Building capacity for diagnosis of Primary Ciliary Dyskinesia in South Africa: a descriptive study

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Submitted to the University of Cape Town

In partial fulfillment of the requirements for the award of degree

MASTER OF PHILOSOPHY (MPHIL) (PAEDIATRIC PULMONOLOGY)

(Minor Dissertation)

In the Faculty of Health Sciences

Date of Submission: February 09, 2024

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TABLE OF CONTENTS

Title page……1

Declaration……3

The abstract ……..4

Acknowledgements……5

List of Tables……..6

List of Figures……7

List of supplementary files……8

Abbreviations……9

Chapter 1

1.1: Introduction and methods……10

1.2: Ethical consideration……17

1.3: Author guidelines of the Journal for which the paper has been formatted…17

Plagiarism check declaration……..18

Chapter 2 Dissertation (Publication-ready format…….....19

Appendices……...41

Declaration

I, Dr. Joy Eze, of the Division of Pulmonology, in the Department of Paediatrics and Child Health, University of Cape Town, hereby declare that this research titled '**Building capacity for diagnosis of primary ciliary dyskinesia in South Africa: a descriptive study'** is original based on independent work performed by me. The research is a cross-sectional observational study of children with chronic wet cough who are suspected to have PCD based on pre-established inclusion criteria (HREC ref 762/2020). The findings of this research was presented as abstract in the 2023 Department of Paediatrics and Child Health, University of Cape Town research days, and has not been previously published either in part or whole as an original article in any journal; nor has it been submitted for another degree to any other university for award of any other degree other than the above mentioned degree.

…

Dr. Joy Eze

ABSTRACT

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by abnormal cilia motility. Diagnostic capacity for PCD in sub-Saharan Africa (sSA) is limited; and incidence of PCD and genotype in sSA is unknown.

Objectives: To determine the prevalence of PCD in children and adults with suspected PCD using a range of specialized diagnostic tests; and inform the development of local guidelines for PCD diagnosis.

Methods: A prospective cross-sectional study in Cape Town, SA. Diagnostic tests performed included: nasal nitric oxide (nNO), nasal brushings for video microscopy of ciliary beat; transmission electron microscopy (TEM), immunofluorescence (IF) of ciliary protein antigens and genotyping.

Results: Thirty-three participants (31 children; 2 adults) were enrolled July 2022 to July 2023 [median (IQR) age 5.6 years (3.8, 8.2); 16 (49%) males; 22 (66.7%) non-Caucasian]. The most frequent clinical characteristics were upper respiratory disease with or without hearing impairment (91%, n=30), chronic wet cough (73%, n=24),, bronchiectasis (36%, n=12), neonatal respiratory distress (48%, n= 16), and situs inversus (36%, n=12); 7/17 (41%) participants recorded nNO <77nl/. Ciliary beat and TEM were abnormal in 55% (n=18) and 6.1% (n=2) of the participants respectively; PCD was confirmed genetically in 5/24 (21%), of which two had abnormal IF. Two unrelated black Africans were homozygous for the same pathogenic variant in *DNAAF3*.

Conclusion: Using a range of diagnostic modalities, the study has identified PCD cases who would have otherwise been missed or incorrectly diagnosed.

ACKNOWLEDGEMENTS

I wish to acknowledge my supervisor, Assoc. Professor Marco Zampoli, Co-supervisor A/Prof. Diane Gray, and all the co-authors for the tremendous and unwavering support given to me at all times throughout the stages of this work. In a special way, I acknowledge Prof. Heymut Omran his sacrifices, flying into South Africa from Germany to train me and other co-authors on the procedure for nasal brushing and helping us to set up the equipment/and consumables for cilia beat microscopy. I acknowledge tremendous contribution by Prof. Heymut and his team in the University of Munster laboratory in performing the genotyping for PCD on the participants in this study.

LIST OF TABLES

Table 1: Demographic characteristics and clinical features among participants

Table 2: Participants' routine investigation results

Table 3: Characteristics of genotype confirmed PCD cases

LIST OF FIGURES

Figure 1: Chest CT scan showing extensive bronchiectasis in confirmed PCD case (Case #18)

Figure 2: Abnormalities of cilia on immunofluorescence staining in one of the cases (Case #19).

LIST OF SUPPLEMETARY FILES

Supplement 1: Technique/protocol for nasal brushing and Cilia Beat Microscopy

Supplement 2: Video clip showing immotile cilia in PCD cases compared to normal cilia motility in non-PCD counterparts.

Supplement 3: List of PCD genes tested

Suplementary Table 1: Comparison between PCD confirmed cases and not-PCD cases by PICADAR criteria

LIST OF ABBREVIATIONS

ALI:	Air liquid interface
DNA:	Deoxyribonucleic acid
HIV:	Human Immunodeficiency Virus
HREC:	Human Research Ethics Committee
IF:	Immunofluorescence
LMICs:	Low and middles income countries
nNO:	Nasal nitric Oxide
PCD:	Primary ciliary dyskinesia
RCWMCH:	Red Cross War Memorial Children's Hospital
TEM:	Transmission electron microscopy

CHAPTER 1: INTRODUCTION

1.1 Context

Primary ciliary dyskinesia (PCD) is a genetic disorder of heterogeneous nature characterized by impaired mucociliary clearance due to dysfunctional or immotile cilia which predisposes the affected individual to recurrent respiratory infections.[1, 2] People with PCD have lifelong rhinorrhea, chronic wet cough, and progressive loss of lung function; and eventually develop structural damage of the airways, and bronchiectasis.[3] Other clinical findings that support the diagnosis of PCD are situs abnormalities, a history of neonatal respiratory distress syndrome, a family history of PCD, male infertility, and chronic productive cough in the absence of more common causes of chronic lung disease.[4]

The exact global burden of PCD in both adults and children remains unknown;[5, 6] The disease is heterogeneous, and its genotype may be influenced by ancestry. Previous reports suggested that PCD constitutes the highest burden among children with chronic suppurative lung disease and bronchiectasis in high income countries such as United Kingdom, Ireland, and Italy; with proportions between 1% to 24%; as well as in low and middles income countries (LMICs) like Tunisia, and India with proportions ranging from 3% to 26%.[2, 6] However, a recent global study found that PCD is estimated to be more common than has been recognised, particularly in individuals of African ancestry in whom the spectrum of PCD genes differs to other ancestries. [7] The five most common pathogenic or likely pathogenic PCD genes identified in the African or African American cohorts include *DNAH11, DNAI1, CCDC39, DNAH5, and DNAAF2*. These findings are important for case identification and PCD diagnosis in sub-Saharan Africa.

Being a heterogeneous disease, confirmation of a PCD diagnosis remains challenging as no single diagnostic test has been shown to have 100% sensitivity and specificity, thus a combination of diagnostic tests is needed to confirm the diagnosis.[3, 8] Diagnostic testing for PCD includes nasal nitric oxide (nNO); high-speed video microscopy (HSVM), transmission electron microscopy (TEM); immune-fluorescence staining (IF) and genetics.[1, 9-11] Adopting a universally acceptable reference standard for PCD diagnosis is challenging. Furthermore, many of these tests are not widely available in low-middle income countries (LMIC).

The index study will provide the first diagnostic capacity (including predictive screening tool) for PCD in South Africa, a country with high bronchiectasis burden; and for the first time in Africa in general. The study will in future inform the development of local diagnostic and

clinical guidelines for PCD diagnosis and care that build on international knowledge but are locally relevant and feasible. Quantifying the burden of PCD in South African children with unexplained chronic cough will guide the clinical management of children in this setup and enlighten clinicians on the aetiology of what has been labeled as idiopathic cause of bronchiectasis. In addition, it will build a foundation from which important genetic work will be added to define the genotype of African PCD. Participants in the current study will directly benefit through greater understanding of the aetiology and prognosis of their lung disease and where applicable, genetic counseling will be provided to their families subsequently.

Methodology

This ongoing study in SA employs a range of specialised diagnostic tests PCD in a referred population with suspected PCD. We report the preliminary findings of participants recruited so far using a range of specialized diagnostic tests for PCD that are previously unavailable in SA.

Study site and design

A descriptive prospective cross-sectional study was carried out at Red Cross War Memorial Children's Hospital (RCWMCH), Cape-Town, South Africa. Diagnostic testing capacity for PCD was established de novo at RCWMCH. Training to collect nasal brushing samples, video microscopy and preparation of IF slides was provided on-site through collaborating partners at the University of Munster, Germany.

Study population and participant recruitment

Eligible participants of all ages were consecutively recruited from the outpatient clinics from July 2022 to July 2023. Children and adults with suspected PCD were included in the study if they had: a chronic wet cough or unexplained bronchiectasis and one or more of the following criteria: unexplained respiratory distress syndrome as a term infant admitted to neonatal intensive care unit for respiratory illness; and/or congenital heart disease with or without situs inversus or heterotaxy syndrome; and/or persistent rhinitis and/or chronic middle ear effusion or hearing loss, without a history of recent acute worsening of symptoms in the previous one week.

People with other underlying causes of bronchiectasis or chronic cough such as active or past pulmonary tuberculosis (PTB), cystic fibrosis, HIV, primary immune deficiency, past foreign body aspiration, or post-infectious bronchiolitis obliterans were excluded from the study.

