for years for a lung transplant in the Brazilian city of Curitiba. By 2021, her weight had dropped to 80 pounds, and she no longer had the strength to walk to the bathroom. Family members sold property and scraped together $54,000 to purchase a two-month supply of Trikafta in the United States.

"I took it for a couple of days, and then I just got up and said, 'I think I'll have a shower.' My whole family was in shock," she said. "A few days before I couldn't even lift my arm."

With the data on how the medication had changed her condition, Ms. Pereira used a legal process to get Brazil's public health system to buy a steady supply for her. She's now working toward a career as a soccer commentator.

The obstacles to getting Trikafta in the developing world go beyond the availability of the product. Huge numbers of patients remain undiagnosed. Those who do get diagnosed are unlikely to have access to expensive genetic testing to determine whether they have one of the mutations necessary for the drug to work.

About 90 percent of patients of Northern European ancestry have the most common mutation needed for the drug to work, compared with far fewer people from the Middle East, Asia and Africa. In India, estimates range from 19 to 44 percent.

Vertex is funding an academic project to better understand the genetics of cystic fibrosis patients in poorer countries. Dr. Milan Macek Jr., a geneticist in Prague, is working with doctors in lower-resourced countries to collect and analyze blood samples from willing patients. He has identified hundreds of diagnosed patients in Eastern Europe, the Middle East and Central Asia who have the most common mutation.

Belinda Nell, who is working on the action in South Africa, followed the news about Vertex's drugs closely, as her two sisters grew increasingly frail with cystic fibrosis.

In 2014, Ms. Nell and her sister, Lorryn, who also had the disease, nursed a third sibling, Jennifer, as she was dying of it. Ms. Nell promised Lorryn that she would keep her from the same fate and, in early 2022, managed to obtain a couple months' supply of Trikafta for her. But Lorryn's lungs were too damaged, and she died last October.

"It's vital that children everywhere get access from a young age so they don't endure the end stage like I saw with my sisters," Ms. Nell said.

ATUL LOKE FOR THE NEW YORK TIMES

JOÃO SILVA/THE NEW YORK TIMES

Vihaan, top, is a 5-year-old cystic fibrosis patient living in Hyderabad, India. "They say it's a miracle drug, but it's not a miracle if it is not available to everyone who needs it," said Shwetha Sree, Vihaan's mother, about Trikafta. Above, Belinda Nell with portraits of her sisters Lorryn and Jennifer. Both died of cystic fibrosis in South Africa.

Elif Ince contributed reporting from Istanbul.

DADO GALDIERI FOR THE NEW YORK TIMES

Trikafta costs over $322,000 annually in the U.S. A U.K. analysis said it could be made for $5,700.

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

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Prof B Morrow
Paediatrics Pulmonology
5th floor, ICH Building
Red Cross War Memorial Children's Hospital

Dear Prof Morrow

PROJECT TITLE: CYSTIC FIBROSIS IN SOUTH AFRICA: SPECTRUM OF DISEASE, DIAGNOSIS AND DETERMINANTS OF OUTCOME (PHD CANDIDATE - DR M ZAMPOLI) SUB-STUDY LINKED R007/2018

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee dated 06 March 2019.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 March 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Dr Marco Zampoli will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

02 June 2020

HREC REF: 272/2020

Prof B Morrow
Department of Child and Adolescent Health
Intensive Care Unit-C -Floor
Red Cross Children's Hospital
Email: brenda.morrow@uct.ac.za
Student: m.zampoli@uct.ac.za

Dear Prof Morrow

PROJECT TITLE: SURVIVAL TRENDS OF CYSTIC FIBROSIS (CF) IN THE WESTERN CAPE REGION OF SOUTH AFRICA OVER 45 YEARS -PHD CANDIDATE-DR MARCO ZAMPOLI-SUB-STUDY LINKED TO R007/2018

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020.

Approval is granted for one year until the 30 May 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Dr Marco Zampoli will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
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Expected presentation date	Jul 2023
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