ERS Task Force guideline for the diagnosis of primary ciliary dyskinesia

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Take Home Message: International ERS guidelines recommend a combination of tests to diagnose PCD

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Abstract

The diagnosis of primary ciliary dyskinesia is often confirmed with standard, albeit complex and expensive tests. In many cases, however, the diagnosis remains difficult despite the array of sophisticated diagnostic tests. There is no 'gold standard' reference test. Hence, a task force supported by the European Respiratory Society has developed this guideline to provide evidence-based recommendations on diagnostic testing, especially in the light of new developments in such tests, and the need for robust diagnoses of patients who might enter randomised controlled trials of treatments. The guideline is based on pre-defined questions relevant for clinical care, a systematic review of the literature, and assessment of the evidence using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach. It focuses on: clinical presentation, nasal nitric oxide, analysis of ciliary beat frequency and pattern by high-speed video-microscopy analysis, transmission electron microscopy, genotyping and immunofluorescence. It then used a modified Delphi survey to develop an algorithm for the use of diagnostic tests to definitively confirm and exclude the diagnosis of PCD; also to provide advice when the diagnosis is not conclusive. Finally, this guideline proposes a set of quality criteria for future research on the validity of diagnostic methods for PCD.

Introduction

PCD represents a clinical and genetic heterogeneous group of respiratory ciliopathies, with reduced muco-ciliary clearance of the airways. The various mutations result in different clinical and pathological patterns, contributing to the challenges of diagnosis. There is no single gold standard diagnostic test for primary ciliary dyskinesia (PCD) [1]; current diagnosis requires a combination of technically demanding investigations, including nasal nitric oxide (nNO), high-speed video microscopy analysis (HSVA) and transmission electron microscopy (TEM). Historically clinicians used the saccharine test to screen for PCD, but this is no longer advocated [2]. Furthermore, more sophisticated diagnostic tests that might improve diagnostic accuracy (genotyping, immunofluorescence (IF) of ciliary proteins and EM tomography) are becoming increasingly available.

The availability of tests varies across Europe [3]; this has partially improved recently in response to collaborations including a former Task Force(2006-9) of the European Respiratory Society (ERS) [2–4] and FP-7 funded BESTCILIA. The ERS Task Force published a consensus statement in 2009 [2] to guide diagnostic testing and BESTCILIA has recently introduced diagnostic testing into three countries where services did not previously exist. Since the 2009 statement, a number of groups and consortia have investigated the accuracy of various diagnostic tests for PCD, providing the opportunity to advance the state of diagnostics by developing evidence based guidelines. Therefore, in 2014 a new PCD ERS Task Force consisting of adult and paediatric physicians from pulmonology and ENT disciplines along with diagnostic scientists was established; it aimed to develop evidence based guidelines for the diagnosis of PCD. This is important for the appropriate clinical management and prognosis of individual patients with suspected or eventually confirmed PCD; to ensure patients with PCD are correctly diagnosed whilst avoiding the problems of false positive diagnoses. It should also ensure a definitive diagnosis before PCD patients are enrolled in randomised controlled clinical trials of treatment.

Methods

The methods are described in detail in the supplementary file.

Task Force Composition

In brief, the panel consisted of a multidisciplinary group of clinicians and scientists with recognised expertise in the diagnosis of primary ciliary dyskinesia; junior members/ trainees affiliated to European PCD centres were active members of the committee (Supplementary Table 1). Methodologists from the ERS provided expertise in guideline development following the GRADE approach for diagnostic tests [5]. 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.

Patient-Important Outcomes

The GRADE approach emphasizes the importance of recommendations based on the impact on patient-important outcomes [6]. The patient representatives to the Task Force fully endorsed that an accurate diagnosis was an important outcome, because it leads to a better recognition of their problems by physicians and more effective treatment, and thus improves their health and quality of life. This was confirmed by our questionnaire survey of 352 PCD patients from 25 countries, and 20 in-depth interviews [7]. However, diagnostic accuracy studies do not provide direct evidence for the improvement of patient-important outcomes; consequently, the confidence in results of test accuracy studies can be judged at best as moderate.

Formulation of the Topics and Questions

The Task Force members agreed that six facets of PCD diagnostics should be evaluated: clinical symptoms, nNO, HSV, TEM, genotype and IF labelling of ciliary proteins. We evaluated each test to see whether it should be included in the diagnostic pathway for PCD, 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?" The PICO questions for each test were finalised during several rounds of teleconferences and email discussions (Supplementary Table 2).

The essential inclusion criterion for studies was that they must have included consecutive patients referred for PCD testing, in whom the PCD diagnosis was then either confirmed or excluded; we excluded studies if patients had already had previous diagnostic testing. In the absence of such studies, in the narrative review we discussed case control studies which compared PCD patients with

healthy controls, or with patients suffering from other respiratory diseases (e.g. CF). . Results from such studies cannot be generalised to the clinical situation, where patients with PCD must be distinguished from patients referred for similar complaints, but without PCD. Thus, the results from case control studies are far less relevant for clinical care. The main limitation for this project was the lack of a gold standard diagnostic test for PCD. In the absence of this, we compared the diagnostic performance indicators (e.g. sensitivity and specificity) to the authors' final decision regarding positive/negative PCD diagnosis based on all available tests.

The Task Force also agreed on a list of less structured questions relevant to PCD diagnostics for the narrative discussion. As the evidence for these questions were not formally graded, they were not used for recommendations.

Literature Search Methods

We searched Medline and Embase databases (accessed through Ovid) from 1st January 1996 to 14th March 2016. Full details are provided in the supplementary file. In brief, titles and abstracts were screened; the full text was then reviewed for papers which potentially fulfilled criteria for inclusion. These manuscripts were checked for completeness by the Task Force panel to ensure all data fulfilling the *a priori* inclusion criteria were present. PRISMA flow diagrams show the search process for each WG (supplementary file figure 1).