Data collection

Semi-structured interviewer administered clinical questionnaires were used to collect data including the previously validated PICADAR predictive tool questionnaire (Appendix D).[10] Relevant clinical information included:

i. Demographics: gender, age at assessment, and ancestry
ii. Medical history including neonatal illness or admittance to a neonatal intensive care; chronic (>3 months) cough; middle ear complaints; symptoms suggestive of bronchiectasis; and family history of PCD, bronchiectasis, hearing problems, asthma and consanguinity.
iii. Physical examination findings such as presence of situs abnormalities, congenital cardiac defect, rhinitis, and sinusitis.
iv. Previous investigation results such as sputum microscopy, culture, and sensitivity; lung function (spirometry); chest X-ray; CT scan; and previous routine PCD investigations if available.

PCD diagnostic testing/study procedures

Prior to nasal brushing, tidal breathing nNO was measured by a trained respiratory technologist using the NIOX VERO ® (Circassia AB, UK), and following the European Respiratory Society Technical Standard.[12, 13] The tidal breathing technique for measurement of nNO is recommended as a PCD diagnostic test in cooperative patients aged 4 years and older.[14] Participants who were younger than 4 years were exempted from this test.

The NIOX VERO® is an electrochemical device for hand-held nNO measurements that requires uninterrupted sampling for a fixed time (sampling time of 30 s at 5 mL·s−1).[13] The device is portable, simple to use, requires no calibration. It is relatively inexpensive when compared to the conventional stationary chemiluminescence-based nNO–analysers.[13,15] The device can be easily adapted in small paediatric centres to enhance early targeted case finding in young children with symptoms suspicious of PCD, and allows measurements at different sites. However, measurement of nNO with this device can be problematic in young children due to interrupted

flow (sniffing, crying) or difficulty maintaining the desired technique for the fixed duration of the test.[13] The device does not give a real-time sample display thus it is difficult to detect unreliable measurements such as leak, and lower airway contamination. In addition, the device relies on a cost per test model with the analyser requiring replacement after a set number of tests or a set timeframe. It discourages recording of ambient NO levels and repeated measurements unlike the Chemiluminescence analysers which allows an easier assessment of relevant factors such as nasal permeability and ambient NO levels.(15) Chemiluminescence analysers provides real time display of nNO recordings and allows identification and recording of unreliable measurements (nares leak contamination from lower airways), aiding the validation of the result.[13] It also helps in identification of the nNO measurement end-point such as stable plateau (ER and BH) or regular peaks (TB) without a fixed sample collection, and with minimum time requirement, However, the device is expensive and maintain (high cost per test for centres performing a limited number of measurements, and needs regular calibration and preventative maintenance and requires increased operator training and expertise, Although an "offline method" of measurement allows for remote sampling, it is difficult to transport and loses the advantage of the real-time trace.

All measurements were done in all subjects at one single occasion, within 30 minutes and by one experienced technician in the clinic setting. Patients were interviewed about known anatomical defects or previous nasal surgery, and in such cases the affected side was avoided and the opposite nostril chosen for measurements. Participants were required to blow their nostrils, and their nostrils were checked for any obvious nasal obstruction, or epistaxis. A nasal olive probe with a central lumen connected to the nNO analyser was inserted into one nostril. TB-nNO measurements were then performed by applying normal relaxed tidal breathing during sampling at a sampling time of 30 seconds and flow rate of 5 mL·s−1 respectively. The device displays the test results on the screen. The nNO levels in the two nostrils were measured and checked for variations. For quality control, biological control testing was done prior to each test using a healthy employee with known normal and stable nNO levels.

For the cilia studies, a single nasal brushing with a standard cytology brush (Celletta™; Engelbrecht Medizin-und Labortechnik GmbH, Germany) was obtained from awake participants in the clinic according to prescribed technique and protocol (see Supplement 1).[16]
The nasal brush sample was immediately immersed into the collecting tube containing liquid cell culture medium (RPMI 1640 Medium, Gibco ™, ThermoFisher) maintained at room temperature and shaken vigorously for 20 seconds to release epithelial cells into the medium. A smear of the sample was made onto the slides and the cilia were examined for their presence and beat pattern by direct evaluation under an inverted light microscope at X60 magnification. Ciliary beating was assessed within 5 to 10 minutes of collection of samples and reported as normal, irregular beating, immotile, or inconclusive (see supplement 2 for video examples).
Cilia beat frequency was not assessed as our microscope did not have the complete accessories for digital high speed video analysis.[17]

Additional slides (20 to 25) were prepared from the transport medium fluid and left to air dry and later shipped to collaborating partners at the University of Münster, Germany for direct (IF for ciliary protein antigens. Lastly, the nasal brush tip was immersed and cut-off into glutaraldehyde for TEM studies of cilia ultrastructure.[18]. Whole blood samples (two mls) each were collected from study participants and parents (where possible), stored in an ethylenediamine tetraacetic acid (EDTA) embedded bottles, and shipped to University of Munster for DNA extraction and PCD panel genetic studies following gene sequencing procedures.[19] All codon exons of previously published PCD-causing genes were amplified and sequenced. (see Supplement 3 for list of PCD genes).

Statistical analysis

Descriptive analyses were used to characterise the basic demographic and clinical characteristics of participants. Continuous variables were presented as means and standard deviation (SD), or median and range where applicable, while categorical variables were summarised as frequency counts and percentages.

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1.2 Ethical considerations

Ethics approval to conduct the study was granted by the UCT Faculty of Health Sciences Human Research Ethics Committee (HREC), (REF 762/2020) Appendix A; and the Red Cross War Memorial Children's Hospital (RCC 280/WC_202106_007). The study complied with the recommendations of the latest version of the Declaration of Helsinki 2013.[20] Informed consent were sought from the legal guardian and assent from children aged 7 years and above, in a language that the study participant understood (Appendix B & C). A professional interpreter assisted in the informed consent process where required.

The study involved minimal risks. The participant experienced a little discomfort that lasts for about 3 min when nasal brushings were taken or when nasal nitric oxide measurements were done. Localized pain due to venipuncture and collection of blood sample for DNA testing were minimised by the application of topical anesthetic cream at the skin site beforehand. Study participant's decision was respected if they choose to opt out of the study at any point. The participants were informed that there are no consequences of withdrawal from the study.

1.3 Author guidelines of the Journal for which the paper has been formatted

This manuscript has been formatted in line with the requirements of the African Journal of Thoracic and Critical Care Medicine (AJTCCM)

Interesting findings related to primary ciliary dyskinesia documented in this study have revealed that PCD is not uncommon in the South African population. These findings are best published in a national journal like the AJTCCM which has wide scope covering disorders of the respiratory system and related topics that are particularly of interest to researchers, clinicians and the wider audience in the general public. It is believed that these findings will shape opinions and guide current practice and future research bordering on PCD. The "Instructions to Authors" of the journal are appended (see Appendix E).

Plagiarism Declaration

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Date: 12/04/24

CHAPTER 2: PUBLICATION-READY MANUSCRIPT

Title page

Building capacity for diagnosis of Primary Ciliary Dyskinesia in South Africa: a descriptive study

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Study Synopsis:

What the study adds

The study is the first in South Africa to employ a range of specialised diagnostic tests for primary ciliary dyskinesia (PCD). PCD was confirmed in 21% of the referred study population with suspected PCD.

Implications of the study

Primary ciliary dyskinesia was previously underdiagnosed or missed in South Africa due to limited availability of diagnostic facilities. Our preliminary data suggests that PCD genotype in South Africans may be different from those of PCD cohorts in other countries. The findings of this study will improve clinician's awareness and management of PCD in South Africa. It has laid a foundation to building greater capacity for diagnosing PCD in SA and potentially and identifying PCD genotype linked to African ancestry.

Abstract

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by abnormal cilia motility. Diagnostic capacity for PCD in sub-Saharan Africa (sSA) is limited; and incidence of PCD and genotype in sSA is unknown.

Objectives: To determine the prevalence of PCD in children and adults with suspected PCD using a range of specialized diagnostic tests; and inform the development of local guidelines for PCD diagnosis.

Methods: A prospective cross-sectional study in Cape Town, SA. Diagnostic tests performed included: nasal nitric oxide (nNO), nasal brushings for video microscopy of ciliary beat; transmission electron microscopy (TEM), immunofluorescence (IF) of ciliary protein antigens and genotyping.

Results: Thirty-three participants (31 children; 2 adults) were enrolled July 2022 to July 2023 [median (IQR) age 5.6 years (3.8, 8.2); 16 (49%) males; 22 (66.7%) non-Caucasian]. The most frequent clinical characteristics were upper respiratory disease with or without hearing impairment (91%, n=30), chronic wet cough (73%, n=24), bronchiectasis (36%, n=12), neonatal respiratory distress (48%, n= 16), and situs inversus (36%, n=12); 7/17 (41%) participants recorded nNO <77nl/. Ciliary beat pattern and TEM were abnormal in 55% (n=18) and 6.1% (n=2) of the participants respectively; PCD was confirmed genetically in 5/24 (21%), of which two had abnormal IF. Two unrelated black Africans were homozygous for the same pathogenic variant in *DNAAF3*.

