Primary Ciliary Dyskinesia

A Biopsychosocial Approach

Laura Behan

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LIST OF ABBREVIATIONS

AJRCCM	American Journal of Respiratory and Critical Care Medicine
CASP	Critical Appraisal Skills Programme
CBF	Cilia beat frequency
CBP	Cilia beat pattern
CT	Chest tomography
CF	Cystic fibrosis
COREQ	Consolidated Criteria for Reporting Qualitative Health Research
ERS	European Respiratory Society
ELF	European Lung Foundation
LCI	Lung Clearance Index
IF	Immunofluorescence
ISPOR	International Society for Pharmacoeconomics and Outcome Research
HRCT	High-resolution computed tomography
HRQoL	Health related quality of life
HSVMA	High speed video microscopy analysis
MRI	Magnetic resonance imaging
MID	Minimum importance difference
NPV	Negative predictive value
Nno	Nasal nitric oxide
PCD	Primary ciliary dyskinesia
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analyses Approach
PPV	Positive predictive value
PROM	Patient reported outcome measure
PRMC	Pulmonary radioaerosol mucociliary clearance
QUADAS	Quality assessment of studies of diagnostic accuracy included in systematic review
RBH	Royal Brompton Hospital
ROC	Receiver operator curve analysis

TEM	Transmission electron microscopy
WHO	World Health Organisation

DECLARATION

I declare that this thesis has not been submitted for another degree at this or at any other University. The work, upon which this thesis is based, was carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis.

Signed: Date:

_____ _____

ACKNOWLEDGEMENTS

This thesis would not be completed if it were not for the support of a number of people.

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I feel privileged to also be a part of the BESTCILIA FP7 project that has funded this work and the BEAT-PCD Cost Action project which has provided me with the opportunity to present my work to the international forum. I would also like to thank the AAIR Charity for funding the final months of this study. I would like to thank the PCD Family Support Group in the UK and PCD Foundation in the USA for their involvement in these studies and to the all the study participants who were so open and enthusiastic to be a part of PCD research.

To my parents, I dedicate this thesis to you. Your belief, love and support throughout my life have been unending. Thank you for your constant interest in my work. Dad, hopefully by now you know what the 'D' stands for.

To my husband Johnny, thank you for your endless love and belief in me. Without you I would never have been able to achieve this. Thank you for the countless dinners and for always making me laugh.

THESIS ABSTRACT

Background: Primary ciliary dyskinesia (PCD) is a rare heterogeneous genetic disorder associated with abnormal ciliary structure and function, characterised by progressive sino-pulmonary disease, with symptoms starting soon after birth. There is no 'gold standard' for diagnosing PCD, and testing includes genotyping, and analysis of cilia function and ultrastructure which requires high levels of expertise and expense. Using the biopsychosocial framework, this thesis aimed: to provide an overview of the PCD patient perspective and experience of living with PCD, to address some of the complexities in referring and diagnosing PCD patients, and to provide age specific, PCD-specific validated health-related quality of life (HRQoL) measures to monitor patient outcomes and assess effectiveness of treatments and interventions.

Method: A patient survey to capture patient experience of PCD diagnostic testing was completed in 25 countries (n=365). This was followed by semi-structured interviews with 20 participants, which were analysed thematically. A systematic review was conducted to synthesise evidence on the experience of living with PCD. Through the analysis of prospectively collected data from consecutive patients referred to a PCD diagnostic centre (2007-2013), sensitivity and specificity for individual testing strategies were calculated. Patient clinical characteristics were correlated with diagnostic outcome and using logistic regression, the predictive performance of the best model was simplified into a tool (PICADAR), predicting the likelihood of referrals having PCD based on clinical symptoms. This was externally validated in a second diagnostic centre. A PCD-specific HRQoL measure for adults (QOL-PCD) was developed following a literature review, an expert panel meeting, and semi-structured interviews (n=21) from which content analysis was used to

derive saturation matrices. Items were rated for relevance (n=49) and the preliminary questionnaire was refined following cognitive interviews. Validation followed with patients completing QOL-PCD and generic quality of life measures. Stability and responsiveness to change were assessed.

Results: The most prominent theme in the assessment of diagnostic experiences was the lack of awareness among medical practitioners leading to a delayed diagnosis; 35% visited their doctor >40 times with PCD related symptoms prior to referral. Only 14 PCD studies have presented the PCD patient experience or have used patient-reported outcome measures e.g. generic HRQoL measures. PCD was found to have a negative impact on physical, emotional, and social functioning, and the need for PCD-specific HRQoL measures was highlighted. Outcome data from 641 consecutive referrals for diagnostic testing showed that none of the diagnostic testing strategies were 100% sensitive and specific. PICADAR applies to patients with persistent wet cough and has 7 clinical predictive parameters; it has been shown to have good accuracy and validity to predict diagnostic outcome when validated in a second diagnostic centre. The QOL-PCD HRQoL measure for adults has demonstrated good internal consistency, test-retest reliability, convergent and divergent validity, and responsiveness to exacerbations.

Discussion: Findings from the international survey and interviews have been used to advise on ERS Task Force guidelines for diagnosing PCD. The development of PICADAR will lead to earlier referral of patients and improved awareness among medical practitioner of the signs and symptoms of PCD. The development and validation of QOL-PCD has provided the first disease specific patient-reported outcome measure to allow for the assessment of new and existing treatments. Overall this thesis has led to advances in the field of PCD; from diagnosis to the treatment and management of patients.

1. Introduction

1.1.Introduction

PCD is a rare heterogeneous genetic disorder characterised by impaired mucociliary clearance due to abnormal ciliary function. Symptoms of PCD mostly begin in the first few days of life with unexplained neonatal respiratory symptoms ranging in severity (1-3). Patients continue to have daily chronic wet cough and recurrent upper and lower respiratory tract infections throughout childhood (4). Recurrent infections can lead to bronchiectasis and reduced lung function over time (5-7). This can result in end-stage lung disease with long-term oxygen dependency or require lung transplantation. Male infertility is common in PCD since sperm flagella have a similar ultrastructure to cilia, however the incidence of female infertility and of ectopic pregnancy is unclear but can be explained by immotile fallopian tube cilia. A spectrum of organ laterality defects occur with PCD, including situs inversus totalis which is a mirror-image arrangement of the thoracic and abdominal organs (~40%), and situs ambiguous (~12%) where organ arrangement falls somewhere between normal and mirror image (8). Reported prevalence in PCD varies from 1:2,000 to 1:40,000. Variability in prevalence rates is likely attributed to a lack of access to diagnostic facilities, or to populations where there are high levels of consanguinity (9-11). A European Respiratory Society (ERS) Task Force survey of 26 European countries found that PCD is both under-diagnosed and diagnosed late, particularly in countries with low health-care expenditure (9). Early diagnosis has been associated with improved respiratory prognosis (1, 12).

There is currently no “gold standard” test to diagnose PCD (13). European guidelines recommend that PCD be confirmed in a specialist centre using a combination of tests. Diagnostic tests include nasal nitric oxide (nNO) screening (4, 14), high-speed video microscopy analysis (HSVMA) of ciliary beat frequency (CBF) and pattern (CBP) (15, 16)

and transmission electron microscopy (TEM) analysis of ciliary ultrastructure (17, 18). PCD diagnostic investigations are complex, requiring expensive infrastructure and an experienced team of clinicians, scientists, and microscopists (13, 19, 20). Several articles have reported the accuracy of individual diagnostic tests for the diagnosis of PCD, but none have explored their accuracy when used in combination. There is also a need to promote early diagnosis without overburdening specialist services. As PCD symptoms are variable and non-specific (21), physicians need guidelines on whom to refer for diagnostic testing.

No medications to date have specifically been approved by regulatory bodies for PCD. Treatment strategies for PCD have been applied from other diseases (22, 23), particularly cystic fibrosis (CF). A major obstacle to evaluating new treatments and monitoring disease progression is the lack of disease-specific outcome measures (24).

This thesis aims to address some of the challenges faced in referring, diagnosing, and treating this rare disease.

1.2 Aim

To contribute to the current evidence base in PCD by developing an understanding of the issues that are important for patients with PCD, and by developing and validating tools that will aid diagnosing, monitoring, and treating patients (Figure 1).

1.3 Objectives

The thesis has three main objectives:

1. To provide an overview of the patient's perspective and opinion on PCD diagnostic testing and the experience of living with PCD through:
    A. An international survey and a series of interviews.
    B. Systematically collating and synthesising evidence on PCD patient reported outcomes and experiences.

2. To address some of the complexities in referring and diagnosing PCD patients by conducting an analysis of 641 consecutive patients referred to a national reference centre:
    A. To describe the accuracy of each diagnostic test when used in isolation, in combination, or as a screening tool.
    B. To develop and validate a clinical predictive rule to assess the likelihood of a PCD diagnosis based on clinical symptoms.

3. To provide a disease-specific outcome measure which can be used to monitor patients and assess effectiveness of interventions by:

A. Developing a health-related quality of life measure specifically for adults with PCD (QOL-PCD).
B. Perform psychometric testing to assess QOL-PCD for reliability, validity and responsiveness to change.

1.4. Research Setting

This research was conducted both from University College Cork in the Republic of Ireland, and Southampton University Hospital (UHS)/University of Southampton in the United Kingdom. UHS is a national reference centre for the diagnosis and management of patients with PCD. Data from consecutive referrals have been prospectively and systematically collected here since 2007. Clinical and diagnostic data collected between the years of 2007-2013 was cleaned, collated, and analysed to calculate diagnostic accuracy and to develop PICADAR. To externally validate the predictive score, data was extracted from patient records of nearly 200 referrals at a separate PCD diagnostic centre at the Royal Brompton Hospital in London.

Semi-structured interviews were conducted with a large body of patients to generate the items for the HRQoL measures. Patients were recruited and interviewed in PCD clinics in UHS and in various locations across the UK including Bristol, London and Leeds/Bradford. The author also interviewed patients in their homes both around Southampton, the Isle of Wight and the Republic of Ireland.

Recruitment of patients for the validation of the PCD HRQoL measures required the author to travel to clinics across the UK and attend PCD Family Support Information days to inform

patients of the research that was taking place and to provide them with an opportunity to participate.

1.6. Thesis Outline

The thesis comprises six papers which describe and address some of the complexities of referring, diagnosing, and treating patients with PCD. The patient experience of diagnosis, daily living and quality of life will also be provided. Chapter 2 provides a brief introduction about PCD and the gaps in the knowledge base.

In Part 1 the patient experience is explored.

Chapter 3 provides an overview of the PCD diagnostic journey from referral to follow-up, from the perspective of the patient. Findings are presented from an international survey and thematic analysis of semi-structured interviews. The findings from this study have contributed to an ERS Task Force developing guidelines for diagnosing PCD.

Chapter 4 provides a systematic overview of the literature on the patient experience, health-related quality of life and perspectives of the PCD patient. Perspective from various countries and age groups are presented; comparisons between groups, and factors affecting quality of life are explored.

In Part 2 the challenges of referring high risk patients for diagnostic testing are explored in addition to the accuracy of diagnostic tests.

Chapter 5 provides a detailed analysis of the sensitivity and specificity of each diagnostic test. The accuracy of each test when used in isolation and in combination is presented. This study highlights the challenges of diagnosing PCD which requires highly specialised equipment and expertise, not available in most countries.

Chapter 6 will examine how well a PCD diagnosis is predicted by clinical features and past medical history. The development of PICADAR, a clinical predictive rule for diagnosing PCD is described in addition to the external validation of this rule.
Chapter 7 includes a letter to the editor which was published in the European Respiratory Journal following publication of these two papers. The response to this letter is provided further highlighting the complexities and challenges faced in research of this rare disease where there is no 'gold standard' diagnostic method available.

Part 3 shows the process of developing and validating the first patient-reported outcome measure for PCD.

Chapter 8 presents the developmental phase of this HRQoL measure for PCD adults: QOL-PCD.

Chapter 9 presents the results from the psychometric validation of this measure showing its reliability, validity, and responsiveness to change.

Chapter 10 integrates the main findings of these studies. The main findings are discussed according to the biophysical, psychological, and social impact of PCD. The implications of these findings are provided. Conclusions are drawn and the limitations of the studies outlined. Recommendations are made for future research.

1.7. Author's Contribution

I was lead author of the research papers in Chapters 3, 4, 6 and 9 and co-first author for the papers in Chapters 5 and 8. This involved developing the study protocols, conducting literature searches, data cleaning, data analysis, and drafting of each of these manuscripts.

In Chapter 3, the research question was generated to inform a European Respiratory Society (ERS) Task Force who were developing guidelines for diagnosing PCD. It is important that these guidelines are patient centred. This provided me with an international platform to establish the patient perspective from countries where it has not been investigated before. Supported by my supervisors, I developed the survey based on literature searches and analyses of transcripts of previous interviews with PCD patients. Once developed, members of the ERS Task Force (international) and patient support groups (UK and USA) were contacted for their opinions on the survey and to suggest interactive changes. Once amended, I organised the translation and back translation of the survey into 9 European languages and enlisted the help of members of the taskforce, international patient support groups, and the European Lung Foundation (ELF) to disseminate the survey internationally. I performed descriptive analysis of the survey findings. Dr Bruna Rubbo (a PhD student in the University of Southampton) conducted an analysis of the survey results by country to allow comparisons to be made. I completed 20 semi-structured interviews focusing on the participant's experience of the referral and diagnostic process. Telephone interview were completed with participants from Europe, Australia and the USA. I conducted a thematic analysis of the transcripts and drafted the manuscript. I presented the findings to the ERS Task Force at committee meetings and at the European Respiratory Society Congress in 2015 as a poster presentation. In Chapter 4, I formulated the research question, wrote the protocol, conducted

the data synthesis and wrote the manuscript. I was assisted by Dr Bruna Rubbo who completed the search and data extraction independently to ensure accuracy of reporting.

For Chapter 5 and Chapter 6, I prepared and cleaned the database used for both studies, conducted the data analysis and presented the results. For Chapter 6, I collected the data for the external validation of the score by extracting the data from 200 patient records at the Royal Brompton Hospital. I was responsible for drafting the manuscript.

I participated in discussions and contributed to the writing of the response letter in Chapter 7.

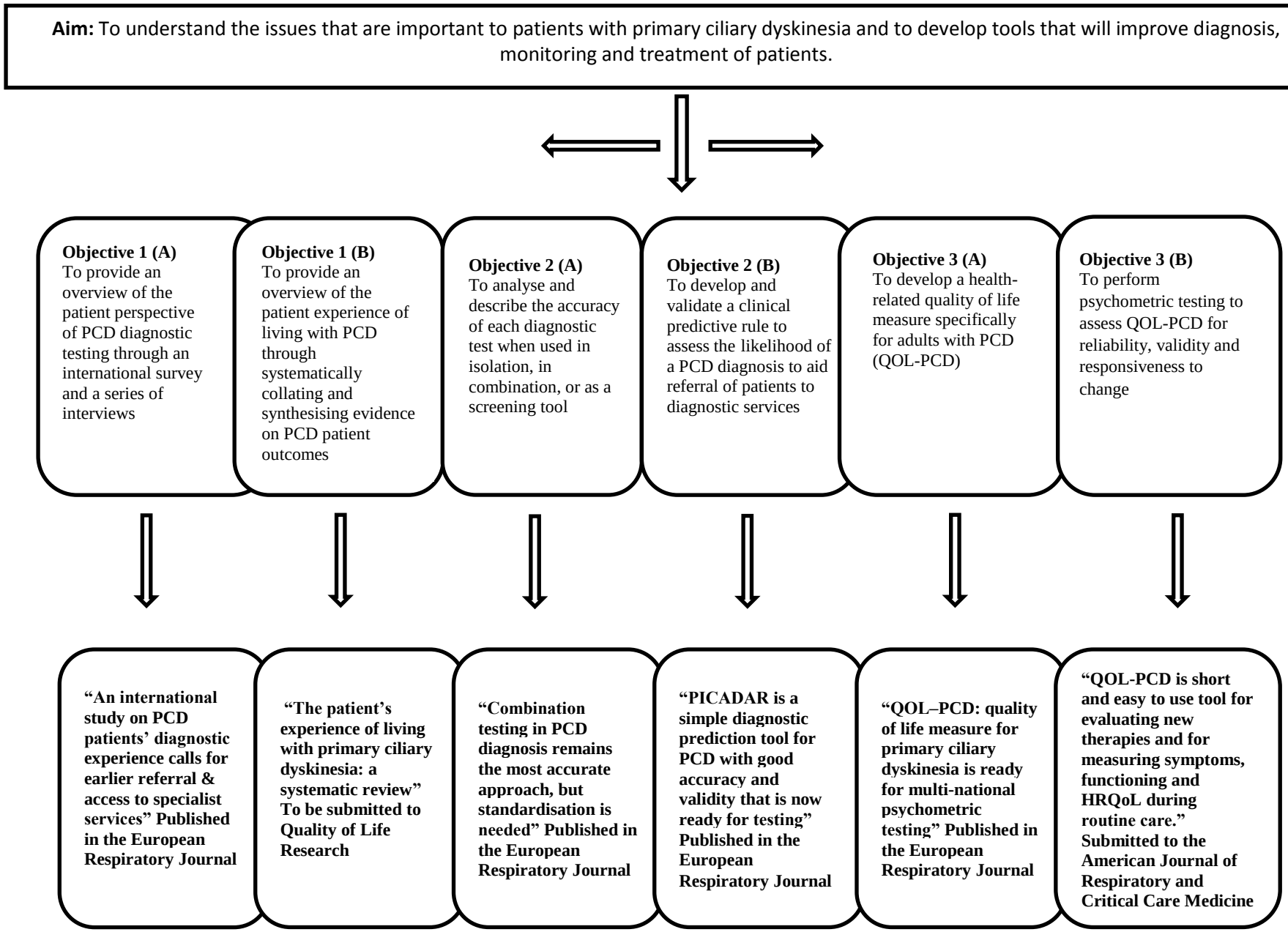
Chapter 8: As a senior research assistant on the BESTCILIA FP7 study, my main task was to develop, validate, and translate age-specific HRQoL measures for PCD patients. These have been developed for adults, adolescence, children and parent-proxy; the adult version is presented in this thesis. I completed semi-structured interviews and cognitive interviews with all age groups in the UK as part of the work package protocol. I conducted the content analysis of transcripts and developed saturation grids for all domains of interest. This has been a major collaboration with partners in North America and throughout Europe; I conducted the co-ordination of the project. I presented the results from the UK interviews, and findings from the clinical relevance scores to our colleagues in North America, who had followed this study protocol in parallel. Results from both countries were triangulated to generate the HRQoL measures (QOL-PCD). This ensured the measures were cross-culturally valid across English speaking populations. I led the comprehensive translations of the QOL-PCD questionnaires into a number of European languages (German (adapted for Swiss German), Dutch, Danish and French). The English, German, Danish and Dutch QOL-PCD measures have been included as a secondary outcome measure in the BESTCILIA

international clinical trial. For this paper, I was responsible for data collection, analysis, reporting the results, and drafting the manuscript.

Chapter 9: Following on from the development of the adult measures, I was responsible for developing the protocol for the longitudinal psychometric validation of the QOL-PCD measures. I recruited patients in the UK and regularly updated the team on the status of the study. I performed the analyses and drafted the manuscript. Throughout this research project of developing, validating, and translating the HRQoL measures for PCD, I have presented findings from each stage of the developmental process at BESTCILIA conferences and meetings, at BEAT-PCD Cost Action training schools and annual conference, and as a poster presentation at the European Respiratory Society Congress.

Recognising the importance of international collaboration in rare diseases such as PCD, for the past three years, I have fully immersed myself in international PCD collaborations such as the European Respiratory Task Force (I am a member of the management committee), BESTCILIA and the Cost Action project: BEAT-PCD, where I am the early-career researcher representative on the Steering Group as well the Irish representative on the management committee. In March 2015, I co-organised The First Young Researchers Conference in PCD, which was held in Bern, Switzerland, bringing together early-career researchers from across Europe with the aim of developing collaborations and providing a platform for early-stage researchers to present their work to peers as well as a panel of PCD experts and to received feedback and recommendations.

Figure 1. Overview of thesis including aim and objectives



2. Background

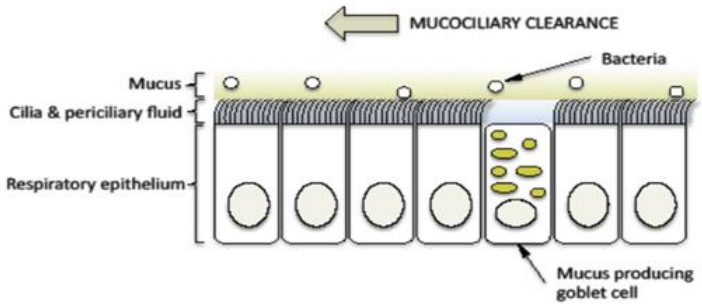
BACKGROUND

This chapter provides an overview of primary ciliary dyskinesia (PCD). It describes the history of PCD, the symptoms, and prevalence rates. Secondly, the pathophysiology of PCD, and the methods used to diagnose this illness are examined. Thirdly, disease progression and prognosis are discussed. The importance of health-related quality of life (HRQoL) in PCD is highlighted and the approaches used for developing and applying tools to measure this multi-dimensional concept. Finally the biopsychosocial model is presented and the need for an integrated approach for diagnosing and treating PCD patients across this three tiered framework is presented.

2.1. Description of the illness

PCD represents a clinically and genetically heterogeneous group of respiratory ciliopathies with absent mucociliary clearance of the airways. Simply explained, the normal bronchial tree, upper respiratory tract, and fallopian tubes are lined by small extrusions from the cell body (cilia) which beat in a coordinated fashion to remove mucus and secretions (often containing bacteria and allergens) from the lungs and sinuses (Figure 2). When cilia are immotile or beat in a dysfunctional or uncoordinated way, secretions build up causing inflammation and leading to recurrent infections (22). Eventually permanent damage occurs to the airway structure, a condition known as bronchiectasis. In addition to recurrent infections, the predominant symptoms of PCD are chronic daily productive cough and chronic persistent rhinitis (1, 10). As the middle ear, paranasal sinuses and nasal pharynx also are lined with ciliated epithelium, defective mucociliary clearance leads to persistent chronic rhinitis, sinusitis, recurrent otitis media and hearing impairment (22, 25).

Figure 2: Ciliated epithelium lines the upper and lower airways. The coordinated beating of the cilia (at 12-18Hz) sweeps mucus and any debris or pathogens via the 'mucociliary escalator' to the pharynx, where it can be swallowed (26).

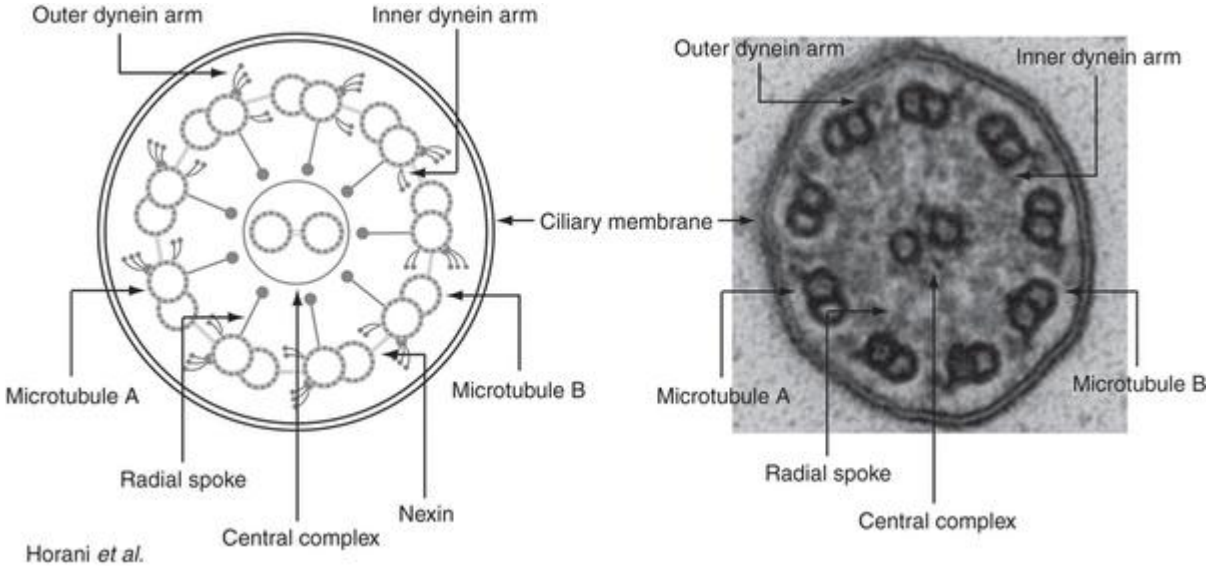


2.2. Pathophysiology of PCD

The ultrastructure of motile cilia found in the upper and lower respiratory airways and fallopian tubes consists of a highly organised '9+2' arrangement: 9 microtubule doublets located around the periphery of the cell which surround a single pair of central microtubules (Figure 3). The structure of the axoneme is maintained by protein cross-links with each doublet microtubule linked to the adjacent by nexin links and to the central pair by radial spokes. The central pair of microtubules are connected together by a bridge. Attached to the microtubule doublets are inner and outer dynein arms. In 70-80% of PCD cases, the ultrastructural defect is a result of missing inner and/or outer dynein arms. Other ultrastructural defects include a loss of microtubule arrangement (radial spokes defect) or where the central pair microtubules are missing and are replaced by a peripheral doublet of microtubules (transposition defect) (17, 27-30). Ultrastructural defects are associated with various types of cilia dysfunction. As dynein is a mechano-chemical protein, it generates force for ciliary beating and bending (28). Abnormalities of the dynein arm affect ciliary

beating i.e. an outer dynein arm defect results in static or extremely slow beating cilia. A transposition defect leads to ineffective circular beat patterns (16).

Figure 3: Diagram of the transverse section of a respiratory cilium axoneme (right) and a transmission electron photomicrograph of cilia ultrastructure, with ultrastructure features labelled. Both diagram and micrograph show normal cilia ultrastructure (31).



In addition to sinopulmonary disease, infertility or subfertility is common (32). For males, infertility occurs since sperm flagella have a similar ultrastructure to cilia so there is often an absence of the sperm tail or impaired motility of the sperm tail (33, 34). The incidence of female infertility and of ectopic pregnancy is less clear and is explained by immotile fallopian tube cilia (32, 35).

Situs inversus totalis or mirror-image organ arrangement is found to be present in nearly half of PCD patients, and at least 12% of patients present with heterotaxy (8, 36, 37). This is a result of cilia dysfunction in embryonic nodal plate cells which results in laterality defects.

This can also lead to complex congenital cardiac defects which is found in 5% of patients (37).

2.3. History of PCD

Primary ciliary dyskinesia was first described by Siewart in 1904 where an association between bronchiectasis and situs inversus was made in a 21 year old male. Kartagener (in 1933) reported on 11 cases with situs inversus and bronchiectasis that were all associated with sinusitis, and described a condition as being made up of a triad of these symptoms (38). He also described this as a hereditary condition which became referred to as 'Kartagener's Syndrome'. It was in the 1970s that Afzelius found infertility was also a feature. He described 4 subjects with immotile sperm, three of whom had frequent bronchitis and sinusitis. He noted the absence of mucociliary transport (Figure 2) and indicated (through electron microscopy) that cilia from the cells of these patients lacked dynein arms (39, 40).

Pedersen continued this work to confirm the unifying role of respiratory tract symptoms in patients with Kartagener's Syndrome and abnormality in cilia at the sub-structural level (41) (Figure 3). Later work by Afzelius found that cilia were immotile (39) and he proposed and defended that 'Kartagener's Syndrome' be changed to 'Immotile Cilia Syndrome'. Later work found that not all patients have immotile cilia, with a proportion of patients having dysfunctional cilia motility (42, 43).

In 1980, Sleigh, supported by 23 researchers, advocated for the name to be changed to 'primary ciliary dyskinesia' (44). 'Primary ciliary dyskinesia' would be the term used for patients with the congenital disease and 'secondary cilia dyskinesia' would be a diagnosis for those who acquired the disease as a result of infection, pollutants or inflammation.

Further work has indicated more levels of heterogeneity in the diagnosis of this condition. Ultrastructural abnormalities in cilia are not confined to the absence of outer dynein arms. Radial spokes (45) and central pair (46) have been found to be missing in some cases. Later again it was found that patients with PCD could have normal cilia ultrastructure but abnormal beating (47). While advances in the diagnosis of PCD continue to be made, the contributions of this early work on identifying PCD through analysis of cilia ultrastructure and cilia motility remain as recommended diagnostic tests in European guidelines (22).

PCD is a heterogeneous disorder. Advances in genotyping have led to the discovery of over 30 mutations causing genes which identify approximately 65% of PCD cases. Cilia consist of more than 250 proteins and thus many genes are involved in ciliary structure and function. The first PCD gene isolated was dynein axonemal intermediate chain 1 DNAH5, identified in 1999 (48). The second gene, isolated in 2000, was dynein axonemal heavy chain 5 (DNAI1) (49). These are the most common mutations and are found to be present in up to 38% of all PCD patients (50). It has been found that DNAH5 mutations are associated with outer dynein arm defects, left–right asymmetry, and male infertility. In patients with this gene defect, cilia are completely immotile. DNAI1 mutations have been shown to cause either immotile cilia or a reduced cilia beat frequency (51).

2.4 Obstacles in diagnosing PCD

Diagnostic challenges begin early with symptoms of PCD being non-specific and although PCD is a rare disease, symptoms such as recurrent upper and lower respiratory tract infections are extremely common, especially in the paediatric population. Therefore, an important but complicated task for clinicians is to identify patients suffering from this condition. Physicians need guidance of whom to refer for diagnostic testing and large multi-

national studies are ongoing to identify the more prominent clinical features to predict PCD diagnosis (21).

Diagnostic testing in PCD requires expensive infrastructure and an experienced team of clinicians, scientists, and microscopists (13, 19, 20). Guidelines recommend that PCD should be confirmed in a specialist centre using appropriate diagnostic testing (22). Diagnosing PCD is difficult with no single 'gold standard' test currently available (52). A combination of complex techniques was recommended in European consensus guidelines in 2009 (22) which include high-speed video microscopy analysis to identify abnormal ciliary function (HSVMA) (15, 16, 29), and transmission electron microscopy (TEM) to define ciliary ultrastructural defects (17, 18). Nasal nitric oxide (which is extremely low in patients with PCD) (14, 53) was recommended as a screening test for PCD. These tests are not readily available or standardised (54) and various models exist to deliver diagnostic services. Up to now, genetic testing has taken a prominent position in some countries such as the USA. In the UK, while genetic testing has recently been introduced, the main diagnostic strategies include measuring nNO, HSVMA, and TEM (21, 55). In the UK, generally there is a network of satellite screening centres accessing a specialist centre (14, 20, 21).

No diagnostic test for PCD is 100% accurate and therefore combinations of tests must be available. Each test has its own limitations. HSVMA is a qualitative test with potential subjectivity. Both false negative and false positive results can occur. False positive results can occur due to recent infection or damage during sampling leading to a proportion of cilia presenting as dyskinetic (62). Therefore PCD scientists require significant experience to distinguish between PCD samples and non-PCD samples.

TEM has been shown to be unreliable when used in isolation as approximately 20% of patients have normal cilia ultrastructure (56, 57). TEM often requires the support of nNO and

HSVMA to make a diagnostic assessment. However, it is a vital part of the diagnostic portfolio in both supporting HSVMA findings and in providing a diagnosis when HSVMA results are inconclusive or when results are not available.

Nasal nitric oxide (nNO) is extremely low in patients with PCD however there is much debate of the best cut-off values for nNO testing. An arbitrary nNO value in the diagnostic centre in Southampton is set at 30 $nL \cdot min^{-1}$. This was determined based on prior experience, however more recent evidence suggests that higher cut-offs may be more useful (53). A USA diagnostic centre has recommended that the cut-off value of 77 $nL \cdot min^{-1}$ be used (14). While nNO is a useful screening test for symptomatic patients (53, 58), UHS uses nNO in parallel to TEM and HSVMA, to support a positive diagnosis. Without a standardised agreed cut-off value, it is considered a risk to exclude a PCD diagnosis if a patient has a nNO measurement of >30 $nL \cdot min^{-1}$.

Although the rate of discovery of new PCD genes has accelerated during the past number of years, clinically available genetic testing is only available for a subset of individuals with PCD as it is estimated that known PCD-causing mutations account for approximately 60% of cases (31). Disease-associated mutations have been identified in over 30 genes with strong correlations between genotype and cilia ultrastructure defect (20, 59).

A more recent European Task Force (2014-2016) for which the author was a junior member, has developed updated evidence based guidelines for diagnosing PCD (Submitted for publication to ERJ, May 2016) (Appendix 8)). The guidelines recommend genotyping to be included in the diagnostic protocol, where a positive test has been diagnosed by other means (e.g. HSVMA, TEM). In the case where a mutation in a PCD causing gene is found in addition to an identified hallmark ciliary ultrastructure defects, a conclusive positive diagnosis can be provided. As genetic testing only identifies approximately 60% of PCD

patients, a negative genetic test cannot exclude PCD. It is now recommended that only a 'highly likely' diagnosis can be provided in patients with a combination of a compatible history of PCD, very low nNO, and HSVMA findings (static or circling cilia) consistently suggestive of PCD, or where a very low nNO is found plus HSVMA consistent with PCD following cell culture.

In more recent times, tests such as immunofluorescence (IF) of ciliary proteins (60, 61) and pulmonary radioaerosol mucocliliary clearance (PRMC) (62) are becoming more increasingly available which may lead to greater accuracy of diagnostic testing in the future. IF of ciliary proteins was developed as a research tool to improve understanding of the impact of disease-causing genes on ciliary proteins. PRMC is a method that draws on the fact that patients with PCD have impaired mucociliary clearance as a result of abnormal ciliary motility. Abnormal clearance patterns can be visualised for the entire lung using a gamma camera to follow the movement of an inhaled radioaerosol (63). Evidence on the sensitivity and specificity of IF and PRMC is too limited for these tests to be included alongside some the more mainstream diagnostic tests, however application of these tests is recommended in patients where the diagnosis is highly likely or remains inconclusive following other investigations.

2.5. Prevalence of PCD

There is limited representative international data on the prevalence of this illness. Estimates of prevalence vary from 1:2,000 to 1:40,000. The highest prevalence rate (1:2,265) has been reported in a South Asian population living in the UK with relatively high levels of consanguinity (11). A survey of 26 countries across Europe examined prevalence rates in pediatric respiratory centres and found that prevalence of PCD varied considerably between countries. The highest rate was reported in Cyprus (1:10,000), Switzerland (1:20,000) and

Denmark (1:20,000). Lower rates were found in countries such as Findland and the Czech Republic, where prevalence rates were approximately 1:200,000. This may reflect true variability or it could be due to under-diagnosis or late diagnosis of patients. It could also be due to a lack of access to PCD diagnostic services in some countries (9).

2.6. Disease progression and prognosis of PCD

There is little known about the clinical severity or the natural progression of this illness (1, 22, 64). Symptoms of PCD mostly begin in the first few days of life with unexplained neonatal respiratory symptoms; these can range in severity from mild transient tachypnoea to pneumonia and significant respiratory failure requiring prolonged respiratory support (1-3). Patients continue to have chronic wet cough and recurrent upper and lower respiratory tract infections throughout childhood. Reduced lung function i.e. mean FEV and forced vital capacity (FVC) have been shown over time in a large number of patients showing the progressive nature of this illness over time (1, 4, 65). Recurrent infection invariably leads to bronchiectasis which has been reported in 25-55% of children (66) and in virtually all adults (6, 7). Severity of bronchiectasis has been shown to correlate with increasing age, decreased lung function, and colonisation with pseudomonas aeruginosa (6, 7). The progression of hearing and upper airways disease is unknown however it has been reported that hearing impairment in PCD children can lead to speech delay and impaired learning at school if not picked up and manged correctly (25, 67, 68).

PCD can lead to end stage lung disease with one study reporting that 25% of adult PCD patients in the USA require long term oxygen or lung transplantation. A USA cross-sectional study showed that FEV_1 (% predicted) decreases with age at a rate of 0.8% per year (1),

however it is unclear if this is influenced by age at the time of diagnosis. An early study has suggested that lung function can improve over time once a diagnosis has been made and treatment has initiated (64). A more recent longitudinal study however, by Marthin et al. (4) described the long term outcome of 74 patients of all age groups, followed over a 9.5 year period. Despite the initiation of state of the art treatments, only 10% showed an improvement of ≥10% FEV_1 in the 10 year period following diagnosis, 57% remained stable and 34% had a decline in lung function of ≥10%. Change in lung function over time was also not correlated with age at diagnosis, although a decrease in lung function did slow-down following diagnosis and initiation of treatment.

2.7. Treatment, management and outcome measures in PCD

There is little evidence to inform PCD management practice. As an orphan disease, PCD has historically been absent from clinical trials evaluating medicines and therapeutic treatments. Most treatment recommendations are extrapolated from cystic fibrosis and non-cystic fibrosis bronchiectasis guidelines, subjective experience of experts, and small observational studies. Due to the underlying impairment of mucociliary clearance, routine daily therapies including regular airway clearance are considered the backbone of prescribed treatments (1). By ridding the airway of excess mucus, the patient is also removing pathogenic debris. Exercise is recommended for aiding physiotherapy and general wellbeing as exercise intolerance has been correlated with decreased lung function in PCD (1).

Routine clinical visits are recommended 2-4 times annually. This allows for review by the multidisciplinary team including physiotherapists and ENT (Ear, Nose Throat) specialists. At routine visits lung function measurements are also taken, as are sputum cultures for the surveillance of microbial organism growth (59). Staphylococcus aureus, Streptococcus

pneumonia, Haemophilus influenza, and Pseudomonas aeruginosa are the most common microbial organisms grown by PCD patients (1, 64, 69). While mild exacerbations are treated with oral antibiotics and increased airway clearance, severe exacerbations can require intravenous antibiotics and inpatient hospitalisation (69). Inhaled antibiotics are often prescribed for patients with Pseudomonas aeruginosa infection or when Pseudomonas aeruginosa is grown in airway sputum culture (70). Other less routine case-by-case treatments include chronic suppressive oral or inhaled antibiotics, DNase, inhaled hyperosmolar agents and inhaled bronchodilators (70).

The use of cystic fibrosis guidelines for the management of PCD has its limitations. PCD has been shown to be clinically distinct from cystic fibrosis with different underlying genetics and pathophysiology. Patients are therefore likely to benefit from different treatment strategies (23). Randomised control trials are needed in PCD. These will allow for treatment efficacy and side effects to be determined and guidelines on treatment plans to be tailored for this disease specifically. The first PCD randomised controlled clinical trial is currently ongoing as part of the BESTCILIA FP7 Study. This trial is evaluating the use of prophylactic Azithromycin.

Assessment of new treatments is challenging without the availability of a highly sensitive disease-specific outcome measure. Outcome measures used to assess disease severity in PCD to date include spirometry (4), chest computed tomography (66, 71), magnetic resonance imaging (5) and lung clearance index (72-75). These physiological and radiological measures all have limitations in terms of their sensitivity or feasibility to monitor disease progression. Although spirometry is easy to perform, it is an insensitive marker of lung function decline. Normal spirometry has been found in cases where there are substantial structural lung changes as shown by chest computed tomography (CT). Spirometry has also been shown to

be less accurate in detecting changes associated with the progression of lung disease. Assessment of disease severity using high-resolution computed tomography (HRCT) has been shown to be a sensitive method of assessment, however HRCT cannot be used for regular monitoring because of radiation risks (66). Magnetic resonance imaging (MRI) protocols have been developed showing good agreement with HRCT for determining the extent and severity of lung disease in non-CF bronchiectasis. Although MRI is inferior to HRCT with regard to speed, image contrast, and spatial resolution, it is a useful radon-free tool (5). Lung clearance index (LCI) is a measure of abnormal ventilation distribution derived from the multiple breath inert gas washout (MBW) technique. Similar to cystic fibrosis, LCI has shown to be more sensitive than spirometry in detecting pulmonary disease in PCD patients (76). However it has been reported not to be so sensitive in detecting changes in advanced PCD (73). In PCD, longitudinal data for each of these physiological outcomes is either absent or of poor quality. This highlights the need for a sensitive disease-specific outcome measure.

2.8. Psychosocial factors in PCD

Few studies have reported on the psychological and social burden of PCD. However a small case-control study of 10 PCD children and 32 healthy children (77), who underwent the Wechsler Intelligence Scale for Children-III edition and the Child Behaviour Check-List questionnaire (CBCL), suggests that PCD children were more likely to be withdrawn, experience anxiety or depression, and internalise more problems than their healthy peers. PCD has also been found to be associated with higher scores in the Parenting Stress Index-Short Form in mothers of children with PCD compared to mothers of healthy children. Qualitative research findings have shown the emotional impact this illness can have, with

prominent themes including anger and frustration on account of the constant symptoms, treatment burden, and frequent exacerbations associated with PCD. Patients reported feeling anxious when thinking about their future health (78). Feelings of embarrassment were prominent and were attributed to the need to cough or blow nose in public or when producing sputum in social contexts (79). Patients also spoke about not being able to physically keep up with other family members and peers due to fatigue caused by PCD (78).

2.9. Health-Related Quality of Life (HRQoL)

The term 'quality of life' itself has no strict definition, however the World Health Organisation (WHO) defines it as an "individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns" (80). Thus, quality of life assesses physical health, psychological state, levels of independence and social relationships, but also a broader range of conditions such as environment, wealth, safety, crowding etc. (81).

HRQoL is a less general term than quality of life. This term was intended to narrow the focus to the effects of health, illness, and treatment on quality of life. Cella et al's (82) definition for HRQoL is 'the extent to which one's usual or expected physical, emotional, and social well-being are affected by a medical condition or it's treatment.' The US Food and Drug Administration (FDA) defines HRQoL as the patient's perception of how they "survive, feel, and function"(83). HRQoL is a multi-dimensional construct evaluating physical, psychological, and social components that may be impacted by a disease or medical condition. HRQoL is evaluated from the patient perspective.