Quality of Evidence and Strength of Recommendations

We used the GRADE approach through the entire process, from grading the quality of evidence, to deciding on the strength of the recommendations [8, 9]. Full details are provided in the supplementary file including reasons for downgrading the confidence in the evidence (summary of evidence tables)Recommendations were made based on the strength of evidence and other factors such as overall accuracy of the test (sensitivity and specificity), confidence in the net accuracy (range of sensitivity/sensitivity from included studies and/or confidence intervals of net sensitivity and specificity) and considerations such as patient acceptability of the test, feasibility of testing and how accessible the test is. The four tests where evidence based recommendations were made, were all acceptable to the patient, feasible and acceptable."

Consensus statement for confirming or excluding PCD

We used a modified Delphi survey to reach a consensus regarding the use of diagnostic tests to definitively confirm and exclude the diagnosis of PCD; also to provide advice regarding patients who

do not have a definitive diagnosis but diagnostic tests suggest that the diagnosis is highly likely or inconclusive. The methods are detailed in the on-line supplementary file.

Results

The results of the evidence assessment gave rise to the recommendations in Table 1.

Recommendations
Which patients should be referred for diagnostic testing? Based on MODERATE confidence in the evidence: 1. We recommend that patients are tested for PCD 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 (strong recommendation). 2. Patients with normal situs presenting with other symptoms suggestive of PCD (as listed in recommendation 1) should be referred for diagnostic testing (strong recommendation). 3. Siblings of patients should be tested for PCD, particularly if they have symptoms suggestive of PCD (as listed in recommendation 1) (strong recommendation). 4. We recommend the use of combinations of distinct PCD symptoms and predictive tools (e.g. PICADAR) to identify patients for diagnostic testing (weak recommendation).
In patients suspected of having PCD, should nasal nitric oxide be used as a diagnostic tool? Based on MODERATE confidence in the evidence, we recommend that: 1. Nasal nitric oxide measurement should be used as part of the diagnostic work-up of schoolchildren over 6 years and adults suspected of having PCD, preferably using a chemiluminescence analyser with a velum closure technique (strong recommendation). 2. In children under 6 years suspected of having PCD, we suggest nasal nitric oxide measurement using tidal breathing as part of the diagnostic work-up (weak recommendation). Remark - we suggest that patients presenting with a strong clinical history should undergo further testing, even if nNO is normal (weak recommendation).
In patients suspected of having PCD, should HSVA be used as a diagnostic tool? Based on LOW confidence in the evidence, we recommend: 1. High speed video analysis, including ciliary beat frequency and beat pattern analysis, should be used as part of the diagnostic work-up of patients suspected of having PCD (weak recommendation). 2. Ciliary beat frequency should not be used without assessment of ciliary beat pattern in diagnosing PCD (strong recommendation). 3. To improve diagnostic accuracy of HSVA, CBF/P assessment should be repeated after ALI culture (strong recommendation).

In patients suspected of having PCD, should TEM be used as a diagnostic tool? Based on LOW confidence in the evidence, we recommend: 1. Ciliary ultrastructure analysis by transmission electron microscopy should be used as part of the diagnostic work-up of patients suspected of having PCD (strong recommendation). 2. Further diagnostic investigations should be performed in patients with normal ultrastructure if the clinical history is strong (strong recommendation).[1] 3. In patients with hallmark ciliary ultrastructure defects for PCD further confirmatory diagnostic investigations are not required[2]. (strong recommendation).
In patients suspected of having PCD, should genotyping be used as a diagnostic tool? There were no studies that fulfilled inclusion criteria to answer this question. Statements to assist the clinician are made in the genetics sections but these are NOT evidence based. Therefore, we could not make formal recommendations as for other diagnostic procedures. However, we have provided a list of taskforce statements on genetics, which is based upon agreement between experts rather than upon published evidence.
In patients suspected of having PCD, should IF be used as a diagnostic tool? There were no studies that fulfilled inclusion criteria to answer this question. Statements to assist the clinician are made in the IF sections but these are NOT evidence based. Therefore, we could not make formal recommendations as for other diagnostic procedures. However, we have provided a list of taskforce statements on immunofluorescence, which is based upon agreement between experts rather than upon published evidence.

Table 1. Evidence-based recommendations for the use of each of the six tests considered for PCD diagnosis. [1] normal ciliary ultrastructure, as resolvable by transmission electron microscopy, does not exclude the diagnosis of PCD (16% PCD positive patients have TEM without a detectable defect). [2] patients with hallmark ciliary ultrastructure defects for PCD (absence of outer dynein arms, combined absence of inner and outer dynein arms, inner dynein arm absence combined with microtubular disarrangement), assessed by TEM, almost always have PCD (false positive results are very rare ≈0.7%)

Clinical features

Summary of recommendations

Which patients should be referred for diagnostic testing?
Based on MODERATE confidence in the evidence:

1. We recommend that patients are tested for PCD 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 (strong recommendation).

2. Patients with normal situs presenting with other symptoms suggestive of PCD should be referred for diagnostic testing (strong recommendation).
3. Siblings of patients should be tested for PCD, particularly if they have symptoms suggestive of PCD (strong recommendation).
4. We recommend the use of combinations of distinct PCD symptoms and predictive tools (e.g. PICADAR) to identify patients for diagnostic testing (weak recommendation).

Review of evidence directly addressing the question "in patients suspected of having PCD, which clinical features predict a positive diagnosis"?

Our search identified 1269 studies of which two directly answered the question and were included in the quantitative synthesis (Supplementary Figure 1) and an additional 6 contributed to the narrative review. We excluded 1217 publications based on titles and abstracts. After full text review we excluded 44 of the remaining 52 studies because they did not fulfil the inclusion criteria (Supplementary table 3).