Conclusion: Using a range of diagnostic modalities, the study has identified PCD cases who would have otherwise been missed or incorrectly diagnosed.

Key words: Primary ciliary dyskinesia, cilia beat pattern, diagnosis, genotyping, immunofluorescence, South Africa

Primary ciliary dyskinesia (PCD) is a genetic disorder of heterogeneous nature characterized by impaired mucociliary clearance due to dysfunctional or immotile cilia which predisposes the affected individual to recurrent respiratory infections.[1, 2] People with PCD have lifelong rhinorrhea, chronic wet cough, and progressive loss of lung function; and eventually develop structural damage of the airways, and bronchiectasis.[3] Other clinical findings that support the diagnosis of PCD are situs abnormalities, a history of neonatal respiratory distress syndrome, a family history of PCD, male infertility, and chronic productive cough in the absence of more common causes of chronic lung disease.[4]

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Objectives and methods

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i. Demographics: gender, age at assessment, and ancestry
ii. Medical history including neonatal illness or admittance to a neonatal intensive care; chronic (>3 months) cough; middle ear complaints; symptoms suggestive of bronchiectasis; and family history of PCD, bronchiectasis, hearing problems, asthma and consanguinity.
iii. Physical examination findings such as presence of situs abnormalities, congenital cardiac defect, rhinitis, and sinusitis.

iv. Previous investigation results such as sputum microscopy, culture, and sensitivity; lung function (spirometry); chest X-ray; CT scan; and previous routine PCD investigations if available.

PCD diagnostic testing/study procedures

Prior to nasal brushing, tidal breathing nNO was measured by a trained respiratory technologist using the NIOX VERO ® (Circassia AB, UK), and following the European Respiratory Society Technical Standard.[12, 13] The tidal breathing technique for measurement of nNO is recommended as a PCD diagnostic test in cooperative patients aged 4 years and older.[14] Participants who were younger than 4 years were exempted from this test.

The NIOX VERO® is an electrochemical device for hand-held nNO measurements. The device is portable, simple to use, requires no calibration, and relatively inexpensive when compared to the conventional stationary chemiluminescence-based nNO–analysers,[13,15] It is easily adapted in small paediatric centres to enhance early targeted case finding in young children with symptoms suspicious of PCD, and allows measurements at different sites. The NIOX VERO® is an electrochemical analyser that requires uninterrupted sampling for a fixed time (sampling time of 30 s at 5 mL·s−1. This can be problematic in young children due to interrupted flow (sniffing, crying) or difficulty maintaining the desired technique for the fixed duration of the test. The device does not give a real-time sample display thus it is difficult to detect unreliable measurements such as leak, and lower airway contamination.[13] It relies on a cost per test model and the analyser requires replacement after a set number of tests or a set timeframe; it discourages recording of ambient NO levels and repeated measurements. On the other hand, the chemiluminescence analysers allows an easier assessment of relevant factors such as nasal permeability and ambient NO than the electrochemical technique.[13] It provides real time display of nNO recordings and allows identification and recording of unreliable measurements (nares leak contamination from lower airways), aiding the validation of the result. It also helps in identification of the nNO measurement end-point such as stable plateau (ER and BH) or regular peaks (TB) without a fixed sample collection with minimum time requirement, However, the device is expensive and maintain (high cost per test for centres performing a limited number of measurements), and needs regular calibration and preventative maintenance and requires

increased operator training and expertise, Although an "offline method" of measurement allows for remote sampling, it is difficult to transport it loses the advantage of the real-time trace.

All measurements were done in all subjects at one single occasion, within 30 minutes and by one experienced technician in the clinic setting. Patients were interviewed about known anatomical defects or previous nasal surgery, and in such cases the affected side was avoided and the opposite nostril chosen for measurements. Participants were required to blow their nostrils, and their nostrils were checked for any obvious nasal obstruction, or epistaxis. A nasal olive probe with a central lumen connected to the nNO analyser was inserted into one nostril. TB-nNO measurements were then performed by applying normal relaxed tidal breathing during sampling at a sampling time of 30 seconds and flow rate of 5 mL·s−1 respectively. The device displays the test results on the screen. The nNO levels in the two nostrils were measured and checked for variations. For quality control, biological control testing is done prior to each test using a healthy employee with known normal and stable nNO levels.

For cilia studies, a single nasal brushing using a standard cytology brush (Celletta™; Engelbrecht Medizin-und Labortechnik GmbH, Germany) was obtained from awake participants in the clinic according to prescribed technique and protocol (see Supplement 1).[16]
The nasal brush sample was immediately immersed into the collecting tube containing liquid cell culture medium (RPMI 1640 Medium, Gibco ™, ThermoFisher) maintained at room temperature and shaken vigorously for 20 seconds to release epithelial cells into the medium. A smear of the sample was smade onto the slides and the cilia were examined for their presence and beat pattern by direct evaluation under an inverted light microscope at X60 magnification. Ciliary beating was assessed within 5 to 10 minutes of collection of samples and reported as normal, irregular beating, immotile, or inconclusive (see supplement 2 for video examples).
Cilia beat frequency was not assessed as our microscope did not have the complete accessories for digital high speed video analysis.[17]

Additional slides (20 to 25) were prepared from the transport medium fluid and left to air dry and later shipped to collaborating partners at the University of Münster, Germany for direct (IF for ciliary protein antigens. Lastly, the nasal brush tip was immersed and cut-off into glutaraldehyde for TEM studies of cilia ultrastructure.[18] Whole blood samples (two mls) were collected from each of the study participants and parents (where possible), stored in an

ethylenediamine tetraacetic acid (EDTA) embedded bottles, and shipped to University of Munster for DNA extraction and PCD panel genetic studies following gene sequencing procedures.[19] All codon exons of previously published PCD-causing genes were amplified and sequenced. (see Supplement 3 for list of PCD genes).

Statistical analysis

Descriptive analyses were used to characterise the basic demographic and clinical characteristics of participants. Continuous variables were presented as means and standard deviation (SD), or median and range where applicable, while categorical variables were summarised as frequency counts and percentages.

Ethical approval

Ethics approval to conduct the study was granted by the UCT Faculty of Health Sciences Human Research Ethics Committee (HREC REF 762/2020); Supplement 4. Informed consent and assent were sought from participants. The study complied with the recommendations of the Declaration of Helsinki 2013.[20]

Results

Results of 33 participants (31 children and 2 adults) enrolled from the outpatient clinics, from July 2022 to July 2023 are presented: median (IQR) age was of 5.6 years (3.8, 8.2), and 16 (49%) were males. Participant ancestry was mixed (45%, n=15), Caucasian (33.3%, n=11), Black African (18%, n=6), and Other (3%, n=1). Twenty-four participants (72.7%) had complete genotyping and immunofluorescence results available at the time of this analysis.

Participant clinical characteristics

Participants' clinical characteristics are presented in Table 1. The most frequent clinical characteristics were chronic rhinitis, and chronic wet cough. In all, 54.5% had PICADAR scores >5; all PCD cases had scores ≥ 8. The PICADAR scores were compared between non-PCD cases and genetically confirmed PCD cases in Supplemetary Table 1.

Most frequent chest findings were inspiratory and expiratory crackles occurring in (27.3%, n=9), hyperinflation (27.3%, n=9) and focal reduction in breath sounds (15.1%, n=5); and respiratory

distress (9.1%, n=3). Wheeze was noted in only one of the participants with confirmed PCD (case #19). The most commonly used medications included anti-allergics (cetirizine and monteleukast); inhaled steroids; inhaled short acting salbutamol; and azithromycin.

Table 1: Demographic characteristics and clinical features among participants, n=33

Median (IQR) Age (years)	5.6 (3.8, 8.2)
Sex	
Male	16 (48.5%)
Female	17 (51.5%)
Ancestry (freq, %)	
Caucasian	11 (33.3%)
Black African	6 (18.1%)
Mixed	15 (45.4%)
Other	1 (3.0%)
Clinical features	
Chronic rhinitis	27 (82%)
Chronic wet cough	24 (73%)
Chronic sinusitis	21 (63%)
Chronic otitis media	17 (52%)
Neonatal respiratory distress	16 (48%)
Bronchiectasis	12 (36%)
Situs inversus*	12 (36%)
Hearing impairment	7 (21%)
PICADAR score**	
<5	15 (45.4%)
6-9	10 (30.3%)
>10	8 (24.3%)

*Other abnormalities co-existing with situs inversus were double outlet right ventricle

(DORV), congenitally corrected transposition of the great arteries, atrial or ventricular septal defect, atrioventricular valve regurgitation, left ventricular outlet obstruction, and pulmonary or aortic valve stenosis.