There are two main types of HRQoL instruments: generic and disease-specific. Generic HRQoL instruments are not specific to any particular disease and are therefore useful for comparing HRQoL across different conditions. They are however, more 'general' and less sensitive to the particular problems associated with a condition. Disease-specific HRQoL questionnaires focus on issues pertinent to one disease and provide an in-depth picture of the day-to-day concerns of patients (84). They also have an improved ability to capture small changes in HRQoL that may occur as a result of clinical or therapeutic treatment (84, 85). Disease-specific HRQoL measures have become an increasingly important tool for measuring outcomes, particularly in the context of chronic diseases (13).

Data is sparse in PCD regarding disease progression and life expectancy. It is important that medical interventions not only focus on preventing disease progression but also focus on improving the patient's HRQoL. With healthcare moving more and more towards a patient-centred approach, the utilisation of PROMs such as HRQoL measures have become increasingly important. While traditional medical models focus merely on the etiology, pathogenesis, signs, symptoms, treatment and prognosis of the disease (86), the biopsychosocial approach focuses on the psychological and social components of health and disease. The biopsychosocial model does not provide a straightforward testable model to explain interactions or causal influences by each of the three components (biological, psychological, social) but it does provide a general framework to guide theoretical and empirical exploration (87, 88).

2.10. Measuring heath related quality of life

Substantial progress has been made in the past three decades in defining and measuring HRQoL. There is extensive agreement that assessment of HRQoL should encompass a

minimum of physical, social and emotional well-being which allow for a multidimensional, systematic measure of the impact that the illness and its treatments have on the individual (89, 90). Measures should be capable of capturing changes in disease progression over time. They must also be able to discriminate between subjects with varying levels of severity of symptoms and other criteria such as age differences (91).

The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) strongly promote the use of PROMs in clinical trials in addition to physiological measures (92-94). There are several recommended stages in the development and testing of a HRQoL measure. For example, Kirshner and Guyatt described six stages of development: (1) choosing an initial item pool, (2) selection of the "best" items from that pool, (3) reduction of the number of items, (4) questionnaire format, (5) pretesting the instrument and demonstrating the reproducibility of the instrument, and 6) assessing the validity of the instrument and it's responsiveness to change (91). Guyatt et al. (95) describes these stages as either involving a rigorous process (termed the 'Rolls-Royce model') that will establish the clinical relevance, responsiveness and validity of the instrument, or a less costly approach that does not test for reproducibility, responsiveness and validity (the 'Volkswagan' model). The FDA guidelines for developing PROMs for use in clinical trials (83) recommend an iterative process consisting of: (1) hypothesising the conceptual framework, (2) adjusting conceptual framework and draft instrument, (3) confirming the conceptual framework and accessing other measurement properties, (4) collecting analysing and interpreting data, and (5) modifying the instrument. For this study we aimed to adopt a rigorous and time consuming approach and ensured that FDA guidelines were adhered to in the development of the PCD HRQoL measure. This will allow for the assessment of special states and concerns unique to this diagnostic group and will be an important asset in trials evaluating the effectiveness of

treatments and interventions. The EMA recommends that PROMs are validated in therapeutic exploratory trials before their implementation in therapeutic confirmatory trials. The PRO instrument should be validated by testing and documenting validity, reliability, responsiveness and interpretability for the specific condition/setting.

2.11. Cross-cultural approach to HRQoL

In rare diseases a leading problem can be the recruitment of a requisite number of study participants. To overcome this, international collaborations are often required (96). It is important that PROMs used in these trials are translated and tested comprehensively in each language. Even within countries, there may be differences in dialects and in what is considered culturally acceptable. It is important that steps be taken in the translation process to ensure that validity and reliability remains intact (97). In 2005, recommendations for good practice for the translation and cultural adaptation of PROMs from an International Society for Pharmacoeconomics and Outcome Research (ISPOR) Task Force were released. ISPOR proposed a 10 step process of translation. These steps include (1) preparation, where an outline is provided on what this work involves, (2) forward translation using more than one forward translator (a native speaker of the language to be translated) who would translate their versions independently, (3) reconciliation, a translation panel consisted of project manager and translators would discuss discrepancies between measures and arrive at a consensus forward translation version, (4) back translation of the measure into its original language by a native speaker of the translated language, (5) back translation review, (6) harmonisation, a panel consider all versions and all changes to the translation with the original version, (7) cognitive debriefing, where a number of patients complete the measures and are asked to provide feedback on the items included, (8) review of cognitive debriefing

results and finalisation: a review of the cognitive debriefing results against the original version of the instrument is completed to ensure cultural relevance, (9) proofreading to ensure minor mistakes are corrected early on, and (10) final reports: to provide a report on all translations and decisions which may be useful when interpreting datasets or informing other translation of the same instrument (98). QOL-PCD have been translated to ensure all these steps are taken.

2.12. Summary

PCD is a rare heterogeneous genetic disorder characterised by impaired mucociliary clearance due to abnormal ciliary function. Diagnosis is difficult with no single 'gold standard' test available. A combination of complex techniques is recommended including HSVMA to identify abnormal ciliary function, TEM to define ciliary ultrastructural defects, and measurement of nNO levels. Standardised testing is needed.

Symptoms of PCD mostly begin in the first few days of life with unexplained neonatal respiratory symptoms ranging in severity. The most predominant symptoms of PCD are chronic daily productive cough, chronic persistent rhinitis, sinusitis, recurrent otitis media and hearing impairment. As these symptoms are extremely common, especially in the paediatric population, physicians often miss the signs and symptoms of PCD. Patients are often diagnosed late. Guidance to physicians of whom to refer for diagnostic testing is required.

Living with PCD has been found to have an impact on physical, emotional and social functioning across the age groups. Associations between HRQoL and increasing age, and age at diagnosis have been reported. While it is suggested that diagnosis and subsequent

commencement of treatment can lead to health improvements, there are no sensitive outcome measures to confirm this.

Outcome measures that have been used to assess disease severity in PCD to date include spirometry, chest computed tomography, magnetic resonance imaging and lung clearance index. These physiological and radiological measures all have limitations in terms of their sensitivity or feasibility to monitor disease progression. There is a need for a sensitive PCD specific outcome measures to evaluate new treatments and monitor disease progression. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) strongly promote the use of PROMs in clinical trials.

Part 1: An Overview of the Patient Experience of Primary Ciliary Dyskinesia

3. DIAGNOSING PRIMARY CILIARY DYSKINESIA: THE PATIENT PERSPECTIVE (PAPER 1)

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Author's Contribution

The research question was generated to inform a European Respiratory Society (ERS) Task Force who were developing guidelines for diagnosing PCD. It is important that these guidelines are patient centred. This provided me, the lead author, with an international platform to establish the patient perspective from countries where it has not been investigated before. Supported by JSL and ADG, I developed the survey based on literature searches and analyses of transcripts of previous interviews with PCD patients. Once developed, members of the ERS Task Force (international) and patient support groups (UK (FC) and USA (MM)) were contacted for their opinions on the survey and to suggest interactive changes. Once amended, I organised the translation and back translation of the survey into 9 European languages and enlisted the help of members of the taskforce, international patient support groups and European Lung Foundation (ELF) to disseminate the survey internationally. I performed descriptive analysis of the survey findings. BR conducted an analysis of the survey results by country to allow comparisons to be made. I completed 20 semi-structured interviews focusing on the participant's experience of referral and diagnostics. Telephone interview were completed with participants Europe, Australia and the USA. I conducted a thematic analysis of the transcripts and drafted the manuscript. I presented the findings to the ERS Task Force at committee meetings and at the European Respiratory Society Congress in 2015 as a poster presentation.

3.1. Abstract

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterised by progressive sino-pulmonary disease, with symptoms starting soon after birth. An ERS Task Force aims to address disparities in diagnostics across Europe by providing evidence-based clinical practice guidelines. We aimed to identify challenges faced by patients' when referred for PCD diagnostic testing.

Method: A patient survey was developed by patient representatives and healthcare specialists to capture experience. Online versions of the survey were translated into 9 languages, and completed in 25 countries (n=365) of which 74% were PCD positive, 5% negative and 21% uncertain/ inconclusive. We then interviewed 20 parents/patients. Transcripts were analysed thematically.

Results: 35% of respondents visited their doctor >40 times with PCD related symptoms prior to referral for testing. Furthermore, the most prominent theme among interviewees was a lack of PCD awareness among medical practitioners and failure to take past history into account leading to a delayed diagnosis. Patients also highlighted the need for improved reporting of results and a solution to the "inconclusive" diagnostic status

Conclusion: The greatest frustrations related to delayed referrals and the need for improved communication when receiving results. These findings will be used to advise on ERS Task Force guidelines for diagnosing PCD.

3.2. Introduction

Primary ciliary dyskinesia (PCD) is a rare heterogeneous condition. Genetic mutations cause abnormal ciliary function which is associated with abnormal ciliary ultrastructure in 70% of cases (16, 40). PCD is characterised by impaired mucociliary clearance of upper and lower airways. Symptoms usually present soon after birth and range in severity from mild transient tachypnoea to significant neonatal respiratory failure requiring prolonged respiratory support (1-3). Chronic and progressive chest symptoms persist throughout life and include daily wet cough and recurrent chest infections which almost consistently lead to bronchiectasis (1). Upper airway symptoms include rhinosinusitis and recurrent serous otitis media with hearing impairment (99). Situs inversus is seen in approximately 40% of cases and situs ambiguous is seen in approximately 10% of cases (3).

Reported prevalence varies from 1:2,000 to 1:40,000 which could be due to a true variability across demographics but is likely to reflect differences in access to diagnostic facilities (9-11). A European Respiratory Society (ERS) Task Force survey of 26 European countries found that PCD is under-diagnosed or diagnosed late, particularly in countries with low health-care expenditure (9). In addition to improving respiratory prognosis (4, 12, 64) early diagnosis also facilitates appropriate management of the associated upper airway disease since management is different to non-PCD related serous otitis media and sinusitis (21). Diagnosis also allows genetic counselling for the family.

There is no "gold standard" test to diagnose PCD and diagnostic pathways vary between/within countries (13, 100, 101). European guidelines recommend that PCD should be confirmed in a specialist centre using appropriate diagnostic testing (22). PCD diagnostic investigations are complex, requiring expensive infrastructure and an experienced team of

clinicians, scientists and microscopists. Due to inadequate samples or inadequate results, several samples may need to be taken until a conclusive result is achieved and the time until a conclusive result is reached can vary greatly between patients (13, 19, 20). Various models exist to deliver diagnostic services for this rare disease, generally with a network of satellite screening centres accessing a specialist centre (14, 20, 21).

A European Respiratory Society (ERS) Task Force was established in September 2014 to develop clinical practice guidelines on diagnosing or refuting the diagnosis of PCD. An important element to consider in developing practice guidelines is the patient perspective and experiences of the diagnostic process. There has been no previous international research evaluating PCD patients' experiences and their perspective on the diagnostic process. This study was therefore undertaken to address this gap and to inform the Task Force.

Over 25 years ago the authors of a pioneering study to characterise PCD concluded "*All children with unexplained chronic respiratory disease, in particular those with symptoms starting in the neonatal period should be investigated for ciliary dyskinesia"* (102). However, access of patients with these symptoms to diagnostic services remains problematic. The present study used a mixed methods (qualitative and quantitative) approach to more fully understand the perspectives of patients and to identify the most prominent issues and concerns arising for patients at any stage in the diagnostic pathway.

3.3.Methods

The study combined an international web-based survey and semi-structured interviews, ensuring both breadth and depth. Ethical approval for semi-structured interviews was provided by Southampton and South West Hampshire Research Ethics Committee A 07/Q1702/109. Audio-recorded and/or written consent was obtained from interviewees and electronic consent recorded for survey participants.

We conducted an international survey to investigate the patients' perspectives on diagnosis between April and November 2014. We designed the survey based on findings and expertise from three sources: (1) semi-structured interviews with patients from a previous study to understand factors impacting on quality of life in patients with PCD (103); (2) consultation with patient representatives in the UK, USA and Switzerland; and (3) consultation with the European Lung Foundation (ELF). Survey topics included measures of demographics, age of diagnosis, methods of diagnosis, the patient opinions on their diagnostic experience and how diagnostic services should be delivered (Box 1). To ensure it reflected wider international issues, the prototype survey was amended by ERS Task Force panel members from 7 countries (UK, France, Switzerland, Germany, Italy, Ireland and Belgium). The finalised online survey (Appendix 1) was translated into 9 European languages (Italian, Polish, Russian, Portuguese, Czech, Dutch, German, French and Greek) using forward and back translations. Translation and dissemination of the survey was done according to countries with established PCD diagnostic services in addition to advice from the ELF and ERS Taskforce. Links to the survey were distributed by patient organisations, clinicians and through the ELF via newsletters, targeted emailing and social media channels. The survey was hosted by University of Southampton (https://www.isurvey.soton.ac.uk).

Survey topics: the survey allowed participants to rate the importance of topics relating to their diagnostic experience
The ability to meet, discuss and have their samples analysed by PCD experts
Repeat testing for inconclusive results
The availability of information before testing
The availability of information after diagnosis
Travelling long distances for diagnostic testing
The level of agreement on whether their condition, treatment and quality of life had improved since diagnosis
A free text option to allow for comments was provided at the end of the survey

The survey sought the opinions of patients with PCD and parents of children with PCD. We included all patients who had been considered for PCD testing irrespective of their diagnostic outcome *i.e.* positive /negative/inconclusive or by their diagnostic protocol and the tests that they received if any *i.e.* nasal nitric oxide (nNO), transmission electron microscopy (TEM), high speed video microscopy (HSVM), genetic, diagnosis based on symptoms etc.

The survey was anonymous; however, respondents were invited to provide their email address if they wished to take part in a telephone interview. Over 80 participants provided their email address and expressed interest to be interviewed. Twenty of these respondents were recruited. Participants had to be able to speak English to be included in the interviews. Measures were taken to ensure interviewees represented different age groups, different diagnostic status and countries. The semi-structured interviews were conducted by the lead author (L.B.) who is trained and experienced in semi-structured/ open-ended interviews and qualitative analyses. Topics of discussion included: when they first heard of PCD, medical providers seen, experience with diagnostic services and any problems encountered. Telephone interviews were used to ensure a geographical representation, to provide a degree of anonymity and to relieve pressure on participants to provide the socially acceptable answer

(104) *i.e.* discussing challenges experienced with diagnostic services and medical professionals.

3.3.1. Data analysis

We used simple descriptive statistics to investigate comparisons of the demographic characteristics across the range of diagnostic outcomes and between countries. Data were checked for normality and two-tailed parametric (t-test) or non-parametric (Mann-Whitney) tests were used to assess differences among mean and median values for the group as a whole and across each diagnostic outcome. Positively skewed data were transformed, were appropriate, prior to performing statistical analysis. Categorical variables were presented by frequency and percentage. Statistical analyses were performed with IBM SPSS Statistics (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

Semi-structured interviews were recorded, transcribed, verified for accuracy, and identifying information was removed. In order to maintain thoroughness, a COREQ checklist was used throughout the study (105). Notes were maintained at all times to track decisions and help to verify the link between the original data and the findings (105). Thematic analysis was used where patterns within data were identified using an inductive or 'bottom up' approach. By using an inductive approach, the themes identified were strongly linked to the data themselves (106). The interview transcripts were single-coded by L.B. Themes and subthemes were discussed with the senior author J.S.L. in order to confirm the validity of the interpretations that were being developed. Data was coding using NVivo qualitative data analysis Software (QSR International Pty Ltd. Version 10, 2012). Themes and subthemes were presented in three stages, pre-diagnosis, diagnosis and post-diagnosis (Figure 4).

3.4. Results

3.4.1. Study population

The survey was completed in 9 European languages by 365 participants from 25 countries. The majority of respondents had been diagnosed with PCD (74.3%), with a smaller percentage of respondents distributed across other diagnostic outcome groups. 54.5% of the respondents were parents of children and 41.6% were adults who had been tested for PCD. 49.8% of PCD positive respondents had situs inversus and 11.8% were born with a cardiac defect. Only 1 (6%) of the PCD negative respondents had situs inversus and 0% had a cardiac defect (Table 1).

Respondents were resident in Germany (21%), United States (18%), France (15%), United Kingdom (13%), Belgium (11%), Denmark (5%) and others (17% including Italy, Australia, Switzerland, Canada, Cyprus, Ireland, Greece, South Africa, Austria, Chile, Portugal, Qatar, Poland, Iran, Norway, Spain, Portugal and Algeria). Semi-structured interviews were conducted with 20 participants from 7 countries (40% from United Kingdom) and were diverse in age group, diagnostic testing, and diagnostic outcomes (25% were negative/inconclusive/still waiting). 25% were parents of children between the ages of 0-4 years, 35% were parents of children 5-17 years, and 40% were patients tested at ≥18 years. The majority (70%) were from English speaking countries (Appendix 1; Table A1). Saturation was reached after the 14th interview.

3.4.2. Pre-diagnosis: journey to referral

Most survey respondents had repeatedly visited medical professionals with PCD related symptoms prior to referral; 37% of positive patients had >40 visits and only 3% had been referred soon after birth. 61% of PCD negative respondents had ≤20 visit prior to referral for

testing. 76.4% of respondents were older than one year of age when they were tested for PCD. The overall median age at testing was 6 years (IQR =11) (Table 2)**.** The mean age at testing slightly younger for those with situs inversus with the mean age at testing slightly younger for those with situs inversus (mean 2.38 +/-2.04 *vs* 2.97 +/-1.76 for those without, p=0.016) and congenital cardiac defect (mean 2.01 +/-1.78 *vs* 2.76 +/-1.91 for those without, p=0.036). The most common reasons for diagnostic referral of interviewees was an episode(s) of pneumonia i.e. x-rays and CT-scan showing changes (6/20), and self-referral following reading about PCD online (3/20).

Interviewees attributed a delay in referral for diagnostic testing to a lack of awareness and a poor attitude among healthcare professionals (14/20) (Figure 4). 11/12 parents reported that their child had respiratory distress at birth but 8/11 were not referred until repeated visits to their physicians sometimes over many years. Participants felt that the underlying cause of symptoms was not considered nor was past medical history taken into account. Adult participants felt they were not taken seriously while parents reported being treated as over-cautious and over-protective. Many participants were continually treated for other common conditions e.g. asthma and hay-fever, despite treatments not having any effect. (Box 2: A, B, C, D). Older participants (>30 years at diagnosis) felt the cause of their illness was not seen as a priority for investigation due to their age with focus instead being on the treatment of COPD/bronchiectasis (Box 2: A, B). 99% of survey respondents felt that an improvement in PCD awareness amongst general practitioners (GPs) and local doctors is needed to encourage early referral (Figure 5).

Box 2: Sub-themes and example participant quotes regarding experiences prior to diagnostic testing: journey to referral

Past medical history was not considered

A: It was a wait, but it was more the fact that no-one was looking at the history….she'd been in neonatal for eleven days when she was born, she was full-term…she couldn't get her sats up….how many times I'd been in, you know and no one put two and two together. *Participant 010, parent, positive PCD*

Symptoms not taken seriously – parents treated as fussy/over protective

B: ....and all this time you know…time's going on…I don't think that I was listened to as a mum, it was a bit like, I was you know maybe of over worrying, things like that. *Participant 004, parent, positive PCD*

C: I think the lateness in the diagnosis has caused the bronchiectasis and…other problems and I think that could have been prevented if someone would have taken me a bit more seriously. *Participant 010, parent, positive PCD*

Mistreated for other illnesses

D: ...he treated her for asthma, but the picture never made sense, and she didn't look like an asthmatic child. *Participant 015, parent, positive PCD*

Older patient treated without investigation for route cause

E: There seems to be, among pneumologists, that they think in adult people or at least people who are more than forty years old, it doesn't matter anymore which is the reason for this COPD, and they don't even think about testing it. *Participant 011, adult, positive PCD*

3.4.3. Diagnosis: experience and impact of diagnostic testing

The most common diagnostic procedures experienced by survey respondents were nasal scraping (79%), nasal nitric oxide testing (44%) and genetic testing (37%). 88% of survey participants rated it as important to have multiple tests if this ensured an accurate result and to

see a PCD expert even if it means travelling long distances (Figure 5). This was echoed overall in the interviews (Box 3: A-C); however, some age-specific variances emerged with parents describing nasal scraping as a moderately distressing experience. This distress was heightened if their child needed a repeat brushing (Box 3: B, C). Participants reported that testing was carried out in an efficient and empathetic manner with the appropriate amount of information provided once they reached specialist services (8/20) (Box 3: D, E).

Box 3: Sub-themes and participant quotes for diagnosis: Experience of diagnostic testing

Importance of meeting a PCD expert to be tested and to discuss results

A: And everything just went really smooth for us when we were over there. Having to go over there, it wasn't great, but you know it's the facilities are not in Ireland, so you just have to do it. *Participant 003, parent, inconclusive PCD*

Distress from nasal scrape

B: ...it's a little bit distressing for parents….it's got to be done…. you know you're holding a child still and you don't want to do it, but you have to, for their best. *Participant 004, parent, positive PCD*

C: It was a bit uncomfortable… I mean I don't think she was expecting it to be quite as uncomfortable as it was, but having said that she went along with it okay, she knew she had to get it done. *Participant 002, parent, positive PCD*

Communication of testing procedure

D: First of all it was an absolutely painless experience and the people doing it explained it so well, I wouldn't change a thing at all. *Participant 001, adult, positive PCD*

E: I mean it's all very new for me…but… the amount of information that I got… which explained the process and explained what was being done and how it was going to be achieved, I think was very well done, and the staff involved were very empathetic, very sympathetic. *Participant 008, adult, inconclusive PCD*

3.4.4. Diagnosis: awaiting and receiving results

A total of 67% of survey respondents received their result within a 6 months period and 12% reported that they received confirmatory results within 1 week of testing (Table 2). Opinions varied on what was deemed to be a 'long time' to wait for results (Box 4: A, B). Ninety-four percent of survey participants rated having samples analysed by a PCD expert as important or very important and 97% rated the as important or very important the opportunity to discuss results with a PCD expert (Figure 5).

Participants referred to encountering problems when results for tests such as transmission electron microscopy (TEM) and genetics were received much later than others *e.g.* high speed video microscopy and nNO test. Explanations of what the results mean in combination were incorrectly relayed back to the patients. To overcome this, participants recommended that it would be helpful if they could receive an overall report explaining what the results mean in combination and to have the opportunity to discuss this with a PCD expert (Box 4: D, E).

Box 4: Sub-themes and participant quotes for Diagnosis: awaiting and receiving results

Long-time taken to receive results

A: I had to ring up months after, chasing up the results… we should have got the results sooner than what we did….like the testing was done in the January and probably should have got the results probably March, April. Ended up being like June, July, because I had to actually chase up myself to get the results. *Participant 019, parent, positive PCD*

B: No, definitely a month is a long time to wait. To know what's wrong with your child or significant other or something, but it takes as long as it takes; I have gotten used to that. *Participant 017, parent, positive PCD*

Experience of communication of diagnostic results

C: And I got the results, let's say in drops, maybe one year later I got the information about the electron microscopy was or maybe it was eighteen months later. *Participant 011, adult, positive PCD*

D: I got the information from my doctor that my son…probably has PCD….the cilia didn't move…about already one month later…the report that the electron microscopy was normal, and the report from the electron microscopy said we cannot prove primary ciliary dyskinesia based on this. The doctor told me, 'well then it's not primary ciliary dyskinesia'. *Survey participant, open text response*

E: Before they did any testing we had a good discussion about what it was and what it meant, and then when we got the results they explained what it was that wasn't working….It's usually better if somebody talks to you rather than it being written down. *Participant 010, parent, positive PCD*

3.4.5. Post-Diagnosis: impact of diagnosis

Participants who experienced an inconclusive diagnostic result discussed the practical constraints of such an outcome e.g. insurance constraints and not being included in research (Box 5: A, B). The emotional impact of a positive diagnosis was described by many as leading to a profound sense of validation that their symptoms were down to a specific named condition (Box 5: C, D). Participants also reported feeling some distress that their/their child's diagnosis process took such a long time and felt that an earlier diagnosis could have made a significant difference to the state of their current and future health; relief was expressed once they had a label and could pursue getting the correct treatment (Box 5: E, F). Relief was also described when a negative diagnosis was received (Box 5: G).

Box 5: Sub-themes and participant quotes for Diagnosis: Impact of diagnosis

Impact of an inconclusive diagnostic result

A: I have suffered from serious upper and lower respiratory infections all my life. I have an 'inconclusive' status re: diagnosis of PCD….there must be a category of PCD that includes a variant of PCD as a diagnostic category. This is crucial for people who have insurance challenges. *Survey participant, open text response*

B: I hate the probable PCD diagnosis. 4 years ago my 3 kids were given that. All classic symptoms with bronchiectasis and low nasal nNO. But cilia appeared normal. It infuriates me they can't be included in research. *Survey participant, open text response*

Emotional response to positive diagnosis: feeling of validation

C: Like to have the confirmed diagnosis…well I am just really excited they were finally able to prove it's not in my head. You know like I didn't make it up. *Participant 016, adult, positive PCD*

D: I actually felt like shouting Alleluia once she was diagnosed, because it was just years and years of worry and thinking what's going on and why, and I just, you kind of doubt yourself as a mum. *Participant 010, parent, positive PCD*

Emotional response to positive diagnosis: relief but a sense of sadness that diagnosis wasn't sooner

E: You know that's kind of like oh wow, finally I'm no longer a bookcase. But then I have all this damage that you know at least, you know at least I have a diagnosis, twenty years later, but it's a little late. *Participant 009, adult, positive PCD*

F: Well it was a certain relief, first of all in that you know we can explain now…. we can help her in the way that she needs. So really it was just a huge kind of relief really, just knowing that we have an explanation now. Along with you know a bit of sadness that we didn't catch onto it earlier. *Participant 002, parent, positive PCD*

Impact of a negative diagnosis: relief

G: I was very relieved of course. I didn't know anything at all about it until I was told by the consultant I was going to be sent for the test. And then I received some documentation in advance with the appointment. And I also read a little bit about it online…I was quite concerned, because

one of the things I heard was that it could affect your hearing and you can go deaf…. I was quite anxious, so the length of time being referred and actually having the tests, I was very worried. *Participant 007, adult, negative PCD*

3.4.6. Post diagnosis: follow up care and support available

Participants expressed a sense of being overwhelmed by the prescription of medication, treatments and clinic appointments following a positive diagnosis, particularly when newly diagnosed. The benefits of a diagnosis to the reported current state of health varied among survey participants with 21% reporting no improvement to health since diagnosis (Figure 5). There was a significant difference was found between those who were diagnosed in childhood (0-12 years) and those who were diagnosed in adolescences/adulthood (>13 years) on the level of agreement that health has improved since diagnosis ($p=0.041$). Similar variability was found among the interviewees with some participants feeling that their/their child's health had benefited greatly since starting treatments (Box 5: A-C) and others reporting no major change since diagnosis despite adhering to the prescribed treatments (Box 5: D).

In the survey 91% of respondents strongly agreed/agreed that it is important to have a patient organisation in each country (Figure 5). The role of PCD patient support groups following referral was further elaborated in the interviews as important in terms of meeting other families affected by PCD and having an opportunity to share experiences (Box 6: E, F). Participants in countries without a PCD support group spoke of the need for support services enabling discussions about experiences specific to their country's health system and in their native language (Box 6: G- I).

Box 6: Sub-themes and participant quotes for Post diagnosis: Follow up care and support available

Treatment burden and effect on condition

A: "It was a bit of a shock…. I was probably in my mid-thirties then, to suddenly be told, right, you've got to do twenty minutes of physio twice a day, you've got to take this blue puffer, and the brown puffer… as soon as you get a chest infection you've got to take really strong antibiotics, I rebelled against that, just because it was too much all at once." *Participant 006, adult, positive PCD*

B: "For me it made a huge difference in my treatment when I finally knew the diagnosis. And I am certain that I wouldn't be as well as I still am when I would have continued in the way as I did before it" *Participant 011, adult, positive PCD*

C: "She was sick every month. Once we had a diagnosis… she gets sick, but not as severe as… before. Definitely milder…you know we have a treatment plan and even when she starts to get sick; those medications are changed so we tend to catch that right away rather than after that. *Participant 015, parent, positive PCD*

D: "It's strange, since being diagnosed we are now bombarded with a very heavy burden of care, she uses the nebulisers three times a day, and she has physio twice a day…and yet she's not clinically, she's no different to before she was diagnosed. *Participant 004, parent, positive PCD*

Support from PCD patient organisations

E: Well we go, after the diagnosis…through the PCD Foundation, and that was a huge support. Also there is like chat groups that we go on. We've gone to family days. So we've met quite a few other families that also have PCD…yeah, it's a huge, huge help really. Sometimes it's even just finding out what they did in certain situations. So things you can bring up to your doctor, so that's helpful as well. *Participant 015, parent, positive PCD*

F: I got connected with the PCD group online …without the people in this group… I would be terribly lost…they've been my life support. *Participant 009, adult patient, PCD positive*

Need for PCD patient organisations in each country

G: In Spain, we would like…a Patients Association…., in the UK there will be one of these, you know this association, but in Spain we don't know anything about the illness and the patients.

Participant 013, parent, PCD positive

H: There isn't any in Ireland….I don't know anybody else that has it, and if I did know somebody that would be handy…. like you could see…what does the future hold for us in a few years' time, really we're only going day-by-day, and I know every case is different, but it would be nice to kind of know other issues some people have experienced. And the mistakes I made, maybe you could try this, which might help. *Participant 003, parent, inconclusive PCD*

I: I think it's difficult. There is no information in Bulgarian for example….and I would speak English so I can get a lot of information from the internet, but if you are a Bulgarian and you do not speak English, it's very, very hard for you to get that information. *Participant 012, adult patient, probable PCD*

3.5. Discussion

This study represents findings from an international survey and in-depth semi-structured interviews specifically designed to understand the experiences, concerns and needs of patients requiring diagnostic testing for PCD. It describes the experiences and perspectives of 365 adult patients and parents from 25 EU and non-EU countries, 20 of whom were interviewed to obtain an in-depth insight to their experiences and opinions of being tested for PCD.

When discussing the journey to referral, the overarching theme was the lack of PCD awareness by medical professionals thus leading to a delayed diagnosis. This was also reflected in the survey, with 34% of the total respondents reporting that they visited their doctor on >40 occasions for PCD related symptoms before PCD was considered a possible diagnosis. There is a clear need to reiterate to non-expert clinicians the guidelines (102) published a quarter of a century ago, which stated that individuals with chronic upper and lower airway symptoms should be investigated for PCD. Previous research has found a sense

of isolation and mistrust in medical care among PCD patients which is heightened by a lack of PCD awareness by the patient's GP (79). Participants were concerned that delayed diagnosis had adversely affected their health. All adult interviewees were diagnosed late in life (>30 years of age) and felt that their current state health might have been better had they been diagnosed earlier. This finding supports previous research that found that delayed PCD diagnosis led to poorer subsequent quality of life (107). To prevent a delayed diagnosis, participants felt that medical professionals need be to be better informed about PCD, its signs and symptoms and relevance of past medical history.

When receiving a diagnosis, patient and parent interviewees who visited specialised diagnostic centres were satisfied with their experience. The implications of normal electronic microscopy wrongly being interpreted as negative diagnosis (101) by non-PCD experts was highlighted. Respondents felt that being able to discuss their test results with a PCD expert as well as receiving a written report of all test results was important and contributed to a sense of certainty and assurance.

Due to the importance of societal, health and cultural differences between countries, we aimed to recruit participants from diverse geographical regions. We first examined the results from all participants and then analysed countries separately. A quantitative comparison of countries grouped by general government expenditure on health (the sum of outlays for health maintenance, restoration or enhancement paid for by government entities) or grouped by countries with and without specialist diagnostic services was prevented due to the limited numbers of respondents from some areas. We have, however, included the analyses from the UK, Germany and the USA to show comparisons with differing approaches to delivery of diagnostic services and availability of tests. The percentage of respondents who had 40 or

more visits to their doctor with symptoms related to PCD before being referred for testing was significantly higher in the USA (44%) compared with the UK (24%) and Germany (35%) (Appendix 1: Table A2). The interviews also revealed country-specific variances. Participants from non-English-speaking countries strongly advocated that support groups be set up in their country and for information to be available online in all languages. The inconclusive PCD diagnosis also had country-specific implications, with participants from countries including the USA requiring a definitive diagnosis in order to access insurance for treatments. A resolution to the problems associated with an inconclusive result or a "probable" PCD diagnosis was called for.

3.5.1. Strengths and Limitations

This is the first international study of the perspective of patients referred for PCD diagnostic testing. Questions for the survey involved the contribution of an international panel of PCD experts and patient representatives. However, our study does have limitations: response varies from country to country with the main percentage of respondents from countries with established diagnostic centres *i.e.* UK, US, Germany and France.

The survey also aimed to include patients who were referred and still waiting for results, who were negative for PCD or who have been found to be inconclusive for PCD and are still going through testing however 74.3% of survey respondents were PCD positive with PCD negative representing only 4.9%, inconclusive 8%, and still waiting 3%. It is especially low considering that international results have found that between 11.5%-18.6% of referrals are eventually diagnosed with PCD (29, 58, 101, 108). It is perhaps not surprising that patients with ongoing interest in the disease (PCD-positive) were more likely to respond to the survey. This limits our capacity to assess the perspective of the population with an inconclusive or negative outcome.

Although participants were asked about the tests that had been performed, their results cannot be verified and there is therefore diagnostic uncertainty. In addition, a number of participants' diagnoses were based on tests that are not considered robust, e.g. the saccharine test. It is therefore likely that some patients may have been diagnosed who do not have PCD and vice versa. Since we were interested in the experiences and outcomes of patients from diverse clinical settings it was important to include groups where diagnostic status was uncertain. This inherently means that allocation of patients to diagnostic outcome groups was defined by the participants' understanding rather than the diagnosis that might be provided by a highly specialist centre; this is at the same time a strength and a weakness of the study.

3.5.2. Implications and conclusion

This study concludes that there are a number of recommendations for the improvement of PCD diagnosis from the patient's perspective and that are needed across Europe and internationally. (1) Samples should be analysed by PCD experts. (2) Results should be delivered by a PCD expert. Patients should have the opportunity to discuss their results with a PCD expert and to ask questions. (3) Repeat testing should be completed if needed, to ensure an accurate result. (4) Measures must be introduced to prevent late diagnosis such as better knowledge of PCD by medical practitioners, including relevance of past medical history. (4) A resolution to the "inconclusive" diagnosis result status. (5) Establishment of a patient support group in each country. (6) Availability of online translated information on PCD in all European languages.

This is the first international study evaluating PCD patients' experiences and their perspective on the diagnostic process. The findings from this study will be used to advise the

ERS Task Force (TF-2014-04) as they develop clinical practice guidelines on diagnosing or refuting the diagnosis of PCD. We anticipate that the results will inform stakeholders with responsibility for improving existing diagnostic provision and for expanding services for this rare disease. It should feed into the new European Reference Networks (ERNs) for rare diseases, particularly the PCD ERN.

3.6. Conclusion

This is the first international study evaluating PCD patients' experiences and their perspectives on the diagnostic process. The findings from this study will be used to advise the ERS Task Force TF-2014-04) as they develop clinical practice guidelines on diagnosing or refuting the diagnosis of PCD.

Figure 4: Themes and subthemes from semi-structured interviews

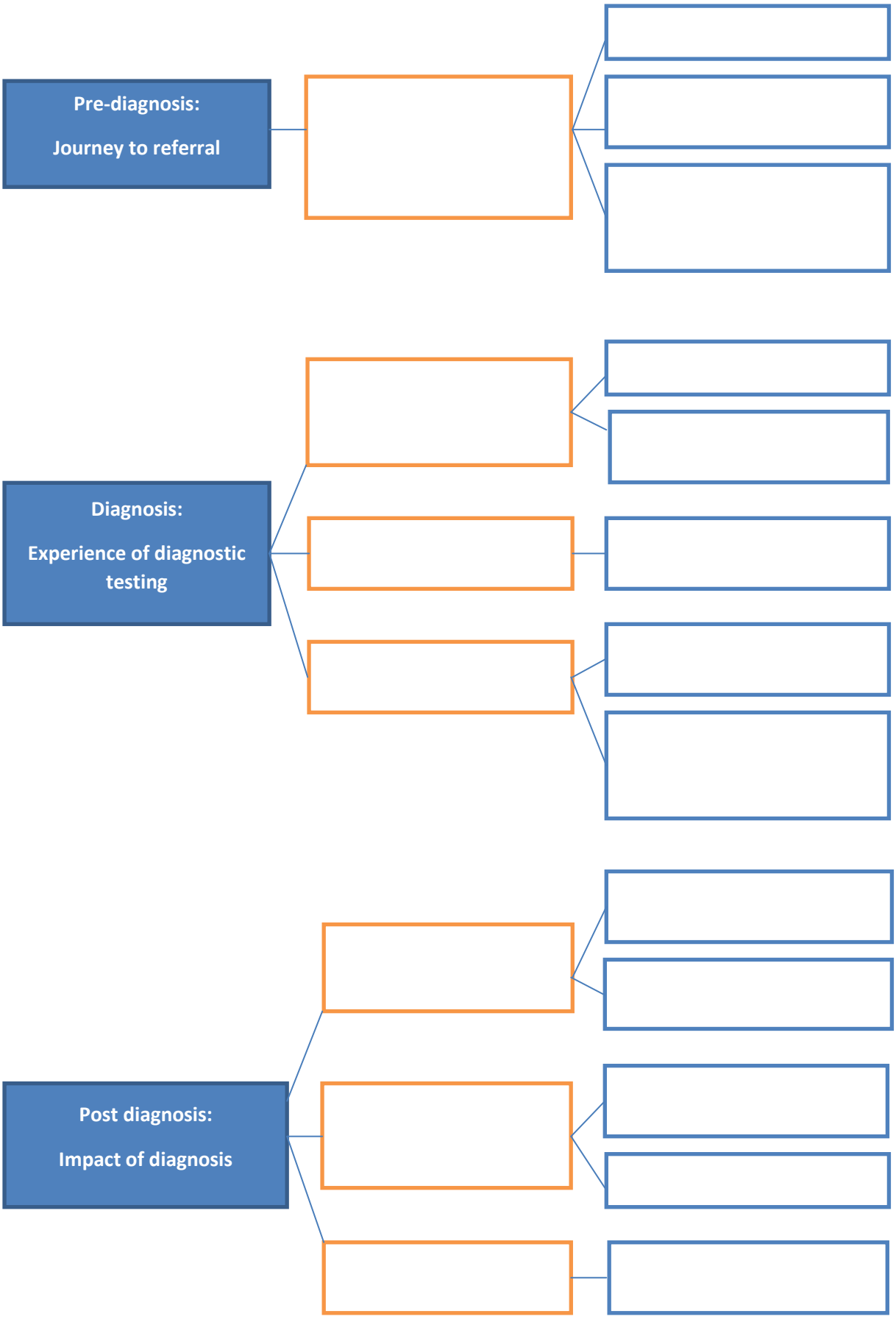
Pre-diagnosis:
Journey to referral
Diagnosis:
Experience of diagnostic testing
Post diagnosis:
Impact of diagnosis

Figure 5: Survey findings on patient opinion of diagnostic effect on health and the importance of expert service provision

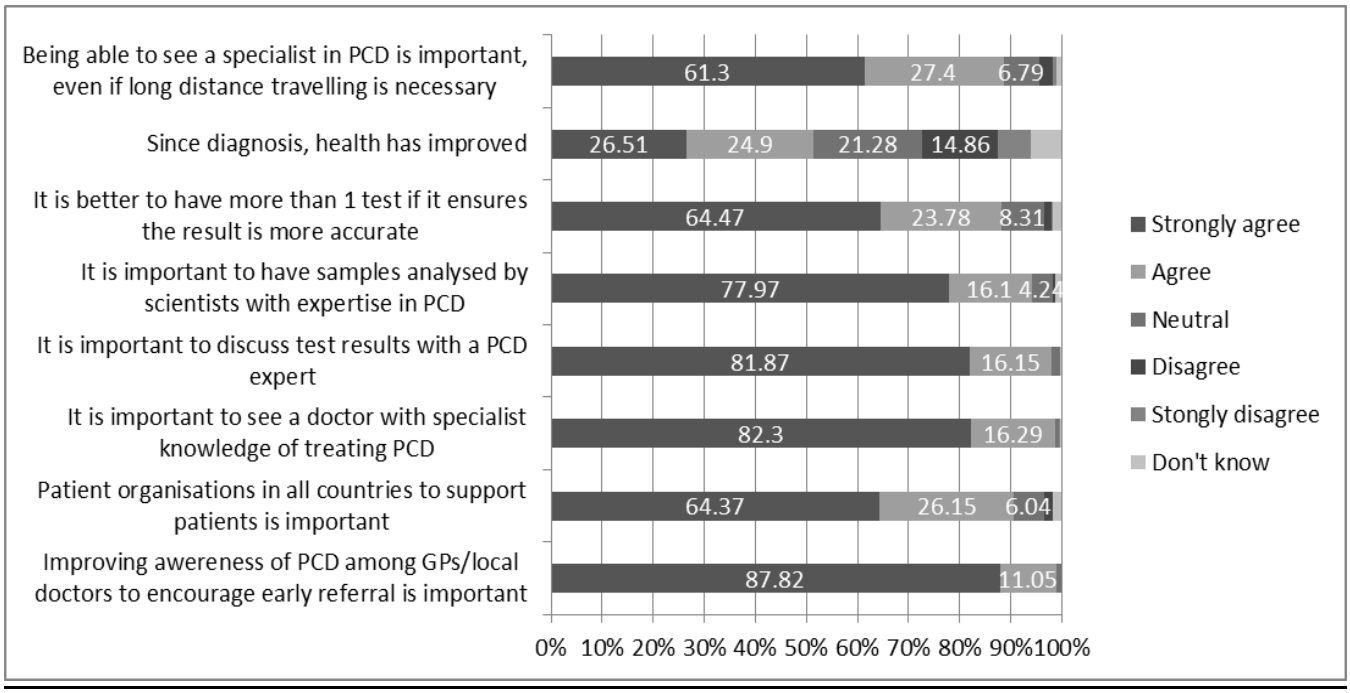

Table 1: Characteristics of participants in the survey stratified by PCD disease status

	Disease status				
	Total	**PCD positive**	**PCD negative**	**Inconclusive**	**Other***
	365 (100%)	**271 (74.3%)**	**18 (4.9%)**	**29 (8.0%)**	**47 (12.9%)**
Respondent's status					
Patient tested for PCD	152 (41.6%)	115 (42.4%)	8 (44.4%)	8 (27.6%)	21 (44.7%)
Parent of child tested for PCD	199 (54.5%)	152 (56.1%)	10 (55.6%)	21 (72.4%)	16 (34.0%)
Missing	14 (3.8%)	4 (1.5%)	-	-	10 (21.2%)
Gender					
Male	156 (42.7%)	114 (42.1%)	8 (44.4%)	14 (48.3%)	20 (42.6%)
Female	195 (53.4%)	154 (56.8%)	7 (38.9%)	14 (48.3%)	21 (44.7%)
Missing	14 (3.8%)	3 (1.1%)	3 (16.7%)	1 (3.5%)	6 (12.8%)
Age of suspected PCD patient at time of survey					
< 5 years	54 (14.8%)	38 (14.0%)	2 (11.1%)	8 (27.6%)	6 (12.8%)
5-12 years	88 (24.1%)	61 (22.5%)	7 (38.9%)	10 (34.5%)	10 (21.2%)
13-17 years	52 (14.3%)	42 (15.5%)	1 (5.6%)	4 (13.8%)	5 (10.6%)
18-35 years	70 (19.18%)	58 (21.4%)	3 (16.67%)	2 (6.9%)	7 (14.9%)
36-50 years	52 (14.25%)	45 (16.61%)	-	2 (6.9%)	5 (10.6%)
51-65 years	26 (7.12%)	19 (7.01%)	1 (5.56%)	1 (3.45%)	5 (10.6%)
> 65 years	11 (3.01%)	4 (1.48%)	4 (22.22%)	2 (6.9%)	1 (2.1%)
Missing	12 (3.29%)	4 (1.48%)	-	-	8 (17.0%)
Situs inversus	157 (43.0%)	135 (49.8%)	1 (5.6%)	6 (20.7%)	15 (31.9%)
Congenital heart defect	41 (11.2%)	32 (11.8%)	-	4 (13.8%)	5 (10.6%)

*Other includes participants still waiting for results and those who received a false positive result as well as missing values.

