International ERS guidelines for the diagnosis of PCD

Supplementary File

Methods

Task Force and Work Group Composition

The membership and roles of the Task Force panel are summarised in Supplementary Table 1. Jane Lucas and Angelo Barbato (Chairs) were responsible for the governance and integrity of the work conducted in this TF. A leadership group of four (Jane Lucas, Claudia Kuehni, Angelo Barbato, Andy Bush) were responsible for chairing meetings, providing support to the work groups and monitoring progress. This leadership group also coordinated the writing of the practice guideline and oversaw the editing. Work Groups (WG) leaders were proposed and agreed at the first meeting of the task force, based on their expertise. Following training from ERS methodologists in GRADE, systematic reviewers drafted protocols for the searches, conducted systematic reviews, extracted data from the chosen manuscripts, assessed the quality of the data and finally synthesised the data using narrative and if appropriate meta-analysis.

The TF panel comprised experts and trainees in the field of PCD from multidisciplinary backgrounds including pulmonologists, ENT, cell scientists, electron microscopists and geneticists. Their expertise included clinical phenotyping, screening tests including nasal nitric oxide (nNO), ex-vivo and in-vivo ciliary function tests including high-speed video microscopy analysis (HSVA) and radioaerosol mucociliary clearance [1], transmission electron microscopy (TEM), cell culture (submerged [2]and at air-liquid interface- ALI[3]), lung physiology and imaging [4–7], epidemiology[8]and qualitative research[9] . Some members of the panel lead national diagnostic centres, and there were members from countries where diagnostic facilities are limited. Members of the panel volunteered to participate in WG activities based on their expertise and interests. The ERS provided support to the panel from two methodologists, an advisor for dissemination and a junior committee member; the methodologists did not participate in the votes of the recommendations, the dissemination advisor and junior committee member were paediatric pulmonologists and did contribute to WG activities, panel discussions and voting.

A larger group with interest in PCD has met annually at ERS Congresses since 2006. The opinions of this group of over 60 clinicians, nurses, scientists and allied health professionals were sought and taken into account when deciding which tests to evaluate and which questions needed answering by the TF.

Two patient representatives (Beatrice Redfern and Bernhard Rindlisbacher) participated in the first task force meeting, helped in the project design, contributed to the writing of the practice guideline and the dissemination of the report. The European Lung Foundation contributed to the first meeting. An international survey and semi-structured interviews were conducted by Laura Behan to understand the patient perspective [10]

Supplementary Table 1: **Task force and Work group composition, presented in alphabetical order.** Membership of TF panel for duration unless dates provided. ** contributed to the work but not members of the task force panel. Additionally, David Rigau and Thomy Tonia are ERS methodologists who supported the project.

Task Force member	Speciality/ expertise	Role/ (Work Group membership)
Barbato, Angelo (Italy)	Paediatric pulmonology and PCD	Co-chair, leadership team (genetics and IF)
Behan, Laura (UK/Ire)	Social scientist, PhD candidate.	Investigated patient perspective
Bush, Andy (UK)	Paediatric pulmonology. PCD diagnostics. Clinical & translational research.	Leadership team.
Caudri, Daan (Netherlands)	Paediatric pulmonology. Epidemiologist.	Junior Member Guidelines Working Group of ERS (clinical features, nNO), second data extraction nNO
Collins, Samuel (UK)	Clinical PhD candidate: Paediatric pulmonology.	Systematic reviewer: (HSV, genetics). Writing team. Internal communications.
****Dell**, Sharon (Canada)	Paediatric pulmonology. PCD. Epidemiology.	Second data extraction clinical features WG

Eber, Ernst (Austria)	Paediatric Pulmonology	Dissemination (clinical features, nNO)
Escudier, Estelle (France)	Paediatrician, Diagnostic scientist, PCD diagnostics with HSV and EM	(TEM) 2015-16
Goutaki, Myrofora (CH)	Clinical PhD candidate: Paediatric pulmonology. Epidemiology.	Systematic reviewer: (clinical features)
Hogg, Claire (UK)	PCD Diagnostics. Paediatric pulmonology.	(clinical features, genetics)
Jorissen, Mark (Belgium)	ENT. PCD diagnostics with expertise in cell culture	(HSV, TEM)
Kennedy, Marcus (Ire)	Adult pulmonologist. Previously working in USA (genetics and EM), now Ireland (no specialist PCD diagnostic facilities)	(genetics, TEM) 2014-15
Kuehni, Claudia(CH)	Paediatric pulmonologist. Epidemiologist.	Leadership team; WG leader: (clinical features)
Latzin, Philipp (CH)	Paediatric pulmonologist, Respiratory physiology	(clinical features)
Legendre, Marie (France)	Clinical molecular geneticist, PCD diagnostics, genetics.	(genetics) 2015-16
Leigh, Margaret (USA)	Paediatric Pulmonology, Diagnostics, EM, genetics. American perspective	(HSV, genetics)
Lucas, Jane S (UK),	PCD Diagnostics. Paediatric pulmonology.	Chair of Task Force, leadership team, WG leader nNO (clinical features, nNO, HSV, IF, TEM)
Midulla, Fabio (It)	Paediatric Pulmonologist	(clinical features, nNO)
Nielsen, Kim G (DK)	PCD Diagnostics. Paediatric pulmonology.	(nNO)
Hirst, Rob (UK)	Diagnostic scientist with expertise in cell culture	(high speed video, TEM, genetics)

Omran, Heymut (DE)	PCD Diagnostics. Paediatric pulmonology.	WG leader: Genetics (IF)
Papon, Jean-Francois (France)	ENT. PCD diagnostics.	WG leader: HSV
Pohunek, Petr (CZ)	Paediatric pulmonology.	(clinical features)
Redfern, Beatrice (UK)	Patient representative	
Rindlisbacher, Bernhard (CH)	Patient representative	
Santamaria, Francesca (Italy)	Paediatric pulmonology. PCD diagnostics	(nNO)
Shoemark, Amelia (UK)	PCD scientist, clinical scientist in ultrastructural pathology	Work group leader: TEM Second extractor TEM IF
Snijders, Deborah (Italy)	Paediatric pulmonology.	Systematic reviewer: IF and genetics
****Titieni**, A (Germany)	Junior scientist in PCD/ Resident in Pediatrics	Second extractor IF
Walker, Woolf (UK)	Paediatric pulmonology.	Systematic reviewer: TEM 2014-16
Werner, Claudius (Germany)	Paediatric pulmonology.	Work group leader IF 2014-16

Disclosure of Conflicts of Interest

Panel members disclosed potential conflicts of interest according to ERS policies at the start of the Task Force and prior to publication of this manuscript. Following review of these statements, the Chairs (Lucas, Barbato) and ERS Guidelines committee considered it unnecessary for any panel member to abstain from decisions for any of the recommendations.

The ERS provided meeting facilities during their annual conference for meeting of the whole committee in 2014 and 2015. Meeting rooms in Lausanne were provided by ERS in January 2015 for training of a core group to undertake the literature searches and evaluation. The views and interests of ERS had no influence on the final recommendations.

Patient important outcomes

The GRADE approach emphasizes the importance of recommendations based on the impact on patient-important outcomes. GRADE methodology is usually used to assess quality of evidence for therapeutic interventions, where important outcomes might include improvement in quality of life, mortality etc. Such outcomes are not directly assessed in diagnostic studies and we therefore used diagnostic accuracy as a surrogate measure. An accurate diagnosis was endorsed as an important outcome by the patient representatives to the Task Force, as well as responses to a survey of 352 patients (25 countries, 9 European languages), and 20 in-depth interviews. Patients were particularly frustrated by delayed referrals often due to poor knowledge of general practitioners about PCD. They were happy to travel for assessment to specialist units, valuing the opportunity for staff with expertise to conduct specialist tests.

Formulation of the Topics and Questions

The panel met with a wider group of professionals (n=80) interested in PCD during ERS Congress 2014. A semi-structured discussion led to understanding of current diagnostic pathways and tests across Europe, and the questions that clinicians and scientists need answering. These discussions informed a closed meeting of the TF panel. The panel agreed that six diagnostic tests (clinical symptoms, nasal nitric oxide- nNO, high speed video-microscopy- HSV, transmission electron microscopy- TEM, genotype and immunofluorescence labelling of ciliary proteins-IF) would be evaluated using a 'PICO' structured question: "**P**atients suspected of having PCD, **I**nvestigated by

nNO, TEM etc, when **C**omparing patients with a final positive or negative diagnostic outcome, what was the diagnostic accuracy (**O**utcome) of the test?" We primarily aimed to identify studies of consecutive patients referred for PCD testing, in whom the PCD diagnosis was either confirmed or excluded. In the absence of sufficient literature of this study design, it was agreed that the comparator group might include healthy controls, or patients with other respiratory diseases (e.g. CF, asthma) from case control studies, but this would down grade the level of evidence. Lack of a gold standard diagnostic test for PCD was a limitation for this project. Diagnostic performance indicators (e.g. sensitivity and specificity) were therefore compared to the authors' final decision regarding positive/ negative diagnosis based on available tests. The PICO questions were refined during teleconferences and email discussions (supplementary table 2).

Several less structured questions were agreed to provide the basis of a narrative synthesis, but these questions were not used to provide recommendations.

	Clinical features	High speed video microscopy	Nasal nitric oxide	Genetics	Immunoflorescence	Electron microscopy
Work Package Question[1]	In patients suspected of having PCD, which clinical features (symptoms, signs, measurements) are associated with a diagnosis of PCD? Findings will help clinicians to define the group of patients, who should be referred for: a) PCD screening (with nNO); b) PCD confirmatory tests, even if nNO is normal?	In patients suspected of having PCD, should ex-vivo assessment of ciliary function be used as a diagnostic test?	In patients suspected of having PCD, should nasal NO measurement be used as a diagnostic tool[6]?	In patients suspected of having PCD, should genetic analysis be used a diagnostic test[6]?	In patients suspected of having PCD, should immunofluorescence analysis of protein mislocalisation be used as a diagnostic test?	In patients suspected of having PCD, should assessment of ciliary structure with transmission electron microscopy[10], be used as a diagnostic test?
Patient group	Patients suspected of having PCD	Patients suspected of having PCD	Patients with clinical suspicion of a	Patients with clinical suspicion of a	Patients with clinical suspicion of a diagnosis of PCD	Patients with clinical suspicion of a

			diagnosis of PCD. Subgroups: <1 year, <5 years ≥5 years. [7]	diagnosis of PCD		diagnosis of PCD
Investigation	Presence and severity of different clinical characteristics easily available in primary and secondary care: symptoms, signs, and simple measurements (spirometry, FeNO, chest X-ray, allergy tests etc). Subgroups by age (<1; 1-4; 5-15; 16-25; >25 years) and sex (for aspects of the reproductive system)	Ex-vivo analysis[4] of ciliary function Sub-groups: CBF, CBP[5]	Measurement of nasal NO. Subgroups: by analyser type; by breathing manoeuvre.[8]	Detecting mutation in PCD causing genes	Detecting protein mislocalisation by IF	Analysis of ciliary ultrastructure by a) transmission electron microscopy b) electron tomography
Comparator Group	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome[2].	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome	In patients with a positive diagnostic outcome in comparison to a negative diagnostic outcome
Outcome	Diagnostic performance measures	Diagnostic performance measures (including	Diagnostic performance	Diagnostic performance	Diagnostic performance measures (including sensitivity,	Diagnostic performance

	(including sensitivity, specificity)[3].	sensitivity, specificity)[3].	measures (including sensitivity, specificity).	measures (including sensitivity, specificity). Correlation of mutations with specific outcomes from other diagnostic tests: ciliary function (CBP and CBF), nNO, TEM, IF[9].	specific). Correlation of IF findings with specific outcomes from other diagnostic tests: ciliary function (CBP and CBF), nNO, TEM, genotype[9].	measures (including sensitivity, specificity).