Two studies, Behan *et al* [10] and Shapiro *et al*[11], were suitable to provide evidence for our recommendations. They included 1408 patients (Table 2).

Clinical Manifestation	**Sensitivity (95% C.I.)**	**Specificity (95% C.I.)**
Neonatal manifestations		
Neonatal chest symptoms	0.75 (0.63-0.84)	0.83 (0.79-0.84)
Neonatal rhinitis	0.27 (0.17-0.38)	0.94 (0.91-0.95)
Neonatal respiratory support	0.41 (0.30-0.53)	0.93 (0.90-0.95)
Neonatal unit admission	0.61 (0.49-0.72)	0.86 (0.83-0.89)
Upper respiratory manifestations after the postnatal period		

Chronic rhinitis	0.81 (0.70-0.89)	0.43 (0.38-0.47)
Chronic serous otitis media	0.57 (0.45-0.69)	0.81(0.77-0.84)
Chronic acute otitis media	0.33 (0.23-0.45)	0.75 (0.71-0.79)
Hearing loss	0.49 (0.38-0.61)	0.84 (0.81-0.87)
Chronic ear perforation	0.12 (0.06-0.22)	0.91 (0.88-0.93)
Ear surgery	0.32 (0.22-0.44)	0.86 (0.82-0.88)
Chronic sinusitis	0.28 (0.19-0.40)	0.76 (0.72-0.79)
Lower respiratory manifestations after the postnatal period		
Chronic wet cough	0.93 (0.84-0.98)	0.15 (0.12-0.18)
Recurrent wheeze	0.48 (0.36-0.60)	0.62 (0.57-0.65)
Previous pneumonia	0.41 (0.30-0.53)	0.65 (0.61-0.69)
Bronchiectasis	0.29 (0.20-0.41)	0.68 (0.64-0.72)
Other manifestations (various ages)		
Situs anomalies**	0.51 (0.46-0.56)	0.94 (0.92-0.95)
Congenital heart disease	0.08 (0.03-0.17)	0.98 (0.97-0.99)
Developmental delay	0.11 (0.05-0.20)	0.94 (0.91-0.96)
Hydrocephalus	0.01 (0.00-0.08)	0.99 (0.98-1.00)

Subfertility*	0.91 (0.57-1.00)	0.82 (0.74-0.87)
Family history (any age)		
Of PCD in siblings	0.24 (0.15-0.35)	0.98 (0.97-0.99)
Of PCD in extended family	0.05 (0.02-0.14)	0.99 (0.97-1.00)
Of asthma	0.16 (0.09-0.27)	0.66 (0.62-0.70)
Of bronchiectasis	0.04 (0.01-0.12)	0.96 (0.93-0.97)
Of otitis media	0.07 (0.02-0.16)	0.89 (0.86-0.92)
Clinical scores		
PICADAR (score>5)	0.90 (0.81-0.96)	0.75 (0.70- 0.80)

Table 2. Summary of reported clinical manifestations in studies included in the quantitative analysis. All data from Behan *et al* (641 eligible referrals, 75 (12%) had PCD)[10] but data on subfertility* are from a subgroup of 152 referrals where 11 (7%) had PCD). Data on situs anomalies** are from Behan *et al* and Shapiro *et al* (767 referrals) [11].

Behan *et al* analysed data from 868 consecutive paediatric and adult patients [10]. Those with inconclusive or incomplete results (227) were excluded, leaving 641 for analysis. All patient data were collected through a proforma completed by a clinician prior to the diagnostic testing. They reported sensitivity and specificity of a large range of clinical features (Table 2 and Supplementary Table 3). Wet cough did not discriminate well between PCD positive and negative patients (sensitivity 0.93, specificity 0.15), because it was the main reason for referral, so was present in virtually all patients. Neonatal chest symptoms and neonatal rhinitis had a high specificity (0.83 and 0.94), but a lower sensitivity (0.27 and 0.75). The sensitivity and specificity for 25 clinical features are summarised in Table 2 and described in detail in the supplementary file.

In addition to reporting on single symptoms, Behan *et al* developed a 7-point questionnaire-based prediction tool (PICADAR), to help predict the likelihood that a patient referred for evaluation of

persistent wet cough has PCD. PICADAR was internally and externally (in a second cohort) validated and is the first clinical prediction tool developed for PCD. The score ranged from 0 to 14; sensitivity and specificity of a score of >5 were 0.90 and 0.75 respectively; clearly better than single symptoms.

Shapiro *et al* analysed data from 767 consecutive paediatric and adult patients [11]. Information on situs 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).

Situs abnormalities were reported by Behan and Shapiro for a total of 1048 patients. The pooled sensitivity for the two papers (for any situs abnormality) was 0.508 and specificity 0.939 (Supplementary Table 3).

Narrative review of additional evidence

Leigh *et al* described a prospective cohort of 534 children with high suspicion of PCD, among whom many had a pre-existing diagnosis of PCD. 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 [12]. Noll *et al* described a retrospective cohort of 323 patients with chronic cough referred for ciliary function analyses, and reported high specificity (>0.9) for neonatal respiratory distress (NRDS), persistent otitis media, situs inversus and bronchiectasis[13]. Chin *et al* reviewed retrospectively records of 118 patients referred for electron microscopy because of suspected PCD, and compared combinations of symptoms between patients with abnormal and normal EM, while excluding uncertain cases. They found more sino-nasal, middle ear and pulmonary symptoms in the abnormal group [11]. Beucher *et al* compared patients with abnormal and normal EM in a retrospective cohort of 89 children suspected of PCD, excluding 21 uncertain cases and found that only situs inversus differed significantly between the groups[14]. Pifferi *et al* compared clinical symptoms in 98 patients with primary PCD versus secondary ciliary dyskinesia; statistically significant differences were found for situs inversus and severity of bronchiectasis [15]. Mullowney *et al* , in the only publication that focused on neonates, compared neonatal symptoms between 46 PCD patients and 46 controls with a history of NRDS[16], and found that lobar collapse, situs inversus and prolonged oxygen need were more common in infants with PCD. The combination of situs inversus, lobar collapse, or oxygen need for >2 days had 87% (95% CI 74–94) sensitivity and 96% (85–99) specificity for PCD. A systematic review by Goutaki *et al* describes other case-control or case series studies on prevalence of clinical symptoms in PCD, which do not fulfil the inclusion

criteria for this study [17]. All studies are from developed countries, and it is probable that the predictive value of some symptoms would be different depending on geographical region; for example, sensitivity and specificity of bronchiectasis will be different in sub-Saharan Africa where bronchiectasis due to TB is common.