Table 2: Age at testing, time taken to receive test results, and number of visits to doctor before referral stratified by disease status for survey participants.

	Disease status				
	Total	**PCD positive**	**PCD negative**	**Inconclusive**	**Other***
	365 (100%)	**271 (74.3%)**	**18 (4.9%)**	**29 (8.0%)**	**47 (12.9%)**
Age at initial testing					
< 1 year old	42 (11.5%)	30 (11.1%)	-	4 (13.8%)	8 (17.0%)
> 1 year old	279 (76.4%)	229 (84.5%)	14 (77.8%)	20 (69.0%)	16 (34.0%)
Missing	44 (12.1%)	12 (4.4%)	4 (22.2%)	5 (17.2%)	23 (48.9%)
Median age (IQR)			6 years (11) (n = 226)		
Number of visits to doctor before diagnosis					
Referred soon after birth	12 (3.3%)	9 (3.3%)	-	2 (6.9%)	1 (2.1%)
1-10 visits	64 (17.5%)	46 (17.0%)	6 (33.3%)	5 (17.3%)	7 (14.2%)
11-20 visits	56 (15.3%)	45 (16.6%)	5 (27.8%)	3 (10.3%)	3 (6.4%)
21-40 visits	68 (18.6%)	52 (19.2%)	4 (22.2%)	8 (27.6%)	4 (8.5%)
>40 visits	123 (33.7%)	101 (37.3%)	1 (5.6%)	8 (27.6%)	13 (27.7%)
Other	22 (6.03%)	13 (4.8%)	1 (5.6%)	3 (10.3%)	5 (10.6%)
Time to diagnosis after testing for PCD					
<1 week	45 (12.3%)	39 (14.4%)	1 (5.6%)	1 (3.5%)	4 (8.5%)
1-4 weeks	76 (20.8%)	61 (22.5%)	8 (44.4%)	3 (10.3%)	4 (8.5%)
1-6 months	122 (33.4%)	105 (38.8%)	8 (44.4%)	4 (13.8%)	5 (10.6%)
6-12 months	10 (2.7%)	8 (3.0%)	-	1 (3.5%)	1 (2.1%)
> 1 year	28 (7.7%)	24 (8.9%)	1 (5.6%)	2 (6.9%)	1 (2.1%)
Still waiting	28 (7.7%)	-	-	15 (51.7%)	13 (27.7%)
Not sure	36 (9.9%)	31 (11.4%)	-	2 (6.9%)	3 (6.4%)
Missing	20 (5.5%)	3 (1.1%)	-	1 (3.5%)	16 (34.0%)
Diagnostic procedures					
Nasal nitric oxide	157 (43.0%)	110 (40.6%)	14 (77.8%)	17 (58.6%)	16 (34.0%)
Nasal scraping	283 (77.5%)	221 (81.6%)	16 (88.9%)	28 (96.6%)	18 (38.3%)

Bronchoscopy	134 (36.7%)	102 (37.6%)	6 (33.3%)	12 (41.4%)	14 (29.8%)
Genetics	134 (36.7%)	105 (38.8%)	1 (5.6%)	13 (44.8%)	15 (31.9%)
Saccharine test	19 (5.2%)	14 (5.2%)	1 (5.6%)	2 (6.9%)	2 (4.3%)
Nuclear medicine scan	25 (6.9%)	19 (7.0%)	2 (11.1%)	1 (3.5%)	3 (6.4%)
X-ray or CT scan alone	8 (2.2%)	2 (0.7%)	-	-	6 (12.8%)
Symptoms only	12 (3.3%)	6 (2.2%)	-	-	6 (12.8%)

*Other includes participants still waiting for results and those who received a false positive result as well as missing value.

4. THE PATIENT'S EXPERIENCE OF PRIMARY CILIARY DYSKINESIA: A SYSTEMATIC REVIEW (PAPER 2)

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THIS HAS BEEN SUBMITTED IN THE JOURNAL OF QUALITY OF LIFE RESEARCH AND IS CURRENTLY UNDER REVIEW

Author’s contribution

I had the concept for this study and formulated the research question. I wrote the protocol, conducted the data synthesis and wrote the manuscript. I was assisted by BR who completed the search and data extraction independently to ensure accuracy of reporting.

4.1 Abstract

Background: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterised by progressive sino-pulmonary disease, with symptoms starting soon after birth. The aim of this study is to critically review, analyse, and synthesise the literature, in order to understand the experiences of patients with primary ciliary dyskinesia (PCD) and the impact on health-related quality of life (HRQoL).

Method: MEDLINE, EBSCO, Cumulative Index to Nursing and Allied Health Literature (CINAHL), PsycINFO and EMBASE were searched according to the inclusion criteria. A qualitative analysis of 14 studies was conducted.

Results: Fourteen studies were included in the review, five with qualitative methodologies. Studies originated from the UK, USA, Italy, Denmark and Belgium, one study included a survey distributed internationally. Significant relationships were found between age and worsening of respiratory symptoms, physical, and mental domains of HRQoL, with a greater decline compared with reference populations. Variations between the UK and Italy were found for HRQoL and its correlation with time since diagnosis. PCD was found to have a physical impact in all age groups: patients found it difficult to keep up with others, and found energy levels were easily depleted compared to family or peers. In terms of social impact, symptoms lead to embarrassment and a sense of isolation, with patients concealing symptoms and/or their diagnosis. In turn, isolation was also linked with the lack of public and medical knowledge. In relation to emotional impact, anxiety was reported in a number of qualitative studies; patients were anxious about getting sick or when thinking about their future health. The burden of treatment and factors influencing adherence were also discussed in depth.

Conclusion: HRQoL decreases with age in patients with PCD. For all age groups, PCD was found to greatly impact physical, emotional, social functioning, and treatment burden. More research needed on the psychosocial impact of the illness, disease burden, and its effect on quality of life.

4.2. Background

Primary ciliary dyskinesia (PCD) is a rare, inherited lung disease affecting cilia motility such that mucociliary clearance is impaired. Individuals with PCD often present with unexplained neonatal symptoms such as neonatal cough, rhinitis transient tachypnoea, and pneumonia, often requiring respiratory support (1, 3, 109). Patients continue to have persistent sino-pulmonary symptoms in infancy. Chronic and progressive chest symptoms persist throughout life and include daily wet cough and recurrent chest infections which almost consistently lead to bronchiectasis (4, 66). By adulthood, bronchiectasis is present and some patients develop respiratory failure (1). Upper airway symptoms include rhinosinusitis and recurrent serous otitis media with hearing impairment (37). Situs inversus is found in approximately 50% of cases and situs ambiguous is seen in approximately 10% of cases (8, 37).

Assessment of the prevalence, burden of disease, and prognosis of PCD patients is difficult to determine due to a lack of representative international data. Reported prevalence varies from 1:2,000 to 1:40,000; this could reflect true variability or could be a result of poor access to diagnostic facilities in some areas and countries (110-112). A European Respiratory Society (ERS) Task Force survey of 26 European countries found that PCD is both under-diagnosed and diagnosed late (110).

As in most orphan diseases, research has focused on describing the pathophysiological mechanisms of the illness and improving diagnostics. Few studies have examined the psychosocial impact of the illness, disease burden, and its effect on health-related quality of life. This was highlighted by McManus back in 2003 (113), where a systematic search found that no studies reporting data from the patient perspective or on the impact of PCD on daily functioning, mental health and well- being.

The overall aim of this study was to synthesise the results from both qualitative and quantitative studies which examine the psychosocial impact of PCD. Through this synthesis, we evaluated qualitative studies documenting the experiences and views of PCD patients, the impact of the condition on their daily lives, in addition to HRQoL and any influencing factors. We included all age groups (adult, child, adolescent) and parents of PCD children. The qualitative studies allowed us to identify the most salient themes among age groups through interviews and focus groups analysis. The quantitative studies allowed us to compare patient-reported outcome PROMs and factors influencing variability. Finally, through this synthesis, we assessed the quality of the studies and made recommendations on future research needs.

4.3. Method

4.3.1. Search Strategy

The systematic review was conducted using the Preferred Reporting Items for Systematic Review and Meta-Analyses Approach (PRISMA)(114). The following electronic databases searched for papers published in the English language from inception until September 2015: MEDLINE –EBSCO, Cumulative Index to Nursing and Allied Health Literature (CINAHL), PsycINFO and EMBASE. Key words and subject headings/MeSH terms searched in titles and abstracts using various combinations included: “ciliary dyskinesia, primary”, “ciliary motility disorder”, “Kartagener’s syndrome”, "primary ciliary dyskinesia", “perspective”,

"perception", "knowledge", "opinion", "psychological", "experience", "attitude", "impact", "view", "idea", "quality of life", "QOL", "HRQL", "patient report", "belief", and "awareness".

4.3.2. Inclusion and Exclusion Criteria

Inclusion criteria were primary studies that reported on experiences and perspective of PCD patients of all age groups, or where patients completed patient reported outcome measures (PROMs) as primary or secondary outcomes. Quantitative, qualitative, and mixed methodologies were considered equally. PROMs were operationalised as generic HRQoL questionnaires, e.g. Short-Form-36 (SF-36), and disease-specific HRQoL questionnaires, e.g. St George's Respiratory Questionnaire (SGRQ), Leicester Cough Questionnaire (LCQ). Measures of psychological distress, e.g. Child Behaviour Checklist questionnaire and Parenting Stress Index-Short Form were also included. Qualitative studies and mixed-methods studies with a significant qualitative component were considered for inclusion if the number of participants was greater than one, and if sufficient methodological details and data were provided. Non–primary research articles (letters, commentaries, and reviews) were excluded.

4.3.3. Search Outcome

The initial database search generated records from which articles were initially identified through screening of titles and abstracts (LB and BR). Following removal of duplicates, full text papers were read by two authors (LB and BR) to determine eligibility for inclusion. Discrepancies about whether a paper met the inclusion criteria were discussed with a third author (JSL) and a final decision was based on consensus. References of the full text articles

assessed for eligibility were hand checked to identify further papers that satisfied selection criteria.

4.3.4. Data Extraction and analysis

The following data from included papers were extracted: author, date and location of study, aim, sample, design and methods, data collection and analysis, and results.

Data from included studies were systematically extracted using a standardised tabulated form (Table 4) by LB and BR independently, and then discussed and combined. Data was extracted on the results from HRQoL measures and PROMs. For qualitative studies, extracted data was compared across studies and grouped into themes to describe the issues pertinent to PCD patients.

4.3.5. Quality Appraisal

Quality appraisals of data from both the qualitative and qualitative studies were independently assessed by LB and BR. The criteria for assessing the quality of quantitative studies as previously used by researchers (115-117) included: study design, participants and recruitment, comparison group, number of participants, and quality of instrument used (Table 3). The total quality score ranged from 0-15 with each of the 5 criteria being scored from 0 to 3. Quality assessment of qualitative studies was performed using the Consolidated Criteria for Reporting Qualitative Health Research (COREQ) (105).

4.4. Results

4.4.1. Study selection

The initial database search generated 260 records from which 32 articles were initially identified through screening of titles and abstracts as potentially relevant (Figure 6). Removal of duplicates resulted in 26 papers of full text. Fourteen papers were identified for inclusion, two of which were conference abstracts where the full results were not available. No further papers were identified where references of the full text articles were hand checked.

Studies included samples from the UK (78, 79, 113, 118), Italy (77, 107, 119, 120), Denmark (121), and Belgium (122, 123) with a collaborative study including participants from the UK and North America (103, 124) and an international study including participants from 25 countries (112). Six studies consisted of cross-sectional surveys, two of which compared the PCD sample results with reference population norms (107, 113, 118, 119, 122, 123), one was a longitudinal survey given at two time points (120), two were case-control design including health samples for comparison (77, 121). Four used a qualitative approach (78, 79, 103, 124) and one used a mixed method approach (112). Three of the UK studies (79, 113, 118) were carried out using the same study population. The two Belgian studies were also conducted on a shared sample. Sample sizes ranged from 5 to 270. Apart from one study, gender was reported and all studies included both male and female participants.

4.4.2. Methodological quality

Quality appraisal of the quantitative studies points to deficits, in particular to study design, and recruitment and inclusion of a comparison group (Table 5). Psychometric properties of the measures were cited in five of the quantitative studies; however the internal reliability of the measures, i.e. Cronbach's alpha within the sample population was not reported in any of the studies. The application of the measures was not clear for all studies. For some studies, measures used were not developed/adapted and validated specifically for younger children.

Most studies were surveys (with one being longitudinal) and although it was apparent that these studies were cross-sectional, this was not stated explicitly in all. The study which ranked the highest had a score of 8 out of 15 points; this was a cross-sectional survey study where 78 patients completed a questionnaire which collected information on age of diagnosis, symptoms and likely PCD-specific problems, in addition to disease specific and general HRQoL (using the St George's Respiratory Questionnaire and the Medical Outcomes Study Short Form-36 (SF-36)). Use of the SF-36 allowed for scores to be compared with the healthy Italian population.

For the five qualitative studies, criteria of the COREQ-32 item checklist were generally adhered to (Table 4). The main deficits in reporting were: the characteristics of the research team and the relationship between interviewee and interviewer, description of the coding tree, and the provision of feedback to the interviewee following transcription (Table 6).

4.4.3. Methodologies of Quantitative and Qualitative Studies

Six studies assessed HRQoL in PCD patients. HRQoL measures are generic or disease specific. Disease-specific measures assess special states and concerns of different diagnostic groups and are important for the detection of small clinically important changes. The most commonly used disease specific HRQoL measure in this review was the St George's Respiratory Questionnaire (SGRQ) for chronic obstructive pulmonary disease (n=6). Other disease-specific outcome measures used included the HRQoL measure for cystic fibrosis (CFQ-R) (n=1), a HRQoL measure for sinonasal conditions: The Sino-Nasal Outcome Test (SNOT-22) (n=1), and the Leicester Cough Questionnaire (LCQ) (n=1). To assess the impact of PCD on HRQoL, related to mental health and wellbeing, the Medical Outcomes Study Short Form 36 (SF-36) was used in five of the studies.

Other patient-reported measures other than HRQoL included the Wechsler Intelligence Scale for Children, the Child Behaviour Checklist questionnaire, the Parenting Stress Index-Short Form, and the Self-reported Physical Activity Measure. One study included a questionnaire measuring Stigma (118). This was developed by the author and stigma was assessed by the patient's response to 11 items on embarrassment about symptoms, feeling a nuisance to friends or family, concealment of condition etc. Four of the studies were cross-sectional, single-occasion, single-centred studies. One study was a cross-sectional, single-occasion, single-centred case-control study, and one was a longitudinal, single-centred study with measures repeated after 1 year.

4.4.4. Main themes

4.4.4.1. Factors influencing HRQoL

In a cross-sectional UK survey (113), a slight decline in HRQoL was found for all three domains of the SGRQ (Activity, Impacts and Symptoms) until the age of 25 years after which a more rapid decline occurred. The physical component score of the SF-36 also showed a continual decline with age so that by the age of 40 onwards, the health status of PCD patients was one and a half standard deviations below the population mean. In contrast, the mental component score also declined with age however the declining health status broadly parallels that found in the general population as a whole, and was, at the most, one third to one half a standard deviation below the population norms. Age was also an important factor in an Italian cross-sectional survey study (107), where all three subscales of the SGRQ and the physical and mental component scores of the SF-36 declined significantly greater than norms for the corresponding Italian population. These declines, however, were found to be earlier in age than those reported in the UK study (113), where deterioration mainly

occurred prior to and during adolescence. In the UK study, little abnormality was found for the childhood and adolescence study population when compared to standard measures of the SF-36.

Both studies found that patients with an earlier diagnosis had better scores for the SGRQ Impact and Activity subscales, suggesting the importance of early medical intervention for HRQoL. The Italian group found a clear majority of patients (71.8%) considered their HRQoL significantly or slightly improved after diagnosis; however there remained a progressive worsening of the disease over time. This was in contrast to the UK group who reported stable scores for patients after diagnosis.

4.4.4.2. Physical Impact

Ten studies addressed the impact of PCD on physical functioning (78, 103, 107, 112, 113, 118-121, 124). The physical impact of PCD was reported by children, teenagers, and their parents in a qualitative study using phenomenological analysis methods (78). Coughing was regularly mentioned by all participants in their accounts of daily activities, as was the impact of their cough on activities when both well and unwell. Symptomatic relief of chest symptoms was reported as leading to a sense of freedom at being able to undertake activities without restrictions. Patients reported feeling limited in their ability to keep up with peers because of coughing, breathlessness, fatigue and low energy levels. Similar themes arose in two collaborative qualitative studies, which included interviews with patients from the UK and North America (103, 124). Children and teenagers reported they became tired quickly when engaging in physical exercise and needed to request more breaks than their peers. This

theme relating to the physical impact of PCD was also found in adult interviewees, where patients reported not being able to keep up with others when walking or exercising (Box 1).

Box 1: Patient experiences of the physical impact of PCD

A: "I go running again and then cough a bit and then I'll stop" Child (78)

B: "I had to tell the group not to worry because I start huffing and spluttering as I'm walking." Adult (103)

"My air goes out because I'm running and I can't speak and then I'm not speaking and sometimes my air goes down a bit and then I can't, and then I just can't, I can't, I can't take it." Child (78)

"…if he's playing in school and …he needs to run around, then he gets more tired than other kids and they're still running around and he's stopping." Parent (124)

In a quantitative study, 10% of patients were found to be moderately-to-highly limited by respiratory symptoms in everyday activities, and 52% of cases had moderate-to-severe limitations in performing vigorous activities (119). This was in contrast to a Dutch study where 34% of patients reported being moderately-to-highly limited by sinopulmonary symptoms in activities of everyday-life, and 39% reported moderate-to-severe limitations in performing vigorous activities. None of the healthy controls reported any limitations in physical abilities.

As reported previously, a continual decline according to age in scores on the physical domain of the SF- 36 reflected a moderate degree of morbidity on normal physical functioning which is progressive across the lifespan (113). Cough, on almost all days of the week in the last 12 months, was the most frequently reported symptom (48.7% of patients) regardless of age, together with excessive sputum (57.7% of patients) (107).

4.4.4.3. Emotional impact: Frustration, anxiety and stress

The emotional impact was explored in depth in three of the five the qualitative studies (78, 103, 124). Interviews in the UK and North America, explored the emotional impact of PCD

in all age groups (103, 124). In the paediatric group, frustration relating to treatment burden was a prominent theme. Children and adolescents reported feeling frustrated about getting sick regularly and about the chronic nature of their symptoms. In addition, a sense of injustice and sadness about having this condition was reported. A UK qualitative study (78) found that children and teenagers became anxious when thinking about their health in the future. The positive changes which had arisen from their diagnosis and effective health care, while appreciated, induced a level of doubt and anxiety as to how these improvements could be sustained. Such feelings of anxiety were also found in a series of interviews with adult patients (103). This was especially the case when thinking about their future and future health. They reported feeling anxious about being able to conceive children as well as being well enough to care for their family (Box 2).

Box 2: Patient experiences of the emotional impact of PCD

A: "I was sick on and off…it's just frustration. Because there's no cure." Adolescent (124)

B: "Sometimes, when he sees his friends running around and he can't tag them, then he feels like 'why do I have PCD?' Parent (124)

C "It…just wastes all of my energy, it makes me feel like I don't want to wake up in the mornings" Child (124)

D "I'm so frustrated with this illness, I just want it to go away, but, unfortunately, that's how I have to live." Adult (103)

E "…if you go to the doctor [and] you're feeling pretty good and you know your numbers are not good; that can be a big cause of anxiety." Adult (103)

F "Finding out that I possibly can't have kids; that are when it started to panic me a little bit." Adult (103)

G "I'm still very uncertain if I ever wanna have children because I don't know how me having this illness will affect them." Adult (103)

Carotenuto et al., conducted a behavioural and psychological evaluation of children with PCD and compared the results to healthy children (77). The findings showed no clinically relevant scores for both healthy and PCD groups. However, higher scores were found in the PCD group for factors such as withdrawnness, somatic complaints, anxious/depressed items, attention span, and internalising problems items ($p<0.05$). This study also found that total stress levels (assessed through the parenting stress index-short form (PSI/SF)) in mothers were significantly higher in the PCD group than in mothers of healthy controls ($p<0.01$), and that all PCD mothers had high levels of stress.

4.4.4.4. Social impact: Stigma, embarrassment and concealment

In the qualitative studies, symptoms such as coughing, sputum production, and ear drainage were reported as causing embarrassment among paediatric patients (78, 103, 124). Acceptance of coughing was found to be variable among participants and depended on severity. There was also a sense of revulsion from coughing up sputum. Symptom relief led to patients feeling 'normal'(78) (Box 3). Paradoxically reluctance to adhere to treatments was also attributed to wanting to feel normal (122, 123). Adult patients also reported feelings of embarrassment (79). In a study assessing stigma (measured using a questionnaire developed for this study) (118), 75% of the sample agreed that their coughing or breathing was embarrassing in public. It also found that stigma correlated with symptoms and impact of illness from the SGRQ but not with activities. It also correlated with the mental health component scores of the SF-36 but not for the physical component scores.

Paediatric patients were found to be reluctant to share their PCD diagnosis with teachers and peers or even to talk about their condition at home (78). In a separate UK study of patient ≥10 years, 45% of patients agreed in a study specific questionnaire that they have sometimes felt

they had to hide their condition from other people (118). Following on from this, a qualitative study (79) found that some patients felt frustrated by lack of knowledge of PCD in the general public. While some interviewees were keen to educate others and were open to discuss their illness, others were more censored, and avoided describing their symptoms. The likelihood of disclosure may be dependent on context, since some patients felt under pressure to disclose their diagnosis, for example to teachers or work managers on an account of needing time off when ill. There were other patients who reported avoiding open disclosure, particularly when at school (Box 3).

C: "I feel like I'm being judged by other people because I constantly sniff and…cough." Teenager (118)

D: "If she has a speech problem or…coughing constantly…when they're in school, it might become embarrassing." Parent (118)

4.4.4.5. Lack of PCD awareness among medical practitioners

A mixed method study (112) reported the accounts of 20 adult patients and parents of children and teenagers from 9 different countries on their experience of being diagnosed with PCD or going through the diagnostic process. The most prominent theme reported among interviewees was frustration due to the lack of PCD awareness among medical practitioners,

manifesting initially in the failure of general practitioners (GPs) to refer them for PCD diagnostic testing. This was also found in a UK based qualitative study, using grounded theory analytical methods (79) where failure to diagnosis PCD until later in life left some patients feeling distrustful of medical care. Themes such as distrust in GPs, difficulty getting antibiotics, and isolation due to poor communication between GPs and specialists was reported by both studies (79, 112).

4.4.4.6. Treatment adherence and treatment burden

Barriers to completing treatments included being too busy, forgetting about treatments, family issues, and treatments taking too much time. A range in the levels of agreement were found between self-reported and prescribed treatment, ranging from 39% for eardrops, to 71% for antibiotics, and 89% for physiotherapy (122, 123). For adolescents, 57% agreed that their PCD team do not understand how difficult it is to follow treatments, and 43% felt that having to follow the PCD treatments meant less freedom in life. The difficult of fitting treatments in on a daily basis was reported by 12/20 adolescences interviewed across the UK and North America (124). Interviews with adult PCD patients also reported the challenges of completing their treatments (103, 112) (Box 4).

There was also agreement among parents of children with PCD (76%) that barriers to completing treatments meant less freedom in life (122). Parents expressed how other commitments, such as siblings and employment, could limit their ability to complete daily treatments (78, 124) (Box 4).

Patients did report that following a PCD diagnosis, treatments could reduce symptoms providing sensations of relief. There was a subjective perception of physiotherapy treatments, corresponding to fluctuating levels of motivation. There was also a variance in the different

levels of PCD health literacy knowledge in the preventative nature of physiotherapy among children and teenagers (78). In a cross-sectional survey study (122), 86% agreed that they had difficulty complying with treatments because they made them feel physically worse; however 96% of patients acknowledged their health would decline without treatments. In the mixed methods study by Behan et al. (112), in a study specific survey a significant difference was found between those who were diagnosed in childhood (0-12 years) and those who were diagnosed in adolescences/adulthood (>13 years) on the level of agreement that health has improved since diagnosis (p=0.041).

Box 4: Patient experiences of treatment burden

A: "I think it just requires more planning. I need to wake up earlier or start getting ready for bed earlier, I need to come home from work and do this; it's just more planning." Adult (103)

B: "It was a bit of a shock…. I was probably in my mid-thirties then, to suddenly be told, right, you've got to do twenty minutes of physio twice a day, you've got to take this blue puffer, and the brown puffer… as soon as you get a chest infection you've got to take really strong antibiotics, I rebelled against that" Adult (118)

C: "She was sick every month. Once we had a diagnosis… she gets sick, but not as severe as… before." Parent (112)

D: Definitely milder…you know we have a treatment plan and even when she starts to get sick; those medications are changed so we tend to catch that right away rather than after that." Adult (118)

4.5. Discussion

This systematic review identified 14 studies focussing on the perspectives, opinions, and attitudes of patient with PCD. Most of the quantitative studies consisted of small cross-section surveys and the methodological quality of these studies was generally low (Table 5). While the qualitative studies provided a deeper insight into the patient experience, only a small number of these studies exist, and mostly include patients from the UK and North America. Notwithstanding these weaknesses, the evidence assembled from the studies makes an important contribution to understanding the PCD patient experience and associated influences relating to quality of life.

Two cross-sectional studies suggested a correlation between age and worsening of respiratory symptoms, general physical and mental quality of life. Within these two studies, variances exist with Pifferi et al.(107) reporting an early decline in HRQoL and McManus reporting little abnormality in standard measures of SF-36 during childhood and adolescence. Also the variances between the two studies could be due to differences in the age of participants involved or a result of their limited sample size. It could also be due to cultural differences between the countries (UK and Italy), access to specialist diagnostic, and management services or treatment adherence may also account for differences. Caution must be exercised in the interpretation of these findings. Cross-sectional studies do not take into account confounding factors such as differences between adult and child participants and experience which may affect changes over time i.e. diet, tobacco smoke exposure etc. The progressive

nature of PCD and the deterioration of health have been described in other studies through physiological methods such as spirometry (4). Werner et al. (2016)(65) has shown the percentage predicted forced expiratory volume in 1 s (FEV1 % pred) values versus age exhibited a mean annual decline of 0.59%. The results show interesting trends however highlight the need for large longitudinal international studies before more reliable conclusions can be made. The Genetic Disorders of Mucociliary Clearance Consortium (GDMCC), the iPCD cohort and the BESTCILIA registry are examples of ongoing large-scale studies that will contribute to this aim.

The physical impact of PCD was a prominent theme in both the qualitative and quantitative studies. This was defined by the most prominent feature of this illness: coughing. Coughing was regularly mentioned by interviewees of all age groups (78, 103, 124). It was the most frequently reported symptom in a survey of 78 participants, where 48.7% reported having to cough nearly all days of the week for the past 12 months (107). Persistent presence of cough was found to be far less prevalent that in other studies (112, 125) where it was found to be as high as 93%-100%. This could be as a result of the way in which the question was phrased or the method of data collection used, i.e. patient reporting at home or reporting to a clinician in a hospital setting. Severity of symptoms might also reflect different data collection points, with patients on their first referral appointment prior to diagnosis and commencement of treatments exhibiting more severe symptoms. The physical impact of PCD was expressed by patients in the qualitative studies, as not being able to keep up with other family members and peers due to fatigue (78, 103, 124).

Questionnaire findings (77) showed that PCD children were more likely to be withdrawn, experience anxiety or depression, and internalise more problems than the healthy population. PCD was found to affect the parent also with significantly higher stress being reported in

mothers of children with PCD. No other PCD study reports on these factors however studies in children and parents with cystic fibrosis have also reported elevated levels of depression, stress and anxiety compared to healthy populations (126, 127). The synthesis of the qualitative studies allows the researcher to conclude possible reasons for this. PCD impacts greatly on the emotional functioning of patients in all age groups. Children described the frustration of having constant symptoms and recurrently getting sick. Patient anxiety was expressed, especially when thinking about the near and distant future. Children reported feeling worried about their health and of getting sick. A sense of sadness was reported because of their awareness of being different from other children. There is a need for further exploration on how PCD causes stress in developmental ages and the psychological effects of PCD on intra-familiar relationships.

Concealing PCD symptoms such as cough and blowing nose in public were reported across the qualitative studies (78, 79, 103, 124). Embarrassment was mostly from coughing and producing sputum in public however ear drainage was also reported as an embarrassing symptom in one of the paediatric studies(124). The stigma questionnaire (which included items on embarrassment from symptoms and concealment) correlated with mental health and the social impact of symptoms. Although the impact of PCD on school functioning were expressed by patients (103, 124), no differences in educational level or IQ were found between PCD children and healthy children. School functioning instead could be related to patients' reluctance to disclose their PCD diagnosis with teachers and peers. Such concealment of symptoms and illness disclosure has been reported across chronic illness (128-131). Results from a cystic fibrosis study (131) found patients were more likely to disclose to romantic partners and close friends than to casual friends, bosses, or co-workers

and disclosure was associated with higher social support, social functioning, and medication adherence self-efficacy.

Poor adherence to treatments can often be a conscious decision in PCD, however it can be the result of not making any decisions at all e.g. worry about having PCD could lead to attempts to avoid thinking about it. Poor adherence however is likely to lead to raised anxiety about the consequences, which often leads to attempts by the individual to minimise the risks (132). This process is known as cognitive dissonance which refers to the widespread observation that in any situation where people who feel uncomfortable about a choice they have made, also hold a strong desire to resolve this discomfort. Its' resolution is central to motivating patients to change (133). Cognitive dissonance has been reported in cystic fibrosis and has been shown to be the case in PCD through this synthesis of literature. It was reported that symptom relief led to patients feeling 'normal'(78) but there was paradoxically reluctance to adhere to treatments which was also attributed to wanting to feel normal (122, 123).

Patients reported in one study that treatments could reduce symptoms providing sensations of relief. In a cross-sectional survey study (122), 85.7% agreed that they had difficulty complying with treatments because they made them feel physically worse; however 96% of patients acknowledged their health would decline without treatments. There was also a variance different levels of PCD health literacy knowledge in the preventative nature of physiotherapy among children and teenagers (78). The perception of physiotherapy treatments, which corresponding to fluctuating levels of motivation, highlighted the need for patient centeredness and personalised medicine.

4.5.1. Limitations

The review has limitations. Papers included were limited to those published in the English language. It is possible that there are relevant studies published in other languages. Overall the evidence of this review is based on a small number of heterogeneous studies (n=14) that are limited in size. The quality assessment of the quantitative studies revealed them to be of low quality with scores no greater than 8 points. Until recently, no disease-specific age appropriate HRQoL measures were available for PCD patients (103, 124) and to date, studies have used general HRQoL tools such as the SF-36 and disease specific tools for cystic fibrosis and COPD. These studies have also included child participants to complete measures that are not age appropriate without psychometric validation. Studies have included results where young children had help from a parent to complete these measures which may lead to bias (129). Only one of the studies performed analyses with and without the children who needed help completing the questionnaire. In addition, limited psychometric data was presented on the validity of the HRQoL used, with some studies reporting validity but never for all of the scales. As with any review, the quality of studies included can only be assessed by what was reported in the final manuscript; e.g. missing information on any of the adopted criterion might reflect unclear reporting as opposed to a limitation in study design.

4.5.2. Recommendations

To date, no medications to treat PCD have been approved by regulatory bodies (125) and current physiological outcome measures, such as spirometry, chest computed tomography, and lung clearance index have been reported to have limitations in terms of their sensitivity and feasibility for evaluating new therapies or disease progression (66, 74, 76, 134). These physiological measures also do not reflect the impact of the disease on patients' daily symptoms or functioning (e.g., physical, respiratory, social) as required by the Food and Drug

Administration (94) and the European Medicines Agency (92, 93). This study has highlighted the need for large multi-national and longitudinal studies to be conducted using PCD specific HRQoL measures (103). Studies are underway and QOL-PCD has been translated comprehensively into five European languages. These tools have been included in the first international RCT azithromycin study(65). The measures are also being included in an international PCD registry developed as part of the BESTCILIA FP7 study, providing an international platform to systematically collect data on incidence, clinical presentation, treatment, and disease course. Qualitative studies that reflect different ethnicities and cultures are important and necessary to establish the needs and opinions specific to these groups.

4.6. Conclusion

The findings of this review indicate the physical impact, emotional and stigmatising impact of PCD. They highlight the need for well designed, quantitative studies using PCD specific health-related quality of life measures to accurately determine the factors that impact PCD. There is also a need for the experience of patients to be further examined across ethnicities to evaluate various nuances between cultures. This will lead to better care, management, and outcomes for PCD patients.

Figure 6: PRISMA Flow Diagram for search to investigate PCD from the patients' perspective.

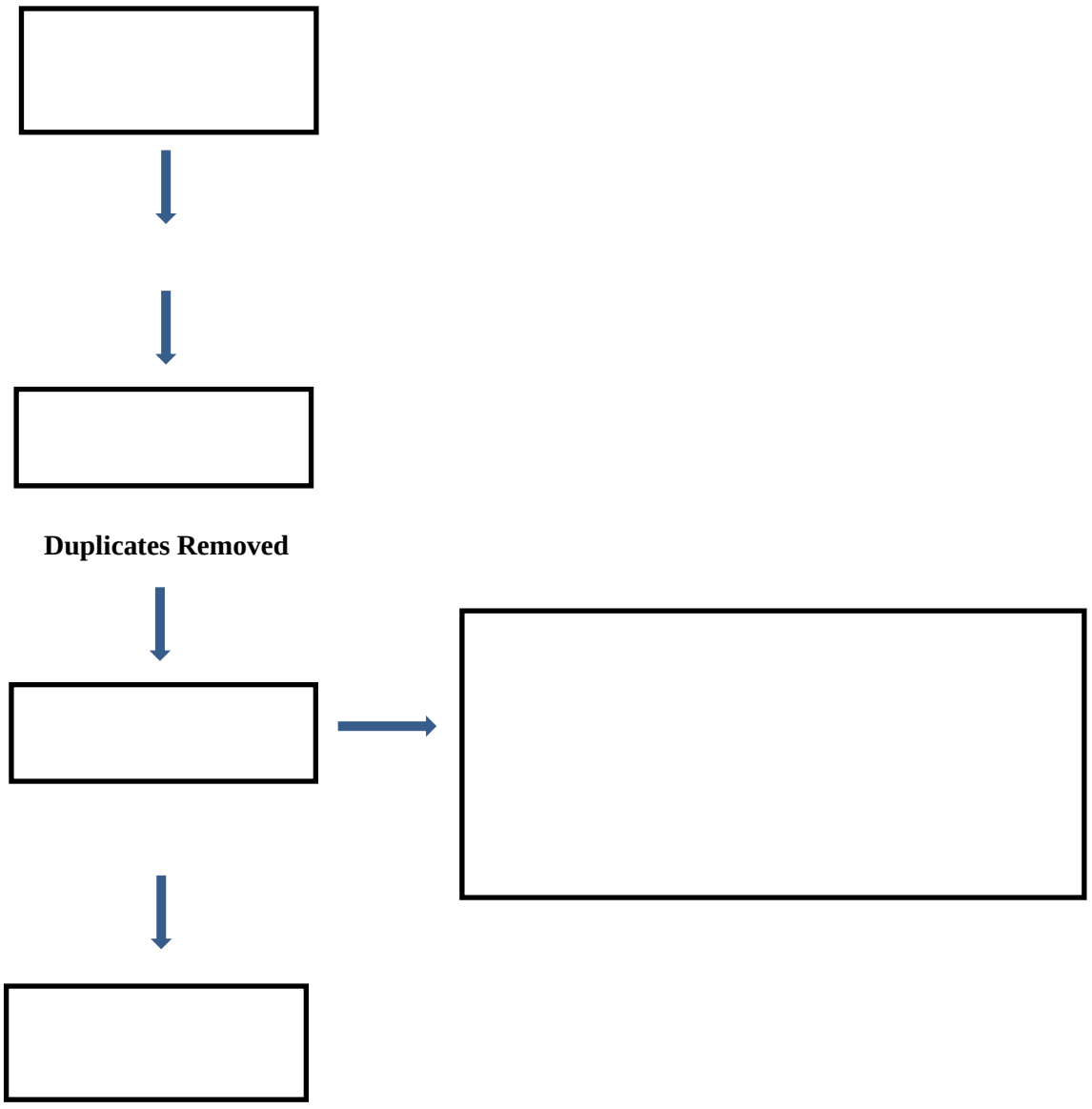

Table 3: Criteria for Rating Methodological Quality of Quantitative Studies

Study parameter	Rating	Criteria
Study design	3	Longitudinal prospective design (explicitly stated)
	2	Retrospective or mixed design (explicitly stated)
	1	Cross-sectional (explicitly stated)
	0	Survey or did not report
Participants and recruitment	3	Description of the population, (2) Eligibility of participants, (3) precise details of the recruitment process, (4) accounted for the number recruited, (5) loss to follow up.
	2	Minimal description of at least four criteria
	1	Two criteria missing
	0	More than two criteria missing
Comparison group	3	Healthy, age appropriate comparison (i.e. adolescents/young people 13-25 years)
	2	Reference sample
	1	Other comparison group (i.e. adults)
	0	No comparison group
Number of participants	3	n>100
	2	n=50-100
	1	n<50
	0	Did not report
Instruments used	3	Psychometrically sound report of instruments used
	2	Some weak psychometric properties reported
	1	Psychometric properties of instruments reported as inadequate for measuring HRQoL or IQ, physical functioning etc.
	0	No psychometric properties reported

Adapted from previously reported studies (115-117)

Table 4: Summary of the 14 studies included in this systematic review, including the aims, study design, analysis, and findings

Study, Year, Country	Aims	Sample	Design	Data collection and Analysis	Findings
Behan et al.(112) (2015) International	Investigate patient opinions about the PCD diagnostic process internationally.	-Survey: 270 PCD patients from 25 countries. - Age: not specified. - Gender: 114/271 males - Interviewed 20 parents/patients. - Gender: 6/20 males - Age: not specified.	Survey, cross sectional and semi-structured interviews	-A patient survey was developed by patient representatives and healthcare specialists to capture experience. - Information collected included age, gender, age at diagnosis, time since diagnosis, diagnostic procedures, number of visits to GP before referral, questions relating to patient's perception on diagnostic process. - Semi-structured interviews were conducted and fully transcribed and thematically analysed	-35% of respondents visited their doctor >40 times with PCD related symptoms prior to referral for testing. -Lack of PCD awareness among medical practitioners and failure to take past history into account leading to a delayed diagnosis. -In the diagnostic process, improved reporting of results and a solution the 'inconclusive' diagnostic status were considered as needs. -A significant difference was found between those who were diagnosed in childhood (0-12 years) and those who were diagnosed in adolescences/adulthood (>13 years) on the level of agreement that health has improved since diagnosis (p=0.041) -Difficulty getting antibiotics, and isolation due to poor communication between GPs and specialists was reported after diagnosis.
Carotenuto et al.(77) (2013) Italy	To perform behaviour and psychological evaluation of children with PCD compared to controls of healthy children. To assess if PCD effects and impacts the quality of family functioning and the psychological equilibrium of children.	-10 PCD and 34 healthy school-aged children -PCD: 7/10 males. -PCD Age range: 8-16 years -Healthy: 24/34 males -Healthy Age range: 6-16 years	Survey; case-control, cross-sectional	-Standardised questionnaires -Children completed Wechsler Intelligence Scale -Parents completed Child Behaviour Checklist questionnaire and Parenting Stress Index-Short Form	-No significant differences between age, gender or BMI, or mother's age and educational level between the 2 groups. -No difference in IQ (WISC-III) between the two groups. -For CBCL no clinical relevant scores were found for both group -Higher scores were found in the PCD group compared to the healthy group for withdrawn, somatic complaints, anxious/depressed items, attention and internalising problems items ($p<0.05$). -Parent Stress Index Short-Form mean scores relating to parental distress, child parent interaction and total stress in mothers was significantly higher in the PCD group -All PCD mothers had high levels of stress.
*Dell et al. (124) (2014)	To develop harmonised (North America, Europe) paediatric HRQoL questionnaires for children (6-12 years), adolescents (13-18 years), and parent respondents.	Age range: 6-17 years Gender: 20/40 males	Focus groups and open-ended interviews.	-Literature review, focus groups (clinician and patient) and semi-structured interviews with children, adolescents and their parents. -Transcripts were content-analysed. -item relevance survey. - Questionnaires refined following cognitive interviews.	-This led to the development of four age-specific preliminary instruments measuring HRQoL in PCD patients. -These consist of a: 1) Child version (6-12 years) (37 items); 2) Adolescent version (13-17 years) (43 items); and 3) Parent version (children aged 6-12 years) (41 items). -Measures consisted of 8-10 scales including: Impact to Physical, Emotional and Social functioning, Vitality, School Functioning, Lower and Upper Respiratory Symptoms, Impact of ear symptoms/hearing loss, Impact of treatment burden, Impact to Eating and Weight.