**See Appendix D for PICADAR Scoring Criteria

Supplementary Table 1: Comparison between PCD confirmed cases and not-PCD cases by PICADAR Criteria, n=45

	NOT PCD 35 (%)	PCD CONFIRMED 10 (%)
Term		
Yes	25 (71.4%)	9 (90%)
No	10 (28.6%)	1 (10%)
Neonatal Respiratory Distress		
Yes	13 (37.1%)	8 (80)
No	22 (62.9%)	2 (20)
Neonatal Admission		
Yes	23 (65.7%)	9 (90%)
No	12(34.3%)	10 (10%)
Situs inversus		
Yes	7 (20%)	6 (60%)
No	28 (80%)	4 (40%)
Congenital Heart Defect		
Yes	6 (17.1%)	3 (30%)
No	29 (82.9%)	7 (70%)
Persistent Perennial Rhinitis		
Yes	25 71.4%)	10 (100%)
No	10 (28.6%)	0 (0)
Chronic ear or hearing problem		
Yes	11 (31.4%)	7 (70%)
No	24 (68.6%)	3 (30%)
Median PICADAR score	**4**	**10**

Routine laboratory investigations

Table 2 shows the investigation results. Microbiological cultures of any of the following respiratory tract samples: sputum. Bronchoalveolar lavage (BAL), tracheal aspirate, cough swab were positive in 39% (n=13), including *Pseudomonas s*pecies in three (9%), *Staphylococcus aureus* in three (9%) and *H Influenzae* in three (9%) of the participants. Other organisms found

included Moraxella catarrhalis, Klebsiella pneumoniae, Stenotrophomonas maltophilia, and *Streptococcus pneumoniae.*

Imaging Studies: chest x-ray and computerised tomography (CT) scans

Predominant chest x-ray findings included increased broncho-vascular markings, air trapping, focal lung infiltrates, bronchial wall thickening and cystic changes. Variable findings were noted on CT scan. Bronchiectasis involving both right middle/lower lobe (RML/RLL) and left lower lobe (LLL) were noted in 7/12 (58.3%) of cases while a few others had right upper lobe (RUL) and lingula bronchiectasis. Figure 1 depicts extensive bronchiectasis in one of the confirmed PCD cases (Case #18).

Nasal nitric oxide (nNO) measurement

Tidal breathing nNO was measured in 17/33 (51.5%) of the participants; the remaining 16 participants were either under age for the procedure, or uncooperative and could not produce acceptable results. The median nNO was 104.9 (range 5.9 - 232) nl/min; 7/17 (41%) participants recorded <77nl/min suggesting possible PCD diagnosis.

Cilia studies

Ciliary beat microscopy was considered abnormal in 55% of the participants (n=18). These abnormalities ranged from sluggish circular movement to immotility. Four (80%) of five confirmed PCD by genotyping had either immotile cilia or cilia with sluggish circular movements. Attached video clip shows immotile cilia in compared to normal cilia motility in non-PCD counterparts (Supplement 2)

Lung function measurements

Lung function measurements using spirometry was performed 66.7% of the participants, (n=22). Majority of them, 68.2% (n=15) had normal spirometry. About 32% (n=7) of them had airflow limitations that was mostly mild. The median FEV_1, FVC, and FEV_1/FVC ratio of the study participants were 1.07, 1.50 and 0.83 respectively. The median FEV_1 z-score was -1.9 (min -5.1, max 1.1).

Table 2: Participants' routine investigation results, n=33

Microbiological cultures	
Positive	13 (39.4%)
Negative	20 (60.6%)
Chest X-ray findings	
Increased broncho-vascular markings	**4 (12.1%)**
Focal lung infiltrates	**3 (9.1%)**
Lobar atelectasis	**5 (15.2%)**
Bronchial wall thickening	**3 (9.1%)**
Cystic changes.	**6 (18.2%)**
Air trapping	**5 (15.2%)**
CT scan finding, n=12	
Bronchial wall thickening	15.1%
Bronchiectasis	36% (varied pattern)
Mosaic attenuation	15.1%
Median (IQR) nasal nitric oxide; n=17	104.9nl/min (16.2, 132.0); range 5.9-232
Ciliary beat abnormal or immotile	18 (55%)
Reported TEM findings	
Insufficient sample	11 (33.3%)
Normal	16 (48.5%)
Abnormal	2 (6.1%)
Not done	4 (12.1%)
Lung function (Spirometry), n=22	
Median FEV_1 litres	1.07
Median FVC litres	1.50
Median FEV_1 z-score	-1.9 (min -5.1, max 1.1)

Genotyping, immunofluorescence, and transmission electron microscopy

Of the 24 participants with completed genotyping and IF, PCD was confirmed genetically in five out of 24 (21%) by identification of known pathogenic variants, of which two had abnormal localisation by IF. Immunofluorescence staining were for DNAH5, GAS8, RSPH9, CCDC39 and CCDC40.Two unrelated black Africans (Cases 19 and 24) with confirmed PCD respectively had homozygous deletion of the same gene loci on *DNAAF3* (*DNAAF3*:c.1396del, p.(Ala466ProfsTer9). A rare X-linked frame-shift deletion on DNAAF3, ZMYND10; and an X-linked frame-shift deletion OFD/:c.2652del, p.Glu884AspfsTer4 were found in two respective participants. Figure 2 shows the abnormalities of cilia on immunofluorescence staining in one of the participants (Case #19).

On EM, the cilia ultrastructure was normal in 16/33 (44.5%) and abnormal in only two (6.1%) of the participants. One of the participants with abnormal EM had extensive cilia defects including absence of cetnral microtubule pair, reduplication of peripheral microtubule, deletion, and reduplication of central pair of microtubule doublet in some cilia. Cross sections of the some of the cilia in the other participant demonstrate a 9+2 microtubule doublet configuration with the presence of dynein arms. Scattered cilia show distorted doublet configuration. The defect noted included an extra central pair of microtubule, some misplacement of central microtubuli, incorrect positioning of one or more of the peripheral pair of microtubuli and absence of the central pair. The root processes appear abnormal but cytoplasmic microvilli are within normal limits.

Three out of the 16 participants with normal cilia ultrastructure were genetically confirmed for PCD, Interestingly, none of those with abnormal cilia ultrastucture had genetic mutations linked to PCD. Eleven (33.3%) of those studied had no cilia detected on EM sample and samples were deemed insufficient for analysis. EM was not done in the remaining four of the 33 (12.1%) participants due to logistical and technical failures.

All the genetically confirmed cases presented with chronic sinusitis/otitis media and wet cough and reported a preceding history of respiratory distress in the neonatal period; three out of five (60%) has situs inversus with or without associated complex cardiac abnormalities; The basic characteristics, genotype and IF results of confirmed PCD cases are presented in Table 3.

Table 3: Characteristics of genotype confirmed PCD case, n=5/24 (21%)

Case ID	Age (yrs.), sex	Ancestry	PICADAR score	Pathogenic genotype confirmed by segregation	Nasal NO (nl/min)	Ciliary beat pattern on microscopy	TEM	IF pattern
02#	8.1, M	Mixed	9	Hemizygous Frameshift deletion OFD-1/:c.2652del. X-linked PCD inheritance	55.7	Circular & Sluggish	Normal cilia ultrastructure. 9+2 microtubule doublet with dynein arms	Normal DNAH5/GAS8/RSPH9
17*#	4.9; M	Caucasian	12	Heterozygous DNAAF4	15.1	inconclusive	NA	DNAH5 staining weak on IF
18#	29.1; M	Caucasian	8	Homozygous ZMYND10	13.53	immotile	Normal microtubule doublet configuration. Unable to assess dynein arms.	NA
19*#	7.0; F	Black African	14	homozygous DNAAF3	5.94	immotile	Sample not surviving processing	Abnormal DNAH5
24*#	6.9; F	Black African	12	homozygous DNAAF3	8.91	immotile	Normal cilia ultrastructure. 9+2 microtubule doublet with dynein arms	NA

* Neonatal RDS # Organ laterality defects

Discussion

Primary ciliary dyskinesia is a rare disease that affects all populations but the nature of the disease is yet to be fully elucidated in the African population. The diagnostic tests for PCD such as TEM, nNO, high-speed video microscopy (HSVM), genetics and immune-fluorescence staining (IF),[1, 9-11] are highly sophisticated and require expertise which are lacking in LMIC. We started a comprehensive PCD diagnostic center in sSA in collaboration with an international partner with established expertise in PCD diagnosis.

Primary ciliary dyskinesia was confirmed by genetic studies in 21% of participants in this study. Primary ciliary dyskinesia was previously regarded as a disease of populations in high income countries and not readily found in the African populations. But recent studies suggest that PCD may be more common than has been recognised, particularly in individuals with African ancestry.[7, 21] A recent study in another city in South Africa documented a prevalence rate of 43% among clinically suspected PCD cases using the TEM modality.[22] The apparent lack of uniform diagnostic modality observed between the index study and the other South African study gives no room for adequate comparisons.

The genes most commonly implicated in PCD in these studies differed across ancestries and contrasted to those in European and North American studies.[7] Pathogenic variants in *DNAH11, DNAI1, CCDC39, DNAH5, and DNAAF2* are predicted in population studies to be more common in individuals with African ancestry.[7] We could not verify this due to small participant numbers, but did observe a homozygous deletion for the same loci on *DNAAF3* (*DNAAF3*:c.1396del, p.(Ala466ProfsTer9) in two unrelated black African individuals. Similarly, in a recent report from South Africa a reported the same variant in homozygous state in two Black African siblings; and two different heterozygous pathogenic variants in the *DNAAF1* were detected in two other siblings.[21] Thus, findings of this study provide an early signal that *DNAAF3* may be more prevalent in black Africans in SA and possibly in sSA. The findings are important and could potentially impact case identification, particularly in sSA where this mutation may be more common than elsewhere in the world due to founder effect.