Key unanswered questions and research needs

Further research is needed using prospective cohort studies of patients referred with suspicion of PCD, in whom clinical features are assessed in a standardised way before they are diagnosed. Analyses must be stratified by age. In particular, there is a need for prospective studies of neonates with NRDS. In addition, it might be helpful to combine information into clinical prediction scores, using state of the art approaches [16]. Validity of the different clinical features is also likely to vary depending on the population under evaluation. For instance, positive and negative predictive values of the symptoms depend strongly on the prevalence of the disease in the referral population and will be poorer in populations with a lower prevalence (i.e. in primary or secondary care compared to PCD referral centres). Sensitivity and specificity (shown in the table) do not depend on prevalence. Nevertheless the mix in PCD phenotypes, and therefore the usefulness of different symptoms for prediction of PCD is likely to differ between patients attending specialised clinics (e.g. ENT, pulmonology, cardiology). 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. Another factor to consider is that clinical features might differ between genetic variants (e.g. patients with CCNO variants show no situs anomalies but increased female infertility), so results vary with differences in prevalence of specific mutations in the evaluated population. Studies therefore must be done in specific health care settings and study populations, and consider age groups, sex, and genetic abnormalities.

Summary

Relevant literature answering our question was extremely scarce (two papers only, of which one reported only on situs inversus), and results did not allow to take severity of symptoms into account or stratify by age. Overall confidence in their results is moderate mainly because diagnostic performance does not inform downstream consequences of further clinical management based on the assessment of these symptoms.

Results suggest that clinical symptoms may help to distinguish between patients with and without PCD, but the positive predictive value (how many patients with a specific symptom do have the

disease) of single symptoms is low. Instead, the combination of suggestive symptoms might discriminate better, but this needs further studies in different populations.

Wet cough starting in early childhood has been used as the initial selection criterion in most PCD studies. Therefore it has a low discriminative value due to its high prevalence in both PCD positive and negative individuals recruited to these studies.

Nasal Nitric Oxide

Summary of recommendations

In patients suspected of having PCD, should nasal nitric oxide be used as a diagnostic tool?
Based on MODERATE confidence in the evidence, we recommend that:

1. Nasal nitric oxide measurement should be used as part of the diagnostic work-up of schoolchildren over 6 years and adults suspected of having PCD, preferably using a chemiluminescence analyser with a velum closure technique (strong recommendation).
2. In children under 6 years suspected of having PCD, we suggest nasal nitric oxide measurement using tidal breathing as part of the diagnostic work-up (weak recommendation).

Remark - we suggest that patients presenting with a strong clinical history should undergo further testing, even if nNO is normal (weak recommendation

Explanation of the diagnostic test

Nasal nitric oxide (nNO) is extremely low in PCD when compared to healthy and disease controls, for unknown reasons [19]. The accuracy of nNO as a diagnostic test in PCD varies by type of analyser, sampling method and age of patient [20].

Current guidelines recommend aspiration of gas from one nostril with gas entrained via the other naris to measure nNO using a stationary chemiluminescence analyser during a velum closure, such as breath hold or oral exhalation against resistance. The reading should be obtained from a technically acceptable plateau reading[21]. Whilst measurement during velum closure by chemiluminescence analyser is considered the 'gold standard', this manoeuvre is not possible in all situations. In young children measurements during tidal breathing have been reported[22, 23]. Electromechanical portable analysers [23] are used where stationary chemiluminescence analysers are not available. There is currently no consensus over what threshold constitutes a positive or negative cut-off.

Publication	Study population (n=)	Sampling method (n, threshold nl/min)	Sensitivity (95% CI)	Specificity (95% CI)

Marthin *et al* 2011 [24]	117 referrals PCD 14	Breath hold (n=58, 52.5) Oral exhalation against resistance (n=37, 72.6) Tidal breathing (n=97, 47.4)	0.92 (0.62 to 0.998) 1.0 (0.54 to 1.0) 0.93 (0.66 to 0.998)	0.96 (0.85 to 0.995) 0.94 (0.79 to 0.99) 0.80 (0.69 to 0.88)
Leigh *et al* 2013[25]	155 referrals PCD 71 Indeterminate 84	Oral exhalation, velum closure (n=155, 77)	0.99 (0.92 to 0.9996)	0.75 (0.64 to 0.84)
Beydon *et al* 2015 [22]	86 referrals PCD 49 Non-PCD 37	Velum closure (n=74, 82.2) Tidal breathing – 5 peaks (n=86, 40)	0.91 (0.79 to 0.98) 0.90 (0.78 to 0.97)	0.86 (0.68 to 0.96) 0.97 (0.86 to 0.999)
Jackson *et al* 2016 [26]	301 referrals PCD 34 Non-PCD 267	Velum closure (breath hold or oral exhalation) (n=301, 30)	0.90 (0.74 to 0.98)	0.95 (0.90 to 0.98)

Table 3. Summary of diagnostic accuracy of nasal nitric oxide (nNO) from measurements in consecutive patients suspected of PCD.