*Lucas et al.(103) (2015) UK and North America	To develop a PCD-specific HRQoL instrument for adults with PCD	-21 PCD adults -3/21 males -Age range: ≥18 years	Focus groups and open-ended interviews.	-Open-ended interviews - Content analysis yielded the most important items for each of the 10 domains based on the frequency with which they were mentioned. Saturation confirmed when no new themes emerged. - Item relevance survey. - Questionnaires were refined following cognitive interviews.	-10 domains based on frequency with which they were mentioned across adult group. -Cognitive interviews provided 6 additional items. -The final prototype instrument contained 49 items across ten domains and included impact of respiratory symptoms, impact of sinus symptoms, impact of ear symptoms/hearing loss, impact to physical functioning, impact to emotional functioning, impact to social functioning, vitality, health perception, role functioning, impact of treatment burden.
Madsen et al. (121) (2013) Denmark	To assess peak oxygen uptake (VO2peak) to compare these values with those of healthy subjects; and assess if VO2peak is associated with parameters of pulmonary function, self-reported physical limitations, and weekly physical activity.	-44 PCD adults and children matched with 33 healthy controls. -PCD: 17/44 males. -PCD age range: 6.0-29.7 years. - Healthy: 17/33 males -Healthy age range: 6.2-28.8 years	Survey, cross-sectional, case control study	-3 questions about physical activity and limitations were extracted from standardised questionnaires; St George's Respiratory Questionnaire, Cystic Fibrosis Questionnaire, Sino-Nasal Outcome Test-22 and the Medical Outcomes Study Short Form-36	-In response to questions relating to physical activity, 34% of patients reported being moderate to highly limited, 44% were slightly limited while 21% were not limited at all by sino-pulmonary symptoms in activities of everyday life. -39% reported moderate to severe limitation in performing vigorous activities, while 30% only reported only slight difficulties and 30% denied having any difficulties. -VO2peak was significantly lower in patients reporting severe limitations in performing vigorous activities compared to patients without limitations. -VO2peak was significantly lower in patients who reported being highly limited by sino-pulmonary symptoms in everyday-life compared to patients who were not limited at all.
Maglione et al. (2014) Italy	To verify HRQoL in respiratory disorders correlate with spirometry or a 6-minute walk test.	-20 PCD patients. -Gender not specified. -Age range: 12.0-33.4 years	Survey, longitudinal.	-Standardised questionnaires were completed by patient: St George's Respiratory Questionnaire, Leicester Cough Questionnaire, Medical Outcomes Study Short Form 36 at two time points. - Data on spirometry and the 6-minute walk test at 2 time points also collected.	-Spirometry and 6 minute walk test were not significantly related to any of the HRQoL assessment tools at baseline or 12 months later. -Over the 12 month period, no significant changes were found in any of the HRQoL outcomes or in spirometry of 6 minute walk test. -HRQoL tools used reported as suboptimal to longitudinally track HRQoL in PCD.
ŧMcManus et al. (113)(2003) UK	To examine the effect of PCD on the overall health status of patients.	-93 members of UK PCD Family Support Group. -34/93 males. -Mean age = 22.7 (SD 16.8) and median 16.5 (IQR 10.8 - 31.3)	Survey, cross-sectional	-Standardised questionnaires were completed by patient; St George's Respiratory Questionnaire and Medical Outcomes Study Short Form 36. -Separate versions of the questionnaire were provided for adults and children (<16 years).	-SGRQ domains; Symptoms, Activity and Impact scores correlated significantly with age and declined more rapidly after 25 years and more rapidly than population norms. - SGRQ domain: Impact and Activity show effect of time since diagnosis. -Almost all patients reported 'a runny nose and nasal congestion', 'pain over my sinuses' and 'a headache' affected patients a few days a month.

ŧMcManus et al.(118) (2006) UK	To describe the influence of demographic factors, respiratory symptoms, physical and mental health status and stress upon stigma experienced by patients and their relationship with the Big Five measures of personality.	-71 members of UK PCD Family Support Group. -23/71 males. -Mean age = 27.7 (SD 16.2) and median 20.1 (IQR 15.6 - 38.7). (Only respondents' ≥10 years were included.)	Survey, cross sectional	Standardised questionnaires were completed: St George Respiratory Questionnaire, Medical Outcomes Study Short Form 36 General Health Questionnaire, personality ('Big Five'). Stigma using the authors own questionnaire was also. Separate versions were provided for adults and children (under16 years).	-Stigma had no association with age or age of diagnosis. -It correlated significantly with the SGRQ Symptom and Impact of Illness Score but not with Activity Score. -Stigma correlated with the GHQ stress score and with the Mental Summary of the SF-36 although not the Physical Summary scores. -The stigma score correlated with neuroticism measure -Stigma did not differ between males and females or between those with situs inversus and situs solitus. -High Impact measure on the SGRQ and good mental health (SF-36) and Low Activity (SGRQ) are predictors of Stigma.
Mirra et al.(119) (2015)	To investigate if levels of Vitamin D are associated with quality of life and self-reported activity level, among other outcome parameters.	-22 PCD patients, -15/22 males. -Age range: 2-34 years	Survey, cross-sectional	-Standardised questionnaires were completed: St George's Respiratory Questionnaire and Self-reported Physical Activity levels. Patients underwent serum vitamin D levels measurement, pulmonary function tests, deep throat and sputum culture.	-SGRQ score was 19 (9-65). -For physical activity, 10% of patients reported moderately-to-highly limited, 26% were slightly limited and 63% were not limited at all by respiratory symptoms in everyday activities. -52% of cases reported moderate-to-severe limitations in performing vigorous activities, while 26% had only slight difficulties, 21% had no difficulties at all.
Pifferi et al. (107)(2009) Italy	To assess the impact of PCD on HRQoL in Italian patients. To identify the unmet needs of the patients and the potential diagnostic and therapeutic pitfalls.	-78 PCD patients. -34/78 males. -Age range: 1.7 - 48.5 years	Survey, cross-sectional	-Standardised questionnaires were completed: St George Respiratory Questionnaire, Medical Outcomes Study Short Form-36. -Information on age, gender, age at diagnosis, time since diagnosis, clinical features, compliance with treatment, diagnostic procedures, incidences of surgery in patients, PCD in family members also collected. -A questionnaire on clinical course of the disease management, including a question on the patient's perception on quality of life after diagnosis was completed. -Separate age specific versions used.	-All 3 subscales of SGRQ correlated with age. -Cough on almost all days a week was the most frequent reported symptoms (48.7% of patients). -Significant correlation between time since diagnosis and impacts subscale but not for symptoms subscale (SGRQ). -Breathlessness increased with age. -There was a decline in physical and mental component scores (SF-36) in relation to age in PCD patients, significant only for mental component scores. -Age at diagnosis influence on symptoms, activity and impacts (SGRQ) and mental health (SF-36). -Reduced compliance with treatment is associated with mental component scores (SG-36) and age at diagnosis and time since diagnosis. - The majority (71.8%) considered their quality of life to have significantly or slightly improved after diagnosis.

Schofield et al. (78) (2014) UK	To explore the physiotherapy experiences of patients and their parents within the paediatric PCD population in the UK. To identify patients' needs and to make recommendations for future service developments.	-3/5 males, -Age range: 8-15 years (all Asian ethnicity).	Semi-structured interviews.	-Interpretative Phenomenological analysis. -Pilot interview conducted: themes based on concepts from existing literature. -Subsequent interviews involved the participant recounting their daily routine. -A second validation interview discussed key points of the first interview.	-Experience of day to day life with symptoms and treatment burden. -Diagnosis led to symptoms perceived as abnormal -Symptoms reduced since treatment began. Coughing was variable in its acceptance and depended on severity. -Embarrassment from coughing. Revulsion from coughing up sputum. Anxiety looking towards the future and how long-term improvements could be sustained fuelled anxiety. Freedom emerged from being able to engage in activities without limitation. -Participant's self-awareness and self-assessment of symptoms**.** Knowledge of condition and the preventative nature of physiotherapy varied. Limited sharing of PCD with teachers and peers and even at home. -The role of the family, carers and health specialists in nurturing personal mastery skills. Clinics provide knowledge and treatment skills which were then refined into practices that were personally enjoyable and effective.
*Taelman et al.(122) (2014) Belgium	This study aims to investigate and identify attitudes and barriers related to treatment adherence in children with PCD and their parents.	-25 parents of PCD children (<18 years) -7 PCD adolescents -Age range: 14-18 years.	Survey, cross sectional	-A questionnaire consisting of demographic information and treatment related questions. -A list of 18 barriers and 10 statements of attitudinal patterns. -Adolescents completed questionnaire independently.	-The most commonly reported barriers to treatment were too busy, forgetting, family issues, wanting to be normal, takes too much time. - For adolescents, attitudes influencing non-adherence include PCD team does not understand how tough it is to follow treatments (57.1%), wanting to follow my treatments but sometimes just forget (71.4%), trouble sticking to treatments because they make teenager feel worse (85.7%), having to follow treatments means less freedom in life (42.9%).
*Taelman et al.(123) (2014) Belgium	To examined the impact of PCD on daily life by comparing self-reported and prescribed treatment; investigating barriers and attitudes to treatment and exploring coping styles.	-39 PCD patients (25 parents) -13/39 males. -Mean age=33 years.	Survey, cross sectional	- A questionnaire consisting of treatment related questions. - Age, gender, FEV1, types of treatment completed also completed	-Frequency of treatments varied with 82% parents reported daily use of nebulizer; 64% patients reported daily use of nose spray and 46% reported physiotherapy. -Agreement between self-reported and prescribed treatment ranged from 39% for eardrops to 71% for antibiotics and 89% for physio. - Most patients (96%) did not agree that their health will be OK, even if treatments are not done and parents (76%) agreed that having to follow treatments means less freedom in life. -Burden of treatment is related to time and wanting a normal life.

ŀWhalley et al. (79)(2006) UK	Depth-qualitative interviews aimed to explore themes surrounding the psycho-social impact of PCD. A quasi - experimental design was used for directly validating the stigma questionnaire.	-6 pairs (n=12) of PCD patients. -2/12 males. -Aged range: 27-65 years	Depth qualitative interviews followed by stigma rating.	-Grounded theory analytical approach. - Interviews conducted and fully transcribed. -Initial themes under investigation included diagnosis, symptoms and social perspectives surrounding PCD, including the possibility of stigma. - Before each interview, the previous was transcribed and loosely open-coded, with emerging themes compared with the previous interview data. - Comprehensive open-coded once data collection was complete.	- Other people's lack of knowledge of PCD led to frustration in some but other understood this was due it being a rare disease. Some educate others and are open. Others were more censored avoiding describing the stigmatised symptoms such as productive cough. Some were under pressure to disclose while others at some point avoided disclosure particularly when at school. -Most had at some stage tried to conceal symptoms -Embarrassment from symptoms led to behavioural change - Failure to diagnosis PCD until later in life left some feeling mistrust of medical care. Mistrust in GPs; difficulty getting antibiotics and isolation due to poor communication between GP and specialist. However praise of tertiary specialist centre. -Ratings of stigma scales were in complete concordance.

Ł Same UK study population
* Same Belgian study population
* Both publication are part of the same study with UK and US participants however different age groups, therefore separate study populations

Table 5: Summary of the quality of the data and studies contributing to the quantitative studies included in this systematic review. Scoring was according to Criteria for Rating Methodological Quality of Quantitative Studies adapted from previous studies (115-117)

	Study design	Participants & recruitment	Comparison group	Number of participants	Instruments	Total
Behan et al.(112) (2015) International	0	2	0	3	0	5
Carotenuto et al.(77) (2013) Italy	0	0	3	1	2	6
Madsen et al. (121) (2013) Denmark	1	2	3	1	0	7
Maglione et al. (2014) Italy	3	0	0	1	0	4
łMcManus et al.(118) (2006) UK	0	1	0	2	2	5
łMcManus et al. (113)(2003) UK	0	1	2	2	2	7
Mirra et al.(119) (2015)	1	0	0	1	2	4
Pifferi et al. (107)(2009) Italy	1	1	2	2	2	8
*Taelman et al.(123) (2014) Belgium	0	0	0	1	0	1
*Taelman et al.(122) (2014) Belgium	0	1	0	1	0	2

łSame UK sample
*Same Belgian sample

Table 6: Summary of completeness of reporting for the qualitative studies included in this systematic review using the Consolidated Criteria for Reporting Qualitative Health Research (105)

Reporting criteria	No (%) n=5	References of studies reporting each criterion
Characteristic of research team:		
Interviewer/facilitator identified	4 (80%)	23, 24, 35, 36
Credentials	2 (40%)	23, 35,
Occupation	2 (40%)	23, 35
Gender	0 (0%)	-
Experience and training	2 (40%)	23, 35
Relationship with participants:		
Participation knowledge of the interviewer	2 (40%)	23, 35
Interviewer characteristics	3 (60%)	23, 24, 35
Methodological orientation and theory	5 (100%)	23, 24, 34-36
Participant selection:		
Sampling method (for example, snowball or purposive)	5 (100%)	23, 24, 34-36
Method of approach	5 (100%)	23, 24, 34-36
Sample size	5 (100%)	23, 24, 34-36
Non-participation	2 (40%)	23, 24
Setting:		
Setting of data collection	4 (80%)	23, 34-36
Presence of non-participants	1 (20%)	23
Description of sample	5 (100%)	23, 24, 34-36
Data collection:		
Interview guide	5 (100%)	23, 24, 34-36
Repeat interviews	1 (20%)	23
Audio/visual recording	5 (100%)	23, 24, 34-36
Field notes	3 (60%)	23, 24, 36
Duration	2 (40%)	23, 24

Data saturation	4 (80%)	24, 34-36
Transcripts returned to participant	0 (0%)	-
Data analysis:		
Number of data coders	5 (100%)	23, 24, 34-36
Description of the coding tree	0 (0%)	-
Derivation of themes	5 (100%)	23, 24, 34-36
Protocol for data preparation and transcription	5 (100%)	23, 24, 34-36
Software	4 (80%)	23, 34-36
Participants' feedback or member checking	3 (60%)	23, 34, 35
Reporting:		
Participant quotations presented	5 (100%)	23, 24, 34-36
Data and findings consistent	5 (100%)	23, 24, 34-36
Clarity of major themes	5 (100%)	23, 24, 34-36
Clarity of minor themes	5 (100%)	23, 24, 34-36

Part Two: Diagnosing Primary Ciliary Dyskinesia

5. ACCURACY OF DIAGNOSTIC TESTING IN PRIMARY CILIARY DYSKINESIA (PAPER 3)

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Author's contributions

JSL had the concept for this study. My main tasks were to prepare and clean the database used for this study. I also conducted the data analysis and presented the results. I interpreted the results for the discussion section. Together with CLJ and JSL, I was involved with drafting this manuscript. At each peer review, I undertook the recommendations of the reviewers and conducted the analysis accordingly. JSL, WTW, HJE and AH completed clinical assessments (history, nNO, nasal brushing); CLJ, JLC, JT and ECA were responsible for HSV and ALI-culture; PMG and AP were responsible for TEM. All authors contributed to iterations and approved the final version of the manuscript.

5.1. Abstract

Background: Diagnosis of primary ciliary dyskinesia (PCD) lacks a 'gold standard' test and is therefore based on combinations of tests including nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA), genotyping and transmission electron microscopy (TEM). There is little published data on the accuracy of this approach.

Method: Using prospectively collected data from 654 consecutive patients referred for PCD diagnostics we calculated sensitivity and specificity for individual testing strategies. Accuracies of combination testing strategies were compared in a subset of 180 patients who underwent all tests.

Results: HSVMA had excellent sensitivity and specificity (100%, 93%), TEM was 100% specific but 21% of PCD patients had normal ultrastructure. nNO (30nL/min cut-off) had good sensitivity and specificity (91%, 96%). Simultaneous testing using HSVMA and TEM was 100% sensitive and 92% specific.

Conclusion: Combination testing was found to be a highly accurate approach for diagnosing PCD. HSVMA alone has excellent accuracy but requires significant expertise and repeated sampling or cell culture is often needed. TEM alone is specific but misses 21% of cases. nNO (≤30 nL/min) contributes well to the diagnostic process. In isolation nNO screening at this cut-off would miss ~10% of cases but in combination with HSVMA could reduce unnecessary further testing. Standardisation of testing between centres is a future priority.

5.2. Introduction

Recent advances in the diagnosis of patients with primary ciliary dyskinesia (PCD) have included networks of specialists developing protocol-driven testing (14, 19, 20, 135) international consensus guidelines (22), and rapid expansion of known PCD-related genes (20).

There is no 'gold-standard' test for PCD, hence European consensus guidelines (2009)(22) recommend a combination of tests including nasal nitric oxide (nNO) screening (14, 58), high-speed video microscopy analysis (HSVMA) of ciliary beat frequency (CBF) and pattern (CBP)(16, 29, 136, 137), and transmission electron microscopy (TEM) analysis of ciliary ultrastructure (17, 18).

Reanalysis following submerged (138) or air-liquid interface (ALI) (139) 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. The 2009 guidelines (22) also suggest potential adjuncts to diagnosis including immunofluorescence labelling of cilia proteins (60), pulmonary radioaerosol mucociliary clearance (62, 140) and genotyping. Since 2009 there have been rapid advances in the discovery of genes responsible for PCD (20, 135) allowing genetic testing to take a prominent position in some countries, but it is currently not funded in the English public healthcare system. The English PCD service (135, 141) diagnoses PCD using nNO, HSVMA and TEM, with reanalysis following ALI-culture for inconclusive and positive samples.

Several manuscripts have reported the accuracy of individual tests for the diagnosis of PCD, but none have considered all available diagnostic data. Most reports have failed to include

the significant numbers of 'inconclusive' results (142). The aim of this study was to determine accuracy of PCD diagnostic tests (nNO, HSVMA, TEM) when used singularly or in combination based on a large prospective study of consecutive patients referred for diagnostic testing.

5.3. Methods

Local and national research and development and ethical approvals were obtained (Southampton and South West Hampshire Research Ethics 07/Q1702/109).

5.3.1. Participants

Eight hundred and sixty-eight consecutive subjects were referred to the national PCD centre at University Hospital Southampton (UHS) for diagnostic testing between 2007 and 2013; 654 had adequate data and samples for inclusion. The population served by the centre is predominantly White and non-consanguineous. Patients attended UHS or samples were couriered to UHS from satellite referral centres, with no pre-screening of nNO.

5.3.2. Diagnostic testing

The pathway leading to diagnostic outcomes is summarised in Figure 7. Details of the method are provided in Appendix 2.

5.3.2.1. Patients and samples

Patients were required to have been free of infection for ⩾4 weeks. At UHS, demographic and clinical history was recorded using a standard form. At UHS nNO was measured using a chemiluminescence analyser (NIOX Flex; Aerocrine, Solna, Sweden) aspirating nasal air from the nostril at 0.3 L·min−1 during a breath-hold manoeuvre. Based on experience, since

2007 we have considered an arbitrary cut-off of $\leqslant$30 nL·min^{-1} . Following nNO measurement, a nasal brush biopsy provided epithelial cells for HSVMA, TEM and ALI culture. Satellite centres completed patient proformas and brush biopsies were couriered to UHS. Cells for HSVMA and ALI culture were transported in buffered medium within 3 h, while fixed samples for TEM were accepted with longer transportation times.

5.3.2.2. Laboratory analyses

HSVMA and TEM were analysed in blinded fashion by PCD-specialist microscopists (Appendix 2). At least six healthy strips of ciliated epithelium were recorded at 500 frames per second (fps). Sequences were played back at 30 fps to observe the CBP and calculate CBF. CBP was qualitatively assessed as normal, dyskinetic (static, uncoordinated, rotational, reduced beat amplitude, slow or hyperfrequent), valid-inconclusive despite adequate sample or invalid-inconclusive due to inadequate sample. HSVMA was only reported normal if both CBF (normal range 11–20 Hz) and CBP were normal. TEM analysis was carried out if HSVMA was abnormal or inconclusive. $\geqslant$100 cilia were imaged in transverse section at ×60000 magnification for the assessment of axonemal structure. Using in-house normative data, quantitative analysis determined ciliary ultrastructure as normal, abnormal, valid-inconclusive or inadequate-inconclusive. HSVMA and/or TEM were reanalysed following ALI-culture or repeat biopsy unless results were normal.

5.3.2.3. Diagnostic decisions

Data were reviewed at multidisciplinary team meetings, attended by a clinician, an HSVMA microscopist and a TEM microscopist. All clinical and diagnostic data were considered when agreeing the diagnostic outcome as PCD-positive, PCD-negative or inconclusive. Positive diagnosis was reported in patients with typical clinical history, usually with at least two

abnormal diagnostic tests (TEM, HSVMA and nNO), but in patients with a strong history (e.g. sibling with PCD or "full" clinical phenotype (e.g. neonatal respiratory distress at term followed by daily wet cough, persistent rhinitis and glue ear, often associated with episodes of upper and lower respiratory tract infection)), we occasionally reported a positive diagnosis based on "hallmark" TEM or repeated HSVMA consistent with PCD. CBP was considered positive if the pattern was typical of PCD rather than secondary ciliary dyskinesia, determined either from two brushing biopsies or from one brushing biopsy with reanalysis following ALI culture. Negative diagnosis was reported if 1) HSVMA with or without TEM was normal or 2) HSVMA and TEM abnormalities were consistent with secondary rather than primary dyskinesia and normal nNO (if available). A valid-inconclusive diagnosis was reported if, on repeated testing, adequate samples had subtle abnormalities not "classical" for PCD but outside the range of our experience of secondary defects. It was considered that these patients might have subtle or rare variants of ciliary phenotype. Patients were therefore told that the diagnosis was equivocal, with the recommendation that they received appropriate treatment (e.g. airway clearance or treatment of exacerbations). They were investigated for other causes of their symptoms (e.g. cystic fibrosis genotype and immunology) and were kept under review for further testing as new tests become available (e.g. new PCD-associated mutations).

If TEM and HSVMA were inconclusive due to inadequate samples, e.g. sparse cilia, diagnostic outcome was invalid-inconclusive and patients were invited for repeat testing. Patients with normal TEM in isolation were considered invalid-inconclusive, since TEM misses 20-30% of PCD (143). Patients with nNO ≤30 nL/min were deemed likely to have PCD, but it was never accepted as a lone diagnostic test.

5.3.3. Statistical analysis

Data were prospectively recorded in an Access database (Microsoft, Redmond, WA, USA) and exported to SPSS Statistics 21 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6 (La Jolla, CA, USA) for analysis. Additional details are listed in Appendix 2.

The distribution of clinical data was examined by univariate analysis. Prevalence of categorical variables was presented as percentages and Chi-squared and Fisher's exact tests assessed proportional differences. For continuous variables mean±SD with two-tailed parametric (t) or nonparametric (×2, Mann–Whitney) tests were presented. $P<0.05$ was considered statistically significant.

For repeated sampling, the most recent test result was used, although all data were considered by the multidisciplinary team when deciding final diagnostic outcome (Figure 7). Patients with inadequate inconclusive outcomes were excluded for analysis of test accuracy. The sensitivity and specificity of the individual tests were determined firstly based on definite positive or negative diagnostic outcome, and then assuming all valid-inconclusive outcomes to be truly positive or negative (using multidisciplinary diagnosis as a reference standard). Receiver operating characteristic (ROC) curves were constructed for nNO and CBF. Further accuracy analysis was completed using HSVMA and TEM as the reference standards.

Additionally, sensitivity, specificity and predictive values were calculated for those who underwent all diagnostic tests (n=180) and compared with the whole study population (using multidisciplinary diagnosis as a reference standard); the whole population was then further stratified into: 1) the full protocol at UHS or 2) partial protocol when samples were couriered

to UHS (nNO measurements not taken). We also allowed for the fact that those aged <5 years did not have nNO readings measured.

For those who underwent all three tests (n=180), theoretical combination testing approaches (144) were used to determine net sensitivity and specificity of simultaneous (≥2 tests in parallel; positive result if any test was abnormal) and sequential (second test only performed if first test(s) abnormal) diagnostic protocols. Net sensitivity/specificity used the addition rule of probability for simultaneous tests and the multiplication rule of probability for sequential tests.

5.4. Results

5.4.1. Study population

We assessed 868 patients between April 2007 and December 2013 (48% male; median (range) age 7 (0–79) years). 517 (60%) attended the UHS clinic in person (Figure 8a) and 351 samples were delivered by courier (Figure 8b). 75 (9%) patients had a positive diagnosis, 566 (65%) had a negative diagnosis and 13 (1%) had inconclusive diagnostic outcome despite adequate samples. 214 (25%) patients had invalid-inconclusive results due to inadequate data at the time of the study, of whom 113 (13%) patients had only TEM samples sent from satellite clinics. Invalid-inconclusive outcomes were excluded from the analyses, resulting in a study population of 654, of which 641 had a definitive positive or negative outcome. The characteristics of the positive, negative and inconclusive patients are shown in Table 7.

5.4.2. Accuracy of individual diagnostic tests

Analysis was dependent on the quality of the sample and many patients required repeat biopsies: 17% (113 out of 654) required one repeat; 2% (11 out of 654) required two repeats;

and 0.4% (three out of 654) required three repeats. Analysis of diagnostic accuracy was based on the final successful test completed.

5.4.2.1. Nasal nitric oxide

nNO was measured in 301 (47%) patients with a positive or negative diagnosis. nNO was significantly lower in PCD-positive patients (17±20 $nL \cdot min^{-1}$, 95% CI 10–23 $nL \cdot min^{-1}$) than negative patients (172±94 $nL \cdot min^{-1}$, 95% CI 160–183 $nL \cdot min^{-1}$) ($p<0.001$) (Appendix 2, Figure A2). ROC curve analysis showed low nNO to be a strong predictor of a multidisciplinary diagnosis of PCD (area under the curve 0.97, 95% CI 0.94–1.00) (Figure 9). A cut-off of 30 $nL \cdot min^{-1}$ was sensitive (0.91, 95% CI 0.76–0.98) and specific (0.96, 95% CI 0.93–0.98) (Table 8). Inclusion of eight valid-inconclusive results as PCD-positive (109.7±119 $nL \cdot min^{-1}$, 95% CI 10–209 $nL \cdot min^{-1}$) reduced sensitivity to 0.78 (95% CI 0.62–0.89) (Appendix 2, Table A3).

5.4.2.2. High-speed video microscopy analysis

HSVMA was performed in 625 (98%) patients including 60 PCD-positive and 565 PCD-negative cases. HSVMA was abnormal in all 60 positive patients tested. Of 565 PCD-negative patients, 39 had abnormal or equivocal HSVMA results (17 had abnormal CBF and 22 had abnormalities of CBP). HSVMA had excellent sensitivity (1.00, 95% CI 0.94–1.00) and specificity (0.93, 95% CI 0.91–0.95) (Table 8). Since our definition of PCD includes abnormal ciliary function (pattern or frequency), sensitivity would be expected to approach 1.00. Inclusion of valid-inconclusive results as PCD-positive kept sensitivity high at 0.97 (95% CI 0.90–1.00) (Appendix 3, Table A3).

Subgroup analysis (e.g. UHS versus courier-delivered or age <5 years) made little difference to the sensitivity, specificity or predictive values (Tables 8 and 9). The mean CBF for PCD-positive patients (2.3±5.2 Hz, 95% CI 0.4–4.3 Hz) was significantly lower than for PCD-

negative patients (15.4±2.3 Hz, 95% CI 15.2–15.6 Hz) (p<0.001) (Appendix 3, Figure A3). ROC curve analysis showed CBF to discriminate well between PCD-positive and -negative patients (AUC 0.92, 95% CI 0.79–1.00) (Figure 9). However, it was not possible to derive a reliable CBF for 31 (41%) PCD positive patients with variable CBP.

5.4.2.3. Transmission electron microscopy

TEM was performed on samples from 368 (57%) patients including 72 PCD-positive and 297 PCD-negative cases. 57 (79%) out of 72 PCD-positive patients had hallmark ultrastructural defects of PCD: 31% with outer dynein arm (ODA) and inner dynein arm (IDA) defects; 26% with an ODA defect; 10% with an ODA defect and suspected IDA defect; 7% with microtubule disarrangement and IDA defect; 4% with an intermittent central pair microtubule defect; and 1% with a microtubule transposition defect. 21% had "normal" ciliary ultrastructure. None of the 297 PCD-negative patients had ultrastructural changes suggestive of PCD, but secondary changes (e.g. swollen membranes or compound cilia) were fairly frequent. TEM sensitivity was 0.79 (95% CI 0.68–0.88) and specificity was 1.0 (95% CI 0.99–1.00) (Table 8). Again, subgroup analysis made little difference to the sensitivity, specificity or predictive values (Tables 8 and 9).

5.4.2.4. Air-liquid interface culture

ALI culture was done on 808 samples and 241 ciliated (30%). Ciliary function was reanalysed following ALI-culture in 152 (24%) patients. ALI-samples confirmed a persistent abnormality of CBP in 21 out of 21 PCD-positive patients. Out of 124 PCD-negative patients, 123 had a normal CBP following ALI culture, and one patient had uncoordinated cilia, perhaps due to variable cell health.

5.4.3. Accuracy of combinations of tests

Various combinations of diagnostic tests are undertaken at our centre (Figure 2) whilst alternative combinations are used in other centres (23). We calculated accuracy for the combinations of tests in 180 patients who had undergone all diagnostic tests, allowing us to consider theoretical scenarios (Table 10). If nNO had been used as a screening test followed sequentially by TEM, 36 patients would have proceeded to TEM, but three out of 31 PCD patients would have been "missed" by not proceeding to further testing due to false negative nNO results; TEM would have subsequently failed to identify nine PCD patients. The net specificity for this combination of tests was excellent (100%), but net sensitivity was poor, failing to identify PCD in 12 (39%) out of 31 patients. Alternatively, if nNO had been used as a screening test followed sequentially by HSVMA, three out of 31 PCD patients would not have proceeded to HSVMA; however, HSVMA would have subsequently identified all 28 positive patients. Therefore, the net sensitivity and specificity were 90% and 100%, respectively. Excellent net sensitivity and specificity were achieved upon simultaneous testing of HSVMA with nNO (100% and 87%, respectively) or HSVMA with TEM (100% and 92%, respectively) or all three tests (100% and 87%, respectively).

5.4.3.1. Accuracy of individual tests using HSVMA or TEM as the 'reference standard'

We calculated the accuracies of individual tests assuming HSVMA and TEM to be the reference standard for diagnosing PCD. When TEM analysis was considered as the reference standard, HSVMA sensitivity and specificity were 1.00 and 0.78, respectively, and nNO ($\leqslant$30 nL·min^{-1}) sensitivity and specificity were 0.95 and 0.89, respectively. When HSVMA was considered as the reference standard, TEM sensitivity was 0.37 (95% CI 0.29–0.47) and specificity was 1.00 (95 % CI 0.98–1.00); nNO ($\leqslant$30 nL·min^{-1}) sensitivity was 0.50 and specificity was 0.96 (Appendix 2, Table A4).

5.5. Discussion

Our large cross-sectional study provides prospectively collected outcome data following a comprehensive range of PCD diagnostic tests. Our diagnostic algorithm varies from some centres, (13) but the findings may contribute to the development of international consensus.

A strength of this study was analyses of consecutive referrals within a national diagnostic program, as this is likely to yield the most valid estimates of diagnostic accuracy. Although not all patients underwent all tests, this pragmatic study reflects the real patient journey. The major limitation is the lack of a 'gold reference standard'; we therefore used a surrogate standard of expert multidisciplinary consensus. Since each test contributes to the final decision, sensitivity and specificity might be over-estimated. Also, genetic testing does not currently form part of our diagnostic pathway and this is a rapidly expanding area that is used for diagnosis in many countries.

11.5% of patients with adequate samples were diagnosed as PCD-positive, which is slightly lower than some centres,(29, 58, 145), but is similar to or higher than others (Switzerland, Amsterdam and London (Claudia Kuehni, Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland; Eric Haarman, VU University Medical Center, Amsterdam, the Netherlands; and Claire Hogg, Royal Brompton & Harefield NHS Foundation Trust, London UK; personal communications). Higher positive rates may be seen at centres with nNO pre-screening, in consanguineous populations (11) or if access to testing is restricted to those who are extremely likely to have PCD. The prevalence in referral populations will not affect the sensitivity or specificity but will alter the positive and negative predictive values of the tests.

5.5.1. High-Speed Video Microscopy Analysis

HSVMA was sensitive and specific for diagnosing PCD; however, if used as a reference standard, this would lead to a high number of false positive results. In line with many European centres we consider HSVMA a first-line test, which might inflate the sensitivity; this needs further investigation in blinded studies. Since HSVMA is a qualitative test with potential subjectivity, results are regularly validated by external experts, but this does not exclude the possibility of some false negative findings. PCD-negative patients all had predominantly normal HSVMA, but often included a proportion of dyskinetic cilia probably due to recent infection or damage during sampling (146). Some PCD patients had areas of apparently normal ciliary function, highlighting that PCD-scientists require significant experience of the qualitative and quantitative range of PCD and non-PCD samples.

HSVMA standardisation is challenging, and robust training, data validation, external audit and continued learning is in place. Our data cannot be generalised to centres where different standards apply. HSVMA requires expensive high-speed video equipment, high optical magnification and digital resolution for accurate CBP analysis, without which errors are likely. CBF is pH and temperature dependent, and we conduct analyses using pH-stable medium equilibrated to 37°C (147, 148).

Inadequate samples and inconclusive results were a common issue for HSVMA. Recent reports of PCD-causing genes associated with subtle changes at HSVMA and TEM (149, 150) have confirmed our suspicion that some inconclusive results might represent disease. Moreover, samples providing inadequate cilia, may be caused by mutations causing a syndrome similar to PCD associated with sparse but normal cilia.(151, 152) It is possible that some patients excluded from analyses due to inadequate samples will fall into this

category. However, in our experience, inadequate samples are commonly adequate on repeat brushing following antibiotics and when patients are well. We always recommend repeat testing for inadequate samples; some invalid-inconclusive cases did not return for testing because symptoms had resolved or an alternative diagnosis was identified.

5.5.2. Transmission Electron Microscopy

Approximately a fifth of PCD patients had apparently normal ciliary ultrastructure confirming that TEM is unreliable in isolation (143, 153). However, it is a vital part of the diagnostic portfolio, supporting HSVMA findings and providing a diagnosis when HSVMA is not available or inconclusive. Analysis of ciliary ultrastructure requires expensive equipment and electron microscopists experienced in the range of normality and abnormality. Some abnormalities are straightforward (e.g. ODA defect), but we have diagnosed several patients with subtle abnormalities of microtubules, supported by nNO and HSVMA, that would only be detected by an experienced microscopist analysing sufficient numbers of cilia in transverse and longitudinal section.

5.5.3 Nasal Nitric Oxide

nNO is a recommended screening test for symptomatic patients, (22, 53, 58). At the outset of the prospective data collection in 2007, a cut-off of 30 $nL \cdot min^{-1}$ was arbitrarily set based on prior experience. Recent evidence suggests that higher cut-offs may be more useful (14, 53), and the accuracy of nNO cut-offs for screening/diagnostics needs to be standardised based on emerging evidence. 30 $nL \cdot min^{-1}$ was used clinically throughout data collection and so it is on this basis that we have analysed the data. The sensitivity and specificity of this cut-off were 0.91 and 0.96, respectively. Therefore, 9% of cases might be missed if further testing was excluded on the basis of this test in isolation. 77 $nL \cdot min^{-1}$ has recently been

recommended as a cut-off (14); this cut-off improved sensitivity in our population to 96%, but reduced specificity to 83%. In Leigh et al's study (14) sensitivity to detect patients with PCD diagnosed by TEM or genetics was 0.98 whilst specificity was >0.75, similar to our findings. In our centre we are confident to use a cut-off of 30 $nL \cdot mL^{-1}$ because it is always alongside a HSVMA result. In our opinion, if nNO is used by referral centres to decide who to refer for testing, the higher cut-off with greater sensitivity should be used, but it is notable that 4% of cases might still be missed. We use nNO to support a positive diagnosis in patients with consistent subtle abnormalities of CBP who might otherwise be labelled inconclusive. We would be cautious to exclude a diagnosis of PCD in patients with nNO $\leqslant$30 $nL \cdot min^{-1}$, and these patients are more likely to be considered inconclusive and therefore undergo repeated testing. Only 47% of the study population underwent nNO testing, because it is not available at satellite centres and the breath-hold manoeuvre is usually technically acceptable only in those aged >5 years. However, the present article reports nNO data from 301 participants, which constitutes the largest study to date in a PCD diagnostic clinic population.

5.5.4. Accuracy of combinations of tests

Data from patients who had undergone all tests (n=180) was used to calculate the accuracy for possible combinations of tests. Two-stage testing based on nNO pre-screening followed by TEM potentially missed ~40% of PCD cases, because both tests were required to be positive for a positive diagnosis (144). However, our 30 $nL \cdot min^{-1}$ cut-off is probably too low for use as a screening threshold [34] and the previously discussed subjectivity of HSVMA needs to be taken into account. Simultaneous testing requires one positive test result for a positive diagnosis and conversely all tests to be negative for a negative outcome (144). Using all three tests simultaneously (where any abnormal test leads to a positive result) sensitivity

was 100%, but specificity reduced to 87%, compared to our multidisciplinary approach where no test was considered in isolation (100% sensitivity and 92% specificity).

5.6. Concluding comments

Advances in understanding the molecular genetic basis of PCD have been made in recent years, to the extent that genetic testing is now able to detect ~65% of PCD cases. However, genetic testing for PCD is not yet available in the UK except as a research tool (135). As more genes are identified, genetic testing by multi-gene panel (135) will make genotyping a cost-effective approach. Characterisation of ciliary structure and function will continue to have a place within diagnostic processes, similar to the need for functional tests to confirm the diagnosis of cystic fibrosis (154). Moreover, a thorough definition of disease phenotype by cilia ultrastructure, cilia beat pattern, nNO production rate will be extremely helpful in guiding genetic analyses in this genetically heterogeneous disease. The English public healthcare system does not fund immunofluorescence (IF) staining of ciliary proteins as a diagnostic test. This method is currently only able to detect abnormalities that are evident by TEM, and would therefore not improve our diagnostic accuracy. However, we anticipate that as more antibodies become available, IF will prove a time and cost efficient diagnostic test.

There is no single diagnostic test that can be used universally to diagnose PCD. Recent reports of PCD-causing genes (RSPH1) associated with subtle HSVMA and TEM abnormalities with normal nNO demonstrate the skill and expert microscopists needed for accurate diagnoses (149, 150, 155). Importantly, the conduct and reporting of tests used to diagnose PCD are not standardised. We believe that the time is right to develop consensus standards for equipment, staff experience and protocols.

Figure 7: PCD diagnostic pathway for patients and samples. Diagnostic tests included: nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA) and transmission electron microscopy (TEM). Not all patients underwent all tests.

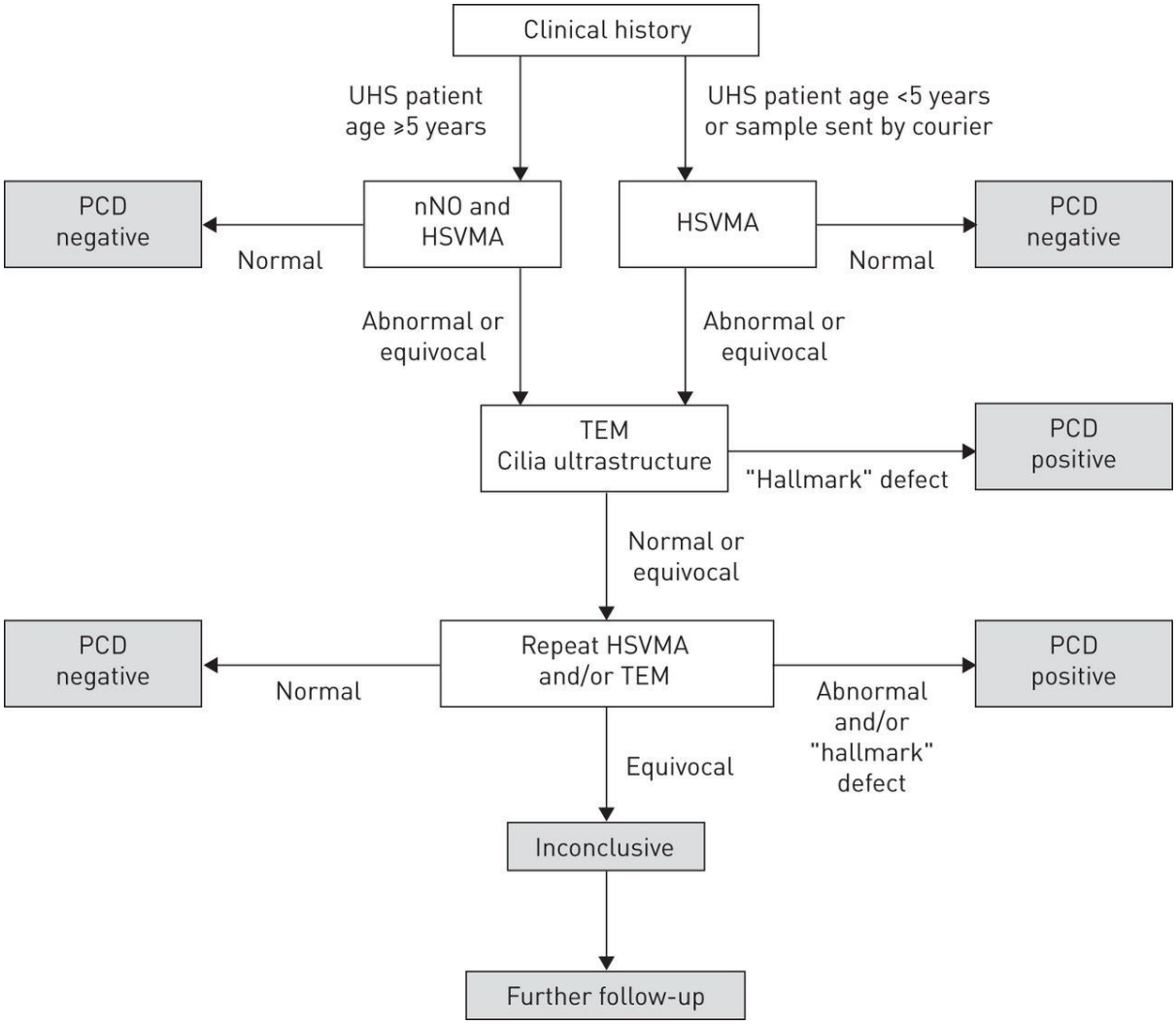

Figure 8: The diagnostic investigations and outcomes of patients seen (A) at the diagnostic centre UHS or (B) having had samples couriered to UHS from a satellite respiratory clinic. Patients were diagnosed as PCD-positive, PCD-negative or valid-inconclusive (VI). Invalid-inconclusive (II) results due to inadequate samples or data are shown but subsequently excluded from accuracy analyses. Diagnostic tests included: nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA) and transmission electron microscopy (TEM).