Supplementary Table 2. Search terms used by Work Groups (WG) to address the PICO driven questions. (comments linked to superscripts in footnotes)

Footnote Comments:

1. Diagnostic tests which are not included in a systematic review will have a narrative comment in the practice guideline, but recommendations cannot be made e.g. radioaerosol mucociliary clearance, saccharine test.
2. Ideally, we will identify manuscripts of consecutive patients referred for PCD testing, in whom the PCD diagnosis is either confirmed or excluded. In the absence of sufficient literature of this study design, the comparator group might include healthy controls, or patients with other respiratory diseases (CF, asthma, …) from case control studies.
3. A limitation is the absence of a gold standard diagnostic test. Diagnostic performance indicators (e.g. sensitivity and specificity) will therefore firstly be comparing the inclusive decision regarding positive/ negative diagnosis. We will determine the hierarchal diagnostic criteria once we have reviewed the literature and will repeat the sensitivity/ specificity using these criteria if sufficient data exists.
4. Narrative comments can be made about obtaining samples e.g. nasal versus bronchial brushing.

5. Further sub-groups may be identified following literature search. For example, analysis of ciliary function by HSVA, by oscillometry, by computerised systems.

6. The term ‘diagnostic test’ is used to mean that the test is being used in a person with clinical symptoms of disease, rather than a screening tool for the general population. Some manuscripts may use the term “screening” to describe this, since the patient will require further confirmatory tests.

7. nNO is low in healthy infants, hence sub-group analyses <1 year, <5 years >5 years.

8. Sub-groups: by analyser type (chemiluminescence, hand-held); by breathing manoeuvre eg velum closure, tidal.

9. Collaboration between IF and genetics groups to tabulate associations.

10. Use of tomography to be included with TEM.

Systematic review

We searched the OVID Medline and Embase databases using the search terms outlined in supplementary table 2 to address each PICO focussed question. In a first step, at least two researchers from each WG screened the titles and abstracts, to exclude manuscripts that clearly did not address the PICO or the WG's additional questions. In a second step, two searchers (one for genetics due to lack of researchers) reviewed the full texts of the remaining papers, to identify manuscripts that addressed the PICO and fulfilled the inclusion criteria. Third, the committee and WG members received the lists of identified papers and were asked to report any additional studies not identified by the search. All data fulfilling the a priori inclusion criteria were included. PRISMA flow diagrams show the search process for each WG (supplementary Figure 1a-f).

We included all peer reviewed manuscripts from 1996 to 14th March 2016 with no language limitations. It was decided that manuscripts predating 1996 would be unlikely to reliably diagnose PCD versus non-PCD according to current standards. We excluded conference proceedings, grey literature and studies in non-humans.

Data extraction tables were designed to capture information required for each WG. These were circulated for editing to the TF panel. Each WG decided what data was required a) to answer the PICO b) to answer additional questions. Data was extracted by two independent researchers with the exception of genetics WG which used single extraction due to lack of researchers. Since there is no reference standard for diagnosis of PCD, details of how diagnosis was confirmed/ excluded was extracted for all studies and acceptability agreed by the TF panel.

Quality of evidence leading to recommendations

Grading of Recommendations Applicability, Development and Evaluation (GRADE) is a method for systematically assessing the quality of evidence for a diagnostic test and then making recommendations for use of the test based on the quality of this evidence. Using the GRADE approach we rated the overall quality of evidence for each question as high, moderate, low or very low, based on the following criteria: risk of bias, directness, consistency, precision and publication bias, are rated as none, not serious or serious.

The identified manuscripts were assessed on the following criteria –

1. **Study design** – for example a randomised controlled trial (although very few exist in diagnostics) would be a higher level of evidence than prospective cohort studies and these would be higher than case-control studies.
2. **Risk of bias** – We assessed risk of bias using the Quadas-2 tool for the quality assessment of diagnostic accuracy studies, based on four domains (a) patient selection; b) conduct or interpretation of index test; c) selection, conduct or interpretation of reference standard; and d) patient flow)[11].
3. **Directness**- This refers to the existence of a direct link between the diagnostic test and patient important outcomes. For intervention studies, intermediate outcomes, such as accuracy of diagnostic tests, are always considered "indirect" evidence and thus reduce the quality. Therefore, directness was graded as "potentially serious" in all WGs.
4. **Consistency**- This refers to the degree to which reported study results (e.g., sensitivity, specificity) from included studies are similar; thus heterogeneity of results was reported as inconsistency.
5. **Precision** – Precision refers to the degree of certainty concerning the estimates of each test performance (quantified by the width of confidence intervals around estimates).
6. **Publication bias** – This indicates that studies may have been published selectively and pooled estimates of published studies might not reflect the truth (e.g. negative findings have not been published, or are unavailable).

Criteria 2-6 are assessed as either serious or very serious. Grading of the evidence as HIGH, MODERATE, LOW or VERY LOW was based initially on the study design and then downgraded appropriately based on the other factors. The final grading of the evidence helped to inform the final recommendations as either STRONG (should always be done) or WEAK (should be performed in certain circumstances). For reaching recommendations, the Committee took into account the quality of the evidence; the balance between benefits and harms; the patients' values and preferences and other factors such as costs, feasibility, accessibility etc. Evidence profiles were discussed with and across WGs electronically and by telephone conferences throughout the duration of the TF and discussed in a face-to-face meeting of the entire TF panel at the 2015 ERS Congress in Amsterdam. Sections of the manuscript were written by WG leaders and members of their groups, and again discussed and amended electronically across WGs and within the committee. Evidence that was of a lower quality than that used for recommendations was commented on in the guideline but was not used to make recommendations [12–14].

Consensus statement for diagnostic outcomes

We conducted a modified Delphi survey in four rounds to develop consensus regarding the contributions of diagnostic tests to confirm or refute a diagnosis of PCD. Only members of the Task Force with relevant expertise participated by completing online questionnaires (https://www.isurvey.soton.ac.uk/). Respondents were anonymous to others with the exception of the Chair (JSL) who could identify participants. Before each round participants reviewed the results of previous surveys, including a summation of comments with reasons underlying opinions and recommendations for iterations. The first round of the survey aimed to understand if any individual tests could definitively confirm or exclude a diagnosis of PCD. In the second round each Delphi participant was asked to review the summary of responses from round 1; they were then invited to consider combinations of tests that might confirm or exclude a diagnosis when the diagnosis is considered clinically very likely, or only modest. In round 3 and 4 there were further iterations. A consensus was reached when 80% of participants were in agreement.