Although analysers report readings in parts per billion (ppb), this is influenced by the machine sampling rate, so the concentration is converted to nanolitres/min (nl/min) by the formula nl/min=ppb x sampling rate in l/min.

Review of evidence directly addressing the question "in patients suspected of having PCD, should nNO be used as a diagnostic tool?"

Our search identified 98 studies, of which 23 met inclusion criteria for qualitative assessment. Of these, four papers (n=588 patients) assessed nNO in a cohort of patients suspected of PCD who eventually received either a positive or negative diagnosis, directly addressing the question (Table 3 and Supplementary Table 4). The other 19 papers were excluded from informing the recommendations but contributed to the narrative review (Supplementary Table 5).

Marthin *et al*. measured nNO during breath hold, exhalation against resistance and tidal breathing. Sensitivity ranged from 0.92 (for breath hold) to 1.0 (oral exhalation against resistance) and

specificity ranged from 0.80 (tidal breathing) to 0.96 (breath hold)[24]. Leigh *et al* developed a threshold of 77nl/min using data from a PCD specialist centre and then trialled this cut-off in 155 consecutive patients at other sites. Comparing PCD positive to indeterminate patients provided a sensitivity of 0.99 and specificity of 0.75[25]. Lower specificity was because the diagnostic protocol only included electron microscopy and genetics, thus missing a number of true PCD cases ('indeterminate' rather than PCD negative). Beydon *et al* reported a sensitivity of 0.91 and specificity of 0.86 for velum closure (cut-off 82nl/min) and 0.90 and 0.97 for tidal breathing (40nl/min; mean of 5 peaks)[22]. Jackson *et al* used a cut-off of 30nl/min, and reported sensitivity of 0.91 and specificity of 0.96 to distinguish 34 PCD positive from 267 PCD negative patients[26]. Further methodological details of these 4 studies are included in Supplementary Table 4.

Overall confidence in this evidence is moderate mainly because diagnostic performance is not informative of downstream consequences of further clinical management

Narrative review of additional evidence

A number of studies that did not meet the inclusion criteria for making recommendations addressed important issues. Several studies used alternative methods for nNO measurement that do not use the ATS/ERS guideline "gold standard" (velum closure, stationary analyser) [21]. Tidal breathing manoeuvres are useful, especially in those unable to perform a velum closure, but may be less discriminative. Marthin *et al's* study of consecutive referrals found breath hold sensitivity of 0.92 and specificity of 0.96 compared to tidal breathing values of 0.93 and 0.80 (thresholds were breath hold – 52.5nl/min, tidal breathing – 47.4nl/min)[23]. Beydon *et al*, however, found increased accuracy of tidal breathing (37.9nl/min threshold; sensitivity 0.94, specificity 0.92) versus velum closure (82.2nl/min, 0.91, 0.86)[22]. Measurement using portable analysers was assessed in two case control studies. Using a portable analyser, Marthin *et al* compared PCD patients to those with CF and healthy controls. They found a 1.0 sensitivity and 0.95 specificity for breath hold (64nl/min threshold) and 1.0/1.0 for tidal breathing (43nl/min) [23]. Harris *et al* studied 13 PCD and 37 disease control/healthy patients using tidal breathing and a portable analyser with a cut off of 30nl/min. Sensitivity was 1.0 and specificity 0.95[27].

Measurement of nNO in young children is possible, however discrimination between PCD patients and controls is reduced as nNO is inversely proportional to age in healthy patients under 12 years[28, 29]. One study showed that velum closure was possible in children as young as 3.9 years [20]. However the majority of very young children are unable to co-operate with velum closure and so tidal breathing measurements have to be used. The studies by Marthin *et al* and Beydon *et al* suggest that tidal breathing may produce similar accuracy to velum closure in adults[22, 23],

however this has not been shown in children; Marthin *et al's* study of 117 consecutive referrals of all ages, found that the false positive rate for children under 6 years using tidal breathing was 39%[23].

There is increasing evidence that some genetic defects causing PCD with subtle beating defects may be associated with nNO levels within the normal range. This includes two studies of PCD individuals with mutations encoding radial spoke head proteins and one in PCD individuals with abnormal nexin link composition due to GAS8 mutations associated with nNO higher than that usually seen in PCD [30–32]. This may partially explain the variability of the results and low diagnostic performance of nNO in some studies

Key unanswered questions and research needs

Current evidence has shown that nNO is a useful test as part of the diagnostic process, however there is no consensus on appropriate thresholds. Likewise, standardised protocols and thresholds need to be developed for tidal breathing, portable analysers and measurements and normative data in younger children, particularly those under 6 years of age. Further work on genotype-phenotype correlation can help in interpretation of nNO levels in cases of diagnostic uncertainty in order to reduce the number of false negative test results. There is no evidence that can lead us to recommend which patients with a normal nNO should be referred for further testing and this requires evaluation.

Summary

Nasal NO is a highly accurate test for PCD when measured via stationary chemiluminescence analyser using velum closure techniques with a sensitivity of 0.90-1.0 and specificity of 0.75-0.97. Tidal breathing technique or use of portable analysers are less sensitive and specific but may contribute to the diagnostic decision. Different studies have used different methods and cut-off values making it difficult to provide definite thresholds.

Nasal NO is not sufficiently accurate to rule in or rule out PCD in isolation but considering that it is relatively easy to perform, non-invasive and affordable, the panel considered that it should be used as part of the diagnostic work-up of patients suspected of having PCD.

High Speed Video Analysis

Summary of recommendations

In patients suspected of having PCD, should HSVA be used as a diagnostic tool?
Based on LOW confidence in the evidence, we recommend:

1. High speed video analysis, including ciliary beat frequency and beat pattern analysis, should be used as part of the diagnostic work-up of patients suspected of having PCD (weak recommendation).
2. Ciliary beat frequency should not be used without assessment of ciliary beat pattern in diagnosing PCD (strong recommendation).