Abnormal ciliary beat patterns ranging from sluggish circular movements to complete immotility were observed in 55% of the participants on video microscopy. However, more than half of those with abnormal cilia beating eventually did not have PCD confirmed. Limited

experience and subjective reporting are important limitations of this test and may explain these findings. In addition, abnormalities of cilia beatings in normal individuals may be secondary to acute or chronic respiratory infections, inflammatory respiratory disease (i.e. asthma), environmental and demographic factors (smoking, pollution and age), or sample handling.[22] For this reason sampling should be avoided when the patient is unwell. If a secondary defect is suspected, repeat sampling is recommended when the individual has recovered fully.[22] Despite the recognized limitations of the test, it is a relatively easy point-of-care test to replicate in low resource settings and may help to exclude PCD if ciliary beat pattern is normal.

Electron microscopy revealed that 48.5% of participants in the current study had normal cilia ultrastructure; abnormal cilia ultrastructure was detected in only two (6.1%) of the participants who eventually did not have PCD by genotyping. Confirmation of PCD diagnosis in this study was based on the finding of known pathogenic variants for PCD, and abnormal localisation by immunofluorescence. It is known that up to 25%- 30% of all genetically PCD cases may have normal ciliary ultrastructure on TEM.[22, 23] On the other hand, a study in Tunisia reported confirmed PCD diagnosis in a cohort of children with or without situs abnormalities based on findings of outer dynein arms (DA) defects in an electron microscope,[24] Similarly, a recent study in SA confirmed PCD in 43% of the clinically-suspected cases by TEM observations (hallmark ultrastructural defects in the dynein arms of the outer doublets). Additional 57% of participants in that study required another PCD testing modality to support ultrastructural observations.[23] Their finding challenges the reports in previous studies which suggest TEM does not have sufficient diagnostic accuracy for PCD. On the other hand, ultrastructural defects due to secondary causes may show overlapping appearance with those seen in confirmed PCD cases.

Important factors that influence interpretation of results of TEM as highlighted in the international consensus guidelines include inadequate samples, individual processing techniques and familiarity with the local appearance of cilia. The initial validation of the guideline revealed that smaller, inadequate samples would most likely lead to a misdiagnosis; hence it is recommended that extensive normal and PCD samples be visualised using local techniques before a diagnostic sample is interpreted. TEM is time consuming and resource-intense and may therefore not be suitable for routine use in low resource settings. It is suggested that where available, TEM in low -resource settings should be reserved when PCD suspicion is high e.g.

high PICADAR score or low nNO. There is need for experts in the field to critically review with the aim of reaching a consensus on the predictive value and applicability of various diagnostic tests for PCD diagnosis within local contexts especially in sSA where most of the advanced techniques for diagnosis such as genotyping and IF may not be readily available. For instance, where there are extensive abnormalities in the cilia ultrastructure in suspected patients, whose clinical characteristics are in keeping with PCD, but no identified gene mutations as were observed in two of our participants, could the clinician boldly say that PCD is definitely ruled out? This forms an important discussion point for various stakeholders especially in sSA, where different centres may have different diagnostic modalities, that are not always genotyping.

A very important concept in PCD diagnostics that had been previously debated is whether the use of cultured epithelial cells at air liquid interface (ALI) to improve yield of diagnostic techniques significantly the cilia sample phenotyes. Independent and silmutaneous studies done at Leicester and Southampton PCD centres recognised exacerbation of the functional defects seen in some subjects with PCD following ALI culture, thus raising concerns about the validity of the technique. [25] However, findings from a robust retrospective study of data generated from a cohort of 54 PCD subjects and 111 non-PCD subjects at the Leicester and Southampton PCD centres suggested that post-culture changes in the phenotype of PCD samples clarifies rather than confuse diagnostic status. The study thus concluded that the post-culture changes actually aid diagnosis as dyskinesia associated with PCD was unchanged or became more prominent in all cases, and secondary dyskinesia in non-PCD group improved,
Other studies have also suggested that reanalysis following submerged or air–liquid interface (ALI) culture may be useful to exclude secondary ciliary dyskinesia or confirm PCD when analysis of the primary sample is abnormal, and may provide additional cilia if the primary sample is inadequate.[26] Perhaps, the use of ALI cell cultures might help to truly define the PCD status of participants in the index study whose TEM results were inconclusive or unclear.

Prior to the cilia studies, we measured participants' nNO levels and lung function. Nasal nitric oxide was measured with the electrochemical device, NIOX VERO® using tidal breathing method. This technique is recommended as a PCD diagnostic test in cooperative patients aged 5 years and older.[14]

Nasal NO as a screening tool in resource constrained settings like ours is valuable as the findings of this study validate nNO as a tool with high sensitivity and specificity for PCD. Consistent

with the ATS/ERS cut-off value for possible PCD diagnosis, all genetically confirmed PCD cases in the index study also recorded nasal nitric oxide levels <77nl/min;[2, 11] with 80% of them having levels <20nl/min.

Although most participants in this study had normal lung function, a significant proportion (30%) had some air flow limitation that was mostly mild. The underlying pathology in PCD is impaired mucociliary clearance due to dysfunctional or immotile cilia which predisposes the affected individual to recurrent respiratory infections, and structural damage of the airways with bronchiectasis with progressive loss of lung function.[1-3] Progressive deterioration in lung function occurs where individuals have not received adequate care before and after diagnosis have been made.

The microbiological cultures of respiratory tract samples revealed polymicrobial organisms commonly associated with RTIs in humans.[27, 28] However, organisms such as *Pseudomonas s*pecies, *Staphylococcus aureus* were top on the list similar to cystic fibrosis associated bronchiectasis. Up to 27% of the participants in the current study were on treatment for chronic sinus and respiratory infections that have not been previously diagnosed as PCD.

The study has highlighted that symptom-based screening tool like the PICADAR scoring tool may be helpful in suspected cases where other diagnostic modalities are not available. The PICADAR tool has an overall sensitivity and specificity of 0.90 and 0.75 respectively for a cut-off score of 5 points for PCD diagnosis in previous studies.[10] In the presence of situs inversus, neonatal respiratory distress, and evidence of upper respiratory symptoms, the suspicion for PCD becomes high. More participants need to be recruited in order to further validate this tool in sSA,

We acknowledge several limitations in this study. First, the small sample size and incomplete data (e.g., IF and genetics) reported from enrolled participants so far may not be an accurate representation of the spectrum of PCD in sSA. Second, we experienced technical challenges with TEM sample preparation and analysis which limited our ability to interpret TEM findings. The inability to detect cilia in many samples sent for TEM highlights the limitation of TEM in settings without the appropriate expertise and experience. The ciliary beat frequency was not assessed as the examination of beat frequency required a high speed video camera which was not readily available. These limitations notwithstanding, clinical practice guidelines recognizes that isolated genetic testing is appropriate in cases with strong clinical PCD phenotypes where other testing modalities pose technical challenges.[29]

Conclusion

Using a range of diagnostic modalities, this study has identified PCD cases in 21% of enrolled participants who would have otherwise been missed or incorrectly diagnosed. Our study suggests that *DNAAF3* may be an important gene linked to PCD in sSA in people with Black African ancestry and should be included PCD diagnostic gene panels in this region. We propose that local diagnostic algorithms for PCD should include clinical findings, nNO, nasal brushings for video microscopy and genotyping. The role of PICADAR scoring tool, IF and TEM in PCD diagnosis in sSA is not yet established and require further exploration.

Declaration. none

Acknowledgement. We acknowledge our German collaborators for the training support given to the SA team

Author Contributions

LG, DG, conceptualized the study. LG, MZ, JNE, JV and AV, KW, HO were responsible for the study's design, questionnaire design, and data acquisition. JNE, MZ, JV, AV, DG, LG, CS, SS, ED, KW, HO, and HO participated in the data analysis and preparation of the manuscript. All authors were involved in proofreading and critically edited the manuscript drafts. All authors read, reviewed, and approved the final draft of the manuscript.

Funding. This research received part funding from;

a. Department of Paediatrics and Child Health Research Committee, University of Cape Town
b. Department of General Pediatrics, University Children's Hospital, Münster, Germany
c. The Harry Crossley Foundation, University of Cape Town.

Conflict of interest. None

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Appendices

Appendix A: Ethics approval letters

Appendix B: Consent form

Appendix C: Assent forms

Appendix D: Questionnaire/data capture instruments

Appendix E: AJTCCM Instructions to authors

Nasal brushing Protocol

Procedure:

- Heat the medium to room temperature. Wet the brush with sterile isotonic saline solution. We use Celletta™brush cell collector with protective tip (product number 9100060; Engelbrecht Medizin-und Labortechnik GmbH, Tiefenbachweg 13; 34295 Edermünde, Germany). Other brushes can also be used.

- Ask the patient to clean his nose. The patient should sit in a chair with the head against a wall so he can´t move the head backwards (figure 1). Alternatively a 2nd person should stand behind him and hold the head. Ask the patient to hold the collecting tube so his hands are occupied with this.