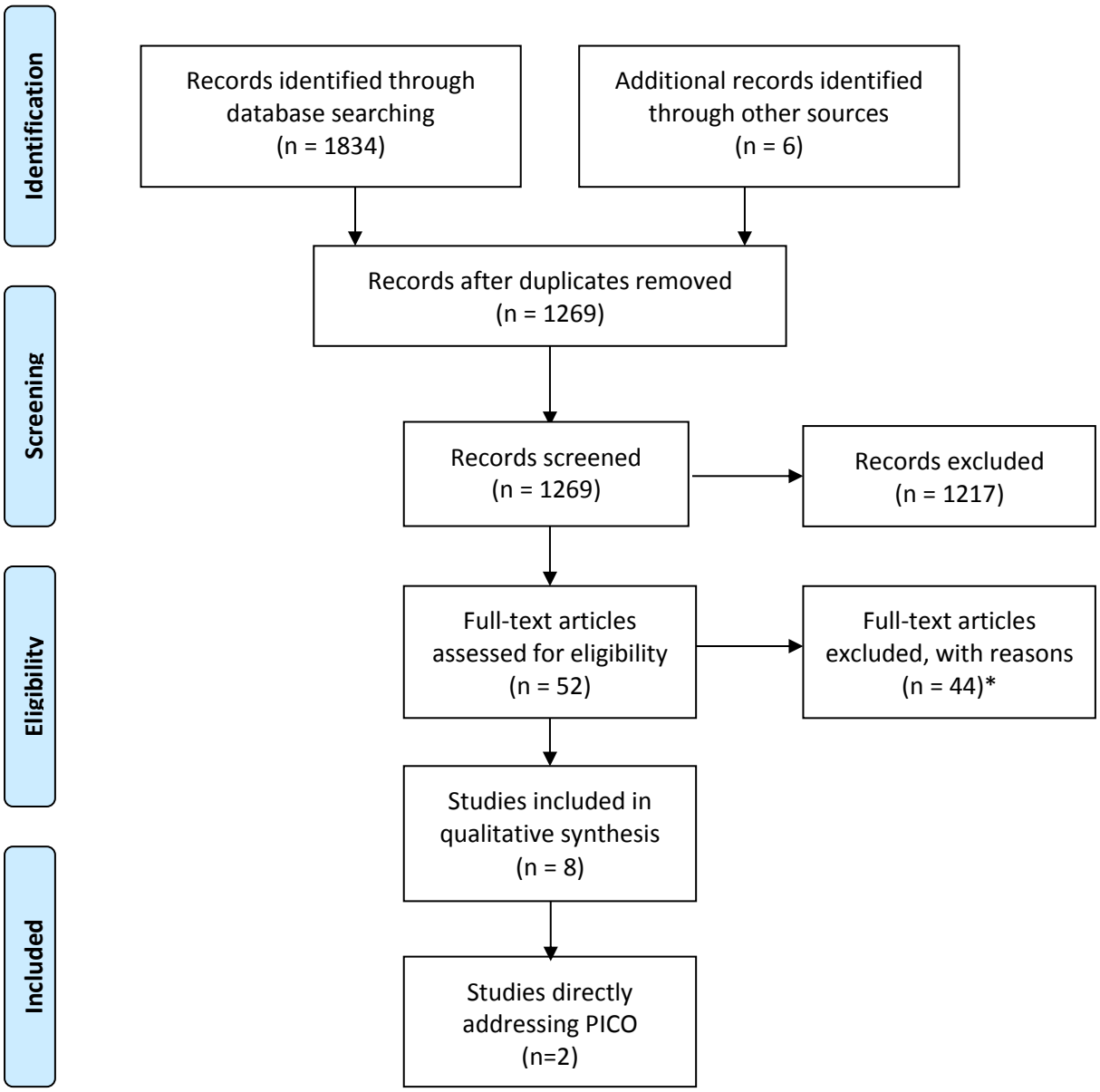
Results

Literature search

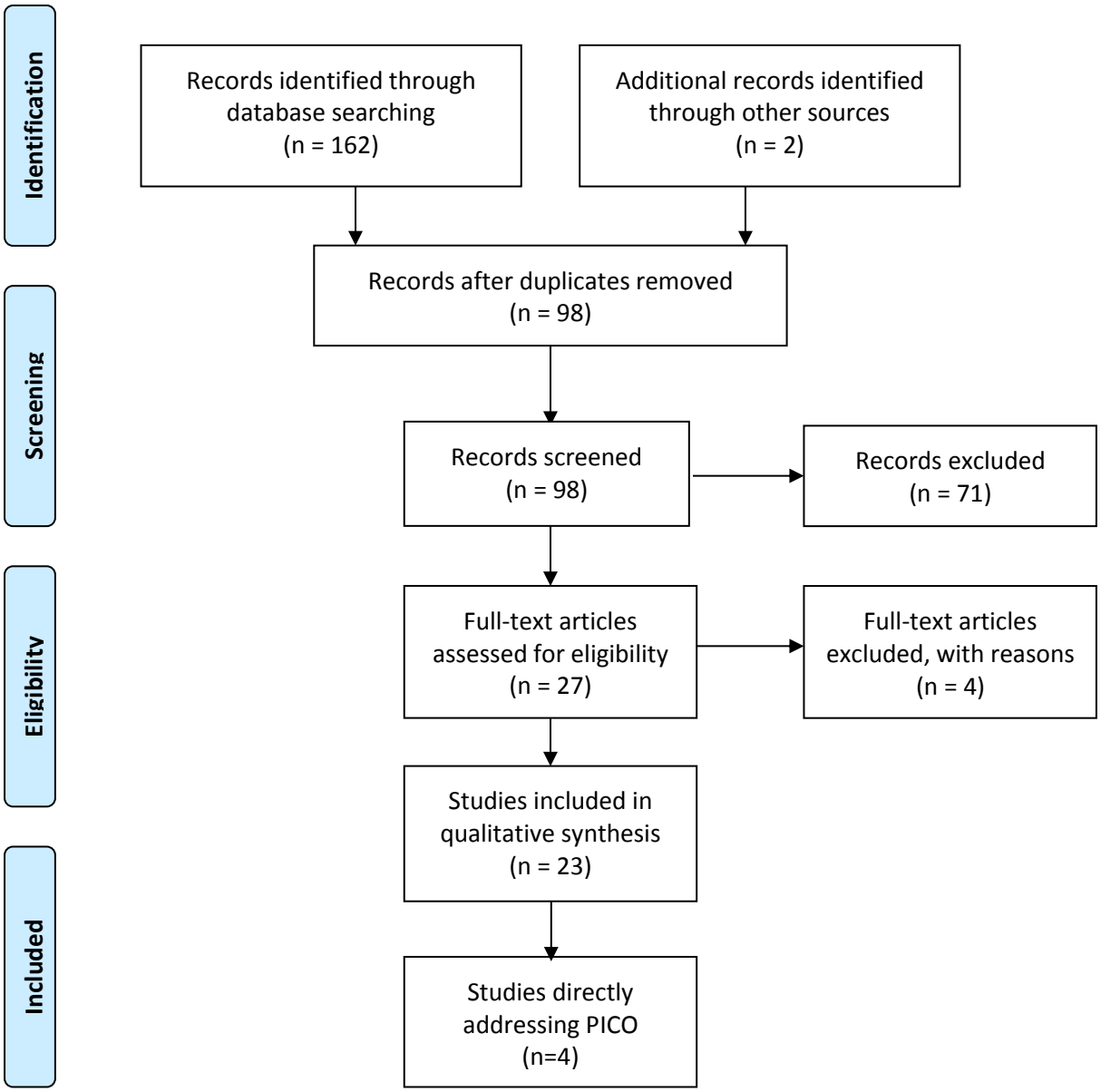
The outcomes of the literature searches for each work group are summarised by PRISMA flowcharts (supplementary figure 1a-f)

Supplementary figure 1 a-f: Identification, screening and inclusion of studies reporting on a) PCD clinical symptoms b) nasal nitric oxide c) high-speed video microscopy d) transmission electron microscopy e) genetics f) immunofluorescence. Flow charts are based on PRISMA guidelines.