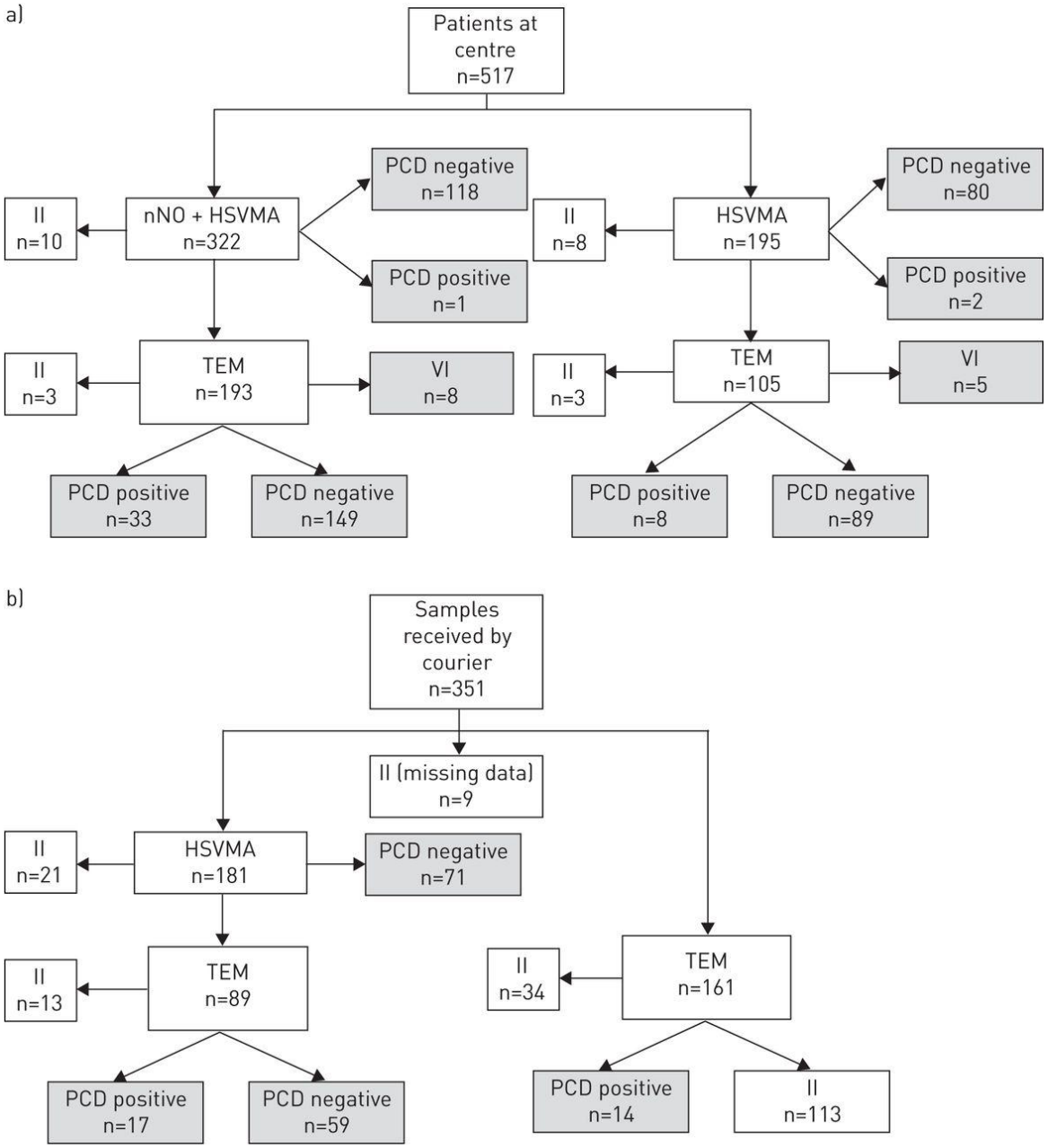

Figure 9: Receiver operating characteristic (ROC) curve analysis for ciliary beat frequency (CBF) and nasal nitric oxide (nNO) for predicting a diagnosis of primary ciliary dyskinesia (PCD) (using multidisciplinary diagnosis as the reference standard). ROC curve analysis showed that nNO ≤30 nL·min^{-1} (area under the curve (AUC) 0.97, 95% CI 0.94–1.00) was superior to CBF (AUC 0.92, 95% CI 0.79–1.00) as predictors of a PCD-positive diagnosis.

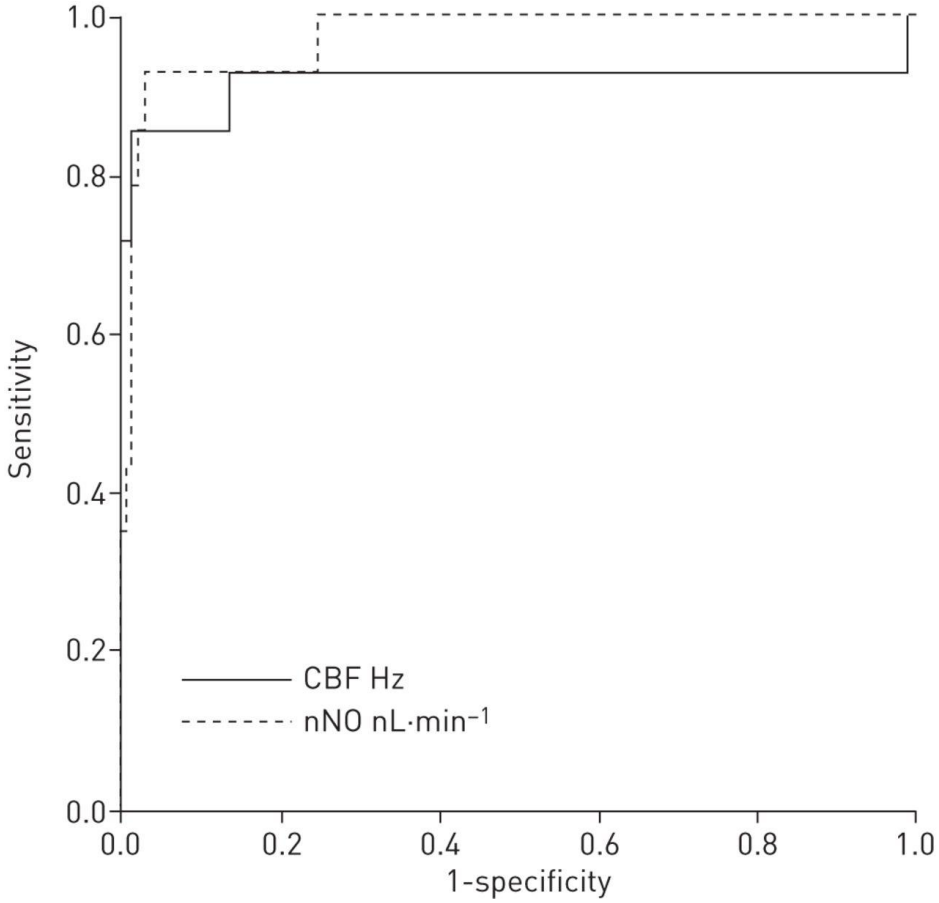

Table 7: Clinical characteristics of the referral population grouped by positive, negative, valid-inconclusive and invalid-inconclusive diagnostic outcomes

	TOTAL (n=868, 100%)			Positive (n=75, 9%)			Negative (n=566, 65%)			VI (n=13, 2%)			II (n=214, 25%)		
	Yes	No	Unknown	Yes	No	Unknown	Yes	No	Unknown	Yes	No	Unknown	Yes	No	Unknown
Gender (Male)	362 (42%)	393 (45%)	113 (13%)	34 (45%)	36 (48%)	5 (7%)	249 (44%)	278 (49%)	39 (7%)	7 (54%)	6 (46%)	0 (0%)	72 (34%)	73 (34%)	69 (32%)
Full term gestation	346 (40%)	102 (12%)	420 (48%)	66 (88%)	7 (9%)	2 (3%)	242 (43%)	80 (14%)	244 (43%)	8 (61%)	2 (15%)	3 (23%)	30 (14%)	13 (6%)	171 (80%)
Sibling with PCD	32 (4%)	792 (91%)	44 (5%)	18 (24%)	55 (73%)	2 (3%)	9 (2%)	529 (93%)	28 (5%)	0 (0%)	11 (85%)	2 (15%)	5 (2%)	197 (92%)	12 (6%)
Neonatal unit	138 (16%)	694 (80%)	35 (4%)	46 (61%)	25 (33%)	4 (5%)	77 (14%)	466 (82%)	23 (4%)	3 (23%)	9 (69%)	1 (8%)	12 (6%)	194 (91%)	8 (4%)
Situs abnormality	70 (8%)	788 (91%)	10 (1%)	33 (44%)	42 (56%)	0 (0%)	22 (5%)	537 (95%)	7 (0%)	4 (31%)	9 (69%)	0 (0%)	11 (5%)	200 (93%)	3 (1%)
Cardiac abnormality	20 (2%)	848 (98%)	0 (0%)	6 (8%)	69 (92%)	0 (0%)	10 (2%)	556 (98%)	0 (0%)	0 (0%)	13 (100%)	0 (0%)	4 (2%)	210 (98%)	0 (0%)
Respiratory symptoms	710 (82%)	158 (18%)	0 (0%)	72 (96%)	3 (4%)	0 (0%)	488 (86%)	78 (14%)	0 (0%)	12 (93%)	1 (7%)	0 (0%)	138 (64%)	76 (36%)	0 (0%)
Rhinitis	477 (55%)	389 (45%)	2 (0%)	61 (81%)	14 (19%)	0 (0%)	325 (57%)	239 (42%)	2 (1%)	9 (69%)	4 (31%)	0 (0%)	82 (38%)	132 (62%)	0 (0%)
Sinusitis	192 (22%)	663 (76%)	13 (2%)	21 (28%)	53 (71%)	1 (1%)	138 (24%)	416 (74%)	12 (2%)	5 (38%)	8 (62%)	0 (0%)	28 (13%)	186 (87%)	0 (0%)

Data are presented as n (%). Patients with invalid-inconclusive (II) outcomes were excluded from the study population for analyses. VI: Valid inconclusive

Table 8: The diagnostic accuracy of nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA) and transmission electron microscopy (TEM) analysis to diagnose primary ciliary dyskinesia. Data were analysed for patients with conclusive positive or negative results (n=641) who underwent the individual tests.

	nNo (≤30nL/min)	HSVMA	TEM
Total n (%) /641	301 (47%)	625 (98%)	368 (57%)
Positive patients n (%) /75	34 (45%)	60 (80%)	71 (95%)
Negative patients n (%) /566	267 (47%)	565 (100%)	297 (52%)
True positive (n)	31	60	56
True negative (n)	257	526	297
False positive (n)	10	39	0
False negative (n)	3	0	15
Sensitivity (95% CI)	0.91 (0.76-0.98)	1.00 (0.94 - 1.00)	0.79 (0.68-0.88)
Specificity (95% CI)	0.96 (0.93-0.98)	0.93 (0.91-0.95)	1.00 (0.99-1.00)
PPV (95% CI)	0.76 (0.60-0.88)	0.61 (0.50-0.70)	1.00 (0.94-1.00)
NPV (95% CI)	0.99 (0.97-1.00)	1.00 (0.99-1.00)	0.95 (0.92-0.97)

Table 9: The diagnostic accuracy of nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA) and transmission electron microscopy (TEM) analysis to diagnose primary ciliary dyskinesia. Analyses were stratified by A. patients seen at University Hospital Southampton (UHS) and B. samples from patients sent by courier to UHS. They were stratified further by age (i) <5 years at time of assessment and (ii) ≥5 years.

	A (i) UHS <5 years n=126			A (i) UHS >5 years n=355		B (i) Courier <5 years n=80		B (i) Courier >5 years n=80	
	HSVMA	TEM	nNO (≤30 nL/min)	HSVMA	TEM	HSVMA	TEM	HSVMA	TEM
Total n (%)	125 (99%)	67 (53%)	301 (85%)	353 (99%)	212 (60%)	71 (89%)	48 (60%)	76 (95%)	41 (51%)
Positive n (%)	8 (6%)	7 (10%)	34 (11%)	34 (10%)	33 (16%)	8 (11%)	17 (35%)	10 (13%)	14 (34%)
Negative n (%)	117 (94%)	60 (90%)	267 (89%)	319 (90%)	179 (84%)	63 (89%)	31 (65%)	66 (87%)	27 (66%)
True positive (n)	8	6	31	34	22	8	15	10	13
True negative (n)	111	60	257	297	179	59	31	59	27
False positive (n)	6	0	10	22	0	4	0	7	0
False negative (n)	0	1	3	0	11	0	2	0	1
Sensitivity (95% CI)	1.00 (0.63-1.00)	0.86 (0.42-0.98)	0.91 (0.76-0.98)	1.00 (0.90-1.00)	0.67 (0.48-0.82)	1.00 (0.63-1.00)	0.88 (0.64-0.98)	1.00 (0.69-1.00)	0.93 (0.66-0.99)
Specificity (95% CI)	0.95 (0.89-0.98)	1.00 (0.94-1.00)	0.96 (0.93-0.98)	0.93 (0.90-0.96)	1.00 (0.98-1.00)	0.94 (0.85-0.98)	1.00 (0.89-1.00)	0.89 (0.79-0.96)	1.00 (0.87-1.00)
PPV (95% CI)	0.57 (0.29-0.82)	1.00 (0.54-1.00)	0.76 (0.60-0.88)	0.61 (0.47-0.74)	1.00 (0.84-1.00)	0.67 (0.35-0.90)	1.00 (0.78-1.00)	0.59 (0.33-0.81)	1.00 (0.75-1.00)
NPV (95% CI)	1.00 (0.97-1.00)	0.98 (0.91-1.00)	0.99 (0.97-1.00)	1.00 (0.99-1.00)	0.94 (0.90-0.97)	1.00 (0.94-1.00)	0.94 (0.80-0.99)	1.00 (0.94-1.00)	0.96 (0.82-0.99)

Data are presented as n or n (%), unless otherwise stated. PPV: positive predictive value; NPV: negative predictive value.

Table 10: Sensitivity and specificity of high-speed video microscopy (HSVMA), ciliary beat pattern (CBP), nasal nitric oxide (nNO) and transmission electron microscopy (TEM) applied as single or combined tests, using simultaneous or sequential testing. *The net sensitivity and specificity was calculated for combined tests.

	A. Single testing			B. Simultaneous testing			C. Sequential two stage testing			
	nNO	**HSVMA**	**TEM**	**nNO + HSVMA**	**HSVMA + TEM**	**nNO + HSVMA + TEM**	**1. nNO n=180 2. HSVMA n=36**	**1. nNO n=180 2. TEM n=36**	**1. nNO +HSVMA n=180 2. TEM n=51**	**1. HSVMA n=180 2. TEM n=43**
Total (n)	180	180	180	180	180	180	36	36	51	43
Total positive (n)	31	31	31	31	31	31	28	28	31	31
Total negative (n)	149	149	149	149	149	149	8	8	20	12
True positive (n)	28	31	20	31	31	31	28	19	20	20
True negative (n)	141	137	149	129	137	129	8	8	20	12
False positive (n)	8	12	0	20	12	20	0	0	0	0
False negative (n)	3	0	11	0	0	0	0	9	11	11
Sensitivity	0.90	1.00	0.65							
(95% CI)	(0.74-0.98)	(0.89-1.00)	(0.45-0.81)							
Specificity	0.95	0.92	1.00							
(95% CI)	(0.90-0.98)	(0.86-0.96)	(0.98-1.00)							
PPV	0.78	0.72	1.00							
(95% CI)	(0.61-0.90)	(0.56-0.85)	(0.83-1.00)							
NPV	0.98	1.00	0.93							
(95% CI)	(0.94-1.00)	(0.97-1.00)	(0.88-0.97)							
***Net sensitivity**				1.00	1.00	1.00	0.90	0.61	0.65	0.65
***Net specificity**				0.87	0.92	0.87	1.00	1.00	1.00	1.00

Data are presented as n, unless otherwise stated. PPV: positive predictive value; NPV: negative predictive value

6. PICADAR: A DIAGNOSTIC PREDICTIVE TOOL FOR PRIMARY CILIARY DYSKINESIA (PAPER 4)

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Author's Contributions

JSL had the idea for this study. This concept was further developed by JSL and BDD. I was involved in developing the study design with BDD. I performed the data cleaning for this study, conducted the analysis and presented the results. To validate the measure, I extracted the data at the external centre and analysed the data. I drafted the manuscript and submitted the manuscript. I dealt with all corrections as recommended during the peer review process. BDD provided statistical supervision for analyses. CK and MG provided further expertise regarding clinical prediction tools. JSL, WTW, HJE, CH and AH undertook clinical assessments and lead diagnostic decision making meetings; SP managed the clinical database. All authors contributed to iterations and approved the final version.

6.1. Abstract

Background: Symptoms of primary ciliary dyskinesia (PCD) are nonspecific and guidance on whom to refer for testing is limited. Diagnostic tests for PCD are highly specialised, requiring expensive equipment and experienced PCD scientists. This study aims to develop a practical clinical diagnostic tool to identify patients requiring testing.

Method: Patients consecutively referred for testing were studied. Information readily obtained from patient history was correlated with diagnostic outcome. Using logistic regression, the predictive performance of the best model was tested by receiver operating characteristic curve analyses. The model was simplified into a practical tool (PICADAR) and externally validated in a second diagnostic centre.

Results: Of 641 referrals with a definitive diagnostic outcome, 75 (12%) were positive. PICADAR applies to patients with persistent wet cough and has seven predictive parameters: full-term gestation, neonatal chest symptoms, neonatal intensive care admittance, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defect. Sensitivity and specificity of the tool were 0.90 and 0.75 for a cut-off score of 5 points. Area under the curve for the internally and externally validated tool was 0.91 and 0.87, respectively.

Conclusion: PICADAR represents a simple diagnostic clinical prediction rule with good accuracy and validity, ready for testing in respiratory centres referring to PCD centres.

6.2. Introduction

Primary ciliary dyskinesia (PCD) is a rare heterogeneous disorder characterised by abnormal ciliary function and associated with abnormal ciliary ultrastructure in 70% of cases (16, 40). Consequences of PCD include impaired mucociliary clearance of the airway causing upper and lower respiratory tract symptoms which usually present soon after birth. Neonatal manifestations range in severity from mild transient tachypnoea to significant respiratory failure requiring prolonged respiratory support (1-3). Patients continue to have chronic, progressive symptoms of persistent wet cough and recurrent chest infections that almost invariably lead to bronchiectasis. Upper airway problems include rhinosinusitis and recurrent otitis media with hearing impairment (99). Motile embryonic nodal cilia are important for left-right asymmetry; approximately half of PCD patients exhibit *situs inversus*, and 6-12% heterotaxic syndromes which may be associated with complex congenital cardiac defects (8, 156). Male Infertility is common since sperm flagella have a similar ultrastructure to cilia; the incidence of female infertility is less clear but can be explained by immotile fallopian tube cilia (157).

The prevalence, burden of disease and prognosis of PCD patients is difficult to determine due to limited representative international data. Reported prevalence varies from 1:2,000 to 1:40,000 reflecting true variability as well as differences in access to diagnostic facilities (10, 11, 110). A survey of 26 European countries found that PCD is under-diagnosed or diagnosed late particularly in countries with low health-care expenditure (110). In addition to expecting to improve respiratory prognosis (4, 12, 64), early diagnosis facilitates appropriate management; management is different to non-PCD related serous otitis media for example (21). Diagnosis also allows genetic counselling for the family.

There is currently no "gold standard" test to diagnose PCD (13). European guidelines recommend that PCD should be confirmed in a specialist centre using appropriate diagnostic testing (22). PCD diagnostic investigations are complex, requiring expensive infrastructure and an experienced team of clinicians, scientists and microscopists (13, 19, 20). Various models exist to deliver diagnostic services for this rare disease, generally with a network of satellite screening centres accessing a specialist centre (14, 20, 21). The symptoms of PCD are non-specific and secondary care physicians need guidance of whom to refer for diagnostic testing (21). To promote early diagnosis without overburdening specialist services, screening tools such as nasal nitric oxide (nNO) are used and has been proved to be an excellent screening measures (14, 53, 158). This however requires expensive equipment and trained technicians to obtain reliable measurements.

The aim of this study was to utilise easily available clinical information from a large prospective population to produce a scoring tool to predict whether symptomatic patients have PCD: PICADAR (PrImary CiliARy DyskinesiA Rule). We aimed to develop a tool that would be quick and easy to use by general respiratory and ear, nose and throat specialists. PICADAR's accuracy was externally validated in a second PCD diagnostic centre.

6.3. Methods

This research was approved by the National Research Ethics Service (NRES-06/Q1702/109).

6.3.1. Study population

6.3.1.1. Derivative group

We analysed data from 641 consecutive patients with a definitive diagnostic outcome from the University Hospital Southampton (UHS) PCD diagnostic centre (2007–2013). A proforma was used to collect patient data, completed by a clinician through a clinical interview prior to diagnostic testing.

6.3.1.2. External validation group

We used data from a sample of 187 patients (93 PCD-positive and 94 PCD-negative) referred for testing to the Royal Brompton Hospital (RBH) to validate the score. An equal number of positive and negative referrals were randomly selected from the overall population of patients referred between 1983 and 2013. Using a similar protocol to UHS, a clinical history proforma was completed before diagnostic testing.

6.3.2. Diagnostic testing

The diagnostic criteria used in the UK (UHS and RBH) have previously been described in detail in Jackson et al.(101) and Lucas and Leigh (13). In brief, a positive diagnosis is usually based on a typical clinical history with at least two abnormal diagnostic tests (“hallmark” transmission electron microscopy (TEM), “hallmark” ciliary beat pattern (CBP), nNO $\leqslant$30 nL·min−1). Occasionally patients with a strong history (e.g. sibling with PCD, “full” clinical

phenotype (e.g. neonatal respiratory distress at term followed by daily wet cough, persistent rhinitis and glue ear), are diagnosed based on either "hallmark" TEM or repeated high-speed video microscopy analysis (HSVMA) consistent with PCD. CBP was only considered positive if the pattern was typical of PCD rather than secondary ciliary dyskinesia either from two brushing biopsies or from one brushing biopsy with reanalysis following air–liquid interface culture.

6.3.3. Clinical Data

Data was collected on gender, date of birth, age at assessment and ethnicity. Neonatal data collected included admittance to a special care babies unit, neonatal respiratory support, neonatal rhinitis or chest symptoms. Data on the presence of situs abnormalities, congenital cardiac defect, chronic (>3 months) cough, rhinitis, sinusitis, ear problems, history of pneumonia and bronchiectasis was collated. Family history of PCD, bronchiectasis, hearing problems, asthma and consanguinity were included. Data on clinical history was coded as yes=0, no=1 or missing=99. For the adult population, subfertility was recorded if the patient had difficultly conceiving but had children, used in vitro fertilisation or if they stated they were never able to conceive.

6.3.4. Model development

Potential predictors were restricted to information readily available in a nonspecialist setting. From the derivation group, 27 potential variables were identified. Two-tailed parametric (t-test) or nonparametric (Mann–Whitney) tests, Chi-squared test or Fisher's exact test (as appropriate) were used to compare the characteristics of positive and negative referrals. Logistic regression analysis was used to develop a simplified practical prediction tool. First, potential predictors were entered into the model individually using forward step-wise

methods. This allowed us to identify and select the significant predictors for PCD and assess their influence on a positive PCD diagnosis. Sensitivity, specificity and overall accuracy (the weighted average of the models sensitivity and specific) from selected significant predictors were interpreted (159).

6.3.5. Model performance

The model's ability to discriminate between those with and without PCD was assessed by plotting receiver operating characteristic (ROC) curve and calculating the area under the ROC curve (AUC). Discrimination was considered moderate if AUC was 0.6 to 0.8 and good if >0.8 (160). The Hosmer Lemeshow goodness-of-fit-test was used to assess the calibration of the model, i.e. how well the predicted probabilities agreed with the prevalence of the outcome in patient subgroups. A Hosmer Lemeshow goodness-of-fit-test (161) result of <0.05 indicates that the predicted probabilities and the actual outcome agree poorly (162). Subjects with missing data were excluded on a case-wise basis; however, to confirm the model's accuracy, multiple imputation was used to check for any biases that can occur in complete case analysis along with a substantial loss of power and precision (163, 164).

6.3.6. Clinical prediction tool

The best model from the logistic regression allowed for the calculation of a diagnostic predictive tool (PICADAR) to estimate the probability of a positive PCD diagnosis based on total score. The score for each predictor corresponds to their regression coefficient rounded to the nearest integer. A ROC curve was plotted to assess the predictive performance of PICADAR for comparison with the original model. Each score has a corresponding accuracy (i.e. sensitivity and specificity) of predicting a positive or negative diagnosis.

6.3.7. Validation in external population

The discriminative ability of the scores in the validation population was assessed using ROC curve analysis. All analyses were performed by using SPSS Statistics for Windows version 21.0 (IBM, Armonk, NY, USA).

6.4. Results

6.4.1. Study population

Of 641 consecutive participants in the derivation group, 75 (12%) were diagnosed with PCD and 566 (88%) had a negative diagnosis. Median (range) age at assessment was 9 (0–79) years and 44% of patients were male.

The validation group was selected to include similar numbers of positive and negative diagnoses. The participants were younger than the derivation group; they were also more likely to be non-white and from a consanguineous background, reflecting the different populations served by UHS and RBH (Table 11).

6.4.2. Clinical and family characteristics

Both PCD-positive and PCD-negative groups had a high prevalence of a persistent daily wet cough throughout life (PCD-positive 93.3%, PCD negative 85.1%, p=0.069). PCD-positive patients were more likely to report neonatal problems requiring admittance to a neonatal unit (61.3%, 13.6%, p<0.001), neonatal rhinitis (26.6%, 6.5%, p<0.001) and neonatal chest symptoms (e.g. wet cough, tachypnoea, oxygen requirement) (74.6%, 17.1%, p<0.001). Symptoms were higher among the PCD-positive group for persistent perennial rhinitis (81.3%, 57.4%, p<0.001), serous otitis media (glue ear) (57.3%, 19.2%, p<0.001) and hearing loss (49.3%, 15.9%, p<0.001). Situs abnormalities (41.3%, 3.9%, p <0.001) and congenital

cardiac defects (9.3%, 2.1%, p=0.001) and were also more common in the PCD-positive group. Fertility data was available for 152 referrals, with significantly higher percentage reporting subfertility in PCD-positive group (90%, 18.4%, p<0.001). Family history of PCD in siblings (24%, 1.6%, p<0.001), or in other family members (5.3%, 1.1%, p=0.012), was significantly higher among the PCD-positive group. Consanguinity (16.0%, 1.4%, p<0.001), was more common in PCD-positive group. Clinical characteristics of the derivation group are summarised in Table 12, and of the validation group in Appendix 3: Table A5.

6.4.3. Development of PICADAR score

Of the 27 binary variables considered for selection, the best logistic regression model included seven significant predictors. In order of importance (based on their corresponding odds ratio) these predictors were situs inversus, birth at full term, neonatal chest symptoms, admission to a neonatal unit, congenital cardiac defect, rhinitis, and ear and hearing symptoms (Table 13). Similar results were found when multiple imputation was applied (Appendix 3: Table A6). The overall accuracy of this model was 90%, and the sensitivity and specificity were 71% and 94%, respectively. The discriminant ability (AUC) of this model was 0.92 (Figure 10). The Hosmer–Lemeshow test showed good agreement between the predicted probabilities and the actual outcome (p=0.64).

PICADAR (Figure 11) was designed as an easily scored predictive tool based on the seven-variable-predictor model. The presence of each clinical factor contributed to the total score following adjustment of its regression coefficient values to an integer between 1 and 4. This adjustment had little effect on discriminative ability (model AUC 0.92, PICADAR AUC 0.91) (Figure 10).

The likelihood of a patient having PCD can be estimated by comparing their score to the probability curve (Figure 11b) or cut-offs could be used. The highest combined sensitivity and specificity (0.90 and 0.75, respectively) was at the cut-off value of 5 points (Table 14). The maximum PICADAR score was 14. This corresponded to a 99.80% probability of having PCD, a score ≥10 had a probability of 92.6%, and a score ≥5 had a probability of 11.10% (Figure 11b and Appendix 3: Table A7). Sensitivity and specificity of the tool were 0.90 and 0.75 for a score of ≥5 points (Table 14). In the UHS derivation group, of the PCD positive, 6.0% had scores ≤5, 58.0% had scores 6-9 and 36.0% had scores ≥10. In the PCD-negative group, 79.4% had scores ≤5, 20.2% had scores 6-9 and only 0.4% had scores ≥10 (Table 15).

6.4.4. Validation of the PICDAR score

Validation of PICADAR used data from an independent set of 93 randomly selected PCD-positive patients and 94 PCD-negative patients from RBH. Data for all PICADAR predictors were available in 157/187 (84%) of the validation group, all of whom were <18 years of age. The remaining 30 were excluded due to missing symptom data. Positive cases accounted for 79 (50%) of the population, 79 (50%) were male; mean age at assessment was 4 years. Scores in the validation group ranged from 0 to 14 (mean±SD 5.9 ±3.3). The mean±SD PICADAR score was higher in the PCD-positive group (PCD-positive 7.9±2.8, PCD-negative 3.8±2.3; $p<0.01$) and distribution of the scores differed between groups (Appendix 3: Figure A4). ROC curve analysis confirmed the performance of the score with AUC 0.87 (95% CI 0.81–0.94) (Figure 12). In the validation group, 18.7% of the PCD-positive group had a score ⩽5, 53.3% had a score 6–9 and 29.1% had a score ⩾10. For the PCD-negative group, 75.6% has a score ⩽5, 20.5% had a score of 6–9 and 3.8% had a score ⩾10 (table 5).

A second predictive tool, PICADAR+S, which includes the variable "siblings with PCD" was developed and validated for patients with one of more siblings. When validated, this tool was also shown to discriminate between positive and negative referrals (AUC 0.94, 95% CI 0.90–0.97) (see Appendix for full description).

6.5. Discussion

6.5.1. Statement of principle findings

We have developed an easy-to-use predictive score for determining the likelihood of an individual having a diagnosis of PCD. The score accurately predicts a positive or negative test result in patients with daily lower respiratory tract symptoms throughout life. PICADAR was developed and validated in patients referred to specialist diagnostic centres; at this stage we would not aim for it to be used in primary care, but anticipate that it could be used by respiratory centres to guide referral to specialist PCD centres. PICADAR should raise awareness of symptoms associated with PCD, and stimulate discussion and research to further refine the tool. PCD centres could use PICADAR to identify patients who should be investigated further following inconclusive or equivocal PCD tests. In resource limited countries with no diagnostic facilities the tool could be used to attach a PCD-likelihood to the patients; this is important for international research registries and metacohorts.

There is no gold standard test for PCD, and testing is restricted to centres with the infrastructure and expertise to analyse and interpret HSVMA or TEM images and genotype data (13, 20, 165).

Measurement of nasal nitric oxide (nNO) provides a good screening tool (14, 53) to differentiate PCD-positive and non-PCD in patients with symptoms. In the study population

which contributed to the development of PICADAR, we previously reported in Jackson et al.(101) that a cut-off of 30 $nL \cdot min^{-1}$ was both sensitive (0.91, 95% CI 0.76–0.98) and specific (0.96, 95% CI 0.93–0.98); of 301 consecutive referrals for diagnostic testing including nNO, 31/34 (91%) of PCD-positive patients had low nNO (true positive), 10/267 (3%) of PCD-negative patients had low nNO (false positive) and 3/34 (9%) of PCD-positives had nNO >30 $nL \cdot min^{-1}$ (false negative). However, the equipment for measuring nNO is not widely available outside specialist centres and needs trained technicians to obtain reliable readings. We designed PICADAR for use outside specialist diagnostic centres. Our data suggests similar accuracy in comparison with nNO, e.g. a cut-off score of $\geqslant 5$ using PICADAR had 90% sensitivity and 75% specificity to differentiate PCD-positive and PCD-negative patients in the derivation group, and had 86% sensitivity and 73% specificity in the validation group. If patients with a score $\leqslant 4$ had not proceeded to further testing, 167 (70.2%) and 57 (73.1%) negative patients would have avoided formal testing at the diagnostic centres; however, two (4.0%) positive patients in the derivation group and 11 (13.9%) in the validation group would have been missed (table 5). The tool therefore should not be used in isolation when deciding who to refer. We believe that promotion of the tool is likely to raise awareness of the most common symptoms associated with PCD. Although PICADAR will "miss" some patients when used in isolation, since diagnosis is currently commonly missed due to lack of physician awareness, widespread use of PICADAR would inevitably increase the number of actual diagnoses.

6.5.2. Strengths and limitations of the study

PICADAR comprises seven predictive variables including full-term gestational age, admittance to a neonatal unit, neonatal chest symptoms, persistent perennial rhinitis, chronic

ear and hearing symptoms, situs abnormalities, and presence of a cardiac defect; such items are easily ascertained and quick to compute in any clinical setting. We did not specify cardiac defects associated with laterality defects within the score because we want PICADAR to be used by nonspecialists. PICADAR was derived in a specialist PCD centre (UHS) and validated externally in another centre (RBH). Although these two diagnostic centres are both situated in Southern England, they have different demographic populations in terms of ethnicity, consanguinity and age at assessment. Good discriminant ability was maintained when used in the validation group with AUC 0.87. The process of developing a clinical prediction rule includes four stages before ever being implemented in routine practice (derivation, internal validation, external validation and impact analysis). If an external validation is done directly in another setting, internal validation is not necessary. Therefore, as of now, we have completed all three of the requested stages of the rule development. Once these results are published, further validations can be done in other countries and settings, by other investigators, so that they can provide additional evidence for its validity for further implementation in practice (166).

PICADAR was developed in a large clinically relevant population. Consecutive patients with a diagnostic outcome were included. Patients had been referred based on symptoms and/or family history. Diagnosis was based on a combination of tests including nNO measurements, HSVMA to assess ciliary function and TEM to assess ciliary ultrastructure (12, 21). A detailed clinical proforma was completed by health professionals before diagnostic testing was started, thus reducing bias. Using PICADAR, patients with a score $\geqslant$10 had a >90% probability of testing positive for PCD. Those with a score $\geqslant$5 had a >11% chance of being diagnosed PCD-positive (Figure 11b and Appendix 3: Table A7). We believe that this guidance will support appropriate referrals of patients for specialist testing, particularly where

patients are geographically remote from a diagnostic centre. Clinicians using the score need to be aware that individuals with low risk scores might still have PCD (table 5). It should be noted that persistent wet cough is not included in the score because virtually all positive and negative referrals had chronic cough; therefore, the score is for use in patients with chronic cough as a precondition. A potential limitation is that a significant amount of data was missing for some variables. For example, a large proportion of the adult population did not know their gestational age and none of the children would yet know their fertility status. Complete case analysis was used to deal with missing data; however, this can lead to bias. To overcome this obstacle, multiple imputation was used to replace missing values within the model's significant variables (163, 164). The pooled result obtained from five imputed datasets showed the best model is accurate (Appendix 3: Table A6). Importantly, it must also be emphasised that PICADAR was developed using a population already referred for diagnostic testing. It was developed with the aim of identifying appropriate patients for referral from secondary care and will now require validation in this setting. Finally, patients with equivocal results were excluded from the derivation and validation populations. This may artificially improve the tool's performance; however, good discrimination was found when the tool was validated in a second diagnostic centre.

6.5.3. Future research

The scores were derived using combined data from adults and children, but we expect that separate scores for adults and children might further improve accuracy. We therefore propose further research in large cohorts of children and adults to derive separate scoring systems. PICADAR includes a number of predictors based in early life, including gestational age and neonatal chest symptoms, which may be difficult to recall in adulthood. Similarly, subfertility

was more common in the PCD-positive group and is likely to be a strong predictor for adult diagnoses. While the derivation group consisted of a wide range of age groups, the majority of referrals in the external validation group were children. Furthermore, the scores were developed and validated in UK specialist diagnostic centres, but referrals originate from nonspecialist services, and therefore future validation will be needed in referral centres and in centres outside of the UK.

6.6. Conclusions

PICADAR provides the first validated tool to aid appropriate referral of patients for diagnostic testing. It was designed to be easily applied in a nonspecialist setting to determine which patients with chronic chest symptoms require PCD diagnostic testing. PICADAR is a simple cost-effective score suitable for use in all clinical settings. The tools are now available for validation in a variety of clinical settings.

Figure 10: PICADAR: receiver operating characteristics curve for the best prediction model (AUC= 0.92; CI 0.87-0.95) and the prediction tool (AUC=0.91; CI 0.87-0.95) in the derivation group.

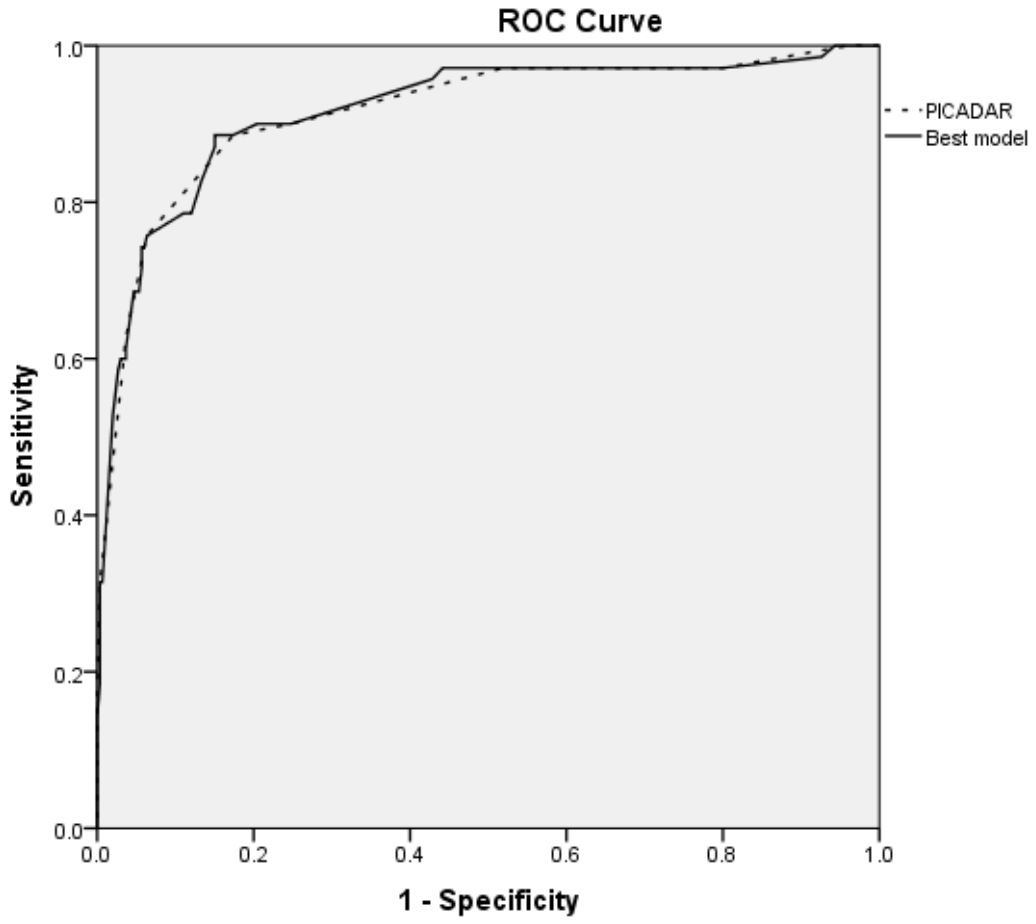

Figure 11: PICADAR is a predictive score with 7 simple questions to predict likelihood of having PCD. It can be used in any patients with chronic respiratory symptoms starting in early childhood. The total score is calculated and the individual probability of having PCD diagnosis can be estimated from Figure 11b.