- Examine the inferior nasal meatus with the rhinoscope (if possible), the mucosa should look clean and healthy.

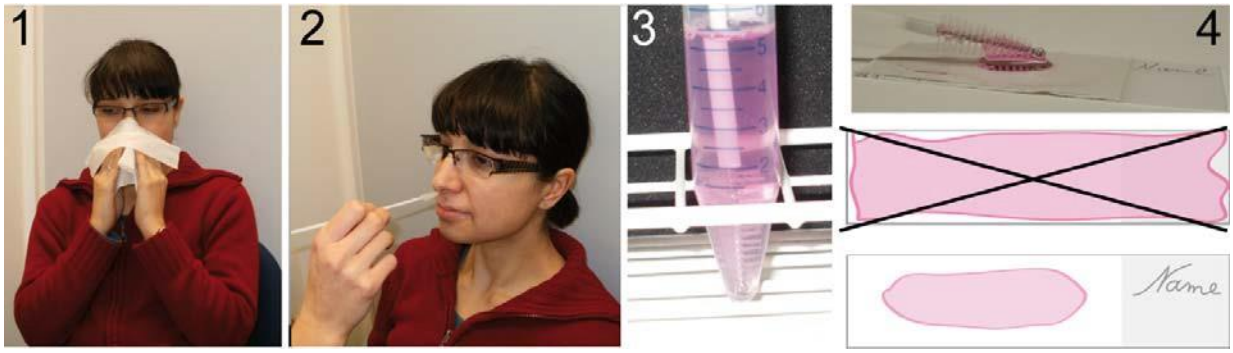
- Take the brush and rub it a few times rapidly against the medial and superior side of the inferior nasal meatus, using rotatory and linear movements (figure 2). The patient feels a local burning and will probably get tears in the eye but the discomfort will diminish rapidly.

- Take the brush out and put it immediately in the collecting tube (figure 3). Shake the brush vigorously within the tube at least 40 times, so that the cells become detached from the brush.

Preparing slides for Immuncytochemistry

- Please include data on direct microscopy (Ciliary function) and electron microscopy findings if available

- Use pre-cleaned slides without additional preparations (ordinary slides). Take the brush to portion out the suspension onto slides (see figure 4). Please prepare ~25 slides until **no fluid** is left in the tube. Let the slides dry completely at room temperature. This will take a few hours. Label the slides with a pencil and send them to our lab address:



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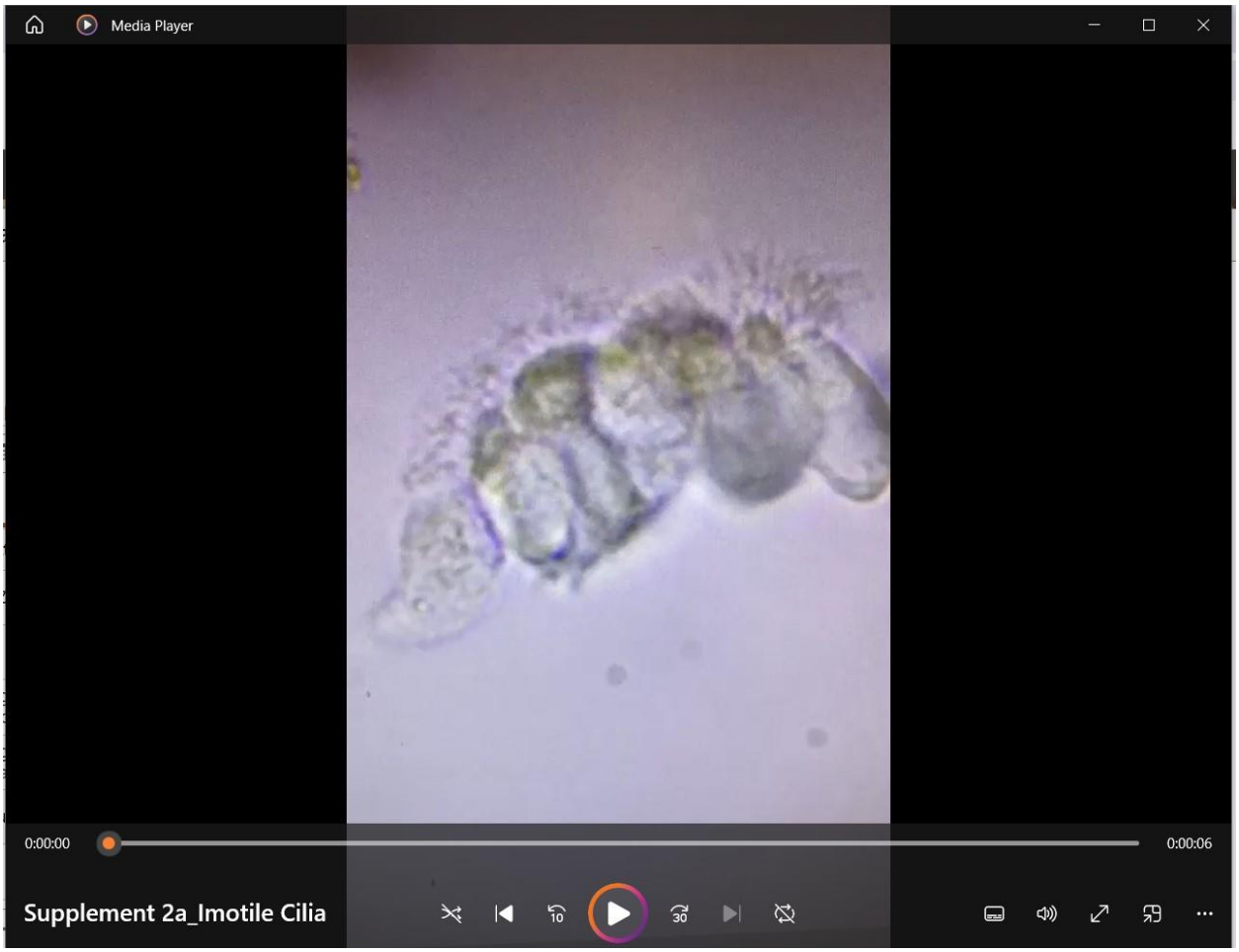
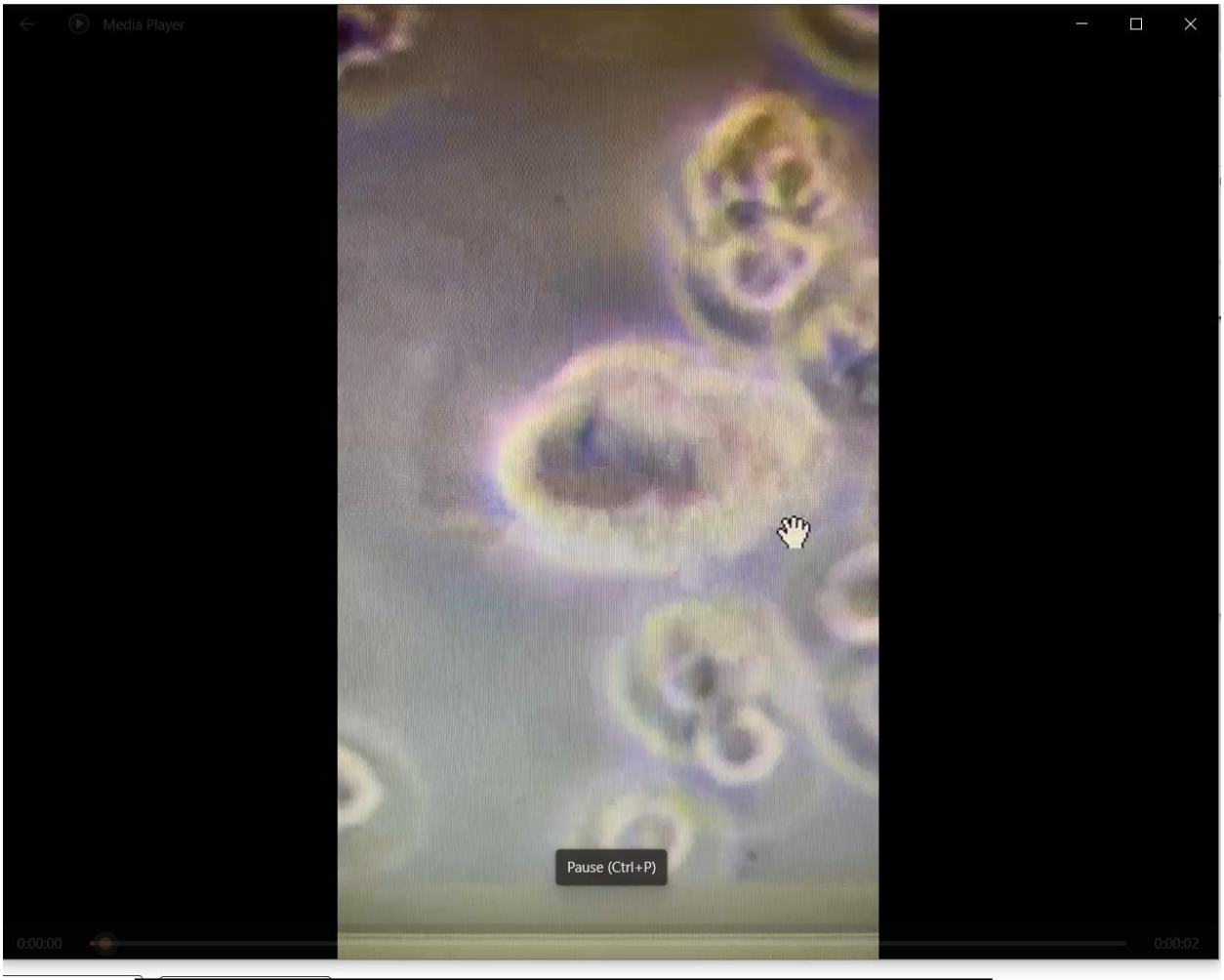

Media Player
0:00:00
0:00:06
Supplement 2a_Imotile Cilia



Supplement 2b: Normal Cilia

Supplement 3: List of previously published primary ciliary dyskinesia causing genes tested in the study (this list is inexhaustive).