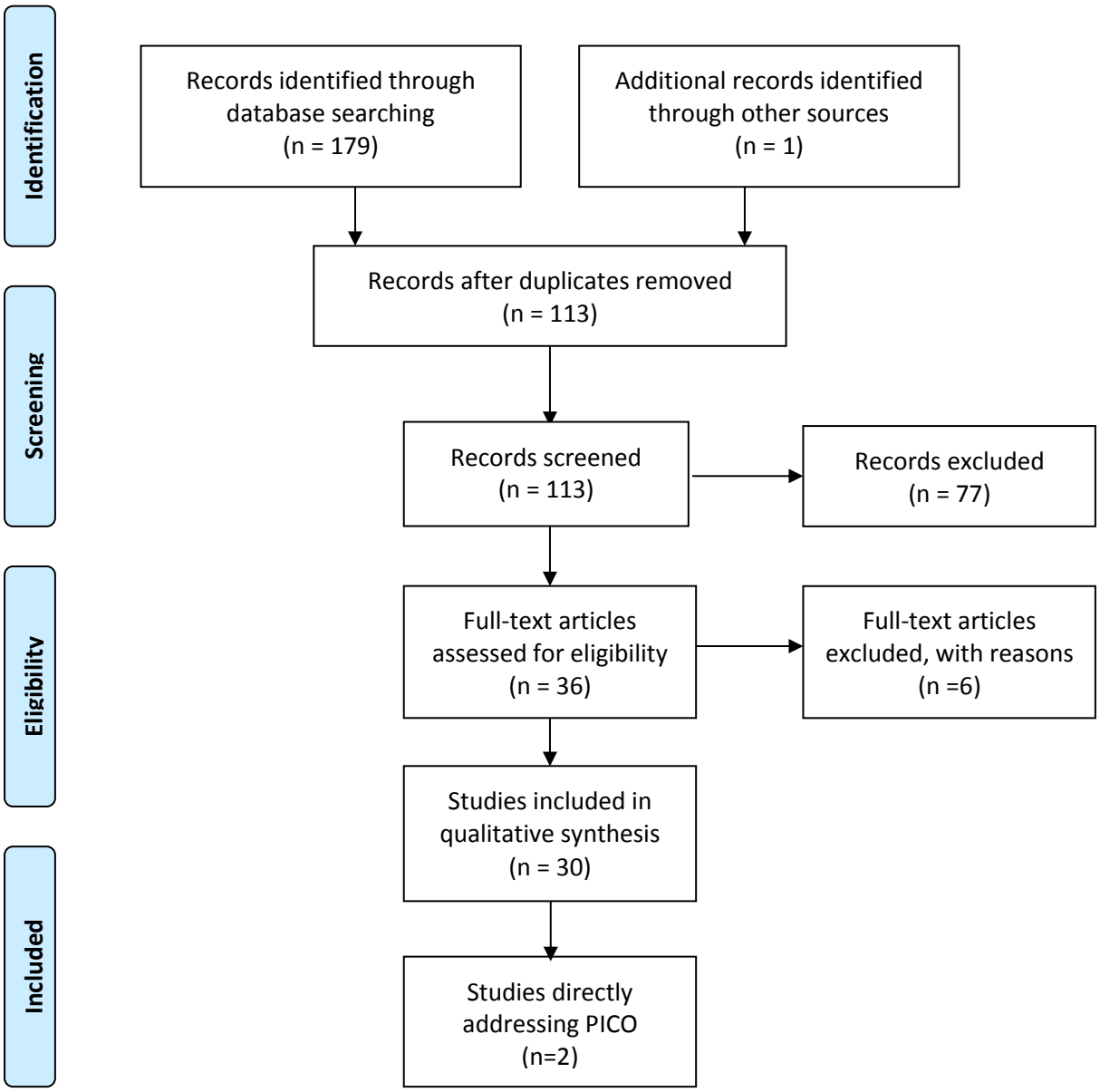
a) Clinical Features



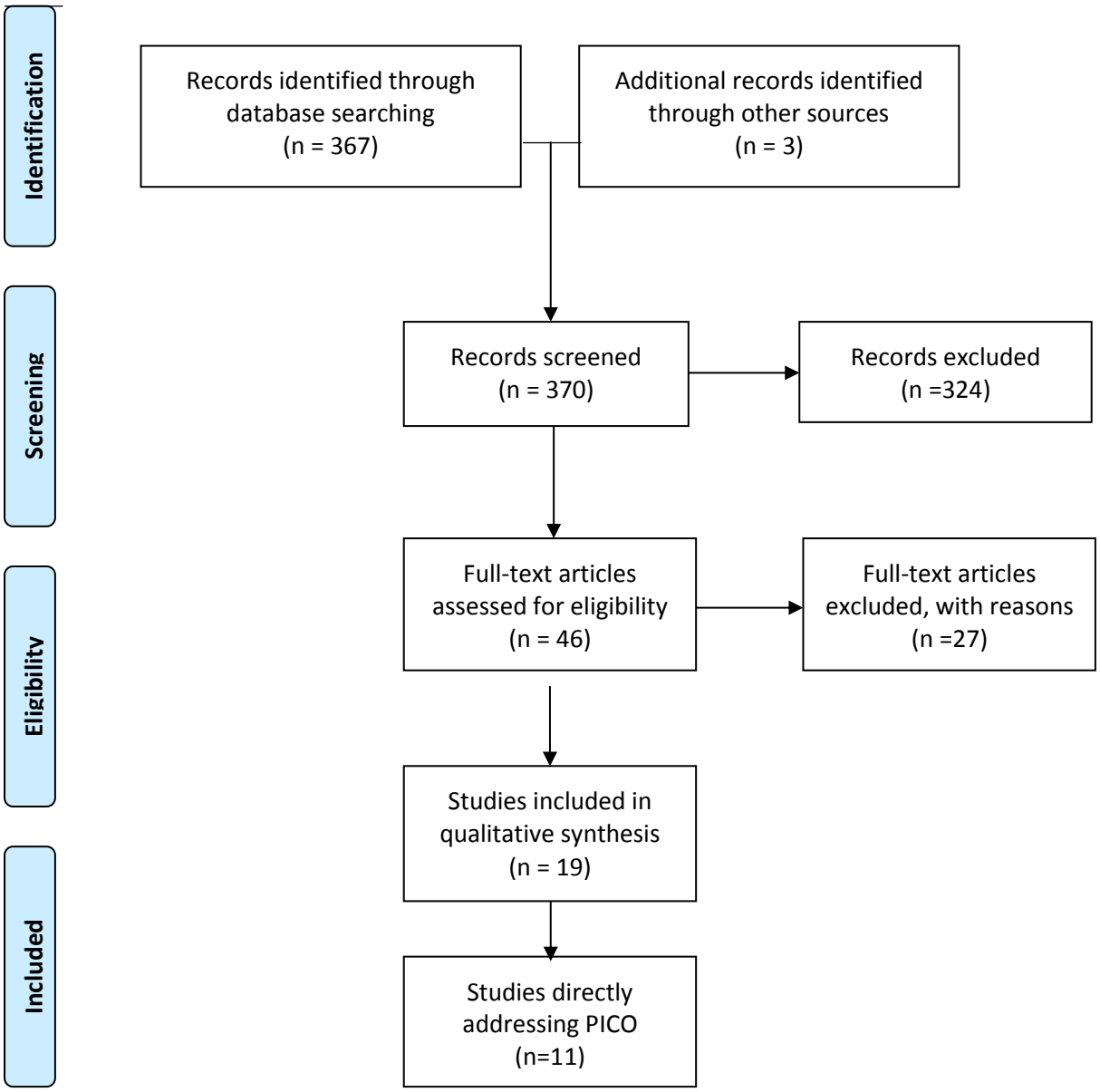
b) Nasal Nitric Oxide



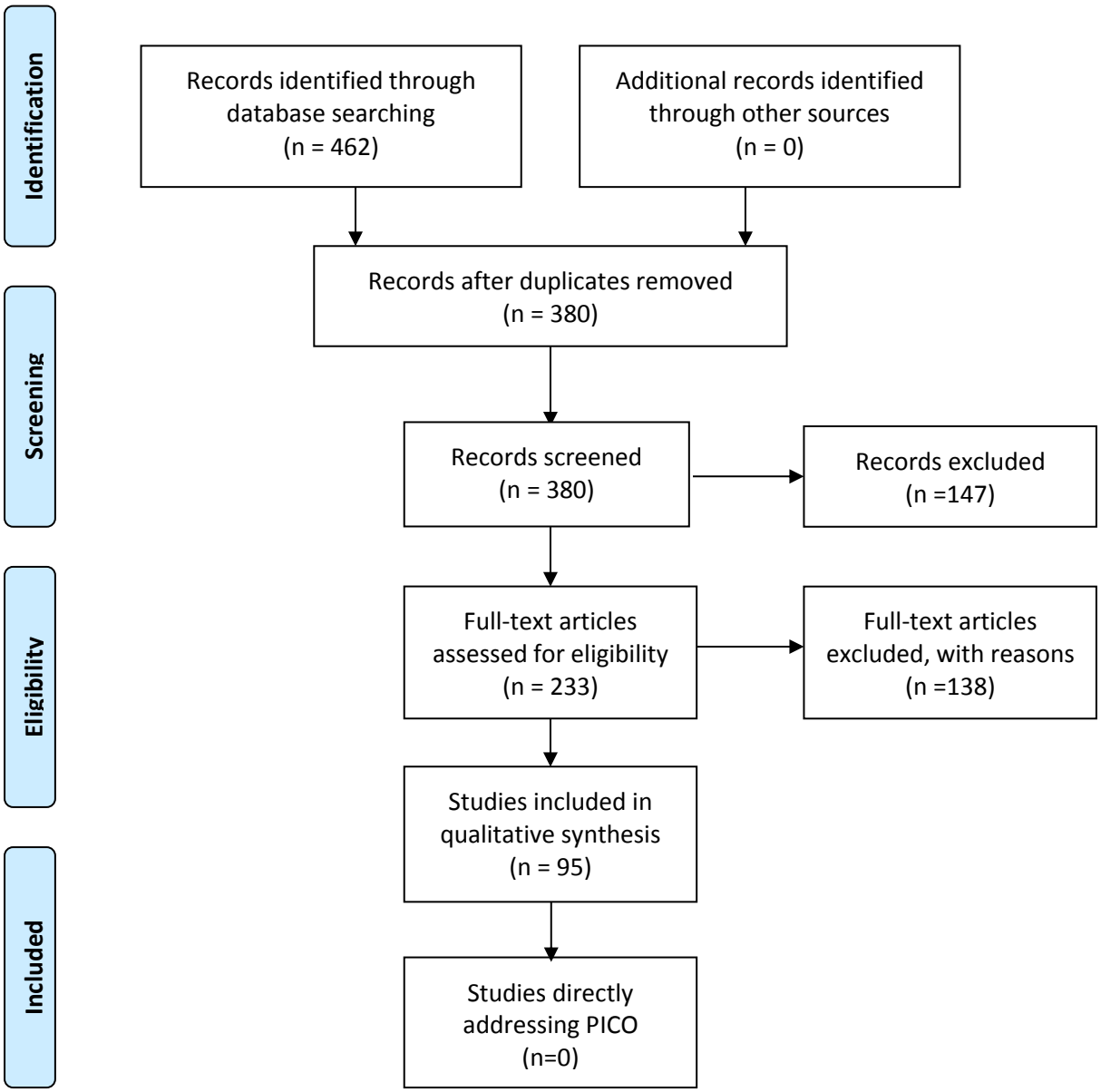
c) High Speed Video



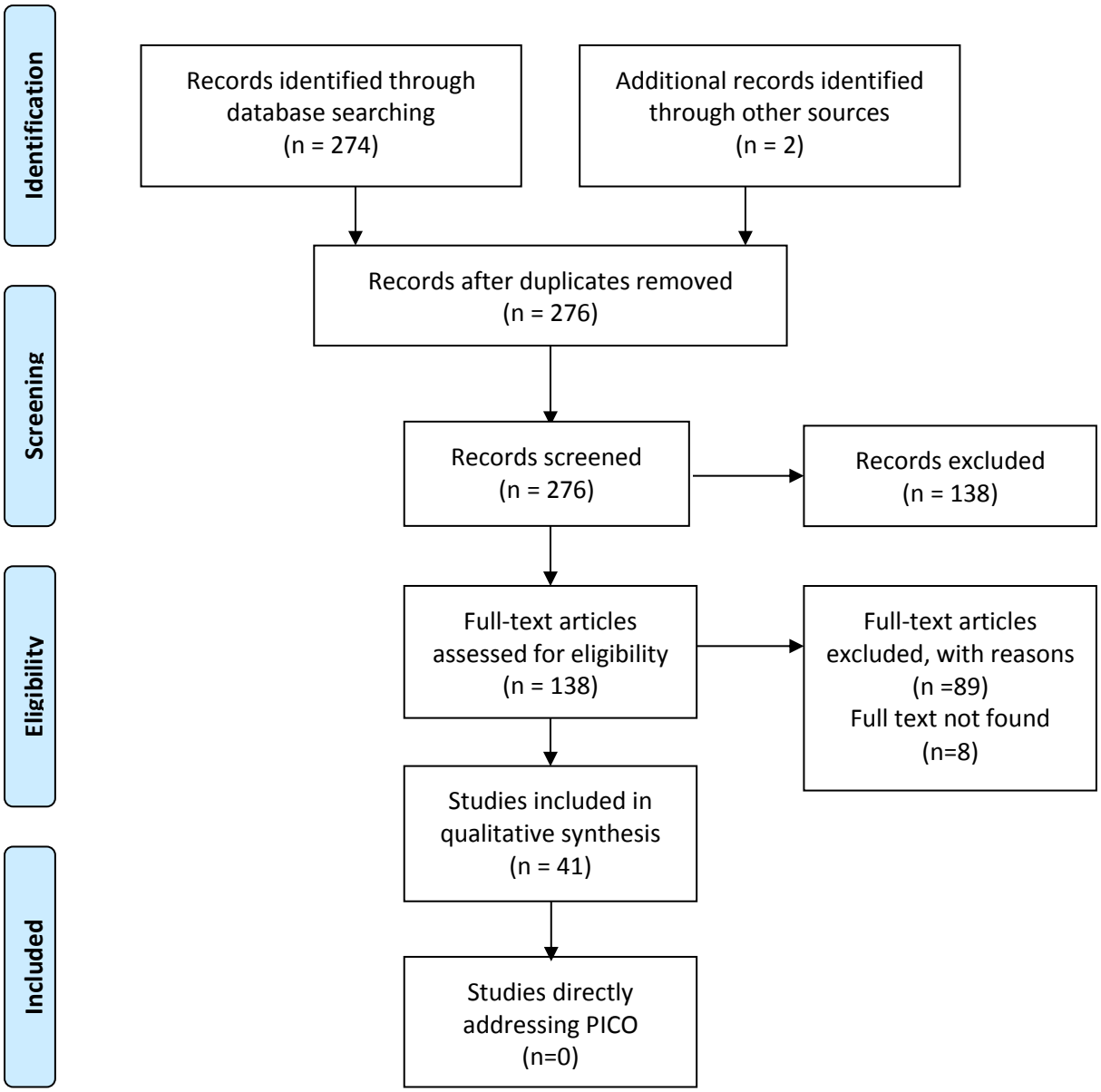
d) TEM


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 367)
Additional records identified through other sources (n = 3)
Records screened (n = 370)
Records excluded (n =324)
Full-text articles assessed for eligibility (n = 46)
Full-text articles excluded, with reasons (n =27)
Studies included in qualitative synthesis (n = 19)
Studies directly addressing PICO (n=11)

e) Genetics


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 462)
Additional records identified through other sources (n = 0)
Records after duplicates removed (n = 380)
Records screened (n = 380)
Records excluded (n =147)
Full-text articles assessed for eligibility (n = 233)
Full-text articles excluded, with reasons (n =138)
Studies included in qualitative synthesis (n = 95)
Studies directly addressing PICO (n=0)

f) IF


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 274)
Additional records identified through other sources (n = 2)
Records after duplicates removed (n = 276)
Records screened (n = 276)
Records excluded (n = 138)
Full-text articles assessed for eligibility (n = 138)
Full-text articles excluded, with reasons (n =89)
Full text not found (n=8)
Studies included in qualitative synthesis (n = 41)
Studies directly addressing PICO (n=0)

Manuscripts contributing to the qualitative review

Each search identified a number of manuscripts which provided relevant information regarding PCD diagnostic testing. The full text was critiqued to establish whether each manuscript fulfilled the criteria needed to contribute to the quantitative analysis (sensitivity and specificity). Those manuscripts which did not fulfil these strict criteria were used to address other important questions regarding PCD diagnostic testing, contributing to the narrative discussion. The summaries of these manuscripts are provided in Supplementary Tables 3-8.

Publication	**Study design**	**Reason for exclusion/ comments**
Ben Khelifa et al 2014	Retrospective cohort study of patients with asthenozoospermia	Cohort with different study population PCD diagnosis only by genetic mutations (DNAH1)
Bouyahia et al 2008	Retrospective cohort study of patients with bronchiectasis	Cohort with different study population Patients with uncertain TEM results were excluded
Coste et al 2004	Prospective cohort study of patients with atypical chronic sinusitis	Cohort with different study population
Garrod et al 2014	Prospective cohort study of patients with congenital heart disease	Cohort with different study population
Goeminne et al 2010	Retrospective cohort study of patients with non-CF bronchiectasis	Cohort with different study population
Guan et al 2015	Prospective cohort study of patients with bronchiectasis	Cohort with different study population
Gurr et al 2009	Retrospective lab study on ear mucosa samples from patients with chronic secretory otitis media	No sufficient information on clinical symptoms
Kim et al 2010	Retrospective cohort study of patients with bronchiectasis	Cohort with different study population
Kumar et al 2015	Retrospective cohort study of patients with non-CF bronchiectasis	No proven diagnosis of PCD PCD is only suspected (use of FeNO) and not diagnosed
Li et al 2005	Retrospective cohort study of patients with non-CF bronchiectasis	Cohort with different study population
Lopes et al 2015	Prospective cohort study of patients with bronchiectasis	Cohort with different study population
Nakhleh et al 2012	Retrospective cohort study of patients with congenital heart disease and heterotaxy	Cohort with different study population
Niu et al	Retrospective cohort study of patients with	Cohort with different study population