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	
Was the patient born preterm or full term?	Term	2
Did the patient experience chest symptoms in the neonatal period (eg. tachypnoea, cough, pneumonia)?	Yes	2
Was the patient admitted to a neonatal unit?	Yes	2
Does the patient have a situs abnormality (situs inversus or heterotaxy)?	Yes	4
Does the patient have a congenital heart defect?	Yes	2
Does the patient have persistent perennial rhinitis	Yes	1
Does the patient experience chronic ear or hearing symptoms (e.g. glue ear, serous otitis media, hearing loss, ear perforation)	Yes	1
Total Score =		

(b)

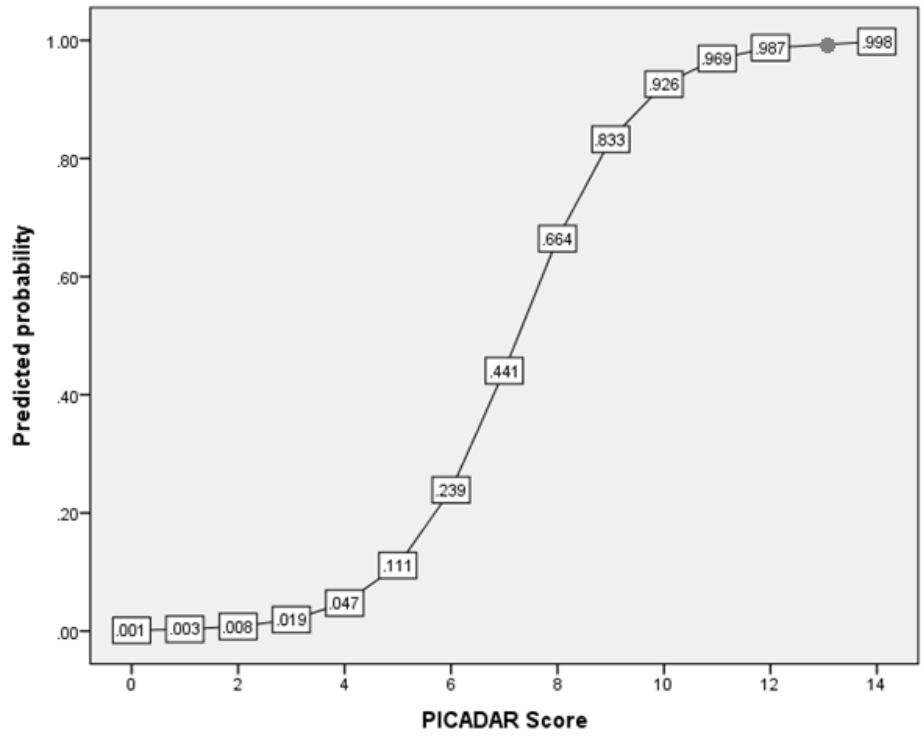
Predicted probability
1.00
.80
.60
.40
.20
.00
.001
.003
.008
.019
.047
.111
.239
.441
.664
.833
.926
.969
.987
.998
0
2
4
6
8
10
12
14
PICADAR Score

Figure 12: Receiver operating characteristics curve for the prediction tool PICADAR (AUC= 0.87; CI 0.81-0.94) in the validation group.

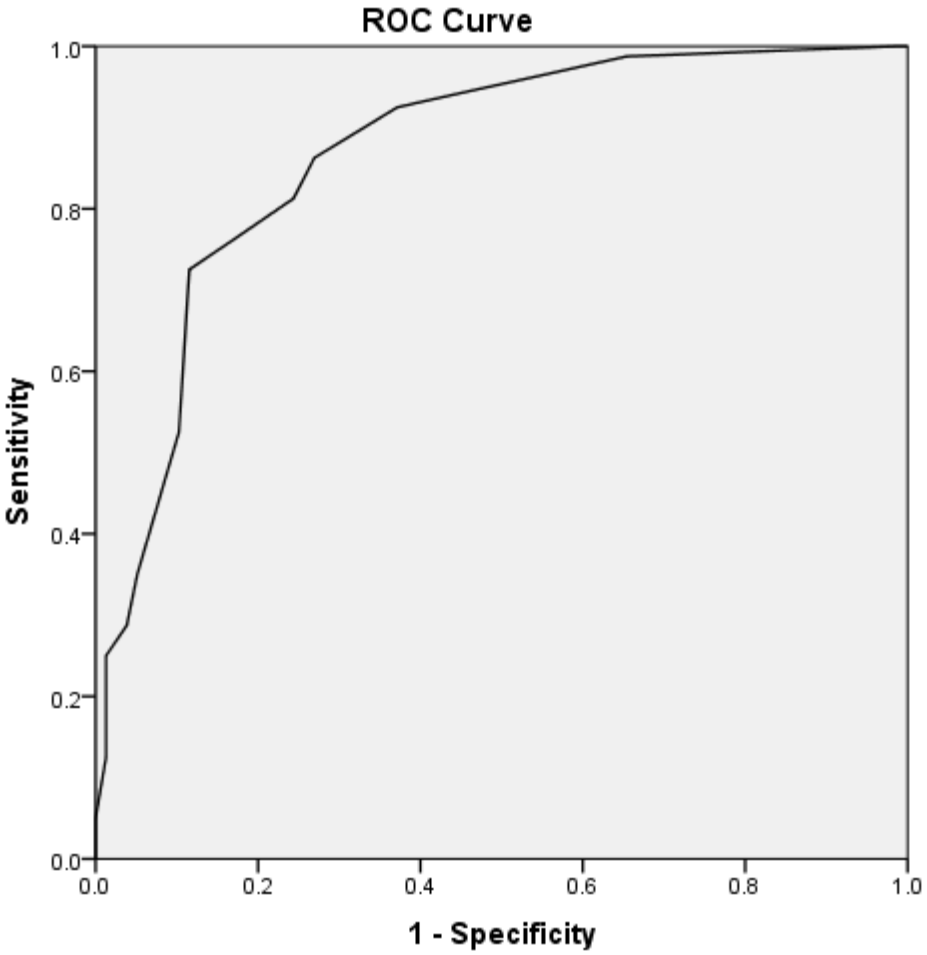

Table 11: Demographical characteristics of the two study populations: the derivation and the validation group.*

	Derivation group (UHS)			Validation group (RBH)		
	Total n=641	**PCD positive n=75**	**PCD negative n=566**	**Total n=157**	**PCD positive n=80**	**PCD negative n=77**
Age at assessment (median, range)	9 (0-79)	6 (0-67)	9 (0-79)	3 (0-18)	6 (0-17)	2 (0-12)
Male (n= %)	283 (44%)	34 (45%)	249 (44%)	78 (50%)	38 (48%)	40 (52%)
Gestational age (mean ±SD)	39 (2.6)	39.6 (1.6)	38.9 (2.7)	39 (2.3)	39 (2.0)	39 (2.5)
Preterm (n= %)	87 (14%)	7 (9%)	80 (14%)	17 (11%)	7 (9%)	10 (13%)
Siblings with PCD (n= %)	27 (4%)	18 (24%)	9 (2%)	27 (17%)	24 (30%)	3 (4%)
Other family with PCD (n= %)	10 (2%)	4 (5%)	6 (1%)	8 (5%)	7 (9%)	1 (1%)
Consanguinity (n= %)	20 (3%)	12(16%)	8 (1%)	38 (24%)	35 (44%)	3 (4%)
Ethnicity						
White	482 (75%)	57 (76%)	425 (75%)	90 (57%)	31 (39%)	59 (77%)
Other	55 (9%)	17 (23%)	38 (7%)	57 (36%)	39 (49%)	18 (23%)
Ethnicity not stated	104(16%)	1 (1%)	103 (18%)	10 (7%)	10 (12%)	0 (0%)

*Missing values not presented

Table 12: Clinical symptom characteristics of the derivation group (n=641)

	Total n=641 (%)	PCD positive n=75 (%)	PCD negative n=566 (%)	Odds ratio	p-value
Neonatal symptoms					
Neonatal respiratory support	72 (11.2%)	31 (41.3%)	41 (7.2%)	9.77 (5.53 - 17.26)	<0.001
Neonatal chest symptoms	153 (23.0%)	56 (74.6%)	97 (17.1%)	13.56 (7.60 - 24.11)	<0.001
Neonatal rhinitis	57 (8.9%)	20 (26.6%)	37 (6.5%)	5.53 (2.99 - 10.23)	<0.001
Respiratory symptoms					
Persistent daily wet cough	552 (86.1%)	70 (93.3%)	482 (85.1%)	2.38 (0.93-6.07)	0.069
Recurrent wheeze	254 (39.6%)	36 (48.0 %)	218 (38.5%)	1.39 (0.86-2.26)	0.176
Previous pneumonia	227 (35.4%)	31 (41.3 %)	196 (34.6%)	1.14 (0.69-1.88)	0.585
Bronchiectasis	202 (31.5%)	22 (29.3%)	180 (31.8%)	0.94 (0.54-1.61)	0.83
Upper airway and ear symptoms					
Perennial persistent rhinitis	386 (60.2%)	61 (81.3%)	325 (57.4%)	3.20 (1.75 - 5.86)	<0.001
Chronic sinusitis	159 (24.8%)	21 (28.0%)	138 (24.3 %)	1.19 (0.69-2.05)	0.52
Hearing loss	127 (19.8%)	37 (49.3%)	90 (15.9%)	5.90 (3.52 - 9.98)	<0.001
Chronic acute otitis media	165 (25.7%)	25 (33.3%)	140 (24.7 %)	1.41 (0.85 - 2.32)	0.117
Serous otitis media	152 (23.7%)	43 (57.3%)	109 (19.2%)	3.24 (2.11 - 4.96)	<0.001
Chronic ear perforation	59 (9.2%)	9 (12.0%)	50 (8.8%)	5.90 (3.52 - 9.98)	0.398
Ear surgery	105 (16.3%)	24 (32.0%)	81 (14.3%)	2.81 (1.64-2.83)	<0.001
Other clinical characteristics					

Neonatal Unit	123 (19.2%)	46 (61.3%)	77 (13.6%)	11.13 (6.46 - 19.17)	<0.001
Situs abnormality	53 (8.2%)	31 (41.3%)	22 (3.9%)	17.19 (9.18 - 32.19)	<0.001
Congenital cardiac defect	19 (2.9%)	7 (9.3%)	12 (2.1%)	4.75 (1.81 - 12.48)	0.001
Hydrocephalus	7 (1.1%)	1 (1.3%)	6 (1.0%)	1.14 (0.13-9.61)	0.903
Developmental delay*	43 (6.7%)	8 (10.6%)	35 (6.2%)	1.68 (0.75-3.82)	0.197
Family history of disease					
Family history PCD in siblings	27 (4.2%)	18 (24.0%)	9 (1.6%)	19.23 (8.24 - 44.87)	<0.001
Family history PCD in extended family	10 (1.5%)	4 (5.3%)	6 (1.1%)	5.22 (1.44 - 18.98)	0.012
Family history of asthma	206 (32.1%)	12 (16.0%)	194 (34.2%)	0.35 (0.18 - 0.67)	0.002
Family history bronchiectasis	28 (4.3%)	3 (4.0%)	25 (4.4%)	0.99 (0.29-3.40)	0.998
Family history otitis media	65 (10.1%)	5 (6.6%)	60 (10.6%)	0.69 (0.26 - 1.80)	0.456
Subfertility (n=152)†	36 (23.6%)	10 (90%)	26 (18.4%)	44.23 (5.42-360.91)	<0.001

* Developmental delay includes those who present with gross motor delay, social delay or language delay

†Subfertility is based on a total of 152 referrals. Subfertility data is available for positive referrals n= 11 and negative referrals n=141

Table 13: Factors for the prediction of PCD selected by stepwise logistic regression

	RC	OR (95% CI)	p-value	*Simplified RC tool
Situs inversus	3.54	34.48 (11.6 to 101.8)	<0.001	4
Gestational age (full term)	2.20	9.06 (2.9 to 27.4)	<0.001	2
Neonatal chest symptoms	1.91	6.79 (2.7 to 16.7)	<0.001	2
Neonatal unit	1.90	6.70 (2.7-16.3)	<0.001	2
Congenital cardiac defect	1.57	4.83 (1.1-22.2)	0.043	2
Rhinitis	1.22	3.40 (1.2 to 8.9)	0.013	1
Ear and hearing symptoms	0.95	2.59 (1.2 to 5.8)	0.021	1

RC, Regression coefficient; *OR*, Odds ratio

*Regression coefficients of the main model are rounded to the nearest integer

Table 14: Performance measures including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the PICADAR prediction tool for different cut-off values calculated from the derivation group and validation group.

	Derivation group (UHS)				Validation group (RBH)			
Cut-off ≥:	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
0	>0.99	<0.01	0.12	NA	>0.99	<0.01	0.51	0.00
1	>0.99	0.01	0.12	1.0	-	-	-	-
2	>0.99	0.04	0.12	1.0	>0.99	0.01	0.51	1.00
3	0.97	0.20	0.14	0.98	0.99	0.35	0.61	0.97
4	0.97	0.48	0.20	0.99	0.93	0.63	0.72	0.90
5	0.90	0.75	0.32	0.98	0.86	0.73	0.77	0.83
6	0.89	0.83	0.41	0.98	0.81	0.76	0.78	0.79
7	0.76	0.94	0.63	0.97	0.73	0.89	0.79	0.79
8	0.63	0.96	0.68	0.95	0.53	0.90	0.85	0.65
9	0.34	0.99	0.82	0.92	0.35	0.96	0.90	0.59
10	0.31	>0.99	0.80	0.92	0.29	0.96	0.88	0.57
11	0.19	>0.99	0.72	0.90	0.25	0.99	0.96	0.56
12	0.13	>0.99	1.0	0.90	0.13	0.99	0.93	0.52
13	0.01	>0.99	1.0	0.88	0.05	>0.99	1.00	0.50
14	<0.01	>0.99	NA	0.88	0.01	>0.99	1.00	0.49

TABLE 15: The distribution of scores (⩽5, 6–9 and ⩾10) in primary ciliary dyskinesia (PCD) positive and PCD-negative participants using PICADAR in the derivation group

	Derivation group n=288				Validation group n=157		
	≤5 n (%)	**6-9 n (%)**	**≥10 n (%)**		**≤5 n (%)**	**6-9 n (%)**	**≥10 n (%)**
PCD+ (n=50)	3 (6.0%)	29 (58.0%)	18 (36.0%)	**PCD+ (n=79)**	15 (18.7%)	42 (53.3%)	22 (28.0%)
PCD- (n=238)	189 (79.4%)	48 (20.2%)	1 (0.4%)	**PCD- (n=78)**	59 (75.6%)	16 (20.5%)	3 (3.8%)

(n=288) and in the validation group (n=157) (only children <18 years included)

7. ACCURACY OF DIAGNOSTIC TESTING FOR PRIMARY CILIARY DYSKINESIA: RESPONSE TO CORRESPONDENCE

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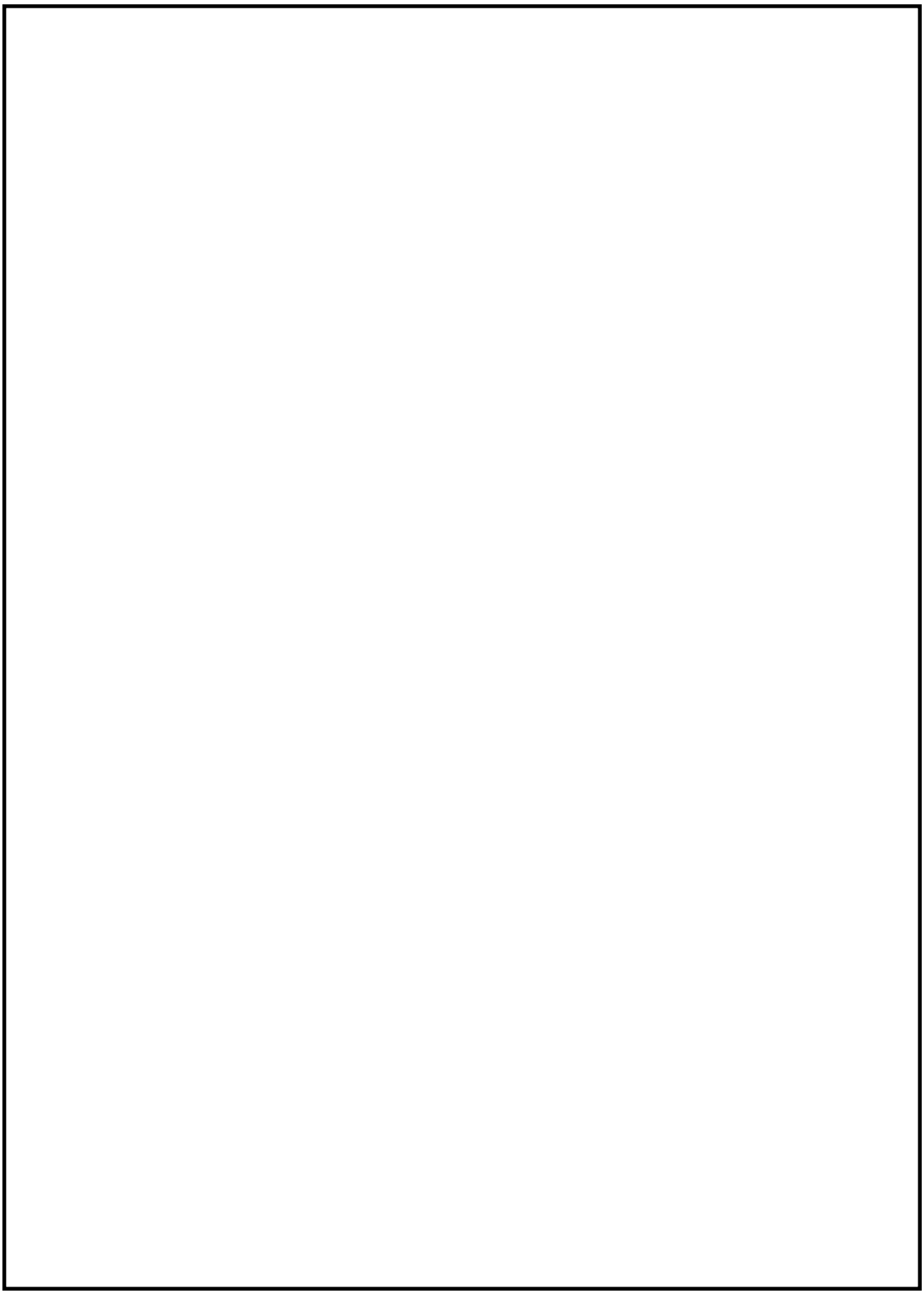
2016



We thank Drs Amirav and Boussuyt for their interest in our manuscripts. We agree that our two studies (101, 167) have some limitations, caused by the lack of a "gold standard" test for diagnosing PCD, but we strongly disagree with their claim that we "did not notify the readers of these design deficiencies". Instead, we had taken great care to highlight these uncertainties and risks of bias. Our first manuscript evaluates the accuracy of different tests used to diagnose PCD: nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVMA) and transmission electron microscopy (TEM), used alone or in combination, against an expert diagnostic consensus based on information from all available tests (101). This was clearly described in the methods section; and several sensitivity analyses tested the robustness of our results by varying different parameters. Most notably, we began the discussion section (101) by describing the lack of a single 'gold reference standard' (second paragraph) as the major limitation of our study. We highlighted that we used a surrogate reference standard, which was an expert multidisciplinary consensus, based on results from all available diagnostic tests. We also cautioned that since each test contributed to the final diagnostic decision, our sensitivity and specificity estimates of the single tests might be overestimated. Their letter is helpful as it highlights the challenges of investigating diagnostic accuracy for diseases where there is no 'gold standard'. This is typical for many diseases, including rare ones like PCD (13), and common ones like asthma. It complicates research, but should not impede it; or else we will never progress. Guidance recommends that in situations without a 'gold standard' researchers can consider constructing a reference standard from multiple test results or use an imperfect reference (168). In our clinical practice, a multidisciplinary panel of specialists considers results of multiple tests to develop a consensus diagnosis of PCD, based on pre-determined rules. In the paper, we used this composite diagnostic outcome as the study's reference standard. Pre-specified rules for deciding the composite diagnostic outcome makes the method transparent and easy to use.

An alternative approach(168) is to use an imperfect reference standard and then adjust the calculated sensitivity and specificity based on existing data about the imperfections. For example, we could have used TEM alone as a single reference standard, and calculated the accuracy of the other tests compared to TEM, taking into consideration that the latter has excellent specificity but limited sensitivity (70-90%). For completeness, we did this for TEM and for HSVMA (Appendix 2: Table A4) (101)), but given that this was not our primary approach and that pre-existing data about the degree of imperfection were highly variable, we decided not to make any adjustments. As discussed in our manuscript (101) and the accompanying editorial by Haarman and Schmidts (169), generalisability of our findings should be considered with caution. Accurate analysis of ciliary function by HSVMA and of ultrastructure by TEM depends on expertise. The UK PCD reference centres regularly audit each other's analyses, and discuss difficult cases (21). We routinely analyse de novo cilia following culture of the original sample at air liquid interface allowing us to differentiate primary and secondary functional and structural defects (139). These methods are technically demanding and not available in many centres. In centres without similar infrastructure subtle cases of PCD are more likely to be missed, and secondary defects incorrectly attributed to PCD; even with these facilities, we may misdiagnose some patients. Introduction of new tests is likely to advance the accuracy of diagnostic decisions; we did not include data on genotype and immunofluorescence because these methods were introduced relatively recently and were not available during the study period. The second manuscript that Amirav and Boussuyt refer to describes the PICADAR tool, a 7-item prediction rule aimed at identifying patients needing referral for diagnostic testing (167). The tool uses information on clinical symptoms at presentation to predict the likelihood (or, risk) of a final PCD diagnosis. As diagnostic outcome, we used the same composite reference standard as in the first study (101) based on results from all available diagnostic tests. A standardised clinical history was taken from all

referred patients, prior to performing any of the diagnostic tests. As detailed in the manuscript, diagnosis was based on positive test results, but not on the clinical history. Symptoms were not part of the composite diagnosis, so there was no incorporation bias in this study. In addition to the published analyses, as strategy to assess potential model overfitting, we performed a bootstrapping testing of the receiver operator characteristic curves of the derivation population, which indicated an expected shrinkage of <3% (Appendix 4). This suggests that there is no significant overestimation for the ROC curves as produced within the predictive logistic regression models. We further published the external validation using an independent patient cohort (167) but pointed out that PICADAR should be further validated, and if necessary modified, in different study populations, in general respiratory clinics and different countries. In summary, we agree that current methods for diagnosing PCD and for assessing diagnostic accuracy are imperfect and require further development and scrutiny. However, we confirm that our manuscripts, which were very transparent about diagnostic pathways, diagnostic and statistical methods, and their limitations, are an acceptable way forward whilst we establish better methods. The two publications are not intended as the definitive answer, but as significant steps in the plight to develop an evidence-base to diagnostic testing. Waiting for a 'gold standard' will not allow us to move forward in the near future.

Part 3: The Development and Validation of a Health-Related Quality of Life Questionnaire for Adults with PCD

8: A QUALITY-OF-LIFE MEASURE FOR ADULTS WITH PRIMARY CILIARY DYSKINESIA: QOL–PCD (PAPER 5)

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Author's contributions

MWL, SDD, ALQ and JSL had the concept for this study. I was involved, with the other authors, in contributing to the study design. My role involved conducting the semi-structured interviews and cognitive interviews with UK patients and analysing the data using nVIVO software. I regularly presented the results of the analyses to MWL, SDD, ALQ and JSL and triangulated the findings with results from the US interviews. Through this discussion, the 49-item preliminary QOL-PCD was agreed. With JSL, I drafted the manuscript and was involved in each stage of the peer-review process.

8.1. Abstract

Background: Primary ciliary dyskinesia (PCD) is characterised by chronic suppurative lung disease, rhino-sinusitis, hearing impairment and sub-fertility. We have developed the first multidimensional measure to assess health-related quality of life (HRQoL) in adults with PCD (QOL–PCD).

Method: Following a literature review and expert panel meeting, open-ended interviews with patients investigated the impact of PCD on HRQoL in the UK and North America (n=21). Transcripts were content analysed to derive saturation matrices. Items were rated for relevance by patients (n=49). Saturation matrices, relevance scores, literature review, evaluation of existing measures, and expert opinion contributed to development of a preliminary questionnaire. The questionnaire was refined following cognitive interviews (n=18).

Results: Open-ended interviews identified a spectrum of issues unique to adults with PCD. Saturation matrices confirmed comprehensive coverage of content. QOL–PCD includes 48 items covering the following seven domains: Physical Functioning, Emotional Functioning, Treatment Burden, Respiratory and Sinus Symptoms, Ears and Hearing, Social Functioning, and Vitality and Health Perceptions. Cognitive testing confirmed that content was comprehensive and the items were well-understood by respondents.

Conclusion: Content validity and cognitive testing supported the items and structure. QOL–PCD has been translated into other languages and is awaiting psychometric testing.

8.2. Introduction

Primary ciliary dyskinesia (PCD) is a rare disease (~1 in 15 000 people) inherited in a genetically heterogeneous, autosomal recessive pattern (20, 21, 170). It is characterised by chronic infection of the upper and lower airways caused by impaired mucociliary clearance as a consequence of abnormal function of motile cilia. In healthy individuals, cilia clear airway mucus, bacteria and debris by coordinated beating. The ciliary dysfunction in PCD leads to a daily wet cough, recurrent chest infections and rhino-sinusitis. By adulthood, bronchiectasis is invariable and many patients develop respiratory failure (1). Motile cilia are important in organ systems besides the airways, such as the embryonic node, sperm flagella and the female reproductive tract. Therefore, patients frequently have problems caused by non-respiratory dysmotile cilia, *e.g.* serous otitis media ("glue ear") leading to hearing impairment and immotile sperm causing infertility). The cilia of the embryonic node, responsible for left–right asymmetry of organs, are similar in structure to respiratory cilia. Dysfunction of embryonic nodal cilia in PCD causes laterality defects, including situs inversus (chest and abdominal organs are mirror image of normal; seen in approximately 40% of cases) and situs ambiguous (disturbance of the usual left and right distribution of the thoracic and abdominal organs which does not entirely correspond to mirror image; seen in approximately 10% of cases) and can be associated with congenital heterotaxic heart disease in approximately 2–3% of cases (8).

Monitoring of disease progression and evaluation of therapeutic options has been hampered by a lack of disease-specific outcome measures. Spirometry is an insensitive marker of progressive lung disease, which is evident using high-resolution computed tomography (HRCT)(66). Although HRCT is a useful staging test, it is an impractical monitoring tool. Lung clearance index (LCI), measured by multiple breath washout, has been investigated as a

potential tool for monitoring at specialist centres using sulphur hexafluoride (SF_6) as a tracer gas (73, 76). However, contrary to findings in cystic fibrosis (CF), LCI does not appear to be a sensitive test of airway disease in advanced PCD (73). Furthermore, physiological measures provide information on objective indicators of health to patients and clinicians, but these measures do not reflect the patient perceptions of the impact of the disease on symptoms as well as physical, social and emotional functioning.

Thus, measures are needed to assess the impact of PCD, from the patient's perspective, on all domains of patient functioning (24, 90, 171). Health-related quality of life (HRQoL) measures have become a vital and necessary component of patient-reported outcomes (PROs) in populations with chronic disease (172). The US Food and Drug Administration (FDA) define HRQoL as the patient's perception of how they "survive, feel, and function" (94). We used a model for HRQoL originally proposed by Wilson and Cleary (173) and revised by Ferrans et al. (174). There is extensive agreement that assessment of HRQoL should encompass, at minimum, physical, social and emotional well-being and symptoms which allow for a multidimensional, systematic measure of how the illness and its treatment impact symptoms and other domains of functioning.

Existing PROs do not assess the disease-specific effects of PCD on daily symptoms and functioning. Most studies have utilised either generic (*e.g.* Short Form 36 Health Survey) or broad-based respiratory questionnaires, such as the St George's Respiratory Questionnaire (SGRQ)(77, 107, 113, 118) and two additional studies have utilised qualitative interview methods (78, 79). These PROs have a number of limitations for assessing HRQoL in adults with PCD. For example, the SGRQ was developed for patients with chronic obstructive airways disease and, thus, has a limited number of respiratory symptoms, no items relevant to ear, nose and throat disease or fertility problems, long and variable recall periods and

considerable respondent burden (*e.g.* nearly an hour to complete). Thus, there was an urgent need to develop a disease-specific measure for adults with PCD. These tools can be used to document the progression of disease, monitor patients clinically and serve as an outcome measure for clinical trials of new therapies.

Our ultimate goal is to develop a PCD-specific HRQoL instrument for use as a primary or secondary outcome measure in large, randomised clinical trials. To recruit adequate numbers of participants, multi-centre and multi-national collaboration was required. Therefore, researchers from the UK, North America and Ireland worked closely to develop age-specific questionnaires (child, adolescent, parent-proxy and adult) using guidance developed by the FDA and European Agency for the Evaluation of Medicinal Products (EMEA)(92-94). Development of the child, adolescent and parent-proxy versions will be reported in a separate manuscript. This manuscript describes the development process for the QOL–PCD Adult version which included the following phases: 1) literature review and expert panels; 2) open-ended interviews with patients in in UK, USA and Canada; 3) item generation; 4) cognitive testing; and 5) refinement of the draft measure. QOL–PCD is now being validated in Europe, USA and Canada; the psychometric reliability and validity will be reported when these studies are complete.

8.3. Methods

The protocol for development of the QOL–PCD complied with FDA requirements. The study was approved by Southampton and South West Hampshire Research Ethics Committee (Southampton, UK) A 07/Q1702/109 and by the University of Miami Institutional Review Board (Miami, FL, USA). Informed consent was obtained prior to interviews.

8.3.1. Literature review and expert panel

First, a systematic literature review was conducted to identify key symptoms and effects of PCD on patient functioning. MEDLINE and EMBASE were searched, and additional references were sought through citations in the identified studies. Abstracts were reviewed and manuscripts sourced for research investigating the effects of PCD on adults.

In the next step, expert clinicians, allied health professionals and researchers met to discuss their own perceptions of the impact of PCD on adults, based on their clinical experiences. These sources of information contributed to a long list of items patients rated for relevance. These items also informed the development of the open-ended interview guide.

8.3.2. Participants

In the UK, participants for the open-ended and cognitive interviews were recruited from PCD clinics and from an advert through the PCD Family Support Group UK. A list of potential items was sent to the Family Support Group in the UK to rate their relevance. Participants in North America were recruited from a cohort of PCD patients evaluated at the University of North Carolina, as well as from the US PCD Foundation's registry of patients. Expert opinion was also sought during the item generation and item reduction phases from members of the European PCD Group.

Criteria for participation in the open-ended and cognitive interviews included age ≥18 years with a diagnosis of PCD. Patients were recruited from English-speaking countries: UK, USA and Canada. UK participants had an existing diagnosis from one of the English diagnostic centres (21, 141) based on clinical phenotype plus high-speed video analysis of ciliary function and/or assessment of ciliary ultrastructure by electron microscopy. North American participants were diagnosed at a specialised PCD research center, based on: a compatible

clinical phenotype plus defect in ciliary ultrastructure and/or identification of biallellic disease-causing mutations in one of the PCD genes.

8.3.3. Open-ended interviews

In-depth interviews were conducted either in-person or by phone to elicit the effects of PCD from the patients' perspective. Interviews were conducted in the UK by L. Behan and in USA and Canada by A.L. Quittner, A. Alpern and A.M. Morris. All participants were fluent in English. We attempted to interview patients who were geographically representative and clinically stable. The audio of all interviews was recorded and transcribed for content analysis using either NVivo (version 8 2008; QSR International Pty Ltd, Daresbury, UK) in the UK or Atlas.ti (Version 7.0; Scientific Software Development, Corvallis, OR, USA) in the USA. Thematic coding was used to identify key symptoms and psychosocial impacts. Two members of each research team coded the interview transcripts using consensus coding. When there was a discrepancy, this was discussed and resolved within the pair of coders. We did not calculate percentage agreement since we used a consensus coding process. These data were then analysed to identify critical items based on frequency of endorsement. Content analysis of these transcripts yielded saturation and indicated that the measure was comprehensive and that all relevant items were included.

8.3.4. Item generation

Questions from the literature review, expert panel and open-ended interviews were also sent by post to respondents of an advert circulated to members of the PCD Support Group and to adult patients at PCD clinics in the UK. A pre-paid envelope and covering letter was included. Participants rated each item using a 5-point Likert scale (1: “not relevant”; to 5: “highly relevant”).

Items for the initial QOL–PCD measure were based primarily on the patient-based content analysis. We discussed these results in a series of teleconferences chaired by J.S. Lucas. Each meeting included clinicians, psychologists and interviewers from Ireland, the UK and the USA. Decisions made using a modified Delphi approach guided by two main principles. The primary criterion for including an item was its impact on HRQoL, measured by the frequency with which items were mentioned across patients and in relation to other items mentioned. There was no pre-determined frequency required for inclusion of an item, but the researchers considered the frequency each item was mentioned relative to the other items in that domain. They also considered the importance interviewees placed on these items. Secondly, patients' ratings of relevance from the UK survey were considered. For each item, decisions were made to include an item based on our discussion. When there was initial disagreement, the chair invited each individual to explain their rationale. Unanimous agreement was achieved within two rounds of discussion. Finally, we determined that all of the content had been identified based on saturation matrices in the UK and US, which showed that no new content emerged after six patients, on average, in each country. We included at least four items on each subscale to ensure adequate internal consistency.

8.3.5. Construct of prototype questionnaire

Agreement on item selection and wording was achieved during multi-disciplinary, multinational conference calls. Selected items were written using patient language obtained in the qualitative interviews and then combined into scales (*e.g.* frequency and severity of respiratory symptoms, perceptions of treatment burden). Where appropriate, the items were formulated into questions based on the Cystic Fibrosis Questionnaire-Revised (CFQ-R), which has been well-validated(172, 175). Some example CFQ-R items that were adapted to the PCD HRQoL measure include: “Did you cough during the day?” and “How often does

CF get in the way of meeting your school, work, or personal goals?" Items were written to ensure conceptual, cultural and linguistic equivalence for North America, the UK and Ireland, by researchers from the USA, England and Ireland. We also adhered to both the FDA and EMEA Guidance (92-94). The FDA guidelines for developing PROMs for use in clinical trials (83) recommend an iterative process consisting of: (1) hypothesising the conceptual framework, (2) adjusting conceptual framework and draft instrument, (3) confirming the conceptual framework and accessing other measurement properties, (4) collecting analysing and interpreting data, and (5) modifying the instrument.

8.3.6. Cognitive interviews

Cognitive interviews were conducted prior to formal psychometric validation of questionnaires to evaluate how respondents process the question and rating options cognitively; *i.e.* What "meaning" do these items have for respondents? Is this the same meaning we intended? What were they considering when rating frequency or impact? Specifically, we wanted to identify any problems with the instructions, organisation of the questionnaire, item interpretation, memory retrieval, decision-making processes and response selection. A "think aloud" procedure was used to investigate the participants' comprehension of the instructions, items and rating scales. They were asked clarifying questions, such as: "What were you thinking of when answering that question?" and "What does X word mean to you?" For example, "What does feeling 'well' mean to you?", "What would have made you endorse a higher/lower frequency for that question?", "How relevant/important is this question for you?", "Are the rating options clear to you?" and "Are they easy to use?"

Participants were first asked to complete the prototype questionnaire independently. Next, they were interviewed using specific cognitive probes, which focused on the clarity of the

question, its meaning, relevance and importance, and what would have shifted their response to an adjacent answer. The audio of all interviews was recorded and transcribed. The results were discussed during a series of teleconferences to determine whether revisions were required for the format, instructions or items. The measure was refined based on the cognitive interviews and then finalised.

8.4. Results

8.4.1. Item generation

Items were generated by the expert panel and the qualitative, open-ended interviews with patients. Characteristics of participants who completed the open-ended interviews (n=21) are shown in Table 16. The majority of participants were female, and among US participants, most were between 18 and 35 years of age. As expected, nearly all adults described a chronic cough and sino-nasal symptoms. Eight (38%) participants described themselves as infertile or had required assisted fertilisation, but 10 (48%) participants had not yet tried to conceive or had their fertility status checked. Selected patient quotes from the open-ended interviews conducted with UK and North American participants are presented in Table 17.

8.4.2. Content analysis and item reduction

Content analysis of the transcripts yielded the most important items for each of the 10 domains based on the frequency with which they were mentioned across adults. Saturation of content, across domains, was confirmed when no new themes emerged (Figure 13). Our results indicated that this was achieved by the 5–7th interview, depending on the specific content area. We also harmonised the content across UK and North America to identify the

most important topics. The items that were considered most important and relevant by the participants who performed the open-ended interviews were also scored as highly relevant by the 49 adult members of the PCD UK support group who completed the survey. Thus, content analysis of the interviews concurred with results of the survey (Appendix 5: Table A12).

8.4.3. Cognitive testing

Cognitive interviews were conducted with 15 adults (UK n=9, USA n=6). Review of these transcripts indicated that patients found the items clear, important and relevant and had no difficulty with the response options. Six items were added, based on patient input, after the cognitive testing phase (Table 18). These topics included: making plans for the future (vacation, attending family events), treatment burden, intimacy, and pain associated with sinus disease. Thus, the final prototype instrument contained 48 items (QOL–PCD v1.2). Following this iterative process, the draft version of the QOL–PCD v.1.2 is ready to be tested in a psychometric validation study.

8.5. Discussion

This process, conducted in the UK and North America, yielded the first HRQoL instrument for adults with PCD, the QOL–PCD. It was developed following the guidelines published by the major regulatory bodies in Europe and the USA (*i.e.* EMEA and FDA) (92-94) and will be submitted to these agencies for consideration as an outcome measure for clinical trials. The most important principle governing its development was our reliance on patient input and their perspective at each phase. Thus, this tool systematically reflects how an adult with

PCD “survives, feels, or functions” (94). Given the rarity of this chronic disease, we developed the content cross-culturally in English-speaking countries (UK, USA and Canada) and found no discrepancies in content across countries. Additional input was obtained from the current literature and from medical experts across Europe and North America.

Open-ended interviews highlighted the importance not only of patients' respiratory symptoms, but the effects of sinus disease and hearing problems on daily functioning. Although sinus disease is also problematic for patients with CF and non-CF bronchiectasis, patients with PCD emphasised the additional impact of their upper respiratory tract symptoms. This highlights that PCD has distinct features from other bronchiectatic diseases (23), and deserves individualised management. Thus, a number of items assessing rhino-sinus and ear symptoms appear on the final instrument, differentiating it from disease-specific HRQoL measures for adults with CF or non-CF bronchiectasis (172, 176).

The study population was not fully representative of the PCD population. In common with many previous studies, it was more difficult to recruit men than women with only three men (14%) participating in the interviews. Approximately 6% of patients with PCD have cardiovascular disease (8). None of the 21 interviewees in this study had cardiovascular disease, but even if we had designed the study population to be representative of the PCD population we would have aimed to have only one patient. Moreover, we would not be able to recruit patients with the diverse spectrum of cardiac disease *e.g.* complex cyanotic heart disease versus simple cardiac anomaly. It is therefore a limitation of the questionnaire that it does not include items relevant to patients with cardiac disease.

A reliable patient-reported outcome measure for PCD is particularly important, given that physiological measures such as forced expiratory volume in 1 s and LCI are not sensitive or

predictive, and HRCT is not suitable for repeat testing due to radiation exposure. Importantly, the QOL–PCD provides a measure of the multidimensional effects of PCD on adults, from their own perspective including its impact on the upper and lower respiratory systems, treatment burden and social and emotional functioning. Reliability and validity studies are currently in process and will be reported in due course.

8.6. Conclusion

In summary, the QOL–PCD was developed and has undergone cognitive testing in adults from several English-speaking countries. It has been translated into Dutch, German, and Danish, with plans to develop translations from major European countries and the Middle East. A multi-national, psychometric field-study is now planned to assess several forms of reliability and validity. Similar processes have been used to develop age-specific HRQoL measures for children and adolescents with PCD and parent caregivers. These instruments will also be translated into other languages and validated in future studies.

Figure 13: Saturation grids for UK and US participants: respiratory symptoms, sinus symptoms, and ear and hearing symptoms. Vertical lines indicate saturation was reached. All items above the horizontal lines were retained for the final questionnaire. Shaded cells refer to the first time the item was mentioned.

[#]: participants did not indicate adverse effects of difficulty smelling; therefore it was not included in the final questionnaire.