ARMCA/ODAD2(NM_001290020,NM_018076), DNAAF11(NM_012472); DNAAF2(NM_018139); DNAAF3(NM_001256714, NM_001256715); DNAAF4(NM_130810, NM_001033560); DNAAF6(NM_001169154, NM_173494; DNAH1(NM_015512); DNAH5; DNAH9; DNAH11; DNAI2; DNAAF1; CCDC39(NM_181426); CCDC40 (NM_017950, NM_001330508, NM_001243342); CCDC103 (NM_213607); CCDC114/ODAD1(NM_144577); CCDC151/ODAD3(NM_145045); CCDC164; HYDIN(NM_001270974, NM_001198542); DYX1C1; ZMYND10(NM_015896); SPAG1(NM_003114, NM_172218)); CFAP298(NM_001350335. NM_021254); GAS8 and *TTC25/ODAD4(NM_031421)*); *DRC4(NM_001481) etc.,* amplified and sequenced.

Appendix A

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001537; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/5/2024
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	17/5/2023

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

HUMAN RESEARCH ETHICS COMMITTEE
16 MAY 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol Information

Date (when submitting this form)	15/05/2023		
HREC REF Number	782/2020	Current Ethics Approval was granted until	30 May 2023
Protocol title	Primary Ciliary Dyskinesia in a South African Tertiary Hospital: A cross-sectional study		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☒ Yes	☐ No	
If yes, could you please provide the HREC Reference number for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.	HREC 350/2022 Validity of the PICADAR score for diagnosis of primary ciliary dyskinesia among South African children with chronic cough		

(Note: Please complete the Closure form (FHS017) if the study is completed within the approval period)

Appendix B: Consent and Assent Forms

Parent /Legal Guardian/Caregiver Information Sheet and Consent Form

Primary ciliary dyskinesia in a South African tertiary hospital: Cross-sectional study

Your child is being invited to take part in this clinical research study because he/she has chest or nose symptoms of frequent or long-standing wet cough or nasal discharge. This study is being done at Red Cross Children's Hospital in the School of Child and Adolescent Health, University of Cape Town. Before you decide if you want to be a part of this study, we want you and your child to know more about the study.

This form gives you information about your child's participation in this study. The research staff will talk with you and your child about this information. You are free to ask questions about this study and discuss any concerns with the staff. If you agree to take part in this study, you will be asked to sign this consent form and your child may be asked to sign a separate assent form.

You will be given copies of these forms.

WHY IS THIS STUDY BEING DONE?

Cilia are tiny hairs, only seen under the microscope, that help your cells move, which helps your body carry out important jobs like breathing and reproducing.

Primary ciliary dyskinesia (PCD) is a rare genetic disorder in which the cilia don't work properly. We know PCD mainly causes problems in your respiratory system. The cilia are tiny hair-like structures, responsible for clearing mucus and bacteria from your airways. If they don't work as they should, mucus and bacteria can be retained in the airways causing recurrent or longstanding wet cough. We are doing this research to understand how your cilia work and to understand if any genes in your body are associated with primary ciliary dyskinesia.

The study will take place over 6 months during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment, we will treat you at Red Cross War Memorial Children's Hospital.

WHO CAN TAKE PART IN THE STUDY?

Any child between 0-18 years old who has symptoms of abnormal cilia function can participate, approximately 50 children will take part in this study.

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend one study visit held at Red Cross War Memorial Children's Hospital. The study visit will last about 2-3 hours. Your child will undergo the following measurements: -

1. You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, chest disease treatment history, and other aspects of health, both in the past and at present.
2. Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic. We will also ask for permission to look at information from your child's health care records at the clinic he/ she currently attends, and other facilities he/she may have attended. We will get information about your child's past health in the past, including whether they attended a clinic or hospital, the treatment received, and laboratory results over time. This information is important to help us understand the results of the tests done as part of this study.
3. In addition to the standard lung function test Your child has at clinic, they will be asked to do a blowing test to measure gas in his/her nostrils called nitric oxide. This test is best carried out when your child has not had flu or common cold in the last 6 weeks. Nitric oxide can be low if cilia are not working properly.
4. Then we shall brush or swab your child's nostrils (nasal swab). This procedure involves putting a small stick with a tiny brush into the lower third of your child's nose to collect some cilia which we look at under the microscope to see the movement of cilia or structure of the cilia. The swabbing of the nostrils can have some minor discomfort that lasts about 3 minutes.

   We will take 3 ml blood sample for genetic or DNA testing.A cream to prevent pain/discomfort on your child's skin will be applied on the small part of the skin of the forearm where about 3mls of blood will be drawn. This will be done by a trained health care worker in a sterile technique. If permission is given some of the blood sample that is taken may be stored for future tests and studies.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

- You may experience a little discomfort that lasts for about 3 min when we swab your nose or when we take a blood sample from your child
- Your name will not be displayed on our research records, but you will be assigned a unique number

- When we receive the results of the genetics test from the lab, we shall communicate the results back to you.
- We will try to make sure that no bad things happen to you when we carry out these tests. You can say 'no' to what we ask you to do for the research at any time and we will stop.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

We think being in this research may help you because we shall test and know if there is a problem with your cilia or not. The genetic testing will also help us know that you or your family members do not carry the gene that causes lack of or abnormal movement of cilia. We are then able to treat your current symptoms appropriately.

Your child's health will be carefully monitored during the study so any new health problems may be picked up early. If we find your child has a problem that needs to be treated, we will refer him/her to an appropriate facility. The information we learn through this study may help to improve the diagnosis of children with primary ciliary dyskinesia in the future.

WILL I RECEIVE ANY PAYMENT?

You will receive R300 per visit when you and your child visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?

If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given consent for him/her participation. Even if you and your child decide not to take part, your child will receive the same health care at the hospital.

CONFIDENTIALITY

Every effort will be made to ensure that your child's information is protected. The study team will keep your child's study information confidential. Your child will be given a study number. The questionnaire and study specimens will be labelled with this study number and not with his/ her name. As a participant in this study, it is very important to be able to contact you and therefore we will need to collect detailed tracing information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you. All data will be stored

in password-protected computer programs which are only accessed by health care workers who are permitted to carry out this research by the University of Cape Town Human Research Ethics Committee.

STORAGE OF SAMPLES

If any of the nasal swabs or blood samples my child has provided for this research project are unused or leftover when the project is completed

□ I give my permission for my child's samples to be stored indefinitely and used in future research of any type which has been properly approved by the Human Research Ethics Committee including genetic testing and other research.

□ I give permission for my child's samples to be stored indefinitely and used in future research except for research about **OR**

□ I wish for my child's samples to be destroyed immediately.

□ I have read this consent form (or have had it explained to me), all my questions have been answered, and I agree for my child to take part in this study.

		DD/MMM/YYYY at - -H - -
Parent/Legal Guardian/Caregiver (Print)	Parent/Legal Guardian/Caregiver (signature/Thumbprint)	Date and Time
		DD/MMM/YYYY at - -H - -
Study Staff (Print)	Study Staff's Signature	Date and Time

Please complete the Witness page if the participant's Parent/Legal Guardian/Caregiver is unable to read or write.

_____ _____ DD/MMM/YYYY at - -H- -

Witness' Name (Print) Witness' Signature Date and Time

Participant Enrolment number: _____

Please complete the following:

□ The caregiver of this child confirms that the biological mother / father (please circle) of this child is alive but does not take ANY responsibility for the care of or decision-making for this child.

□ Not applicable - neither of the child's biological parents are alive.

□ Not applicable - the child's biological parent signed consent.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study, you may ask the study staff or you may contact:

Dr Joy Eze +27747174660

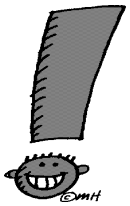
If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town: Prof Marc Blockman at (021) 406 6338

What is a research study?

Research studies help us learn new things. We can test new ideas. First, we ask a question. Then we try to find the answer.

This paper talks about our research and the choice that you have to take part in it. We want you to ask us any questions that you have. You can ask questions at any time.