2011	asthenozoospermia	
Noone et al 2014	Retrospective cohort study of patients suspected of PCD	Reported symptoms only in PCD positive patients
Offen et al 2014	Retrospective cohort study of patients with congenital heart disease and dextrocardia	Cohort with different study population
Pifferi et al 2004	Prospective cohort of patients with history of recurrent lower respiratory infections and bronchiectasis	No sufficient information on clinical symptoms Paper is focused on describing a specific ultrastructure anomaly
Pifferi et al 2009	Prospective cohort study of patients with recurrent pneumonia	Cohort with different study population
Qi et al 2015	Prospective cohort study of patients with bronchiectasis	Cohort with different study population
Santamaria et al 2009	Retrospective cohort study of patients with bronchiectasis	Cohort with different study population
Shapiro et al 2010	Retrospective cohort of patients with suspicion of PCD	Conference abstract
Shoemark et al 2007	Prospective cohort of patients with symptoms suspected for bronchiectasis	Cohort with different study population
Stewart et 2014	Retrospective cohort of patients with congenital heart disease undergoing cardiac surgery	Cohort with different study population
Tsang et 2015	Prospective cohort of patients with bronchiectasis	Cohort with different study population
Welch et al 2004	Prospective cohort of patients with history of recurrent or chronic upper or lower respiratory tract problems	Reported symptoms only in PCD positive patients
Zahid et al 2012	Retrospective cohort of patients with transposition of great arteries	Cohort with different study population
Zahid et al 2014	Retrospective cohort of patients with transposition of great arteries	Cohort with different study population
Zaid et al 2010	Retrospective cohort of patients with non CF-bronchiectasis	Cohort with different study population
Al Saadi et al 2013	Case control study comparing PCD patients with healthy controls	No sufficient information on clinical symptoms
Armengot et al 2012	Case control study comparing PCD patients with healthy controls and patients with SCD	Reported symptoms only in PCD positive patients
Boon et al 2014	Case control study comparing PCD patients with healthy and disease controls	Reported symptoms only in PCD positive patients
Cohen-Cymberknoh et al 2012	Case control study comparing patients with PCD and CF	Conference abstract

Cohen-Cymberknoh et al 2014	Case control study comparing patients with PCD and CF	No sufficient information on clinical symptoms
Irving et al 2013	Case control study comparing patients with PCD and CF	No sufficient information on clinical symptoms
Knowles et al 2014	Case control study comparing patients with different TEM defects and healthy controls	Reported symptoms only in PCD positive patients
Madsen et al 2013	Case control study comparing PCD patients with healthy controls	Reported symptoms only in PCD positive patients
Mahut et al 2006	Case control study comparing PCD patients with healthy controls	Reported symptoms only in PCD positive patients
Oktem et al 2013	Case control study comparing PCD patients with healthy controls	No sufficient information on clinical symptoms
Olm et al 2011	Case control study comparing PCD patients with healthy controls	Reported symptoms only in PCD positive patients
Paff et al 2013	Case control study comparing PCD patients with healthy controls and CF patients	No sufficient information on clinical symptoms
Paraskakis et al 2007	Case control study comparing PCD patients with healthy controls	No sufficient information on clinical symptoms
Phillips et al 1998	Case control study comparing PCD patients with healthy controls	No sufficient information on clinical symptoms
Regnis et al 2000	Case control study comparing PCD patients with healthy controls and CF patients	No sufficient information on clinical symptoms
Santamaria et 2014	Case control study comparing PCD patients with healthy controls	Reported symptoms only in PCD positive patients
Shapiro et 2011	Case control study of patients with heterotaxy, PCD positive and negative	Conference abstract

Supplementary Table 3. Clinical symptoms workgroup. Summary of the 44 excluded full-text studies on clinical manifestations of PCD and the reasons of exclusion. CF: cystic fibrosis, SCD: secondary ciliary dyskinesia

Publication	Study population	Ages	Aim of study	Analyser	Sampling method
Marthin & Nielsen 2011	117 referrals PCD 14	6.9 (0.0-62.4) Median (range)	Evaluate 3 different sampling methods for nNO in consecutive referrals to a PCD service	NIOX Flex (Aerocrine, Sweden)	Breath hold (n=58 Oral exhalation ag Tidal breathing (n
Leigh *et al* 2013	155 referrals PCD 71 Indeterminate 84	PCD 23.3 (5.1-69.0) Indeterminate 31.8 (5.5-79.6) Mean (range)	Use a standard protocol for nNO measurement to establish disease specific cut-offs then validate at 6 other sites.	Sievers, CLD 88SP (ECO PHYSICS/MEDICS, Switzerland), NIOX Flex (Aerocrine, Sweden)	Oral exhalation, v (n=?, 77)
Beydon et al 2015	86 referrals PCD 49 Non-PCD 37	Median 8.9y IQR (5.7-12.8)	Assess the accuracy of velum closure and 3 different tidal breathing measurements in diagnosing PCD	Niox Flex (Aerocrine, Sweden), Endono 8000 (manufacture unknown)	Velum closure (n= Tidal breathing –
Jackson et al	301 referrals PCD 34 Non-PCD 267	Range 6-79 years	Accuracy of nNO screening by velum closure in consecutive referrals for PCD diagnosis	NIOx Flex (Aerocrine, Sweden)	Velum closure (br (n=301, 30)

Supplementary Table 4: Nasal nitric oxide workgroup. Methodological details of the nasal nitric oxide studies directly addressing the PICO.

Publication	Study summary	Comments/ Exclusion reason
Arnal et al 1999	Case control study of nasal polyposis, sinusitis, Kartagener's and healthy controls	PCD – Kartagener's, clinical diagnosis only Not consecutive patients
Narang et al 2002	Case-control study of breath hold nNO in PCD, disease control and healthy	Case-control, not consecutive referrals
Horvath et al 2003	Case control study of PCD, CF, Bronchiectasis and healthy	Case-control, not consecutive referrals
Wodehouse et al 2003	Case-control of PCD, disease control and healthy	Case-control, not consecutive referrals
Corbelli et al 2004	Prospective cohort in symptomatic children	Unclear if consecutive referrals, blinding not stated, inconsistencies in reported numbers
Noone et al 2004	Prospective case control study, PCD, CF and disease controls	Case-control, not consecutive referrals and unclear diagnostic criteria
Pifferi et al 2007	Prospective cohort study of those with recurrent pneumonia	Diagnosis based on TEM only, nNO results used to retrospectively assign diagnosis
Santamaria et al 2008	Case-control study PCD vs Healthy	Case-control, not consecutive referrals
Moreno Galdo et al 2010	Case control of PCD vs healthy and disease controls	PCD based on TEM diagnosis only
Mateos-Corral et al 2011	Case control, PCD, Healthy, other disease controls	PCD diagnosis symptoms and EM only, not consecutive patients
Montella et al 2011	Case control PCD vs disease controls	Comparing different sampling methods not diagnostic accuracy in referrals
Marthin et al 2013	Case control study of different analysers	Case-control, not consecutive referrals
Boon et al 2014	Case-control PCD vs healthy and disease controls	Case-control, not consecutive referrals
Collins et al 2014	Systematic review and meta-analysis of nNO	Covers studies in this review and includes both case-control and cohort studies
Harris et al 2014	Case-control study of differing sampling techniques	Covers studies in this review and includes both case-control and cohort studies
Pifferi et al 2007	Cohort study of recurrent pneumonia (PCD, secondary dyskinesia and healthy controls)	SCD cases determined only in retrospect, sampling method unclear Not consecutive patients
Adams et al 2015	Case-control study of nNO in under 1s	Not consecutive referrals, healthy controls only
Kouis et al 2015	Systematic review/meta-analysis of nNO	Covers studies in this review and includes both case-control and cohort studies
Amirav et al 2016	Cohort study on high speed video	No data on nNO given

Supplementary Table 5. Nasal nitric oxide workgroup. Summary of studies excluded at the full-text stage with reason for exclusion.

Publication	Study summary	Comments/ Exclusion reason
Rayner et al 1996	Case-control study of beat pattern	Saccharine and TEM diagnosis only
Chapelin et al 1997	Nasal brushings in those with recurrent respiratory infections	Beat frequency only
Bent et al 1997	Tracheal biopsies	Subjective movement only, no measurements
Santamaria et al 1999	Case-control study chronic infection vs controls	Subjective motility only
Friedman et al 2000	Retrospective cohort study	Light microscopy only
Jorissen et al 2000	Retrospective cohort study (primary and secondary dyskinesia)	Not consecutive referrals
Pifferi et al 2001	Response of ciliary motion to intensive treatment	
Ahmad et al 2003	Retrospective cohort study	Diagnosis criteria for PCD not clear
Chilvers et al 2003	Cohort of PCD patients	No negative patients
Coste et al 2004	Prospective cohort study	Stroboscopy only
Nuesslein et al 2004	Prospective case-control study in bronchitis patients	Compares nose and bronchus not positive vs negative PCD
Pifferi et al 2007	Retrospective nasal NO study	Not a study of HSV, little detail on ciliary assessment
Pifferi et al 2009	Prospective cohort of PCD, SCD and inconclusive	Comparison of HSV before/after culture
Armengot et al 2010	Case control PCD, SCD and healthy	Not consecutive referrals, unclear criteria for diagnosis of PCD
Hirst et al 2010	Retrospective cohort study of abnormalities after ALI	Correlation before/after ALI
O'Callaghan et al 2010	Retrospective cohort	Epidemiological study, no details of ciliary assessment
Stannard et al 2010	Retrospective case-control study	Diagnosis of PCD by TEM only
Noll et al 2011	Retrospective cohort	Photoelectrical method only
Shoemark et al 2012	Retrospective cohort	Study of TEM findings so little detail of ciliary assessment
Pifferi et al 2013	Prospective cohort of ciliary assessment	Not study of HSV, investigating different ciliary motion parameters
Boon et al 2014	Cohort of PCD positive patients	No negatives

Hirst et al 2014	Case control study of ALI	
Kim et al 2014	Genetic study in PCD cases	Very little HSV data
Parrilla et al 2014	Case control study of ciliary assessment methods	Study of assessment methods
Raidt et al 2014	Prospective cohort	Studying genetic/TEM correlation with beat pattern
Pifferi et al 2015	Prospective case-control study	Not study of HSV
Amirav et al 2015	Retrospective cohort	Not clearly a cohort of suspected PCD, reference test unclear
Quinn et al 2015	Establishing system for computational analysis of CBP/F	Not consecutive referrals

Supplementary Table 6. High speed video microscopy workgroup. Summary of studies excluded at full-text review stage with reason for exclusion.

Publication	**Study summary**	**Comments/ Exclusion reason**
Jorisson et al 2000	Retrospective cohort study	Duplication of cohort data in a study already included in the PICO (The larger study more relevant to TEM has been included)
Escudier et al 2002	Computer assisted analysis aids detection of IDAs	TEM add on technique study
Stannard et al 2010	Retrospective case-control study	Diagnosis of PCD by TEM only
O'Callaghan et al 2011	Retrospective cohort study. IDA defects require repeat testing	TEM only
Olin et al 2011	Diagnostic yield of nasal scrapes Retrospective cohort	TEM only
Boon et al 2014	Cohort PCD positive patients	No negatives
Funkhouser et al,2014	Computer assisted analysis aids TEM performance	TEM add on technique study
Wallmeier et al 2014	Gene discovery study	Not consecutive referrals

Supplementary Table 7. Transmission electron microscopy workgroup. Summary of studies excluded at full-text review stage with reason for exclusion.