To improve diagnostic accuracy of HSVA, CBF/P assessment should be repeated after ALI culture (strong recommendation).

Explanation of the test

PCD is related to abnormal ciliary function [33] which can be analysed ex-vivo by assessment of ciliary activity in respiratory epithelium from the nose or bronchus. Ciliated cells can be observed immediately after sampling[34–36] and again after a period of culture to differentiate PCD from secondary dyskinesia [37–40]. A video attached to a microscope records at high speeds (120 to 500 fps), and is replayed slower (30-60fps) to review ciliary beat pattern (CBP) and measure ciliary beat frequency (CBF)[41]. Most studies have used analysis by expert microscopists, whilst several studies used computer analysis in an attempt to reduce subjectivity/ observer bias. High speed video analysis (HSVA) provides a permanent record that can be used for audit, expert advice, or research.

Review of evidence directly addressing the question "in patients suspected of having PCD, should HSVA be used as a diagnostic tool?"

Our search identified 113 studies, of which 30 met inclusion criteria for qualitative assessment. (Supplementary Figure 1). Two studies (n=650 patients) assessed HSVA in cohorts suspected of PCD who eventually received either a positive or negative diagnosis, contributing to the evidence for recommendations (Table 4). The other 28 papers did not meet the inclusion criteria for informing the recommendations, but contributed to our narrative review (Supplementary Table 6).

Publication	Study population	Cilial assessment method	Sensitivity (95% CI)	Specificity (95% CI)

Papon *et al* 2012 [42]	25 referrals (10 PCD positive)	HSVA beat frequency and quantitative measurements of beat pattern	0.96 (0.89-0.98)	0.95 (0.91-0.98)
Jackson *et al* 2015 [26]	625 referrals (60 PCD positive)	HSVA beat frequency and subjective pattern	1.0 (0.94-1.00)	0.93 (0.91-0.95)

Table 4. Summary of diagnostic accuracy of high-speed video analysis (HSVA) from evaluation in consecutive patients suspected of PCD. Two studies fitted the PICO and inclusion criteria.

Papon *et al*. measured 12 parameters of ciliary beat pattern, including ciliary beat frequency. The distance travelled by the cilia tip weighted by the percentage of beating edges had the best sensitivity (0.96) and specificity (0.95) to distinguish 10 PCD positive from 15 PCD negative patients [42]. Using this parameter in 9 patients with previously inconclusive diagnoses, it was possible to support the diagnosis of PCD in 4 cases and exclude it in 2. Jackson *et al.* found a sensitivity of 1.00 and specificity of 0.93 for the combination of ciliary beat frequency measurement and beat pattern evaluation in a cohort of 625 referrals, including 60 PCD positive [26].

Overall confidence in this evidence is low mainly because diagnostic performance is not informative of downstream consequences of further clinical management based on the assessment of HSV and because of study limitations (HSVA was widely used as part of the reference standard and lack of blinding)."

Narrative review of additional evidence

To date, there is no standardised method for cell processing and analysis. Respiratory epithelium can be collected using brush, curette or forceps, usually from the nose[43]. Ciliary function varies under differing conditions, for example temperature and pH, with some centres measuring at 37^{o}C[26, 40, 42, 44] and others at lower temperatures[35, 37, 45]. This will affect ciliary function and all centres need to define their own normative data until a consensus is reached to allow standardisation of methods and reporting between centres.

Equivocal results or abnormal results require repeat sampling or reanalysis following cell culture [38, 40, 46, 47] since secondary defects are common. In a series of 712 patients, Jorissen *et al* assessed

CBF and ciliary coordination in suspension culture (i.e. spheroids). Twenty percent of non-PCD (n=642) patients demonstrated abnormal ciliary activity before culture, but after culture 100% had normal ciliary activity. Conversely, in biopsies of PCD patients (n=70; evaluable in 56), 20% had a normal CBF and 10% had a coordinated ciliary activity before culture. After culture, a normal CBF was found in 7% of PCD patients but ciliary function was never normally coordinated, making this parameter more sensitive and specific than CBF measurement [47]. Hirst *et al* report CBF and CBP before and after air-liquid interface (ALI) cultures in 158 patients [40]. Before culture, most PCD and non-PCD patients exhibited a degree of functional ciliary abnormalities. After ALI-culture, normal CBP was observed in all non-PCD patients whilst in PCD patients, CBP was uniformly abnormal. Pifferi *et al* assessed the results of CBP and CBF analysis after suspension culture (i.e. spheroids) in 9 subjects with inconclusive results on nasal brushings [36]. After culture, 4 patients had abnormal CBP suggesting PCD diagnosis, 2 had secondary dyskinesia CBP and 3 remained inconclusive. Culture techniques are limited by success rates ranging from 54-83% [26, 37, 40].

CBF measurement does not adequately differentiate PCD from non-PCD unless combined with CBP assessment. Stannard *et al* found a sensitivity of 0.97 and a specificity of 0.95 for the percentage of dyskinetic epithelial edges whilst CBF alone only yielded a sensitivity and specificity of 0.87 and 0.77[48]. Moreover, CBF may be slow, normal or increased in PCD depending on the genotype [49].

Some ultrastructural defects and genetic mutations causing PCD may be associated with specific patterns of ciliary beating. In a cohort of 56 children, Chilvers *et al* reported virtually immotile cilia in patients with either a combined IDA and ODA defect or an isolated ODA defect, stiff beat pattern in patients with either an isolated IDA or radial spoke with an IDA defect, and circular beating cilia in patients with a ciliary transposition defect[44]. Raidt *et al* studied CBP according to the genetic variants of PCD[49]. Although numbers associated with some genes were extremely small, the data supports the linking of PCD causing-gene with particular CBPs: for cilia from patients with mutations in ODA causing genes (*DNAH5, DNAI1, DNAI2, ARMC4*), showed minimal residual movements. In patients with DNAH11 mutations (normal ultrastructure), cilia exhibited a hyperkinetic CBP with reduced proximal axonemal bending. Some genetic defects such as GAS8 mutations can result in so subtle defects hardly detectable by HSVM [30].