UK participants

Respiratory symptoms	1	2	3	4	5	6	7	8	9	10	11	Total
Cough	1	1	1	1	1	1	1	1	1	1	1	11
Shortness of breath	1	1	1	1	1	1	1	0	1	1	0	9
Chest congestion	1	1	1	1	1	1	1	0	0	1	1	9
Cough up mucus	1	0	1	1	1	1	1	0	0	1	1	8
Chest tightness	1	1	0	0	1	0	1	0	0	0	1	5
Wheezing	0	1	1	0	1	0	0	0	0	0	1	4
Cough interrupts sleep	0	1	1	0	0	0	0	0	0	0	1	3
Chest pain	0	0	0	0	1	1	0	0	0	0	1	3
Cough with exertion	0	0	1	1	0	0	1	0	0	0	0	3
Chest pain with exertion	0	0	0	0	0	0	0	0	0	0	0	0
Sleep disturbances from chest pain	0	0	0	0	0	0	0	0	0	0	0	0
Rhino-sinus symptoms												
Runny nose	1	1	1	1	1	1	1	1	1	1	1	11
Stuffy nose	1	0	1	1	0	0	0	1	0	1	0	5
Post-nasal drip	1	0	0	0	0	0	0	1	0	0	0	2
Sinus pain/headache	0	0	1	1	0	0	0	0	0	0	0	2
Sinus surgery	1	0	0	1	0	0	0	0	0	0	0	2
Difficulty smelling	1	0	0	1	0	0	0	0	0	0	0	2[#]
Sinus congestion	1	0	1	0	0	0	0	0	0	0	0	2
Sinus pressure	0	0	1	0	0	0	0	0	0	0	0	1
Difficulty tasting	0	0	0	1	0	0	0	0	0	0	0	1
Ear and hearing symptoms												
Trouble hearing	1	0	1	0	1	0	0	0	1	1	1	6
Ears blocked	1	0	1	0	1	0	0	0	1	0	1	5
Ear ringing	0	0	0	0	0	0	0	0	0	0	0	0
Problems with balance	0	0	0	0	0	0	0	0	0	0	0	0

US participants

	1	2	3	4	5	6	7	8	9	10	Total
Cough	1	1	1	0	1	1	1	1	1	1	9
Chest congestion	0	1	0	1	1	1	1	1	1	0	7
Chest pain	1	1	0	1	1	0	1	1	0	1	7
Cough up mucus	1	0	1	1	0	1	1	0	0	0	5
Shortness of breath	0	0	1	0	1	0	0	1	1	1	5
Chest tightness	1	0	1	1	0	0	0	0	0	1	4
Cough interrupts sleep	1	0	1	0	1	0	0	0	0	0	3
Wheezing	1	0	1	0	0	0	0	0	1	0	3
Cough with exertion	0	1	0	0	0	0	0	0	0	0	1
Sleep disturbances from chest pain	0	1	0	0	0	0	0	0	0	0	1
Chest pain with exertion	1	0	0	0	0	0	0	0	0	0	1
Rhino-sinus symptoms											
Stuffy nose	1	1	0	1	1	1	1	1	1	0	8
Sinus pain/headache	1	1	0	1	1	1	0	1	1	1	8
Runny nose	1	1	0	0	1	0	1	1	1	1	7
Post-nasal drip	0	0	0	1	0	1	0	0	1	1	4
Difficulty smelling	0	1	0	0	1	0	0	1	1	1	5[#]
Sinus surgery	1	1	0	0	0	0	0	1	0	1	4
Difficulty tasting	0	1	0	1	1	0	0	0	0	0	3
Sinus congestion	0	0	0	1	1	0	0	0	1	0	3
Sinus pressure	1	0	0	0	0	0	0	0	0	0	1
Ear and hearing symptoms											
Trouble hearing	1	1	1	1	1	1	1	1	1	1	10
Ears blocked	0	1	0	1	0	0	0	1	1	1	5
Ear ringing	0	0	0	0	0	1	0	1	0	0	2
Problems with balance	1	0	0	0	0	0	0	0	0	0	1

Table 16: Demographics and clinical characteristics of participants

	Study population	UK	USA
Participant n	21	11	10
Sex			
Male	3 (14)	1 (9)	2 (20)
Female	18 (86)	10 (91)	8 (80)
Age (years)			
18-35 years	12 (57)	5 (45)	7 (70)
36-50 years	4 (19)	1 (9)	3 (30)
51-65 years	3 (14)	3 (27)	
Over 65 years	2 (10)	2 (18)	
Age at diagnosis			
<5 years	4 (19)	1 (9)	3 (30)
5-12 years	4 (19)	0	4 (40)
12-18 years	1 (5)	1 (9)	0
>18 years	12 (57)	9 (82)	3 (30)
Race/ethnicity			
UK			
White British		9 (82)	
British Asian		1 (9)	
Asian		1 (9)	
USA			
Caucasian			10 (100)
Hispanic			9 (90)
White non-Hispanic			1 (10)
Symptoms			
Chronic wet cough	21 (100)	11 (100)	10 (100)
Persistent runny nose	20 (95)	11 (100)	9 (90)
Recurrent sinus disease	16 (76)	8 (72)	8 (80)
Infertility	8 (38)	4 (36)	4 (40)
Situs abnormalities	12 (57)	8 (72)	4 (40)

Cardiac disease	0	0 (0)	0 (0)
FEV_1 % predicted #			
Range		31-98	29-102
Mean±SD		64±21	65±25
Employment status			
In paid employment	11 (52)	4 (36)	7 (70)
Student	4 (19)	2 (18)	2 (20)
Retired due to age	1 (5)	1 (9)	0
Retired/left work due to PCD	4 (19)	3 (27)	1 (10)
Carer for dependents	1 (5)	1 (9)	0
Other	0	0	0
Marital status			
Single	5 (24)	3 (27)	2 (20)
Living with partner/ spouse	16 (76)	8 (73)	8 (80)
Separated from partner/ spouse		0	0
Widowed		0	0
Fertility			
Conceived naturally		3 (27%)	0 (0%)
Conceived through IVF		1 (9%)	2 (20%)
Infertility		3 (27%)	2 (20%)
Not yet known		4 (36%)	6 (60%)

Data are presented as n (%), unless otherwise stated. #: forced expiratory volume in 1 s (FEV1) is based on n=5 participants in the UK and n=8 in North America; data were unavailable for telephone interviews.

Table 17. Participant quotes by topic

Topic	Quote	Country of interviewee/gender/ age band (in years)
Impact of respiratory symptoms	"I had to tell the group…not to worry because I start huffing and spluttering as I'm walking…"	UK/female/36-50
	"When I listen to myself breathe I always wheeze."	US/female/18-35
Impact of sinus symptoms	"...I'm always blowing my nose…doesn't matter what weather it is.	UK/female/36-50
	"I always have to blow my nose before I eat if I wanna taste anything."	US/female/36-50
Impact of ear symptoms/ hearing loss	"You have to ask people to repeat themselves…so many times, they're just like oh don't worry about it."	UK/male/18-35
	"I can't go white water rafting because I have tubes in my ears and my ears can't get wet."	US/female/18-35
Impact of fertility issues	"Finding out that I possibly can't have kids…that's when it…started to panic me a little bit."	UK/male/18-35
	"I'm still very uncertain if I ever wanna have children because I don't know how me having this illness will affect them."	US/female/18-35
Impact of treatment burden	"…I don't really want to do it, it's kind of boring and it's not fun and… I'd rather do something else. But obviously you have to do it."	UK/female/18-35
	"I think it just requires more planning…I need to wake up earlier…or…start getting ready for bed earlier, I need to come home from work and do this…it's just more planning."	US/female/18-35
Emotional functioning	"I'm so frustrated with this illness, I just want it to go away, but…unfortunately that's how I have to live."	UK/male/18-35
	"…if you go to the doctor [and] you're feeling pretty good and you know your numbers are not good…that	

	can be a big cause of anxiety."	US/female/18-35
Social functioning	"It has had such a huge impact on my life, and certainly I think it's…contributed to the breakup of my first marriage."	UK/female/50-64
	"…there have been times where I've had to cancel things because I I've gotten sick…getting sick can happen overnight…you're fine one day and the next day you feel awful…"	US/female/18-35

Table 18. Summary of modifications to QOL-PCD after cognitive testing

Issues	Modifications after cognitive testing
Items added to scales	Respiratory Symptoms: Wheezing Chest tightness Sinus Symptoms: Post-nasal drip Sinus pain Physical Functioning: Carrying heavy things, such as books and shopping bags Health Perceptions: I feel healthy Emotional functioning: Felt depressed Felt lonely Social Functioning: Stay at home more often than would like Feel comfortable coughing in front of others Feel comfortable blowing nose in front of others Intimacy with a partner (kissing, hugging, sexual activity) Worried about being exposed to others who are sick Comfortable doing treatments (airway clearance, physiotherapy) in front of others Treatment Burden Physiotherapy/airway clearance made you feel tired quickly
Items deleted from scales	Health Perceptions: I feel in control of my PCD Emotional Functioning: Felt angry Felt limited Felt self-conscious Social Functioning Self-conscious coughing and blowing my nose in public Treatment Burden: Treatments made you feel better Physiotherapy is hard work
Wording modifications	Emotional Functioning: “Felt anxious” changed to “felt worried” “Felt frustrated” changed to “felt frustrated about doing your daily treatments”

9. VALIDATION OF A HEALTH-RELATED QUALITY OF LIFE INSTRUMENT FOR PRIMARY CILIARY DYSKINESIA: QOL-PCD (PAPER 6)

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SUBMITTED TO THORAX AND UNDER REVIEW

Author's contributions

MWL, SDD, ALQ and JSL had the concept for this study. All authors contributed to the study design. I managed the study. I developed the protocol for the study design and communicated with the validation centres throughout the study. I recruited UK patients to complete the measures and issued regular reminders to follow-up completion of measures. I managed all data received from UK and US sites and I completed the analysis of the data. Together with MWL, SDD, ALQ and JSL, we discussed the analysed data and agreed the resultant 40-item QOL-PCD. I was responsible for draftING the manuscript and submitting it to a suitable journal. I was responsible for making the recommended changes during the peer review process.

9.1. Abstract

Background: QOL-PCD is the first disease-specific, health-related quality of life instrument for primary ciliary dyskinesia. The aim of this study is to perform a psychometric validation of QOL-PCD assessing the performance of this measure in adults, including its reliability, validity and responsiveness to change.

Method: Seventy-two adults with primary ciliary dyskinesia completed the 49-item QOL-PCD and generic quality of life measures: SF-36, SNOT-20 and SGRQ-C. Thirty-five participants repeated QOLPCD 10-14 days later to measure stability or reproducibility of the measure, with 10 patients repeating it during respiratory exacerbations to assess responsiveness.

Results: Multi-trait analysis was used to evaluate how the items loaded on 10 hypothesised scales: Physical, Emotional, Role and Social Functioning, Treatment Burden, Vitality, Health Perceptions, Upper-Respiratory Symptoms, Lower-Respiratory Symptoms, and Ears and Hearing Symptoms. This analysis of item-to-total correlations led to 9 items being dropped; the validated measure is comprised of 40 items. Each scale had excellent internal consistency (0.74 to 0.94). Two-week test-retest demonstrated stability for all scales (intra-class coefficients 0.73 to 0.96). The QOL-PCD was responsive to exacerbations, with decreased mean scores on several scales. Significant correlations were obtained between QOL-PCD scores and age and FEV 1. Strong associations were also found between QOL-PCD scales and corresponding generic questionnaires e.g. Lower-Respiratory Symptoms and SGRQ-C ($r=0.72$, $p<0.001$), whilst correlations between measures of different constructs were poor.

Conclusion: QOL-PCD has demonstrated good internal consistency, test-retest reliability, convergent and divergent validity, and responsiveness to exacerbations. QOL-PCD is short

and easy to use, offering a promising tool for evaluating new therapies and for measuring symptoms, functioning and quality of life during routine care.

9.2. Introduction

PCD is a rare, heterogeneous genetic disorder characterised by impaired mucociliary clearance due to abnormal ciliary function. Individuals with PCD usually present with unexplained neonatal respiratory symptoms in the first few days of life (3, 167), have early onset of persistent sino-pulmonary infections, bronchiectasis during childhood (66) and a progressive decline in lung function(4). This can lead to end stage lung disease with a report that 25% of adult PCD patients in the US required long-term oxygen or lung transplantation (1). Male infertility is common since sperm flagella have a similar ultrastructure to cilia; the incidence of female infertility and of ectopic pregnancy is unclear and is explained by immotile fallopian tube cilia. Motile embryonic nodal cilia contribute to left-right asymmetry and nearly half of PCD patients' exhibit situs inversus, and 12% heterotaxic syndromes, sometimes associated with complex congenital cardiac defects (8). Outcome measures that have been used to assess disease severity in PCD include spirometry (4), chest computed tomography (66, 71), magnetic resonance imaging (5) and lung clearance index (73-76). These physiological and radiological measures all have limitations in terms of their sensitivity or feasibility to monitor disease progression. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) strongly endorse the use of outcome measures in clinical trials, assessing the impact of the disease on the patient's daily symptoms and functioning (e.g., physical, respiratory, social) in addition to physiological measures (92-94). Treatment strategies for PCD have necessarily been applied from other diseases (22, 23), particularly cystic fibrosis (CF), since no medications have specifically been tested and approved for PCD. A major obstacle to evaluating new treatments and monitoring disease

progression is the lack of a disease-specific outcome measure (24). Thus, we developed health-related quality of life (HRQoL) measures to assess the impact of PCD for children, teenagers and adults from the patient perspective (103). Here we present the psychometric validation of the English version of QOL-PCD in adults from United Kingdom, United States of America and Canada.

9.3. Method

9.3.1. Participants

Participants were recruited from PCD diagnostic centres across the UK, USA and Canada. Adults (>18 years) with a positive diagnosis of PCD were eligible to participate. Information about the study was provided at a clinic appointment or by telephone. The following inclusion criteria had to be met by patients: (1) diagnosis of PCD in one of the specified diagnostic centres, (2) age ≥18 years and (3) ability to read and speak English fluently. UK participants had been diagnosed at one of the English diagnostic centres (21, 141) based on clinical phenotype plus high-speed video analysis of ciliary function and/or assessment of ciliary ultrastructure by electron microscopy. North American participants were diagnosed at a specialised PCD research center, based on: a compatible clinical phenotype plus defect in ciliary ultrastructure and/or identification of biallellic disease-causing mutations in one of the PCD genes.

9.3.2. QOL-PCD scales

The QOL-PCD Adult version consisted of 49 items (103), which were self-completed electronically at home or in the clinic, where possible; ‘pen and paper’ copies were provided to those without access to internet. Participants were provided with a unique study number and a link to the online survey. No identifiable information was included, and the data were

captured on a server of University of Southampton. Most responses used a four-point Likert scale: "not at all true" to "very true" or "never" to "always". The first time QOL-PCD was completed, participants also completed generic questionnaires: Short-Form 36 Health Survey (SF-36), the shortened St George Respiratory Questionnaire (SGRQ-C), and a measure focusing on rhino-sinus symptoms: SNOT-20. Further details on this protocol and these measures can be found in Appendix 6.

9.3.3. Statistical analysis

Statistical analyses were conducted using the SPSS Statistics (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). $P<0.05$ was considered significant. Firstly, we assessed the distribution of responses for each item and each scale to look for floor and ceiling effects. Next, we used multitrait analysis to examine the relationships between items and their proposed scales. These analyses assessed the extent to which items correlated with their hypothesised versus competing scales; we required item-to-scale correlations ≥ 0.40 with the intended scale and lower correlations with competing scales (177, 178). We considered floor and ceiling effects, using <15% of participants as the threshold for the highest and lowest scores for a scale (179).

9.3.3.1 Reliability

Internal consistency of the QOL-PCD scales were investigated by Cronbach's α values. Cronbach's α gives a score of between zero and one, a value of >0.70 indicating good internal consistency. Items were removed if this led to higher internal consistency (i.e. higher Cronbach's α) to increase the parsimony and efficiency of the instrument (180, 181).

The distributions of responses and multi-trait analyses were reviewed in a series of teleconferences to decide which items could be removed, allowing us to shorten QOL-PCD, taking reliability and clinical relevance into consideration. Test-retest reliability was assessed using intra-class correlation coefficients (ICC) in stable patients who completed the QOL-PCD a second time, 10 to 14 days after completing the baseline measures. An ICC value of >0.60 provided evidence of good stability and >0.75 excellent stability for each scale.

9.3.3.2. Validity

For a questionnaire to be construct valid, all items together should represent the underlying construct (HRQoL). Construct validity can be determined by testing the instrument against hypotheses. We hypothesised *a priori* that clinical features (age, gender, FEV_1 and Pseudomonas status) would correlate with specific scales (e.g. FEV_1 would correlate with Physical Functioning and Upper Respiratory Symptoms (construct validity). Cohen's guidelines for the interpretation of correlation coefficients were used; correlations between 0.50 and 1.00 were interpreted as strong, correlations between 0.30 and 0.50 as moderate, correlations between 0.10 and 0.30 as small and correlations <0.1 as weak (182). We also predicted that QOL-PCD scales would have moderate correlations (>0.3), using Spearman's Correlation, with generic scales (SF-36, SGRQ-C and SNOT20) measuring similar constructs (convergent validity). We hypothesised small or weak correlations (<0.3) with scales measuring different constructs (divergent validity). Details of the hypothesised correlations are provided in Tables 22 and 23.

9.3.3.3. Responsiveness

Participants who contacted the study team towards the start of an exacerbation were asked to complete the questionnaire whilst unwell. An exacerbation was defined as respiratory tract

symptoms leading to prescription of antibiotic treatment, or a decline in FEV_1% predicted ≥ 10%. For patients who had experienced more than one exacerbation, the first exacerbation was used for analyses. For patients who repeated the measure during an exacerbation, we used paired sample t-tests to compare scores when the patient was stable and unwell.

9.3.4. Ethical approval

The study was approved by the National Research Ethics Service, UK (UK 07/Q1702/109), the Research Ethics Board at the Hospital for Sick Children in Toronto, Canada and the Institutional Review Boards at the University of North Carolina, Chapel Hill. Written consent was obtained prior to participation.

9.4. Results

9.4.1. Participants

Between April 2014 and March 2016, 72 adults were recruited. Participant characteristics are shown in Table 18. The 49-item prototype took a mean time of 8 minutes (SD 5) to complete.

9.4.2. Development of scales

We used a multi-trait analysis to generate ten hypothesised scales: Physical, Emotional, Role and Social Functioning, Treatment Burden, Vitality, Health Perceptions, Upper Respiratory Symptoms, Lower-Respiratory Symptoms, and Ears and Hearing Symptoms. Examination of the distribution of responses to items and the multi-trait analyses enabled us to shorten the questionnaire by removing nine items which were redundant, not strongly endorsed or did not correlate strongly with its designated scale. (Appendix 6: Table A14). The final QOL-PCD

comprised of 40 items on ten scales which were subjected to further psychometric analysis (Table 20).

Analyses of the 40-item QOL-PCD confirmed that all items had strong correlations (>0.63) with their intended scales. Few floor and ceiling effects were observed with the exception of: 1) floor effects on the Social Functioning scale (16.7% of respondents had low scores) and 2) ceiling effects were found on the Physical Functioning and Ears and Hearing scales, with 23.9% and 18.1% of respondents scoring the highest values, respectively.

9.4.3. Reliability: Internal consistency and test-retest reliability

The QOL-PCD scales had moderate to strong internal consistency (0.74 to 0.94) (Table 21). Thirty-five participants repeated QOL-PCD after 10-14 days, providing evidence of stability across all scales with intraclass correlation coefficients ranging from 0.73 to 0.96. (Table 21)

9.4.4. Validity

We had hypothesised that older age groups would report worse Physical Functioning, poorer Vitality, and more symptoms (Table 22). Mean Physical Functioning scores were significantly worse in those who were older; average scores for the 18-22 years age group was 88.21 (SD= 15.81), decreasing to 49.33 (SD= 34.88) in the >55 year old group ($p<0.001$). Lower Respiratory Symptoms scores were highest in the youngest (18-22 Year; M=58.76 (SD= 16.72) and oldest age groups (>55 years (M=55.00 (SD= 24.21)) and worst in the 37-55 years age group (M=36.30 SD= 19.90). For patients with reported Pseudomonas aeruginosa, lower mean scores were reported for Physical Functioning and Lower and Upper Respiratory Symptoms however these did not reach statistical significance. For lung function, Physical

Functioning scores were significantly lower in those with poor lung function, however, no significant associations were found on the Upper and Lower Respiratory Symptoms scores.

As hypothesised, females reported significantly worse Lower Respiratory Symptoms ($p=0.004$) (Table 22). Although mean Treatment Burden scores were higher in males, this did not reach statistical significance ($p=0.052$). There was also no significant difference between males and females on the Social Functioning scale (Table 22).

Convergent validity was tested by examining correlations between scales measuring similar constructs on the QOL-PCD with other validated scales: SNOT-20 (upper airway), the SF-36 (generic health status) and the SGRQ-C (lower respiratory) (Table 23). As expected, strong associations were found between the QOL-PCD Upper Respiratory symptoms and the SNOT- 20 total score ($r=0.60$, $p<0.01$). Strong correlations were also found between QOL-PCD Lower Respiratory Symptoms and SGRQ-C Symptoms ($r=0.69$, $p<0.001$). On the SF-36, as hypothesised we found strong correlations between Physical Functioning and QOL-PCD Physical scale ($r=0.83$, $p<0.001$), Role-physical and QOL-PCD Role Functioning ($r=0.83$, $p<0.001$) and Mental health with QOL-PCD Emotional Functioning scale ($r= 0.73$, $p<0.001$). In contrast, as hypothesised, much weaker relationships were found between the QOL-PCD scores and generic questionnaires that measured dissimilar constructs (divergent validity) For example, the Ear and Hearing Symptoms on the QOL-PCD correlated weakly with Role Functioning ($r=0.39$, $p<0.001$) on the SF-36 (Table 23). Similarly the QOL-PCD Lower Respiratory Symptoms scale correlated more strongly than the Upper Respiratory Scale, which focused on sinus symptoms, with SGRQ-C Symptoms.

9.4.5. Responsiveness

Ten patients completed the measure both while stable and when they experienced a respiratory exacerbation. Median time from completing the QOL-PCD when stable to exacerbation was 54 days (range: 3-172 days). A significant reduction in scores with large effect sizes was found for all scales except Emotional and Social Functioning and Ear and Hearing Symptoms (Table 24). Changes in scores were mapped for each individual for Physical Functioning and Lower Respiratory Symptom scales (Figure 14). Most participants reported a large decrease in scores between stable and exacerbation periods with some individuals' scores remaining stable.

9.5. Discussion

We have shown that QOL-PCD (Adult version) is a valid instrument to measure health related quality of life in patients with PCD. Psychometric testing confirmed our measure to be robust, reliable, responsive and valid. QOL-PCD was developed in the UK and North America to ensure cross-cultural equivalence in English-speaking countries (103). The QOL-PCD has already been translated into Danish, Dutch, German (developed and linguistically validated for Germany and Switzerland), Greek and French; translations are progressing well in Turkish, Arabic, Spanish (European), Italian and Spanish (Latin America). This international approach is important for rare diseases since multinational clinical trials are needed to recruit sufficient patients.

Our study involved the recruitment of 72 PCD patients from centres in North America (n=38) and the UK (n=34). All age groups were represented in the study, and participants had a range of disease severity (FEV_1% predicted: 26%-115%). This collaborative effort allowed us

to recruit sufficient participants with this rare disease. However, given the modest size of the study population, we conducted a multi-trait analysis to develop the scales rather than an exploratory factor analysis, which would require over 200 patients. The multi-trait analysis supported the conceptual foundations of 10 scales with 40 items and has been recommended for use in the development of HRQoL measures which are expected to have correlations across domains (e.g., increased respiratory symptoms are likely to lead to decreased physical functioning). QOL-PCD had excellent item-to-total correlations and strong internal consistency across all scales (Cronbach's α 0.74 to 0.94).

All scales were stable in an analysis of test-retest reliability over 10 to 14 days. Despite the small numbers of patients who completed QOL-PCD during an exacerbation (n=10) most scales evidenced worse mean scores than in comparison to these patients' stable state. Scores for three sub-scales did not change during exacerbations (Ears and Hearing Symptoms, Social and Emotional Functioning) perhaps suggesting that chest exacerbations have minimal effects on these domains of functioning. QOL-PCD correlated with generic HRQoL measures (SF-36, SGRQ, SNOT-20). As expected there were stronger relationships between scales assessing similar than dissimilar constructs.

The QOL-PCD was generally sensitive to the changes that have been reported to occur with increasing age and disease severity. As hypothesised Physical Functioning scores were highest for those with FEV_1>75% predicted and those who have never been identified with Pseudomonas aeruginosa although this did not reach statistical significance. Worsening quality of life with age has previously been described in a PCD study using generic measures (113) and decreases with age group were also found in this study for both Physical Functioning and Lower Respiratory Symptoms. Unexpectedly, the lowest QOL-PCD score in the Upper and Lower Respiratory Symptom scales were in our 37-55 years age group, with

scores increasing again in the ≥55 year age group. This may reflect specific challenges for this age group in comparison to those who are older e.g. lack of time to fit in treatments due to careers and families, or perhaps a survivor effect i.e. those who have survived to 55 years may have milder disease. This highlights the kinds of information that can be derived from HRQoL measures, reflecting the impact of disease management (i.e., adherence to prescribed treatments) and progression of disease.

QOL-PCD has advantages over physiological or radiological assessments of disease. By facilitating discussion on issues that are of importance to patients, clinicians can focus attention on their patient's perceptions of their illness, facilitating collaborative care and shared medical decision-making. Moreover, patient's perceptions of improved functioning that are not reflected in other physiological outcomes may be important factors in promoting adherence to treatments.

QOL-PCD has also been developed for children (aged 6-12 years), adolescents (aged 13-17 years) and for parents (proxy) of children aged 6-12 years. QOL-PCD has been translated into six conceptually and linguistically equivalent language versions; a number of further translations are in progress, each following a protocol-led process of forward and back translation followed by cognitive testing. Validation of the remaining age-specific tools and different languages is underway. Studies will also be needed to demonstrate equivalence between computerised and paper administrations. As the QOL-PCD is used in clinical trials we will need to determine the minimal clinical important difference score to allow for interpretation of changes in scores.

The adult version of QOL-PCD is ready for use in clinical trials to assess the benefits of medications or non-pharmacological interventions. It can also be used to understand the natural course and progression of the disease in terms of its effects on physical, emotional,

role and social functioning. We propose QOL-PCD as a tool to be used at annual assessments, providing a broad assessment of well-being, as perceived by the patient.

9.6. Conclusion

In summary, we have developed (103) and validated the first health-related quality of life instrument specific for PCD. QOL-PCD is valid and reliable; it is short and easy for patients to complete and provides a promising outcome measure for use in clinical trials and clinical practice.

Figure 14: Change in Quality of Life-Primary ciliary dyskinesia (QOL-PCD) from stable time point to day of exacerbation. A, Lower Respiratory Symptoms scores B, Physical Functioning. (n=10)

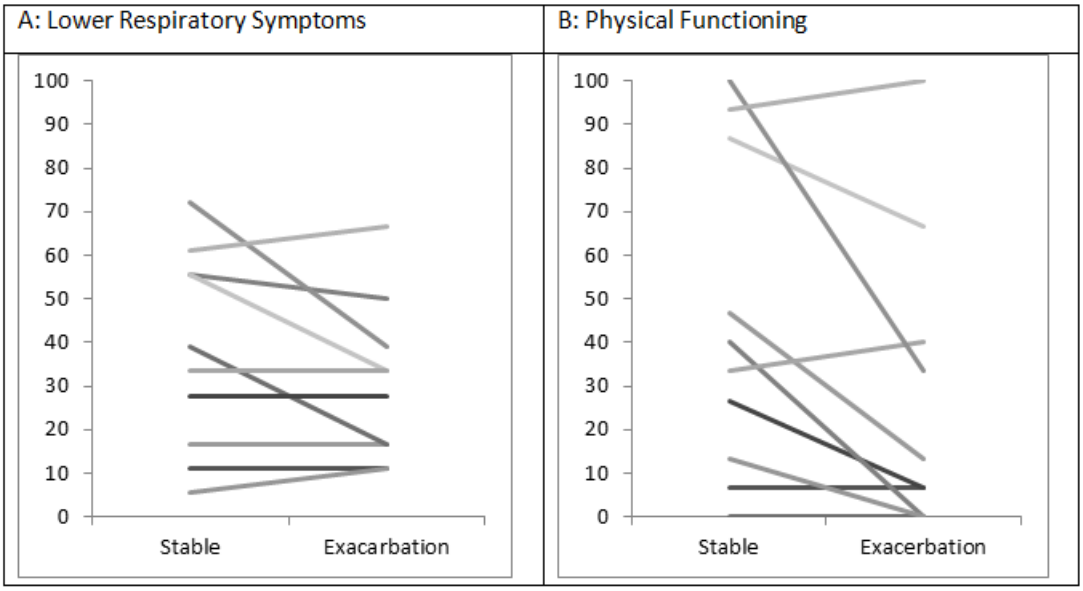


Table 19: Participant characteristics by country of residence

	United Kingdom N=34	USA and Canada N=38
Female n (%)	20 (58.8)	29 (76.3)
Age, mean in years (SD)	34.8 (17.3)	31.0 (12.9)
Range	18-79	18-65
18-32 years n (%)	22 (64.7)	24 (63.2)
33-55 years n (%)	4 (11.8)	12 (31.6)
>55 years n (%)	8 (23.5)	2 (2.6)
FEV_1 % predicted, mean (SD)	72 (26)	66 (19)
Range	26-115	33-101
Missing n (%)	1 (3)	1 (3)
FEV_1 % predicted >80%, n (%)	12 (35)	8 (21)
Missing n (%)	1 (3)	1 (3)
Past/current growth of pseudomonas aeruginosa, n (%)		
Missing n (%)	11 (32)	23 (61)
	2 (6)	1 (3)
Education, n (%)		
Second level or less	11 (32)	8 (21)
Some college	4 (12)	7 (19)
College graduate/post graduate	16 (47)	21 (55)
Missing	3 (9)	2 (5)
Working status, n (%)		

Part time or full time employment	18(52.9)	7 (18.4)
Full time homemaker	1 (2.9)	13 (34.2)
Attending education courses outside the home	7(20.6)	6 (15.8)
Attending education courses inside the home	0 (0)	3 (7.9)
Not working due to health	3 (8.8)	2 (5.3)
Not working for other reason	3 (8.8)	6 (15.8)
Retired	2 (5.9)	1 (2.6)
Ethnicity, n (%)		
White	32 (94.1)	31 (81.65)
Black	0 (0)	0 (0)
Hispanic	0 (0)	2 (5.3)
Asian	0 (0)	2 (5.3)
Other	2 (5.9)	1 (2.6)
Missing	0 (0)	2 (5.3)

Table 20: Multi-trait analysis of QOL-PCD scales showing item to scale correlations: Physical Functioning, Emotional Functioning, Treatment Burden, Social Functioning, Role Functioning, Health Perception, Vitality, Upper Respiratory Symptoms, Lower Respiratory symptoms and Ear and Hearing Symptoms (n = 72, adults≥18 years). We required item to scale correlations ≥0.40 with the intended scale (shaded) and much lower correlations with the competing scales.

	Physical	Emotional	Treatment	Social	Role	Health	Vitality	Upper Respiratory	Lower Respiratory	Hearing
Physical 1	0.880	0.502	0.463	0.408	0.705	0.728	0.527	0.387	0.715	0.474
Physical 2	0.873	0.471	0.445	0.393	0.762	0.688	0.465	0.243	0.542	0.364
Physical 3	0.863	0.445	0.424	0.328	0.629	0.672	0.508	0.229	0.590	0.315
Physical 4	0.801	0.323	0.315	0.266	0.562	0.528	0.371	0.154	0.461	0.359
Physical 5	0.906	0.515	0.573	0.196	0.742	0.711	0.471	0.430	0.659	0.395
Emotional 1	0.495	0.766	0.343	0.247	0.523	0.623	0.514	0.299	0.402	0.239
Emotional 2	0.428	0.782	0.281	0.316	0.408	0.466	0.374	0.267	0.453	0.300
Emotional 3	0.309	0.723	0.281	0.121	0.361	0.452	0.390	0.265	0.273	0.196
Emotional 4	0.307	0.629	0.366	0.220	0.404	0.450	0.295	0.368	0.306	0.345
Emotional 5	0.418	0.757	0.444	0.161	0.416	0.527	0.539	0.471	0.512	0.330
Treatment 1	0.397	0.198	0.598	0.137	0.542	0.497	0.244	0.217	0.259	0.164
Treatment 2	0.423	0.330	0.805	0.236	0.527	0.550	0.409	0.288	0.414	0.333
Treatment 3	0.280	0.308	0.730	0.211	0.407	0.312	0.462	0.381	0.318	0.313
Treatment 4	0.368	0.505	0.736	0.262	0.522	0.596	0.628	0.445	0.472	0.239
Social 1	0.273	0.161	0.284	0.814	0.357	0.452	0.324	0.055	0.286	0.055
Social 2	0.151	0.231	0.220	0.861	0.185	0.306	0.367	0.015	0.138	-0.006
Social 3	0.431	0.206	0.157	0.710	0.447	0.364	0.327	0.100	0.193	0.250
Role 1	0.562	0.336	0.434	0.204	0.731	0.431	0.225	0.432	0.498	0.389
Role 2	0.741	0.508	0.668	0.305	0.868	0.774	0.594	0.359	0.535	0.354

Role 3	0.647	0.493	0.588	0.406	0.899	0.751	0.521	0.382	0.541	0.417
Role 4	0.717	0.548	0.598	0.314	0.812	0.746	0.629	0.372	0.664	0.395
Health 1	0.529	0.506	0.563	0.541	0.628	0.808	0.580	0.328	0.427	0.263
Health 2	0.608	0.563	0.435	0.293	0.686	0.784	0.442	0.388	0.666	0.386
Health 3	0.697	0.642	0.662	0.391	0.725	0.923	0.678	0.307	0.585	0.396
Health 4	0.704	0.524	0.560	0.301	0.582	0.781	0.653	0.364	0.628	0.386
Vitality 1	0.577	0.576	0.458	0.382	0.394	0.533	0.893	0.337	0.410	0.269
Vitality 2	0.681	0.377	0.428	0.400	0.549	0.647	0.749	0.271	0.463	0.396
Vitality 3	0.354	0.526	0.618	0.240	0.526	0.594	0.836	0.322	0.380	0.290
Upper Resp 1	0.311	0.316	0.274	0.041	0.366	0.307	0.221	0.836	0.484	0.281
Upper Resp 2	0.315	0.406	0.462	0.085	0.402	0.373	0.352	0.827	0.452	0.439
Upper Resp 3	0.210	0.322	0.227	-0.074	0.321	0.222	0.267	0.762	0.374	0.287
Upper Resp 4	0.249	0.342	0.366	0.129	0.347	0.380	0.288	0.821	0.456	0.470
Lower Resp 1	0.513	0.428	0.467	0.194	0.543	0.599	0.391	0.472	0.821	0.429
Lower Resp 2	0.463	0.460	0.380	0.079	0.522	0.501	0.362	0.507	0.717	0.414
Lower Resp 3	0.343	0.127	0.323	0.162	0.400	0.352	0.230	0.250	0.649	0.267
Lower Resp 4	0.367	0.240	0.314	0.351	0.336	0.458	0.298	0.281	0.679	0.286
Lower Resp 5	0.461	0.488	0.355	0.144	0.443	0.452	.346	0.503	0.751	0.496
Lower Resp 6	0.711*	0.546	0.490	0.263	0.615	0.643	0.467	0.455	0.694	0.448
Ear and Hear 1	0.416	0.415	0.315	0.107	0.443	0.478	0.424	0.434	0.538	0.931
Ear and Hear 2	0.364	0.232	0.292	0.073	0.357	0.289	0.221	0.408	0.435	0.886

†Item-scale correlation to intended scale
* item correlation with competing scale higher than its correlation with its own scale.

Table 21: QOL-PCD scales' internal consistency measured by Cronbach's alpha and test-retest reliability measured by intraclass coefficients (ICC). Cronbach's alpha >0.7 indicates good internal consistency. ICC >0.6 indicates good stability and >0.75 excellent stability of the scales.

QOL-PCD (Adult) Scales	No. of Items	Mean (SD) of scales	Cronbach's α N=72	ICC (95% CI) N=35
Physical Functioning	5	70.51 (30.37)	0.94	0.94 (0.89-0.97)
Emotional Functioning	5	73.68 (19.56)	0.83	0.91 (0.82-0.95)
Treatment Functioning	4	60.80 (23.70)	0.75	0.92 (0.82-0.96)
Social Functioning	3	38.11 (29.47)	0.74	0.73 (0.47-0.87)
Role Functioning	4	64.23 (28.98)	0.86	0.94 (0.88-0.97)
Health Perception	4	51.16 (26.32)	0.83	0.91 (0.82-0.95)
Vitality	3	53.76 (21.93)	0.79	0.88 (0.75-0.94)
Upper Respiratory Symptoms	4	45.83 (26.76)	0.83	0.91 (0.81-0.95)
Lower Respiratory Symptoms	6	47.30 (15.49)	0.83	0.92 (0.85-0.96)
Eat and Hearing Symptoms	2	61.81 (28.59)	0.79	0.96 (0.87-0.97)

Table 22: QOL-PCD scales mean scores and standard deviations (SD) for participant characteristics where we had hypothesised an association *a priori*.

	Physical Functioning	Social Functioning	Vitality	Treatment Burden	Upper Respiratory Symptoms	Lower Respiratory Symptoms
Gender						
Male (n=23)	_	43.96 (32.74)	_	69.61 (19.75)	_	58.70 (15.32)
Female (n=49)		35.37 (27.74)		57.62 (24.37)		43.20 (20.36)
		p=0.284		p=0.052		p=0.004
Age						
18-22 years (n=26)	88.21 (15.81)	_	58.55 (20.50)	_	46.47 (23.35)	58.76 (16.72)
23-36 years (n=21)	69.21 (29.63)		52.91 (20.20)		43.25 (30.80)	40.21 (19.63)
37-55 years (n=15)	56.88 (32.35)		46.66 (20.30)		42.22 (27.72)	36.30 (19.90)
>55 years (n=10)	49.33 (34.88)		48.88 (23.54)		55.00 (26.41)	55.00 (24.21)
	p<0.001		p=0.355		p=0.655	p=0.001
Pseudomonas						
Yes (n=34)	63.92 (33.43)	_	_	_	43.63 (26.75)	44.12 (20.51)
No (n=35)	76.38 (26.78)				48.57 (27.08)	52.22 (21.86)
	p=0.092				p=0.448	p=0.117

FEV_1 % pred						
<40 (n=11)	41.82 (30.12)	_	_	_	59.09 (24.28)	44.44 (26.99)
≥40<60 (n=14)	63.81 (32.94)				45.83 (26.90)	42.86 (25.17)
≥60<75 (n=16)	77.33 (29.04)				41.67 (28.87)	48.88 (17.21)
≥75 (n=29)	80.46 (23.70)				43.68 (26.51)	51.92 (19.49)
	p<0.02				p=0.364	p=0.563

Table 23: Convergent validity testing: Spearman's rank correlation coefficients between scales from QOL-PCD and generic health related quality of life measures (SNOT-20, SGRQ-C and SF-36). We *a priori* hypothesised scales that would have stronger correlations (closer to 1 or -1); these correlations are shadowed grey.

	SNOT-20	SGRQ-C				SF-36								
Scales of QOL-PCD		**Total**	**Symptoms**	**Activity**	**Impacts**	**Physical functioning**	**Role-Physical**	**Bodily pain**	**General health**	**Vitality**	**Social functioning**	**Role functioning**	**Mental health**	**Total Physical score**	**Total Mental score**
Physical	0.51	-0.83	-0.78	-0.85	-0.74	0.83	0.79	0.54	0.79	0.65	0.62	0.49	0.46	0.84	0.37
Emotional	-0.48	-0.52	-0.04	-0.47	-0.54	0.47	0.55	0.60	0.63	0.61	0.67	0.60	0.73	0.57	0.69
Treatment	-0.61	-0.67	-0.61	-0.54	-0.65	0.54	0.67	0.78	0.66	0.54	0.50	0.54	0.38	0.71	0.36
Social	-0.21	-0.43	-0.40	-0.28	-0.48	0.34	0.35	0.32	0.42	0.46	0.28	0.27	0.34	0.39	0.27
Role	-0.60	-0.80	-0.73	-0.67	-0.78	0.73	0.84	0.68	0.79	0.69	0.72	0.44	0.47	0.86	0.43
Health	-0.64	-0.84	-0.76	-0.72	-0.84	0.71	0.79	0.76	0.87	0.79	0.76	0.50	0.62	0.87	0.56
Vitality	-0.46	-0.63	-0.67	-0.49	-0.59	0.59	0.56	0.60	0.72	0.75	0.52	0.43	0.39	0.69	0.43

Upper respiratory	-0.60	-0.39	-0.31	-0.31	-0.39	0.36	0.51	0.54	0.48	0.35	0.42	0.37	0.39	0.48	0.35
Lower respiratory	-0.53	-0.72	-0.69	-0.61	-0.69	0.59	0.69	0.58	0.75	0.67	0.59	0.43	0.49	0.69	0.49
Ear and Hearing	-0.57	-0.47	-0.40	-0.36	-0.49	0.51	0.49	0.46	0.66	0.55	0.51	0.39	0.46	0.56	0.47

SGRQ = St. George Respiratory Questionnaire (higher score indicates worse HRQoL) and SF-36 = Medical Outcome Survey Short Form-36 (higher score indicates better health status).

Table 24: Responsiveness to change was measured, paired t-tests compared scores (SD) for each scale in 10 patients who completed QOL-PCD whilst stable and during an exacerbation.

	Stable Mean (SD)	Exacerbation Mean (SD)	Mean Difference (95% CI)	P-value	Effect size
Physical	41.33 (37.55)	26.00 (34.20)	15.33 (1.47-29.20)	0.03	0.43
Emotional	67.33 (27.12)	63.33 (23.63)	4.00 (-6.35-14.35)	0.41	0.16
Treatment	49.16 (26.77)	39.16 (23.58)	10.00 (0.05-20.05)	0.05	0.40
Social	38.88 (26.31)	43.33 (21.24)	-4.44 (-20.42-11.54)	0.54	0.19
Role	45.83 (36.05)	31.66 (30.63)	14.16 (4.41-23.92)	0.01	0.42
Health	38.33 (27.8)	27.50 (20.80)	10.83 (20.58-1.08)	0.03	0.44
Vitality	45.55 (25.89)	27.77 (22.98)	17.77 (3.66-31.91)	0.02	0.73

	45.83 (22.65)	36.66 (26.11)	9.16	0.05	0.38
Upper respiratory					
			(0.34-18.67)		
	38.88 (24.14)	28.33 (19.67)	10.56	0.03	0.48
Lower respiratory					
			(0.74-20.37)		
	41.66 (29.65)	43.33 (28.54)	-1.66	0.78	0.06
Ears and Hearing					
			(-14.78 - -11.45)		

10. General Discussion

This thesis has led to major advances in the field of PCD; from diagnosis to the treatment and management of patients. The development and validation of the first predictive score for diagnosing PCD based on clinical symptoms will lead to earlier referral of patients and improved awareness among medical practitioner of the signs and symptoms of PCD. The development and validation of QOL-PCD has provided the first patient-reported outcome measure for this illness for use in clinical trials assessing new and existing treatments. The systematic review, international survey and series of interviews have provided a detailed insight into patient perspectives and experiences with PCD. Based on the results and tools developed in this thesis, a model has been developed showing how the findings link together under the biopsychosocial framework (Figure 15).

10.1. Main findings

10.1.1. Diagnosing PCD

10.1.1.1. Delayed diagnosis

The results presented in the thesis showed that PCD was diagnosed late among study participants. Furthermore, patients attributed their late diagnosis to the widespread lack of PCD awareness among medical practitioners. Patients who suffer from rare diseases often encounter particular difficulties that those diagnosed with more prevalent and common diseases do not. These include barriers in scientific knowledge, organisational barriers, financial barriers, and personal barriers. A report by EURORDIS (Rare Disease Europe) on the experiences and expectations of patients with rare diseases, has shown that a lack of awareness of the clinical manifestations of a rare disease may lead to diagnostic delay, failure of diagnosis, and misdiagnosis (183). In the European Organisation of Rare Diseases, summarised in The Lancet (184), diagnostic delay has been shown to lead to loss of

confidence in the health-care system, feelings of rejection and marginalisation, confusion, worry, and fear. These findings have certainly been echoed in our findings.