Publication	**Study design**	**Reason for exclusion/ comments**
Janitzl et al 1999	Genetic testing for HSET gene mutations in PCD patients	Genetics not used as a diagnostic tool
Pennarum et al 1999	Genetic testing for Loss-of-Function Mutations in IC78	Genetics not used as a diagnostic tool
Witt et al 1999	Candidate careening for chromosome 7 in syndrome di Kartagener	Genetics not used as a diagnostic tool
Blouin et al 2000	Genome-wide linkage analysis in PCD patients	Only linkage study, no diagnostic testing
Maiti et al 2000	Evaluations of the FOXJ1 in patients with PCD	Screening test for possible mutations, no diagnostic testing
Meeks et al 2000	Linkage study chromosome 19	Only linkage study, no diagnostic testing
Omran et al 2000	Candidate gene screening Chromosome 5p and DNAH5	Only linkage study, no diagnostic testing
Pennarun et al 2000	Candidate gene screening DNAI2	Only linkage study, no diagnostic testing
Bartoloni et al 2001	Candidate gene screening DNAH9	Only linkage study, no diagnostic testing
Guichard et al 2001	Genetic testing for DNAI1 Mutations in PCD	Genetics not used as a diagnostic tool
Zariwala et al2001	Genetic testing for DNAI1 in PCD	Genetics not used as a diagnostic tool
Bartoloni et al 2002	Genetic testing for DNAH11 in situs inversus totalis	Genetics not used as a diagnostic tool
Neesen et al 2002	Candidate gene screening of human ortholog of the t-complex-encoded protein TCTE3 in PCD	Only linkage study, no diagnostic testing
Noone et al 2002	Genetic testing for DNAI1 in PCD	Genetics not used as a diagnostic tool
Olbrich et al 2002	Genetic testing for DNAH5 in PCD patients	Genetics not used as a diagnostic tool
Pennarun et al 2002	Candidate gee screening of the Human hPF20Gene Orthologous	Only linkage study, no diagnostic testing
Zhang et al 2002	Identification of Dynein Heavy Chain 7 in bronchial cells in PCD patients	Protein localisation in bronchial cells, no diagnostic testing
Zito et al 2003	Genetic testing for RPGR mutation in patients with retinitis pigmentosa, impaired hearing, and sinorespiratory infections	Genetics not used as a diagnostic tool

Jeganathan et al 2004	Candidate gene screening of chromosome 16p12.1-12.2 and 15q13.1-15.1	Letter, linkage study
Zariwala et al 2003	Investigation of the Possible Role of a Novel Gene, DPCD, in Primary Ciliary Dyskinesia	Genetics not used as a diagnostic tool
Fliegauf et al 2005	Genetic testing of DNAH5 and DNAH9 in Respiratory Cells from Patients with PCD	Genetics not used as a diagnostic tool
Geremek et al 2006	Linkage analysis on chromosome 15q24–25 in Kartagener syndrome	Only linkage study, no diagnostic testing
Gutierrez-Roelens et al 2006	Localization of candidate regions for a novel gene for Kartagener syndrome	Candidate gene search, no diagnostic testing
Hornef et al 2006	Genetic testing for DNAH5 Mutations in PCD with Outer Dynein Arm Defects	Genetics not used as a diagnostic tool
Moore et al 2006	Genetic testing for RPGR in primary ciliary dyskinesia and retinitis pigmentosa	Genetics not used as a diagnostic tool
Zariwala et al 2006	Mutations of DNAI1 in Primary Ciliary Dyskinesia Evidence of Founder Effect in a Common Mutation	Genetics not used as a diagnostic tool
Duriez et al 2007	Genetic testing for TXNDC3 in PCD	Genetics not used as a diagnostic tool
Failly et al 2008	Genetic testing per DNAI1 mutations in PCD	Genetics not used as a diagnostic tool
Geremek et al 2008	Sequence analysis of 21 genes located in the Kartagener syndrome linkage region on chromosome 15q	Only linkage study, no diagnostic testing
Loges et al 2008	Genetic testing for DNAI2 mutations in ODA defects	Genetics not used as a diagnostic tool
Omran et al 2008	Genetic testing for KTU mutations in ODA+IDA defects	Genetics not used as a diagnostic tool
Schwabe et al 2008	Genetic testing for DNAH11 mutations in normal axoneme ultrastructure suspected PCD patients	Genetics not used as a diagnostic tool
Wessels et al 2008	Candidate Gene Analysis in Three Families With acilia Syndrome	Candidate gene search, no diagnostic testing
Zuccarello et al 2008	Mutations in dynein genes in patients affected by isolated non-syndromic asthenozoospermia	no PCD population, only astenozoospermia

Castelman et al 2009	Genetic testing in in Radial Spoke Head Protein Genes, RSPH9 and RSPH4A in PCD	Genetics not used as a diagnostic tool
Duquesnoy et al 2009	Genetic testing for LRRC50 mutations in PCD	Genetics not used as a diagnostic tool
Loges et al 2009	Genetic testing for LRRC50 mutations in PCD	Genetics not used as a diagnostic tool
Lie et al 2010	Founder splice mutation of DNAI1 in Amish community	Genetics not used as a diagnostic tool
Pifferi et al 2010	Genetic testing for DNAH11 mutations in normal axoneme ultrastructure suspected PCD patients	Genetics not used as a diagnostic tool
Reish et al 2010	Founder mutation(s) in the RSPH9 gene leading to primary ciliary dyskinesia in two inbred Bedouin families	Genetics not used as a diagnostic tool
Zietkiewicz et al 2010	Population specificity of the DNAI1 gene mutation spectrum in PCD	Genetics not used as a diagnostic tool
Becker-Heck et al 2011	Genetic testing for CCDC40 mutations in PCD patients	Genetics not used as a diagnostic tool
Berg et al 2011	Next generation parallel sequencing of targeted exomes in PCD for 79 genes	Genetics not used as a diagnostic tool
Mazor et al 2011	Genetic testing for DNAL1 mutations in PCD	Genetics not used as a diagnostic tool
Merveille et al 2011	Genetic testing for CCDC39 mutations in PCD	Genetics not used as a diagnostic tool
Alsaadi et al 2012	WES screening for RSPH9	Genetics not used as a diagnostic tool
Blanchon et al 2012	Genetic testing for CCDC39/CCDC40 mutations in PCD	Genetics not used as a diagnostic tool
Djakow et al 2012	Genetic testing for DNAH5 and DNAI1 in PCD	Genetics not used as a diagnostic tool
Horani et al 2012	Whole-Exome Capture and Sequencing identifies HEATR2 Mutation in PCD	Genetics not used as a diagnostic tool
Knowles et al 2012	Genetic testing for DNAH11 mutations in highly suspected PCD	Genetics not used as a diagnostic tool
Kott et al 2012	Genetic testing for LRRC6 mutations in PCD	Genetics not used as a diagnostic tool
Lucas et al 2012	Genetic testing for mutations in DNAH11 in PCD	Genetics not used as a diagnostic tool
Mitchison et al 2012	Genetic testing for mutations in DNAAF3 in PCD	Genetics not used as a diagnostic tool

Nakhleh et al 2012	NGS screening for 14 PCD genes in heterotaxy patient	Genetics not used as a diagnostic tool
Olbrich et al 2012	Genetic testing for HYDIN mutation in patients with normal ultrastructure	Genetics not used as a diagnostic tool
Panizzi et al 2012	Genetic testing for CCDC103 mutations in PCD	Genetics not used as a diagnostic tool
Zietkiewicz et al 2012	Genetic testing for CCDC39/CCDC40 mutations in PCD	Genetics not used as a diagnostic tool
Antony et al 2013	Genetic testing for CCDC39 and CCDC40 in PCD positive patients	Genetics not used as a diagnostic tool
Bukowy-Bieryllo et al 2013	Genetic testing for RPGR Mutations	Genetics not used as a diagnostic tool
D'Andrea et al 2013	Case report of coinheritance of Glanzmann thrombasthenia and primary ciliary dyskinesia	Genetics not used as a diagnostic tool
Daniels et al 2013	Identification of Founder mutation in RSPH4A in PCD	Genetics not used as a diagnostic tool
Ferkol et al 2013	Genome-wide homozygosity mapping, linkage analyses, targeted mutation analyses, and exome sequencing in Primary Ciliary Dyskinesia	Genetics not used as a diagnostic tool
Hjeij et al 2013	Genetic testing for ARMC4 mutations in PCD	Genetics not used as a diagnostic tool
Horani et al 2013	Genetic testing for CCDC65 mutations in patients normal US and hyperkinetic cilia	Genetics not used as a diagnostic tool
Horani et al 2013	Genetic testing for LRRC6 mutation in PCD patients with dynein arm defects	Genetics not used as a diagnostic tool
Knowles et al 2013	Exome Sequencing Identifies Mutations in CCDC114 as a Cause of Primary Ciliary Dyskinesia	Genetics not used as a diagnostic tool
Knowles et al 2013	genetic testing for SPAG1 mutations in PCD patients with defective ODA and IDA	Genetics not used as a diagnostic tool
Kott et al 2013	Genetic testing for RSPH1 mutations in PCD patients with central-complex and radial-spoke defects	Genetics not used as a diagnostic tool
Moore et al 2013	Genetic testing for ZMYND10 in PCD patients	Genetics not used as a diagnostic tool
Onoufriadis et al 2013	Genetic testing for CCDC114 in patients with ODA defects	Genetics not used as a diagnostic tool

Tarkar et al 2013	Genetic testing for DYX1C1 in PCD patients	Genetics not used as a diagnostic tool
Wirschell et al 2013	Genetic testing for CCDC164 in patients with PCD	Genetics not used as a diagnostic tool
Zariwala et al 2013	Genetic testing for ZMYND10 and LRRC6 mutation in PCD patients	Genetics not used as a diagnostic tool
Ben Khalifa et al 2014	Genetic testing of patients with asthenozoospermia	No PCD population, only astenozoospermia
Hjeij et al 2014	Genetic testing for CCDC151 mutations in PCD	Genetics not used as a diagnostic tool
Kim et al 2014	The Role of molecular genetic analysis in Primary Ciliary Dyskinesia	Genetics not used as a diagnostic tool
Knowles et al 2014	Genetic testing of mutations in RSPH1 in PCD	Genetics not used as a diagnostic tool
Onoufriadis et al 2014	Targeted NGS gene search for mutations in RSPH1 causing PCD	Genetics not used as a diagnostic tool
Onoufriadis et al 2014	Combined exome and whole-genome sequencing for testing mutations i ARMC4 in patients with defects in the outer dynein arm	Genetics not used as a diagnostic tool
Shapiro et al 2014	Genetic testing in patients with Situs Ambiguus and Heterotaxy	Genetics not used as a diagnostic tool
Wallmeier et al 2014	Mutations in CCNO in suspected PCD patients	Genetics not used as a diagnostic tool
Watson et al 2014	Robust Diagnostic Genetic Testing Using Solution Capture Enrichment and a Novel Variant-Filtering Interface	Genetics not used as a diagnostic tool
Zhang et al 2014	Genetic testing for DNAH5 mutations in one PCD family	Genetics not used as a diagnostic tool
Frommer et al 2015	IF analysis and genetic testing for radial spoke defects	Genetics not used as a diagnostic tool
Olbrich et al 2015	genetic testing for mutations in GAS8 in suspected PCD patients	Genetics not used as a diagnostic tool
Kurkowiak et al 2016	Genetic testing for ZMYND10 in PCD patients	Genetics not used as a diagnostic tool
Dougherty et al 2016	Genetic testing for DNAH11 mutation in highly suspected PCD patients with normal US	Genetics not used as a diagnostic tool

Jeanson et al 2015	Genetic testing for RSPH3 mutations in patients with radial spoke defects	Genetics not used as a diagnostic tool
Casey et al 2015	Genetic heterogeneity for primary ciliary dyskinesia in the Irish Traveller population.	Genetics not used as a diagnostic tool
Djakow et al 2015	Combination of sanger and next generation sequencing in diagnostics of primary ciliary dyskinesia.	Genetics not used as a diagnostic tool
Fedick et al 2015	Genetic testing in eight PCD genes in the Ashkenazi Jewish population.	Genetics not used as a diagnostic tool
Imtiaz et al 2015	Genetic testing for DNAH1 in PCD patients	Genetics not used as a diagnostic tool
Lai et al 2016	Gene editing of DNAH11 to restore cilia motility in PCD	Genetics not used as a diagnostic tool
Li et al 2016	Exome sequencing analysis for ciliome mutations in heterotaxy patients. Genetic testing for DNAH6.	No PCD population, heterotaxy, genetics
Marshall et al 2015	Whole-Exome Sequencing and Targeted Copy Number Analysis in Primary Ciliary Dyskinesia.	Genetics not used as a diagnostic tool

Supplementary Table 8. Summary of Genetics studies excluded at full-text review stage with reason for exclusion.