Important things to know…



- You get to decide if you want to take part.
- You can say 'No' or you can say 'Yes'.
- No one will be upset if you say 'No'.
- If you say 'Yes', you can always say 'No' later.
- You can say 'No' at anytime.
- We would still take good care of you no matter what you decide.

Why are we doing this research?

Cilia are tiny hairs, only seen under the microscope, that help your cells move, which helps your body carry out important jobs like breathing and reproducing.

Primary ciliary dyskinesia (PCD) is a rare genetic disorder in which the cilia don't work properly. We know PCD mainly causes problems in your respiratory system. The cilia are responsible for clearing mucus and bacteria from your airways. If they don't work as they should, mucus and bacteria can be retained in the airways causing recurrent or longstanding wet cough. We are doing this research to understand how your cilia work and also to understand if there is any genes in your body that are associated with primary ciliary dyskinesia.

What would happen if I join this research?

We will ask you questions whether you have any symptoms of primary ciliary dyskinesia, then we do a test to measure a gas in your nostrils called nitric oxide which if low may point to ciliary dyskinesia if you have not had flu or common cold in the last 6 weeks. Then we shall brush or swab your nostrils to get some cilia which we look at under the microscope to see movement of cilia. Or structure of the cilia. We will also take a blood sample for genetic or DNA testing. We will use cream to numb the area before we take the blood.

Could bad things happen if I join this research?

You may experience a little discomfort that last for about 3 min when we swab your nose and when we take blood. We will try to make sure that no bad things happen to you when we carry out these tests. You can say 'no' to what we ask you to do for the research at any time and we will stop.

Could the research help me?

We think being in this research may help you because we shall test and know if there is a problem with your cilia or not. The genetic testing will also help us know that you or your family members do not carry the gene that causes lack of or abnormal movement of cilia. We are then able to treat your current symptoms appropriately.

What else should I know about this research?

If you don't want to be in the study, you don't have to be. It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the doctor treating you at the Red Cross Children's Hospital chest clinic. You will be compensated for your travel expenses during the study at R300 per visit. You can ask questions any time. You can talk to the doctor treating you during your chest clinic visit. Ask us any questions you have. Take the time you need to make your choice.

Is there anything else?

If you want to be in the research after we talk, please write your name below. We will write our name too. This shows we talked about the research and that you want to take part.

Name of Participant _____

(To be written by child/adolescent)

Printed Name of Researcher or Doctor: _____

Signature of Researcher _____

_____ _____

Date ***Time***

Interpreter Information *(if applicable)*

_____

Printed Name of Interpreter during initial presentation of study *Date*

Original form to: Research Team File

Copies to: Parents/Guardians

Consent form for DNA testing and storage of DNA material

We will take 2.5ml of blood to obtain a sample for genetic or DNA testing. A cream to prevent pain/discomfort on your child's skin will be applied on the small part of the skin of the forearm where blood will be drawn. This will be done by a trained health care worker in a sterile technique.
If permission is given some of the blood sample that is taken may be stored for future tests and studies.

STORAGE OF SAMPLES

If any of the nasal swabsor blood samples my child has provided for this research project are unused or leftover when the project is completed

□ I give my permission for my child's samples to be stored indefinitely and used in future research of any type which has been properly approved by the Human Research Ethics Committee including genetic testing and other research.

□ I give permission for my child's samples to be stored indefinitely and used in future research except for research about _____.

OR

□ I wish for my child's samples to be destroyed immediately.

□ I have read this consent form (or have had it explained to me), all my questions have been answered, and I agree for my child to take part in this study.

		DD/MMM/YYYY at - -H - -
Parent/Legal Guardian/Caregiver (Print)	Parent/Legal Guardian/Caregiver (signature/Thumbprint)	Date and Time
		DD/MMM/YYYY at - -H - -
Study Staff (Print)	Study Staff's Signature	Date and Time

Please complete the Witness page if the participant's Parent/Legal Guardian/Caregiver is unable to read or write.

_____	_____	DD/MMM/YYYY at - -H- -
Witness' Name (Print)	Witness' Signature	Date and Time

Participant Enrolment number: _____

Please complete the following:

□ The caregiver of this child confirms that the biological mother / father (please circle) of this child is alive but does not take ANY responsibility for the care of or decision-making for this child.

□ Not applicable - neither of the child's biological parents are alive.

□ Not applicable - the child's biological parent signed consent.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study, you may ask the study staff or you may contact:

Dr Joy Eze +27747174660

If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town: Prof Marc Blockman at (021) 406 6338

Appendix D: Data collection form for PCD study

PLACE STICKER

Study number _____

Source of enrolment:_____

Height (cm, 1 decimal)_____ **Weight** (kg, 1 decimal)_____

History/Clinical Sign

Consanguinity	◯yes	◯no	◯unknown	(If yes please provide pedigree)
Neonatal respiratory distress	◯yes	◯no	◯unknown	
Chronic sinusitis	◯yes	◯no	◯unknown	
Chronic rhinitis	◯yes	◯no	◯unknown	
Chronic otitis media	◯yes	◯no	◯unknown	
Hearing impairment	◯yes	◯no	◯unknown	
Chronic wet cough	◯yes	◯no	◯unknown	
Bronchiectasis	◯yes	◯no	◯unknown	
Situs inversus totalis	◯yes	◯no	◯unknown	
Heterotaxy syndrome	◯yes	◯no	◯unknown	
Congenital heart disease	◯yes	◯no	◯unknown	

Previous diagnosis_____

Nasal NO maneuver ◯velum closure tech ◯tidal ◯exhalation against resistance

Concentration: **Ppb**_____ **Production rate nl/min**: __

High Frequency Video Microscopy

Frequency ◯normal ◯decreased ◯increased Hz_____ Temperature ^{o}C_____

Ciliary beat pattern ◯normal ◯stiff ◯immotile ◯circular ◯other

Clinical judgment

PCD ◯highly likely ◯possible ◯questionable

Order:

◯IF (air-dried slides enclosed

◯TEM (sample in TEM fixative enclosed

◯Genetics (blood sample/DNA enclosed

Findings to Prof. Zampoli **m.zampoli@uct.ac.za**
Dr. Joy Eze **joyezedr@yahoo.com**

_____

Signature Physician

PLACE RXH STICKER

PLACE PCD STICKER

PICADAR		
Does the patient have a daily wet cough that started in early childhood?	**Yes** – complete PICADAR **No** – **STOP**. PICADAR is not designed for patients without a wet cough	
1. Was the patient born pre-term or full term?	Term	2
2. Did the patient experience chest symptoms in the neonatal period (*e.g.* tachypnoea, cough, pneumonia)?	Yes	2
3. Was the patient admitted to a neonatal unit?	Yes	2
4. Does the patient have a situs abnormality (situs inversus or heterotaxy)?	Yes	4
5. Does the patient have a congenital heart defect?	Yes	2
6. Does the patient have persistent perennial rhinitis?	Yes	1
7. Does the patient experience chronic ear or hearing symptoms (*e.g.* glue ear, serous otitis media, hearing loss, ear perforation)?	Yes	1
	Total score =	

Other Relevant Clinical & Laboratory data

Household Cigarette smoke exposure?	◯yes	◯no
Clinical examination: (tick where applicable		
Clubbing	◯yes	◯no
Respiratory system	◯normal	◯abnormal **(tick as applicable)** • Chest deformity • Hyperinflation • Chest indrawimg • Tachypnoea • Wheeze • Crackles/Bronchial Breathing

Current Medications	◯ICS	◯Steroid nasal spray	◯Azithromycin
	◯Hypertonic saline	◯Others (specify)_____	

Respiratory pathogen (Recent 3 within 12 months. If none, do sputum or cough swab or TA after nasal brushings			
Date (dd/mm/yy)		**Specimen type**	
◯ No growth/normal	◯ H influenzae	◯ Strep pneum	◯ Klebsiella
◯ Moraxella	◯ MSSA	◯ Pseudomonas	◯ Aspergillus spp
◯ Candida spp	◯ Other (please specify		
Other details:			

Lung function					
Spirometry Date:	Within the last 6 months acceptable as long as no clinical change or deterioration				
	Pre	**Post**	BDR (Change in FEV1 > 12%)		BDR %
FEV1 (L)			Yes	No	
FVC (L)					
FEV1/FVC ratio					
FEV1 0.5 (L)					
MMEF (L/min)					

Chest X-ray	**Date** most recent CXR (dd/mm/yy): Within one year acceptable provided no clinical change in the respiratory assessment since the chest x-ray was done. Otherwise request CXR

Normal	Abnormal	fibrosis	Air trapping	Atelectasis	Other details:
		bronchiectasis	Consolidation	lymphadenopathy	
		Other pathology (specify)			

CT scan done?	Date (dd/mm/yy)			
Normal	**Abnormal**	◯Bronchial wall thickening		◯ Mosaic attenuation
		◯Bronchiectasis	Yes, Location?	no
		Other pathology:		
Bronchoscopy nasal brushing	Date	Findings?		

Additional notes			
Summary of **TEM** result	◯normal	◯abnormal	◯not done/ insufficient sample/poor quality
Summary of **IF** result	◯Normal	◯Abnormal	◯Not done
PCD confirmed genetically	◯yes	◯no	
If yes describe			

Data collection form completed by: _____

Date (dd/mm/yy): _____