Key unanswered questions and research needs

Current evidence suggests that HSVA is a useful test as part of the diagnostic process, but there is no consensus on appropriate cell processing and method of ciliary assessment. Likewise, standardised protocols and thresholds need to be developed for *ex vivo* analysis of ciliary beat pattern. Further

work on genotype/ultrastructural-phenotype correlation can help in interpretation of HSVA parameters in cases of diagnostic uncertainty.

Summary

HSVA is an accurate test for PCD when performed by experienced observers combining ciliary beat frequency measurement and pattern analysis (sensitivity of 0.95-1.00 and specificity of 0.93-0.95). Culturing the respiratory cells may contribute to improve the accuracy of HSV, in particular to rule out false positives.

HSVA is not sufficiently standardised to rule in or rule out PCD in isolation. Considering that optimal conditions in functional evaluation of cilia remains to be defined, the panel considered that HSVA should be performed by experienced staff as part of the diagnostic work-up of patients suspected of having PCD. This might impair the availability of the test in many centres.

Transmission Electron Microscopy

Summary of recommendations

In patients suspected of having PCD, should TEM be used as a diagnostic tool? Based on LOW confidence in the evidence, we recommend:

1. Ciliary ultrastructure analysis by transmission electron microscopy should be used as part of the diagnostic work-up of patients suspected of having PCD (strong recommendation).
2. Further diagnostic investigations should be performed in patients with normal ultrastructure if the clinical history is strong (strong recommendation).[1]

3. In patients with hallmark ciliary ultrastructure defects for PCD further confirmatory diagnostic investigations are not required[2]. (strong recommendation).

Explanation of the diagnostic test

In 1976 Afzelius *et al* demonstrated that transmission electron microscopy (TEM) could be used to detect ultrastructural defects of cilia in patients with primary ciliary dyskinesia[33]. For many years subsequently TEM was considered the 'gold standard' diagnostic test for PCD. However, several genetic studies have demonstrated that an increasing number of distinct genetic PCD sub-types (e.g. due to DNAH11 mutations) cannot be diagnosed by TEM [50]. Thus, TEM cannot rule out PCD diagnosis.

Respiratory epithelium is usually sampled from the inferior turbinate of the nose by brush or curette biopsy or from the lower respiratory tract during bronchoscopy. The epithelium is chemically fixed with glutaraldehyde, processed and embedded into blocks which are sectioned with an ultramicrotome. Staining with heavy metals (lead and uranyl) provides contrast. Assessment of cilia from healthy cells in transverse section is made using a transmission electron microscope[51, 52]. The number of cilia and cells analysed varies between centres; unless sufficient numbers are assessed, defects caused by mutations in genes which cause intermittent defects are likely to be missed. Considerable expertise is required to perform TEM and interpret results; expenditure for equipment and running costs are high.

The normal ultrastructural ciliary arrangement in transverse section is a circle of nine microtubule doublets, each with a pair of dynein arms, plus a central pair of microtubules (Figure 1). There are a number of ultrastructural phenotypes associated with a diagnosis of PCD. The majority of cases are due to a lack of dynein arms; other defects include disorganisation of the microtubular doublets or loss of the central microtubular pair (Table 6). Some patients with PCD have apparently normal ciliary ultrastructure, as resolvable by TEM. Secondary ciliary dyskinesia can be associated with transient ultrastructural abnormalities, such as compound cilia, axonemal blebs or additional

tubules, which must not be confused with PCD.

Figure 1: Diagram of normal ultrastructure of the ciliary axoneme in transverse section

Figure 2: Electron microscopy images of PCD defects. A. Inner and outer dynein arm defect, B. Outer dynein arm defect, C. Inner dynein arm and microtubular disarrangement, D. central pair and transposition defect

Review of evidence directly addressing the question "in patients suspected of having PCD, should TEM be used as a diagnostic tool?"

We identified and screened 370 studies, of which 46 full texts were assessed for eligibility. Of the 17 that met inclusion criteria for qualitative assessment (Supplementary Table 7), 11 papers (n=3200 patients) assessed TEM in a cohort of patients suspected of PCD who eventually received either a positive or negative diagnosis, contributing to the evidence for recommendations (Supplementary figure 1).

Tables 5 & 6 summarise the 11 studies addressing the question. The sensitivity calculated from each study ranged between 0.71 and 1.00 and the specificity between 0.92 and 1.00. There were five false positive patients in two studies. Papon *et al* identified two false positive results; one was a child with severe asthma without recurrent upper airway infection who had short or absent ODA concerning 44% of nasal cilia and 90% of bronchial cilia, the other an adult with situs inversus and nasal polyposis without lower airway symptoms who had absent IDA without microtubular disorganisation (pers comm). Munkholm *et al* identified three false positives in individuals each eventually thought to have a secondary ciliary dyskinesia, two of whose clinical phenotype improved becoming asymptomatic and a third who had severe asthma [53]. Total specificity, when combining all 11 studies, was >0.99.

Quality of evidence was rated low because diagnostic performance is not informative of downstream consequences of further clinical management and because of study limitations (frequent use of TEM in the reference standard and lack of blinding). However, the very low rate of false positives and the very high specificity of TEM led to strong recommendations.