IF

Publication	Study design	Reason for exclusion/ comments
Antony et al 2013	Genetic testing for CCDC39 and CCDC40 in PCD positive patients	IF not used as a diagnostic tool
Austin-Tse et al 2013	Identification of C21orf59 in a PCD patient	IF not used as a diagnostic tool
Becker-Heck et al 2011	Genetic testing for CCDC39 in PCD positive patients	IF not used as a diagnostic tool
Ben Khalifa et al 2014	Genetic testing of patients with asthenozoospermia	no definite diagnosis of PCD
Bukowy-Bieryłło et al 2013	RPGR genetic testing in patients with PCD and RP	IF not used as a diagnostic tool
Fliegauf et al 2005	Genetic testing for patients with ODA defects, control incl. CF and P with recurrent respiratory infections	IF not used as a diagnostic tool
Hieij et al 2013	Genetic testing for ARMC4 mutations in PCD patients with ODA defects	IF not used as a diagnostic tool
Hjeij et al 2014	Genetic testing for CCDC151 mutations in PCD	IF not used as a diagnostic tool
Horani et al 2013	Genetic testing for CCDC65 mutations in patients normal US and hyperkinetic cilia	If used to confirm genetic mutation
Horani et al 2012	Whole-exome capture and sequencing identifies HEATR2 mutation as a cause of primary ciliary dyskinesia	IF not used as a diagnostic tool
Horani et al 2013	Genetic testing for LRRC6 mutation in PCD patients with dynein arm defects	IF not used as a diagnostic tool
Hornef et al 2006	Genetic testing for DNAH5 mutations in PCD patients with outer dynein arm defects	Not used as diagnostic test but as confirmation of genetic testing
Knowles et al 2012	Genetic testing for Mutations of DNAH11 in patients with PCD with normal ciliary US	IF not used as a diagnostic tool
Knowles et al 2013	Genetic testing for SPAG1 mutations in PCD patients with defective ODA and IDA	IF not used as a diagnostic tool
Kott et al 2012	Genetic testing for LRRC6 mutations in PCD patients with outer and inner dynein arm defects	IF not used as a diagnostic tool
Kott et al 2013	Genetic testing for RSPH1 mutations in PCD patients	IF used to confirm genetic mutation

	with central-complex and radial-spoke defects	
Lee et al 2012	CEP41 mutation in Joubert syndrome	Ciliopathy disease, no PCD
Loges et al 2009	Genetic testing for LRRC50 mutations in PCD patients with dynein arm defects	IF not used as a diagnostic tool
Loges et al 2008	Genetic testing for DNAI2 mutation in PCD patients with ODA defects	IF not used as a diagnostic tool
Merveille et al 2011	Genetic testing for CCDC39 in suspected PCD patients	IF not used as a diagnostic tool
Mitchison et al 2012	Genetic testing for DNAAF in PCD patients	IF not used as a diagnostic tool
Moore et al 2013	Genetic testing for ZMYND10 in PCD patients	IF not used as a diagnostic tool
Olbrich et al 2006	DNAH5 testing for PCD patients	Not used as diagnostic test but as confirmation
Olbrich 2012	Genetic testing for HYDIN mutations	IF not used as a diagnostic tool
Omran et al 2008	Genetic testing for KTU mutations in PCD patients	IF not used as a diagnostic tool
Onoufriadis et al 2013	Genetic testing for CCDC114 in patients with ODA defects	IF used to confirm significance of genetic mutation
Onoufriadis et al 2014	Targeted NGS gene search for mutations in RSPH1 causing PCD	IF used to confirm genetic mutation
Onoufriadis et al 2014	Combined exome and whole-genome sequencing for testing mutations in ARMC4in patients with defects in the outer dynein arm	IF not used as a diagnostic tool
Panizzi et al 2012	Genetic testing for CCDC103 mutation in PCD patients	IF not used as a diagnostic tool
Schwabe et al 2008	Genetic testing for DNAH11 mutation in highly selected PCD patients with normal US	IF not used as a diagnostic tool
Tarkar et al 2013	Genetic testing for DYX1C1 in PCD patients	IF not used as a diagnostic tool
Wallmeier et al 2014	Mutations in CCNO in suspected PCD patients	IF used to confirm genetic mutation
Wirschell et al 2013	Genetic testing for CCDC164 in patients with PCD	IF not used as a diagnostic tool
Zariwala et al 2013	genetic testing for ZMYND10 and LRRC6 mutation in PCD patients	IF not used as a diagnostic tool
Diggle et al 2014	Genetic testing for HEATR2 mutations in PCD patients	IF used to confirm genetic mutation

Frommer et al 2015	IF analysis and genetic testing for radial spoke defects	Selective group, no control group, no complete diagnostic tests
Olbrich 2015	genetic testing for mutations in GAS8 in suspected PCD patients	IF used to confirm genetic mutation
Kurkowiak 2016	Genetic testing for ZMYND10 in PCD patients	IF not used as a diagnostic tool
Dougherty 2016	Genetic testing for DNAH11 mutation in highly suspected PCD patients with normal US	IF not used as a diagnostic tool
Jeanson 2015	Genetic testing for RSPH3 mutations in patients with radial spoke defects	IF used to confirm genetic mutation

Supplementary Table 9. Immunofluorescence workgroup. Summary of studies excluded at full-text review stage with reason for exclusion.

Summary of evidence

Data was extracted from manuscripts that fulfilled inclusion criteria for inclusion in the qualitative analysis, and was used to answer the questions regarding accuracy of each diagnostic test. The data is summarised in Supplementary Table 10. We did not identify any studies that fulfilled GRADE criteria for genetics nor IF and they are therefore not included in the table.

Outcome	№ of	Study	Factors that may decrease quality of evidence	Test accuracy

		studies (№ of patients)	design	Risk of bias	Indirectness	Inconsistency	Imprecision	Publication bias	QoE
Clinical Workgroup (All clinical features except situs abnormalities)									
Sens.	See table 1	1 study 641 patients	cohort type accuracy study	not serious	serious [1]	not serious	not serious	undetected	⊕⊕⊕ MODERATE
Spec.	See table 1								
Clinical Workgroup (situs abnormalities)									
Sens.	See table 1	2 studies 1408 patients	cohort type accuracy study	not serious	serious [1]	not serious	not serious	undetected	⊕⊕⊕ MODERATE
Spec.	See table 1								
Nasal Nitric Oxide									
Sens.	0.91 to 0.99	4 studies 588 patients	cohort type accuracy study	not serious	serious [1]	not serious	not serious	undetected	⊕⊕⊕ MODERATE
Spec.	0.75 to 0.96								
High Speed Video Microscopy									
Sens.	0.97 to 1.0	2 studies 659 patients	cohort type accuracy study	serious [2]	serious [1]	not serious	not serious	undetected	⊕⊕ LOW
Spec.	0.83 to 0.93								
TEM									
Sens.	0.71 to 1.0	11 studies	cohort	serious [2]	serious [1]	not serious	not serious	undetected	⊕⊕

Spec.	0.92 to 1.0	3200 patients	type accuracy study						LOW

Supplementary Table 10. Summary of the assessments of the evidence and quality of data contributing to the recommendations. 1no direct patient outcomes assessed 2index test is included in the reference standard

Additional information regarding diagnostic testing.

Information relating to the clinical features associated with PCD and to genetics testing which could not be included in the main document is detailed below.

In patients suspected of having PCD, which clinical features are associated with a diagnosis of PCD?

We aimed to identify all original research papers that describe clinical features (symptoms, signs, results from non-specific examinations e.g. imaging) in patients referred for evaluation of possible PCD, and where a final diagnosis was made using a standard considered appropriate by the Task Force panel. We excluded case-control studies for the quantitative synthesis, because these usually include only very typical patients, and healthy controls or patients suffering from other lung diseases. Results from comparison from these two groups are not useful for distinguishing PCD patients from patients with other conditions within those referred for evaluation of possible PCD.

However, we considered case-control studies for the qualitative assessment. We also excluded studies, in which symptoms were assessed once testing had started, to avoid differential reporting bias by physicians aware of the final diagnosis.

We identified 1269 studies, of which eight met the inclusion criteria for qualitative assessment and two for quantitative synthesis (Supplementary Fig 1a). We excluded publications based for the following reasons: studies that were not topic related (n=514), did not describe any clinical manifestations (n=302), not original studies (n=159), case reports or case series without a comparison group (n=223) and studies describing other rare ciliary syndromes (n=14). Additionally we excluded 5 conference abstracts which did not contain sufficient information. After assessing the full-text of the remaining 52 studies, we

excluded 44 for not fulfilling the inclusion criteria. These studies are summarised in Supplementary Table 3.

From the eight eligible studies, six were excluded from the quantitative analysis because they did not fit the inclusion criteria. They either included a highly selected study population introducing bias (e.g. only patients with abnormal cilia structure or patients who were already diagnosed with PCD) or they were case-control studies (e.g. comparing PCD to healthy volunteers).

The two studies by Behan et al(3) and Shapiro et al(4) were included in the quantitative analysis, including a total of 1408 patients and they are summarised in Table 1.

Behan et al analysed data from 868 consecutive paediatric and adult patients referred to the University Hospital of Southampton between 2007 and 2013. Patients with inconclusive or incomplete diagnostic results (227) were excluded, leaving 641 for the analysis. All patient data were collected through a proforma completed by a clinician prior to the diagnostic testing.

Shapiro et al analysed data from 767 consecutive paediatric and adult patients referred to the Genetic Diseases of Mucociliary Clearance Consortium between May 2006 and September 2012. Information on situs status was determined by physicians at local consortium sites through review of radiology, surgery, and cardiology reports and radiology images from participant medical records. Patients were divided into 3 situs categories: situs solitus, situs inversus and situs ambiguous (including heterotaxy).

Genes associated with PCD

One third of genes identified to date encode outer dynein arm (ODA) components (dynein, axonemal, intermediate chain 1 (DNAI1) and 2 (DNAI2); heavy chain 5 (DNAH5) and 11 (DNAH11); thioredoxin domain containing 3 (NME8/TXNDC3) and DNAL1) [15–22] or components of the ODA docking complex machinery, necessary for the binding of ODAs to axonemal microtubules (Coiled-Coil Domain-Containing Protein 114 (CCDC114), CCDC151 and Armadillo Repeat-Containing Protein 4 (ARMC4))[23–26].

Mutations in genes encoding the Dynein Axonemal Assembly Factors 1-5 that are required for cytoplasmic pre-assembly of axonemal dynein components cause absence of inner and outer dynein arms; DNAAF1/LRRC50, DNAAF2/KTU, DNAAF3, DYX1C1/DNAAF4 (Dyslexia Susceptibility 1 Candidate 1), DNAAF5/HEATR2 (Heat Repeat-Containing Protein 2) are responsible for the absence of outer and inner dynein arms. Mutations in *LRRC6* (Leucine Rich Repeat Containing 6), *CCDC103* (Coiled-Coil Domain-Containing Protein103), ZMYND10 (Zinc Finger Mynd Domain-Containing Protein 10), *SPAG1* (Sperm-Associated Antigen 1) and *C21orf59* (Chromosome 21 Open Reading Frame 59)[27–37] have also been associated to the absence of both dynein arms.