Delayed diagnosis in PCD has been well documented in previous studies (25, 109, 110), however the reasons for and the implications of a delayed diagnosis from the patient's perspective has not been presented until now. In Chapter 3, patients discussed the journey to referral in a series of semi-structured interviews (112). A delayed diagnosis was primarily recognised as being due to a lack of PCD awareness by medical professionals. This was also reflected in the survey, with 37% of PCD international respondents reporting that they visited their doctor on >40 occasions for PCD-related symptoms before it was considered a possible diagnosis. Nearly all parent interviewees (92%) reported that their child had respiratory distress at birth and neonatal symptoms, but only 27% were referred at this stage for testing. Participants diagnosed later in life felt that the underlying cause of their symptoms was not considered nor was their past medical history taken into account when presenting to their medical practitioner with symptoms. All adult interviewees were diagnosed late in life (>30 years of age) and felt that their current state of health might have been better had they been diagnosed earlier. These findings were supported in the systematic review (Chapter 4), where two studies reported that later PCD diagnosis was associated with poorer subsequent health-related quality of life (107, 113). To prevent a delayed diagnosis, participants felt that medical professionals need to be better educated about PCD, its signs and symptoms, and the need to take past medical history into account, i.e. neonatal symptoms. This study shows a clear need to educate non-expert clinicians on guidelines published a quarter of a century ago which stated that individuals with chronic upper and lower airway symptoms should be investigated for PCD, particularly if symptoms began early in life (102).

In order to address the lack of PCD awareness among physicians, PICADAR was developed (Chapter 6) (167). It is hoped that the development and validation of this clinical predictive rule will raise awareness of the clinical features associated with PCD and will aid appropriate referral of patients for diagnostic testing. PICADAR is the first validated tool to predict the probability of a PCD diagnosis based on clinical symptoms. The score applies to patients with persistent wet cough and has seven predictive parameters: full-term gestation, neonatal chest symptoms, neonatal intensive care admittance, chronic rhinitis, ear symptoms, situs inversus and congenital cardiac defect. It is designed to be easily applied in a non-specialist setting to determine which patients, with chronic chest symptoms, should be referral for PCD diagnostic testing. As it has been developed in a tertiary care centre, it is not aimed for use in primary care, however it could serve to raise awareness among general practitioners and neonatologists of the signs and symptoms associated with PCD. It could be then used by respiratory centres as a cost effective tool to guide referral of high risk patients to specialist PCD centres.

PICADAR, when using a cut-off score of $\geqslant 5$ had 90% sensitivity and 75% specificity to differentiate PCD-positive and PCD-negative patients. When validated in a second centre, it had 86% sensitivity and 73% specificity. If patients with a score $\leqslant 4$ had not proceeded to further testing, 167 (70.2%) and 57 (73.1%) negative patients in the development and validation group respectively would have avoided formal testing at the diagnostic centres. However, it would have also resulted in two (4.0%) positive patients in the developmental group and 11 (13.9%) in the validation group being missed. The tool should not be used in isolation when deciding who to refer; however widespread use of PICADAR would inevitably increase awareness and lead to an increase in the number of actual diagnoses.

Since the publication of PICADAR, a study from Leigh et al (185) has developed a clinical screening tool for children and adolescents that is also based on symptoms. Experts defined *a priori* and tested 5 clinical features apparent in early childhood, and found 4 to be alone or in combination predictive of PCD: (1) unexplained neonatal respiratory distress with supplemental oxygen requirement more than 24 hours in term infants; (2) early-onset, year-round, wet cough; (3) early-onset, year-round nasal congestion; and (4) laterality defects. Sensitivity and specificity is based on the number of clinical features. This showed that the presence of 4 features had a sensitivity and specificity of 0.21 and 0.99, respectively. Three features had sensitivity and specificity of 0.50 and 0.96, respectively and 2 features was 0.80 sensitive and 0.72 specific. Adapting PICADAR using these more detailed and extended questions may improve the specificity of our tool; however it is also possible that the sensitivity could be reduced.

PICADAR has been included in European Respiratory Society (ERS) Taskforce Guidelines for diagnosing PCD, for which I was a junior member. These guidelines have recently been submitted to the European Respiratory Journal (ERJ) (Appendix 8). It has been recommended as a tool to be used in identifying patients for diagnostic testing but should only be used in isolation in certain circumstances i.e. specialist respiratory centres.

10.1.1.2. Screening for PCD

To improve early diagnosis in PCD, screening has been explored in a number of studies. Nasal nitric oxide has been shown to provide a good screening tool (14, 53) to differentiate between symptomatic patients with and without PCD. The technique, however, is insufficiently standardised, with different centres using their own reference values and proposed cut-off values range from 25 to 126 nL-min·1(53). nNO is not 100% sensitive,

ranging between 75% and 100% in different studies using different cut-off values (53). The equipment for measuring nNO is not widely available outside specialist centres and it requires trained technicians to obtain reliable readings.

In Chapter 5, nasal nitric oxide was examined as a screening test in a referral population of symptomatic patients (101). Using the arbitrary cut-off value of 30 $nL \cdot min^{-1}$, sensitivity and specificity were found to be 0.91 and 0.96, respectively. This shows that 9% of PCD cases could have been missed if this test was completed in isolation. If a higher cut-off value was used, for example the value of 77 $nL \cdot min^{-1}$ as recommended by Leigh et al (14); sensitivity in our sample would have improved to 96%, but specificity would have reduced to 83%. When nNO was tested using a higher cut-off value, it had a greater sensitivity but showed that still 4% of cases could be missed.

If nNO were to be used more widely as a screening test for PCD, greater guidance would be needed to ensure a cost effective approach and to prevent it from overburdening diagnostic services. In Chapter 5, the positive predictive value (PPV) and negative predictive value (NPV) of nNO at the cut of value of 77 $nL \cdot min^{-1}$ were found to be 42.6% (95% CI 30.2% to 54.5%) and 99.1% (96.6% to 99.9%) respectively. This means that approximately 60% of patients with a positive test did not have the disease.

In order to investigate the possible consequences of screening in different populations, I participated in a further study to model PPV and NPV of nNO in several theoretical scenarios (186) (Appendix 11). The prevalence of PCD in the general population is around 1:10 000. Predictive values are dependent on the prevalence of the disease in the tested population. If PCD was universally screened using nNO, the PPV would only be 0.1%, making it impractical as a screening tool. Previous data from children have suggested that the prevalence of PCD in those with recurrent wet cough is 5% (187), which would give a PPV

of 23.6% and NPV of 99.6%, indicating that this group could a target population for nNO screening. It is also suggested that patients with non-cystic fibrosis bronchiectasis could be targeted for screening with a systematic review of 989 patients showing 9% of this population had PCD (186, 188). PICADAR (Chapter 6) has a similar PPV (49%) to that of nNO at 77 $nL \cdot min^{-1}$ when used in a specialist centre. If PICADAR were used as a screening tool in the general population, it would also prove to be an impractical screen (PPV 0.1%).

Another approach could be to use PICADAR to provide guidance on who to refer for nNO screening. In resource limited countries with no diagnostic facilities, both methods used in combination could be used to attach a PCD-likelihood to the patients. I have conducted the analysis to establish the sensitivity and specificity of using both methods simultaneously (Abstract accepted European Respiratory Congress 2016, (Appendix 10). At a cut-off value of >5 points, PICADAR alone had sensitivity 0.88 and specificity of 0.95. Accuracy varied when combined with nNO thresholds and sensitivities were 0.94, 0.94 and 1.0 using cut-off values of 30 nL min^{-1} , 77nL min^{-1} and 100nL min^{-1} nNO. Respective specificities were 0.89, 0.78 and 0.73 using 30 nL min^{-1} , 77 nL min^{-1} and 100 nL min^{-1}. Using a combined approach, PICADAR and nNO testing can provide a highly sensitive method for patient selection with a 100nl/min nNO threshold giving a sensitivity (and hence NPV) of 100%. The PPV of this method is 0.50. This approach could greatly improve the effective use of PCD diagnostic resources.

10.1.1.3. The importance of combination diagnostic testing

There is no 'gold standard' reference test for diagnosing PCD. In Chapter 5, the importance of combination diagnostic testing was highlighted (101). Data from patients who had undergone all tests; nNO, HSVMA and TEM (n=180) were used to calculate the accuracy for

possible combinations of tests. Two-stage testing based on nNO pre-screening followed by TEM potentially missed approximately 40% of PCD cases showing that 33 nL min^{-1} cut-off is probably too low for use as a screening threshold and that HSVMA needs to be taken into account. Using all three tests simultaneously (where any abnormal test leads to a positive result), sensitivity was 100% and specificity was 87%. This showed the importance of the multidisciplinary combination approach where no test was considered in isolation.

The results from this study have contributed to the new ERS Taskforce Guidelines (Appendix 8). The guidelines are based on an assessment of the following diagnostic techniques: clinical presentation, nasal nitric oxide, analysis of ciliary beat frequency by high-speed video microscopy analysis, transmission electron microscopy, genotyping, and immunofluorescence through a systematic review of the literature. Assessment of the evidence was conducted using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) (189, 190) approach. Subsequently, an algorithm was developed for the use of diagnostic tests to definitively confirm and exclude the diagnosis of PCD. Using modified Delphi approach (191), consensus agreement was reached that a conclusive positive diagnosis can only be determined if a patient has a supportive history of PCD, shows a confirmatory hallmark ciliary ultrastructure defects for PCD through TEM or has mutations in PCD causing genes. It must be stressed, however, that these tests will miss a minority of positive cases. Consensus was not reached (<80% consensus) that any other test in isolation or in combination could provide a conclusive positive diagnosis. A 'highly likely' diagnosis can be provided in patients with: a combination of a compatible history of PCD, very low nNO and HSVMA findings (static or circling cilia) consistently suggestive of PCD on three occasions, or have very low nNO plus HSVMA findings consistent with PCD following cell culture.

Good communication between patients and the diagnostic team is of high importance here highlighting the inter-relatedness of biophysical, social and psychological factors. When diagnosis is 'highly likely' but not conclusive, patients must be told that the diagnosis is likely but not 100% certain and they may need to receive additional confirmation when better tests become available. Patients should also have other causes for their symptoms excluded and should be treated as if they have PCD.

10.1.1.4. Inconclusive diagnosis

An inconclusive diagnosis in any disease can generate feelings of uncertainty, self-doubt, stress and distress (192). A qualitative study by Nettleton et al. (2004) explores the narratives of patients who live with medically unexplained symptoms and who have not secured a diagnostic label. It found that patients represent their illness in terms of "chaos narratives" characterised by "confusion and uncertainty" and "a merry-go-round of hope and despair". It also suggests that a prolonged pre-diagnostic period is stressful and traumatic and may produce serious repercussions on the mental health of the patients involved (193).

According to the European Taskforce Guidelines (Appendix 8), an inconclusive diagnosis can be determined if a patient was found to have equivocal results following nNO, HSVMA, TEM, genetics and repeated HSVMA following cell culture. In the UHS longitudinal database, of the 868 referrals, 13 (1%) had an inconclusive diagnostic outcome despite adequate samples (Chapter 5). Many cases can remain inconclusive despite expert testing because both TEM and genetics are not sufficiently sensitive, and HSVMA and ALI cultures are often unclear due to poor health of the cells causing secondary defects.

In Chapter 3, participants who experienced an inconclusive diagnostic result endorsed the sentiments described by Nettleton; that of uncertainty, self-doubt and stress (112). The practical constraints of such an outcome were also discussed. These were often country-specific with participants from countries such as the USA having difficulties accessing insurance for treatments without a definitive diagnosis. The impact of receiving a confirmatory positive diagnosis following years of having an inconclusive label led to a profound sense of validation and relief; a label had finally been provided.

Disbelief that diagnostic testing in PCD cannot always provide a confirmatory positive or negative diagnosis was evident from the interviews. This frustration relating to inconclusive results has been found in other diseases e.g. genetic testing for *BRCA1* and *BRCA2,* which causes increased risk of breast and ovarian cancer (192). Based on the themes that emerged from Chapter 3, it would seem that the needs of patients who have received inconclusive results are not always met. A number of suggestions for improved care of patients with inconclusive results can be adapted from diseases outside PCD. Hallowell et al. (194), recommended that all persons who were informed that they had an inconclusive test result should have a follow-up session with a clinician either by telephone or in person. Ardern-Jones et al. (192) suggested annual telephone contact be provided to inconclusive patients to update them on advances in diagnostic testing status. This has also been suggested in recent European Consensus Guidelines (Appendix 8) which expresses the importance for PCD clinicians to recall inconclusive patients for further testing as advances in PCD diagnostics are made. As PCD is often diagnosed through outreach clinics where professionals may not immediately be aware of new developments in diagnostics, Ardern-Jones et al. (192) advocates that health professionals caring for patients with inconclusive diagnostic results be also updated continuously on new scientific discoveries.

10.1.2. Prevalence and impact of symptoms

The prevalence of each of the 26 clinical features published in Chapters 5 and 6 (101, 167) were presented for both positive and negative referrals. Persistent daily wet cough (93%) and persistent perennial rhinitis (83%) were the most prevalent symptoms reported by positive PCD patients. In a recent systematic review and meta-analysis of clinical manifestations in PCD (37), prevalence of wet cough varied between 14-100%; however the weighted mean was 88%. Rhinitis, rhinorrhoea or nasal congestion ranged in prevalence from 9-100%, against the weighted mean of 75%. In the UHS dataset, the prevalence of hearing loss was 49%. Again heterogeneity was reported in the meta-analysis where prevalence ranged from 8 to 100% (weighted mean 36%).

A number of reasons could account for such heterogeneity. It could be attributed to differences in the definition of symptoms in each study. It could also be related to differences in diagnostic protocol, study design, or selection of study populations. As most studies originated from specialised clinics, it is expected that patients with more severe manifestations were included and these study populations cannot be considered representative for all PCD patients. The type of clinic and where the study population originated from i.e. from pulmonologists, ENT specialist or fertility specialist, can also influence the prevalence of reported symptoms.

In Chapter 6, situs abnormalities were found in 44% of patients and congenital cardiac defects in 8% of the positive group. The prevalence of situs anomalies in the 43 eligible studies in the systematic review ranged from 11-90% (weighted mean: 49%). Situs abnormalities were reported to be more common in adult patients, probably due to the fact that adults without situs anomalies or severe lung disease were underdiagnosed, especially in

the past. The prevalence of congenital heart disease has less heterogeneous across studies with a range of 3-8%, likely due to the lower possibility of misclassification bias. Fertility data was only available for 23% of the total referrals in UHS because a large proportion was children or young adults. In those with available data, infertility or subfertility was reported in 90% of patients; however this was not stratified by sex. In the systematic review it ranged from 15-79%. Again this could be higher in older study group or with some studies applying the application of sensitive testing for detection e.g. spermatozoa analysis (37).

The clinical findings reported in Chapter 5 and 6, has also been included in the systematic meta-analysis to inform the ERS Taskforce Guidelines. A recommendation (labelled a 'strong' recommendation) has been made to refer patients for testing if they have several of the following features; persistent wet cough; situs anomalies; congenital cardiac defects; persistent rhinitis; chronic middle ear disease with or without hearing loss; a history in term infants of neonatal upper and lower respiratory symptoms or neonatal intensive care admittance.

Here we can see how biological factors can influence the social and psycholoical domains. Open-ended interviews with adults, children, teenagers, and parents conducted during the development of the HRQoL measures (QOL-PCD) in Chapter 8 (and Appendix 7) confirmed the burdensome nature of symptoms such as wet productive cough, runny nose and hearing impairment (103). Adults also reported that these symptoms can sometimes impact on their sense of taste and smell. The impact of symptoms on physical functioning was well documented among adult interviewees who reported the physical difficulty of keeping up with family and friends. Impact on physical functioning has previously been reported in the

paediatric population where children reported not being able to run as fast as other children, not being able to keep up with peers in sports, needing to take regular breaks, and having to stop to cough when engaging in activities (78, 124). All age groups described the impact of respiratory symptoms and the constant need to cough. Getting out of breath from physical activity and from coughing was discussed as well as difficulty breathing when experiencing an exacerbation. For both adults and children, hearing problems were discussed and both groups described the social impact of having to constantly ask people to repeat themselves. Children also reported having ear infections and the embarrassment of ear drainage. All age groups described the impact of upper respiratory symptoms, with children describing rhinitis as ‘a constant sniff’ and sinus pain as ‘a pain that stings and feels like somebody's pushing down on my sinuses’. Among older adults, the decline in respiratory health over the years was reflected upon. Younger adults expressed their worry on the possibility of infertility, while older adults discussed the personal and social impact infertility has had or continues to have on their lives.

The impact of these symptoms on HRQoL has been shown for the first time in the validation of the QOL-PCD (195) (Chapter 9). This manuscript has been submitted to the American Journal of Respiratory and Critical Care Medicine (AJRCCM) and is currently under review. Adults had low mean scores for upper and lower respiratory symptoms at 46/100 and 47/100 points. We did not expect that reported mean scores for respiratory symptoms would be lower (indicating worse quality of life) than mean score reported by cystic fibrosis patients who completed the validation for the cystic fibrosis HRQoL questionnaire (CFQ-R). In the validation of the CFQ-R, respiratory symptoms mean scores ranged between 50-70 across the mild to severe disease groups (172). Lower respiratory symptoms scores were also lower than

non-cystic fibrosis bronchiectasis patients who had scores ranging between 52-60 points in the validation of the HRQoL questionnaire for non-cystic fibrosis bronchiectasis (176).

This study also shows the range of severity and heterogeneity in PCD. FEV1 % predicted ranged from 26% to 113%. As hypothesised, Physical Functioning mean scores were highest (indicating better HRQoL) in those with FEV_1>75% predicted, and in patients who have never grown Pseudomonas Aeruginosa in the past (although this did not reach statistical significance). Worsening HRQoL in PCD patients has been shown to correlate with increasing age (using generic HRQoL measures) (113). This was also found in the validation of QOL-PCD study in Chapter 9. However, the lowest QOL-PCD scores in the Upper and Lower Respiratory Symptom scales were found in the 37-55 years age group, with scores increasing again in the ≥55 year age group. This may reflect specific challenges for this age group in comparison to older age groups e.g. lack of time to fit in treatments due to careers and families. These findings highlight that quality of life is a multi-faceted measure influenced by other factors (e.g. age, treatment adherence).

10.1.3. Psychological impact of PCD

The psychological impact of symptoms has been described above, but also presented in this thesis (103) (Chapters 4 and 8) (Appendix 7) (124). The paediatric groups reported feeling anxious and fearful about getting sick and about the chronic nature of their symptoms. In addition, a sense of injustice and sadness about having this condition when their peers and siblings were healthy was reported. The systematic review also found that PCD children were more likely to be withdrawn, experience anxiety or depression, and internalise more problems than the healthy population (77). PCD was found to also affect the parent with significantly higher stress being reported in mothers of children with PCD compared to mothers of health

children. While anxiety and depression have not yet been measured in the adult population, in the validation of QOL-PCD in adults, mean scores for emotional functioning were higher than other scales at 73.68 (SD: 19.56). Emotional functioning in adults was found to be similar to the mean scores reported in the validation of the HRQoL questionnaire for cystic fibrosis (CFQ-R) which ranged from ~68-81 across mild, moderate and severe disease status groups (172).

10.1.4. Social impact of PCD

Concealing PCD symptoms and embarrassment from symptoms such as coughing and blowing nose in public was reported in the semi-structured interviews with adults and children. In the validation of this measure, social functioning was found to have low mean scores (mean 38.11, SD29.47), lower than mean social functioning scores reported in the validation of the cystic fibrosis HRQoL questionnaire where scores ranged from ~65-80 across mild , moderate and severity groups (172). Embarrassment from symptoms and concealment was reported across all age groups in the systematic review (78, 79). Children and teenagers were found to be reluctant to disclose their PCD diagnosis to teachers and peers. Adult patients were reluctant to disclose to work colleagues and those outside their family and close friends. Such concealment of symptoms and illness disclosure has been reported in other chronic illnesses (128-131). Chronic illness has been shown to have a negative impact on relationships with friends, families and peers. Results from a cystic fibrosis study (131) also found patients were more likely to disclose to romantic partners and close friends than to casual friends, bosses, or co-workers. It reported that disclosure was associated with higher social support, social functioning, and medication adherence self-efficacy. In the systematic review (Chapter 4), many of the papers document a sense of isolation and mistrust in medical care among PCD patients which is heightened by a lack of

PCD awareness by the patient's general practitioner (79). Participants were concerned about the effect the delayed diagnosis has had on their health. Such mistrust of medical practitioners was echoed in our findings described in Chapter 3 (196).

This thesis has aimed to contribute to the biopsychosocial model of PCD which states that the workings of the body, mind and environment all affect, and are affected by each other. This theory suggests that each one of these factors is not sufficient in itself to bring about the optimum outcome for this condition in terms of health and well-being; it is the interaction between them that determines the impact. In effect, the biopsychosocial model suggests that it is important to address all three aspects together. Based on the findings in the present series of studies, Figure 15 shows how the findings of this study link together under this biopsychosocial framework.

10.2. Strengths and Limitations

The strengths and limitations of the six papers in this thesis have also been acknowledged and addressed in the previous chapters. This section provides an overall synopsis of the strengths and limitations using a systematic approach. Checklists for systematic reviews that have been employed to guide the assessment of the quality of these studies i.e. STROBE (The Strengthening the Reporting of Observational Studies in Epidemiology)(197), QUADAS (quality assessment of studies of diagnostic accuracy included in systematic reviews)(198), CASP: Diagnostic Test Study Checklist (199); TRIPOD (Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis) and CASP: Evaluating a Clinical Prediction Rule Checklist (50).

10.2.1. Strengths

This thesis has addressed a number of timely and relevant research questions in PCD today. The European Taskforce Guidelines (2009)(22) called for the urgent need for further research in PCD. The findings from this thesis have contributed to these needs but have also attributed to new consensus diagnostic guidelines from an ERS Taskforce (Appendix 8). The findings from the PCD diagnostic accuracy study (Chapter 5) and the data collected and presented for the development of PICADAR (Chapter 6) has been included in the systematic reviews to inform these European Taskforce Guidelines for diagnosing PCD. The results from the survey and interviews conducted on the international diagnostic patient experience (Chapter 3), have been used to inform all decisions reached in these guidelines.

Chapter 3 is the first international study to be completed on the experience of patients referred for PCD diagnostic testing. Questions for the survey were comprehensively translated into 9 languages and the expertise of an international panel of PCD experts and patient representatives was sought on how the diagnostic experience could be fully captured. This is also the first study to report on the perspectives of patients referred for PCD testing but who were found to have a negative or inconclusive diagnostic outcome. Subgroup analysis per country was applied to further interpret the results and examine interactions i.e. differences between countries were assessed in terms of established diagnostic facilities and government health expenditure.

Chapter 5 and 6 conducted analyses of consecutive referrals within a national diagnostic programme. This is a pragmatic study that reflects the real patient journey. High levels of expertise standardisation, and multidisciplinary consensus was applied in this analyses. We can therefore be confident these results yield the most valid estimates of diagnostic accuracy. Confidence in the strength of PICADAR as a predictive tool is evident through the external

validation in a separate diagnostic centre in the Royal Brompton Hospital in London. Good discriminant ability of the tool was maintained when applied to this sample with an AUC 0.87. Although both diagnostic centres are situated in Southern England, they have different demographic populations in terms of ethnicity, consanguinity and age at assessment.

Chapter 8 and Chapter 9 detail the development and validation of the adult HRQoL measure (QOL-PCD). The strength of this study is that QOL-PCD measure was developed using FDA recommended guidelines for developing patient reported outcomes for use in clinical trials (83). This involved a detailed iterative process which included most facets of Guyatt's 'Rolls-Royce' model (95). QOL-PCD was found to be a valid instrument to measure HRQoL when validated in 72 PCD adults with psychometric testing confirming the measure to be robust, reliable, responsive and valid. Now that the measure is complete, it will be submitted to the FDA and EMEA for consideration as an outcome measure for clinical trials. The most important principle governing the development of QOL-PCD was our inclusion of patient input and their perspective at each phase. A unique strength of this study is that this English version has been developed using a cross-cultural approach through close collaboration with PCD centres in the US and Canada. Thus, this tool systematically reflects how an adult with PCD "survives, feels, or functions" not only in English speaking patients from the UK and Ireland but across English speaking countries i.e. USA and Canada.

The 2009 Taskforce Guidelines for diagnosing PCD expressed the need for high-quality randomised controlled multicentre trials to investigate the effect of different treatments on symptoms, lung function, quality of life and long-term progression of PCD. By developing and validating age-specific HRQoL measures, a disease-specific outcome measure is now available for patients with PCD. Its development will facilitate the assessment of long-term outcomes, spectrum and severity of clinical disease, and functional limitations of PCD. It will

also allow for sensitive capture of change following an intervention or trial. Comprehensive translation of this HRQoL measure has been performed by the author so that QOL-PCD is now available in five European languages (Appendix 10). These measures are currently being used as an outcome measure in the BESTCILIA international randomised control trial in azithromycin; the first international clinical trial in PCD.

10.2.2. Limitations

This thesis has a number of limitations.

In Chapter 3, a survey was distributed internationally and this was followed by a series of interviews assessing the diagnostic experiences of patients referred for PCD testing. Selection bias is possible with the main percentage of respondents originating from countries with established diagnostic centres *i.e.* UK, US, Germany and France. Bias could also have been introduced as only those with access to the internet, or 'informed' participants contacted by ELF and patient support groups, completed the survey. It is therefore possible that the results of the survey section of the study may not truly reflect the opinions of referrals from countries with less established diagnostic services or countries without any services available. The survey aimed to include patients who were referred and still waiting for results, who were negative for PCD or who had been found to be inconclusive for PCD and were still going through testing, however the majority of survey respondents (74%) were PCD positive with PCD negative representing only 5%, inconclusive 8%, and still waiting 3%. It is especially low considering that international results have found that between 11.5%-18.6% of referrals are eventually diagnosed with PCD (29, 58, 101, 108). This limits our capacity to assess the perspective of the population with an inconclusive or negative outcome. Although participants were asked about the tests that had been performed, their results cannot be verified and there is therefore diagnostic uncertainty. In addition, a number of participants'

diagnoses were based on tests that are not considered robust, e.g. the saccharine test. It is therefore likely that some patients may have been diagnosed who do not have PCD and vice versa. Since we were interested in the experiences and outcomes of patients from diverse clinical settings it was important to include groups where diagnostic status was uncertain. This inherently means that allocation of patients to diagnostic outcome groups was defined by the participants' understanding rather than the diagnosis that might be provided by a highly specialist centre; this is at the same time a strength and a weakness of the study. PCD is a rare disease with considerable heterogeneity in prevalence rates, therefore no pre-study considerations of statistical power were completed nor was response rate measured. However, the survey was translated into 9 languages to ensure as much inclusiveness at possible. In the qualitative section, the main limitation was that no feedback was provided back to the interviewees to confirm and further clarify the themes reported.

In the review in Chapter 4, papers included were limited to those published in the English language. It is possible that there are relevant studies published in other languages. Overall the evidence of this review is based on a small number of heterogeneous studies (n=14) that are limited in size. The quality assessment of the quantitative studies revealed them to be of low quality with scores no greater than 8 points. Until recently, no disease-specific age appropriate HRQoL measures were available for PCD patients (103, 124) and to date, studies have used general HRQoL tools such as the SF-36 and disease specific tools for cystic fibrosis and COPD. These studies have also included child participants to complete measures that are not age appropriate without psychometric validation. Studies have included results where young children had help from a parent to complete these measures which may lead to bias (129). Only one of the studies performed analyses with and without the children who needed help completing the questionnaire. In addition, limited psychometric data was

presented on the validity of the HRQoL used, with some studies reporting validity but never for all of the scales. As with any review, the quality of studies included can only be assessed by what was reported in the final manuscript; e.g. missing information on any of the adopted criterion might reflect unclear reporting as opposed to a limitation in study design.

With no established 'gold standard' for diagnosing PCD, in both Chapter 5 and Chapter 6, a positive PCD diagnosis was based on a surrogate reference standard. This surrogate reference standard consisted of an expert multidisciplinary consensus based on results from all available diagnostic tests. In Chapter 5, where the accuracy of each diagnostic test was measured in isolation and combination, it is possible that since each test contributed to the final diagnostic decision, our sensitivity and specificity estimates of the single tests might be overestimated. However, guidance does recommend that in situations without a 'gold standard', researchers can consider constructing a reference standard from multiple test results or can use an imperfect reference (168). There is also a risk of population bias as these tests were completed in highly specialised centre referrals; these results may not be generalisable to non-specialist settings. A final limitation in this chapter is that not all participants received all of the three diagnostic tests being assessed (199). To overcome this limitation however, further analysis was conducted examining the accuracy in the subsample of patients (n=180) that received all three tests.

In Chapter 6, PICADAR, a predictive model for diagnosing PCD was developed and validated. This paper fulfils the majority of the 22 criteria recommended by recently published guidelines for reporting the development and validation of multivariable prediction models for diagnosis or prognosis (TRIPOD)(200). One discrepancy was that the researcher was not blinded to the diagnostic outcome when collecting the predictors for the validation

population. This may inflate the predictive accuracy of the predictors. Further limitations of PICADAR can be identified from the CHARMS checklist: critical appraisal and data extraction for systematic reviews of prediction model studies(201). These include the previously discussed limitation of not having an established diagnostic 'gold standard' test for defining the outcome. A report on how the sample size was arrived at was also not provided. For studies validating prediction models, a minimum of 100 events and 100 non-events have been suggested (201). For the validation of PICADAR, this sample size was sought however approximately 10% had to be excluded due to missing data. A significant amount of data was missing for some variables. For example, a large proportion of the adult population did not know their gestational age and none of the children could yet know their fertility status. Complete case analysis was used to deal with missing data which can lead to bias. To overcome this obstacle, multiple imputation was used to replace missing values within the model's significant variables and the pooled result obtained from five imputed datasets showed the best model is accurate (199).

In Chapter 8, there are some limitations in the development and validation of the HRQoL measure. Guyatt et al (95) stated that sampling should be conducted of the complete spectrum of disease severity under consideration, and inclusion of patients from all subclasses (e.g., those of age, sex and duration of disease). In this study, it was more difficult to recruit men than women with only three men (14%) participating in the interviews. Approximately 6% of patients with PCD have cardiovascular disease however none of the 21 interviewees in the developmental stage had cardiovascular disease. It is therefore a limitation of the measure that it does not include items relevant to patients with cardiac disease. However, if the study population had been designed to be representative of the PCD population, only one patient with cardiovascular disease would have been included. While efforts were made to include all

ranges of severity, it is possible that not all ranges were covered without sensitive tools to measure disease progression. Guyatt et al.(95) also suggested that between 50-100 patients would ideally be interviewed and 50 patients should take part in the second round of interviews, as PCD is a rare disease, recruiting a sample of this size was not feasible in this study. Saturation was reached early in the study, which was followed by a further number of interviews to ensure saturation.

In Chapter 9, this study involved the recruitment of 72 PCD patients from centres in North America (n=38) and the UK (n=34). All age groups were represented in the study, and participants had a range of disease severity (FEV_1 % predicted: 26%-115%). This collaborative effort allowed us to recruit sufficient participants with this rare disease. However, given the modest size of the study population, we conducted a multi-trait analysis to develop the scales rather than an exploratory factor analysis (which would require >200 patients), or more complex analysis such as Rasche analysis (which would require >100 patients) (202). The multi-trait analysis supported the conceptual foundations of 10 scales with 40 items and has been recommended for use in the development of HRQoL measures which are expected to have correlations across domains (e.g., increased respiratory symptoms are likely to lead to decreased physical functioning). QOL-PCD had excellent item-to-total correlations and strong internal consistency across all scales (Cronbach's α 0.74 to 0.94). A more complex analysis including factor analysis and Rasche analysis may be an option in future studies if we are able to recruit larger sample sizes.

10.3. Future research recommendations

In the area of PCD, there are many opportunities to build on the existing research, projects and networks. This research has, and will continue to, contribute to networks of excellence

that focus on research infrastructure such as the COST Action project BEAT-PCD, the international PCD Registry, the iPCD cohort and The Genetic Disorders of Mucociliary Clearance Consortium (GDMCC). This research has and will provide disease-related information at EU level and beyond (through guidelines, diagnosis, and patient experience). These research findings will increase translation of chronic disease understanding into drug development and healthcare innovation. Findings will contribute to the understanding on innovative diagnostic methods, and will lead to the improvement and further validation of existing methods. It is intended that these findings will contribute to guidelines for medical and psychosocial care in patients with PCD (including those patients who have received inconclusive results). Finally, it is anticipated that the findings will lead to future research on the impact of culture, demographics, sex and age in terms of needs, concerns and support.

The results from the systematic review on the PCD patient experience and HRQoL in Chapter 3 illustrates the need for further qualitative studies to be conducted in order to reflect different ethnicities and cultures. This will establish the needs and opinions specific to these groups and will facilitate effective management and treatment. Further explorations are also needed on the psychological effects of PCD on intra-familiar relationships and stress related to PCD across the developmental pathway and in different age groups. Chapter 3 has highlighted the limitations of using generic and non-PCD specific HRQoL measures in PCD studies and highlighted the need for large multi-national and longitudinal studies to be conducted using disease-specific HRQoL measures.

Chapter 4 presented the experiences of patients and parents referred for PCD diagnostic testing. The opinions and perspectives from patients presented in Chapter 4 provide a number of future recommendations to improvement PCD diagnosis. These include the need for

samples to be analysed by PCD experts, the need for results to be delivered by a PCD expert, and the importance for patients to have the opportunity to discuss their results with a PCD expert. Patients recommended that measures must be introduced to prevent late diagnosis, such as better knowledge of PCD by medical practitioners including relevance of past medical history. A resolution to the 'inconclusive' diagnosis result status and research to improve diagnostic methods were highlighted an importance factors for future guidelines. Patients also expressed the need to establish a patient support group in each country and to have online translated information on PCD available in all European languages.

Findings from Chapter 5 have highlighted the importance of combination testing since no individual diagnostic test was 100% accurate when used in isolation. It also highlighted the need for tests to be standardised in both the way in which they are conducted, and in the way they are reported. Data from Chapter 5 has already contributed to Taskforce diagnostic guidelines where consensus agreement has stated that conclusive positive diagnosis can only be determined if a patient has a supportive history of PCD, showed a confirmatory hallmark ciliary ultrastructure defects for PCD through TEM or had mutations in PCD causing genes. A 'highly likely' diagnosis is to be provided in patients with a combination of a compatible history of PCD, very low nNO and HSVMA abnormalities, or very low nNO plus HSVMA findings consistent with PCD following cell culture. Chapter 5 highlights the importance of characterisation of ciliary structure and function and the need for this to continue to have a place within diagnostic processes.

The development of PICADAR (Chapter 6) demonstrates the use of a prospective cohort study of patients referred for PCD diagnostic testing in whom clinical features are assessed in a standardised way before they are diagnosed. As PICADAR was derived using combined

data from adults and children, there is a need for analyses to be completed stratified by age as it is expected that separate scores for adults and children might further improve accuracy. PICADAR includes a number of predictors based in early life, including gestational age and neonatal chest symptoms, which may be difficult to recall in adulthood. Similarly, subfertility was more common in the PCD-positive group and is likely to be a strong predictor for adult diagnoses.

Future work on PICADAR must also take into account the settings in which it is used to refer patients. The sensitivity of the tool is likely to differ between patients attending different specialised clinics. For instance, while chronic cough will not distinguish between patients with and without PCD in a pulmonology clinic, chronic ENT symptoms will not be distinctive in an ENT clinic, where (nearly) every patient has these complaints, and cardiac defects will not distinguish in a cardiology setting. Finally, as PICADAR was developed and validated in a UK specialist diagnostic centres, but referrals originate from non-specialist services, future validation will be needed in referral centres and in centres outside of the UK.

Chapter 8 and 9 have facilitated some of the recommendations put forward in Chapter 2 by developing, validating and translating QOL-PCD into five European languages. The measures are currently in the process of inclusion in an international PCD registry developed as part of the BESTCILIA FP7 study, providing an international platform to systematically collect data. Data in this registry is also being collected on clinical presentation and effectiveness of treatment (65). This will increase our understanding of the natural course and progression of the disease in terms of its effects on physical, emotional, role and social functioning (65). QOL-PCD measures are currently being included in the first international randomised control trial on prophylactic azithromycin. Once the analysis of the randomised control trial is complete, analysis will facilitate the generation of the minimum clinically

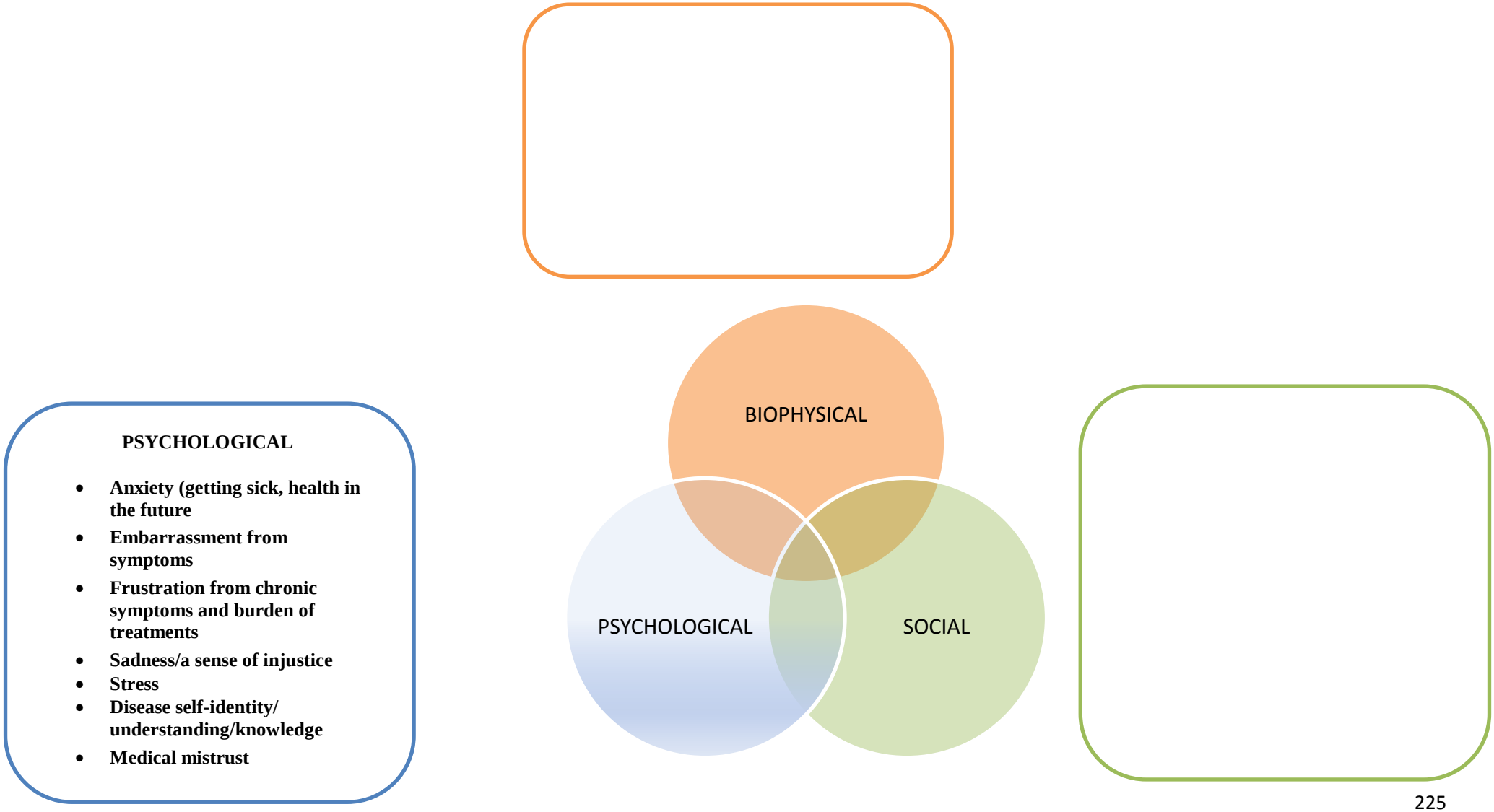
important difference (MCID), which is defined as the smallest difference in score on the HRQoL instrument that patients perceive as beneficial and that would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management (203). This will provide a useful means for future clinical trials to assess the benefits of medications or non-pharmacological interventions and will allow for comparative analysis across different studies and in multiple sites. Finally, it is important that QOL-PCD will also be used a tool at annual assessments, providing a broad assessment of well-being, as perceived by the patient.

11. Conclusion

Findings from this thesis have demonstrated the physical, psychological and social impact of PCD. The author has used a mixed method approach to address some of the identified gaps in knowledge on PCD that fit well with the tenets of the biopsychosocial framework (Figure 15). The work has led to an international study evaluating PCD patients' experiences and their perspective on the diagnostic process, the findings of which were used to advise the ERS Task Force (TF-2014-04) on their development of clinical practice guidelines for diagnosing or refuting a diagnosis of PCD. The author has provided a detailed analysis of the diagnostic pathway in UHS to determine the accuracy of different diagnostic strategies. To overcome some of the barriers to being referred for diagnostic testing identified by patients, the author conducted a detailed analysis of the UHS database and developed the first validated tool to aid appropriate referral of patients for diagnostic testing. PICADAR is a simple cost-effective score suitable for use in referral settings, which will raise awareness among clinicians and lead to earlier referral. The importance of the patient perspective in the assessment of new treatments has led to the development of the first HRQoL measure for PCD (QOL–PCD).

The author has coordinated the translation of these measures for use in major European countries and in the Middle East. QOL-PCD has good reliability, construct validity, internal consistency, stability and responsiveness. QOL-PCD is short and easy to use, offering a promising tool for evaluating new therapies and for measuring symptoms and functioning during routine care. It is hoped that the contributions made in this thesis will lead to improved health-related quality of life and better outcomes for PCD patients around the world.

Figure 15: Venn diagram of the biopsychosocial model showing how biophysical, psychological and social factors can influence health in PCD.



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