Mutations in the genes encoding the radial spoke proteins (RSPH1, RSPH3, RSPH4A, RSPH9), as well as the central pair apparatus associated protein HYDIN have been reported in PCD patients [38–43]. PCD individuals carrying mutations in those genes do not show any laterality defects such as *situs inversus.* Most of their respiratory cilia show normal ultrastructure with central-microtubular-pair abnormalities in a minority of cilia. Cilia of those patients are motile but exhibit subtle abnormalities of their beat pattern [38, 39, 41] which might be missed.

Mutations in genes encoding the ruler proteins CCDC39 and CCDC40 result in severe microtubular disorganisation and IDA defects as well as randomization of left/right asymmetry[44–46]. Both proteins are responsible for the attachment of the nexin links-dynein regulatory complex (nDRC) and inner dynein arms (IDAs) and to the proper spacing of the radial spokes [47].

However, mutations in genes encoding nDRC components such as DRC1/CCDC164, CCDC65 as well as GAS8/DRC4[37, 48, 49] cause PCD with a low percentage of cilia showing axonemal disorganisation and subtle ciliary beating defects detectable by high-speed video microscopy[49] which might be easily missed. Interestingly, so far all reported PCD individuals with isolated nDRC defects showed no laterality defects.

Mutations in two genes, *CCNO* and *MCIDAS*, have been identified as a cause of a PCD-like syndrome referred to as reduced generation of multiple motile cilia (RGMC) with a complete absence or severely reduced numbers of cilia by TEM of respiratory epithelial cells causing impaired mucociliary clearance [50–52]. This condition is somewhat reminiscent of ciliary

aplasia described in the 1980s, especially in cases with complete absence of cilia [53, 54]. To date situs has always been normal in patients with RGMC.

In a minority of cases, X-linked inheritance has been implicated. Retinitis pigmentosa, sensory hearing deficits and PCD have been associated with mutations in the retinitis pigmentosa guanosine triphosphatase regulator gene (*RPGR*), essential for photoreceptor maintenance and viability. In addition, Budny *et al.* described a single family with a novel syndrome that is caused by oral-facial-digital type 1 syndrome gene (*OFD1*) mutations, and characterised by X-linked recessive mental retardation, macrocephaly and PCD [55].

Molecular approaches for genetic testing in PCD

1/ **Sanger sequencing** of all coding regions and flanking intronic regions, ideally targeting to the genes responsible for a specific ultrastructural defect. The numerous genes and the large size of many of them create a problem. However, the yield is good in some cases e.g. *CCDC39* and *CCDC40* explain almost all cases with microtubular disorganization with absence of IDA [46, 56]. Sanger sequencing does not detect deletions encompassing a whole exon or several exons in the heterozygous state. It does not detect homozygous or heterozygous intragenic duplications regarding one or more exons.

2/ **Targeted Next Generation Sequencing** (NGS) of all coding regions and flanking intronic regions. Like Sanger sequencing, this technique can detect point mutations and small indels. This technique can detect insertions/deletions of one or several exons, but this need a specific sensitivity assessment. The coverage and depth may not be optimal for some exons, which should be stated in the molecular report (or the gap should be covered by another sequencing approach).

3/ **Whole exome sequencing**. Coverage and depth are usually lower than targeted NGS. The depth is usually not sufficient to detect deletions or duplications of more than one exon.

4/ **Targeted copy number analysis** consists of semi-quantitative qPCR to characterize large indels that have already been reported[57].

5/ **Whole genome copy number analysis** (SNP array) is a second line technique to detect large rearrangements. Its sensitivity is low for intragenic deletions and relies on the probe density in each region.

6/ **Transcript analysis** on airway epithelial cells from the patient (in patients in whom a single heterozygous mutation has been identified in a relevant gene). It can detect deep intronic mutations (such as those creating pseudo-exons) that are missed by the above mentioned techniques.

The percentage of confirmed PCD with no identified mutation is currently between 25 and 50 % [57–59]. The mutations that are currently missed include:

- deep intronic mutations (except if transcript analysis is performed in specific cases) and mutations in regulatory regions (e.g. promoter)
- heterozygous deletions/insertions encompassing at least one whole exon (for Sanger and exome analysis); they can be detected by targeted NGS analysis and in some cases with targeted CNV analysis
- homozygous duplications of at least one exon (by Sanger and exome); they can be identify by targeted NGS
- homozygous deletions (by exome); they can be detected by Sanger and targeted NGS.
- cases that are not investigated because they are atypical.

The majority of mutations are nonsense or frameshift mutations or result in abnormal splicing, while missense mutations have been reported in a minority of cases. Rare variants in sequence such as missense mutations that change a single amino acid remain difficult to attribute to disease. In order to rate possible pathogenic consequences, the following elements should be considered: evolutionary conservation; allele frequency in control databases such as ExAC (deleterious effect is excluded when the frequency is high); in silico or in vitro assessment of a potential effect on splicing (also true for synonymous variations); functional assessment (eg. Zebrafish, Xenopus); localization of the amino acid in a functional

domain (lower level of evidence); previous description in other PCD patients (lower level of evidence).

Consensus statement for diagnostic outcome

There were four iterative rounds of Delphi Survey. The results of votes are presented in Supplementary Table 11.

Supplementary Table 11: Summary results of four rounds of Delphi Survey (a-d), with voting to reach a consensus for diagnostic outcomes. Consensus was defined by >80% of respondents agreeing or disagreeing a statement (shaded cells)

a) Survey 1 (respondents n=22)	Strongl Agree %
The following test results can be used to CONFIRM a diagnosis of PCD in isolation if conducted in a specialist centre:	
Transmission electron microscopy (hallmark; once)	38
Bi-allelic mutations in PCD causing gene	52
Nasal nitric oxide (persistently abnormal x3)5	14
High speed video analysis (pattern and frequency) once	5
High speed video analysis (pattern and frequency) (consistently abnormal x3)	29
High speed video analysis (pattern and frequency)	5
Immunofluorescence (hallmark; PCD once)	5
The following test results can be used in isolation (i.e. results of the single diagnostic test) to EXCLUDE a diagnosis of PCD if conducted in a specialist center using local reference data:	
Transmission electron microscopy normal	5
Immunofluorescence normal	0
No bi-allelic mutations in PCD causing gene	5
Nasal nitric oxide normal or high	0
High speed video analysis (entirely normal CBF and CBP)	18
High speed video analysis entirely normal following culture (suspension or ALI) if original sample was equivocal	27

b) Survey 2 (respondents n=17)	Strongl Agree %
In a patient with a typical history a diagnosis of PCD is confirmed with the following results:	
Very low nNO PLUS hallmark HSVM consistently on two occasions	12

Very low nNO PLUS hallmark HSVM consistently on three occasions	47
Very low nNO PLUS hallmark HSVM following cell culture	35
Very low nNO PLUS hallmark IF	6
HSVM consistently hallmark abnormal on three occasions	0
HSVM hallmark abnormal following cell culture	6
Where there is only modest clinical suspicion of a diagnosis of PCD and diagnosis can be EXCLUDED:	
High/ normal nNO AND HSVMA normal	18
High/ normal nNO AND HSVM normal following cell culture	24
High/ normal nNO AND TEM normal	6
High/ normal nNO AND IF normal	0
High/ normal nNO AND genetics normal	6
Genetics and TEM normal	6
Entirely normal HSVM following culture	12
Entirely normal HSVM	6
In patients where an expert PCD clinician has a strong suspicion that the diagnosis is positive based on the history (e.g. PICADAR) a positive diagnosis can excluded with the following test results:	
High/ normal nNO AND HSVMA normal	0
High/ normal nNO AND HSVM normal following cell culture	0
High/ normal nNO AND TEM normal	0
High/ normal nNO AND IF normal	0
High/ normal nNO AND genetics normal	0
Genetics and TEM normal	0
Entirely normal HSVM following culture	0
Entirely normal HSVM	0

c) Survey 3 (respondents n=15)	Strongl Agree %
Patients with a clinical history compatible with PCD should have access to a range of diagnostic tests which should include	
nNo	87
HSVM	87
TEM	93
Genetics	40
IF	7
Regarding the diagnosis of PCD	
Tests should be conducted in laboratories with expertise in PCD diagnostics	87
Test results should be interpreted by specialists with expertise in PCD diagnostics	87
Test results should be reported to patients and their non-specialist carers by a PCD specialist clinician	73
Diagnostic tests for PCD are currently imperfect. As our understanding and techniques for PCD diagnosis advance patients should be called back and offered repeated testing to confirm or exclude the diagnosis.	73
A diagnosis of PCD is highly likely if the patient has a compatible history and the test results include:	

Very low nNO PLUS abnormal HSVM consistently on two occasions	20
Very low nNO PLUS abnormal HSVM consistently on three occasions	53
Very low nNO PLUS abnormal HSVM following cell culture	53
Very low nNO PLUS abnormal IF	13
HSVM consistently abnormal on three occasions	13
HSVM abnormal following cell culture	20
Abnormal HSVM twice	0
Abnormal IF	7
Very low nNO	0
Very strong clinical history e.g. Kartagener's syndrome, PICADAR >10 but no access to diagnostic tests	7
If a diagnosis is considered highly likely but can not be definitively confirmed the following statements are true:	
Patients should have other causes for their symptoms excluded	80
Patients should be managed as if they have PCD until the diagnosis can be definitively confirmed or excluded.	53
Patients should be told that the diagnosis is likely but not definite	73
Patients should be invited to have further tests as new tests become available or refinements to existing tests occur.	93
Diagnosis extremely unlikely; the following statements are true:	
Current diagnostic tests for PCD are imperfect	67
As our understanding of the disease improved patients currently considered highly unlikely might be appropriate for further testing if new diagnostic tests become available	40
All patients where the diagnosis is considered "highly unlikely" should be counselled that current diagnostic testing is imperfect	33
The diagnosis of PCD is unlikely in the following circumstances. Given the evidence from the TF review it is acceptable to counsel the patient that a diagnosis is extremely unlikely and stop further investigations until improved diagnostic options are available, unless the diagnosis is considered extremely likely based on the clinical history.	
High/ normal nNO AND HSVMA normal	40
High/ normal nNO AND HSVM normal following cell culture	47
High/ normal nNO AND TEM normal	13
High/ normal nNO AND IF normal	7
High/ normal nNO AND genetics normal	7
Genetics and TEM normal	13
High/ normal nNO AND HSVMA normal	13
High/ normal nNO AND HSVM normal following cell culture	20
High/ normal nNO AND TEM	7
High/ normal nNO AND IF normal	7
High/ normal nNO AND genetics normal	7
Genetics and TEM normal	0

d) Survey 4 (respondents n=19)	Strongl Agree %
The diagnosis of PCD is unlikely in the following circumstance. Given the evidence from the TF review it is acceptable to counsel the patient that a diagnosis is extremely unlikely and stop further investigations until improved diagnostic options are available unless the diagnosis is considered extremely likely based on the clinical history.	
High/ normal nNO Plus normal TEM plus normal genetics	32
If diagnostic tests are inconclusive:	
The decision to repeat tests and/ or conduct different tests should be made by a specialist with expertise in PCD diagnostics.	83
Once all tests are conducted, if still inconclusive the patient should be considered 'possible PCD'	11
Possible PCD patients should have other causes for their symptoms excluded	72
Possible PCD patients should be treated as if they have PCD until the diagnosis is confirmed or excluded.	39

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