Publication	Study Population (n)	Conclusive diagnostic result reached (n)	Sensitivity (95% CI)	Specificity (95% CI)

Jorissen *et al* 2000 [37]	812	468	0.71 (0.61-0.81)	1.0 (0.99-1.0)
Pifferi *et al* 2007 [54]	64	62	0.75 (0.48 -0.93)	1.0 (0.93-1.0)
Pifferi *et al* 2009 [39]	59	56	0.77 (0.50 -0.93)	1.0 (0.91-1.0)
Hirst *et al* 2010 [55]	231	187	1.0 (0.88-1.0)	1.0 (0.98-1.0)
Papon *et al* 2010 [56]	1149	793	0.82 (0.77-0.86)	1.0 (0.99-1.0)
Olm *et al* 2011 [57]	24	24	0.92 (0.62-1.0)	1.0 (0.74-1.0)
Papon *et al* 2012 [42]	34	28	0.83 (0.52-0.98)	1.0 (0.79-1.0)
Shoemark *et al* 2012 [58]	1182	1031	0.88 (0.83-0.91)	1.0 (1.0-1.0)
Hirst *et al* 2014 [40]	165	122	0.96 (0.87-1.0)	1.0 (0.95-1.0)
Munkholm *et al* 2015 [53]	239	61	0.83 (0.61-0.95)	0.92 (0.79-0.98)
Jackson *et al* 2015 [26]	868	368	0.79 (0.68-0.88)	1.0 (0.99-1.0)

Table 5. Sensitivity and specificity of the 11 studies directly addressing the PICO question using transmission electron microscopy to diagnose PCD

	Papon *et al*, 2010 [56] n=190	Stannard *et al*, 2010 [48] n=68	Olin *et al*, 2011 [59] n=155	Shoemark *et al*, 2012 [58] n=214	Boon *et al*, 2014 [60] n=138	Jackson *et al*, 2015 [26] n=57	**Total**
Isolated outer dynein arm defect	33%	26%	54%	41%	59%	46%	**44%**
Inner and outer dynein arm defect	32%	34%	23%	24%	6%	39%	**25%**
Inner dynein arm with microtubular disorganisation	13%	6%	7%	9%	16%	9%	**10%**
Isolated Inner dynein arm defect	4%	21%	15%	13%	0%	0%	**9%**
Central pair defect	19%	13%	1%	12%	14%	7%	**8%**
Other*		3%			5%		**1%**

Total (n=)	190	68	155	214	138	57	

Table 6: Characteristics of the ultrastructural defects described in 9 studies directly addressing the PICO question using transmission electron microscopy to diagnose PCD. * Other defects reported include ciliary aplasia, disorientation and extra microtubules

Narrative review of additional evidence

Assessment of the proportion of TEM defects in patients with PCD was made following review of all manuscripts (post 1996) describing a cohort of more than fifty individuals [26, 42, 48, 58–60] (Table 4). Outer dynein arm defects (26-59%) and combined outer and inner dynein arm defects (6-39%) were the most commonly observed. The recommendations below refer to common hallmark defects (absence of outer dynein arms, combined absence of inner and outer dynein arms, inner dynein arm absence combined with microtubular disarrangement). Isolated inner dynein arm defects by TEM are controversial. Several studies acknowledge that inner dynein arms are difficult to visualise by TEM [42, 61, 62]and repeat analysis has been recommended before confirming a diagnosis [63]. For central pair defects the ciliary defect is usually present in a minority of cilia making the diagnosis difficult especially since patients do not have situs inversus.

Evidence for add on techniques to improve electron microscopy in the diagnosis of PCD was reviewed. Computer-assisted analysis has been reported to enhance the visualisation of dynein arms and consequently improve the sensitivity of electron microscopy [61, 62]. Electron tomography is an advanced TEM technique allowing visualisation of structures in three dimensions. A series of transmission electron microscopy images are acquired by tilting the specimen stage at regular increments around two perpendicular axes. Images from both tilt series are then aligned into a single three-dimensional high-resolution projection. If a structural feature is repeated within a tomogram, it can be enhanced through sub-tomographic averaging; a technique in which software extracts the chosen common features and makes comparison by cross-correlation. Electron tomography has been shown to improve 3D visualisation and resolution of cilia allowing identification of patients with *HYDIN* and *DNAH11* gene defects in a research setting [64]. The use of tomography for diagnosis has not been evaluated.

Our evidence review considered only conclusive results. Reported rates of inconclusive results ranged from 1.7% to 28.6% [26, 37, 39, 58]. This was attributed to poor sampling technique or the presence of secondary changes to the cilia. Seven of the 11 studies reported measures to avoid

sampling during or immediately after an upper respiratory tract infection to improve adequacy and minimise secondary ciliary ultrastructural change.

Cell culture techniques that induce ciliogenesis from human biopsies are used in a number of PCD diagnostic centres. Two techniques to induce basal cell proliferation and ciliated cell differentiation have been described for PCD diagnosis[37, 40]. Jorissen *et al* established a submerged culture technique [37] and the air-liquid interface technique was first described for PCD diagnosis by Hirst *et al*[40]. Both methods have shown that the TEM axoneme structure is conserved after cell culture in normal and PCD subjects, and they have been shown to reduce secondary damage [37, 40]. TEM following culture has the potential to aid diagnosis of reduced generation of multiple motile cilia [65].

Key unanswered questions and research needs

Basic science research must improve the TEM technique and identify PCD in those with 'normal ultrastructure'. The diagnostic community requires standardised protocols and consensus on terminology, especially regarding the number and proportion of cilia required to make a diagnosis. True relevance and prevalence of inner dynein arm and other rare defects needs confirming.

Summary

Transmission electron microscopy is a highly specific test to confirm a diagnosis of PCD and is a key part of the diagnostic work. However, some patients with PCD have apparently normal ultrastructure and therefore TEM should not be used in isolation to exclude a diagnosis.

All 11 studies were retrospective analyses of cohorts of patients with clinical suspicion of PCD, the largest of which spanned time periods of twenty years[56, 58]. Further downgrading was due to use of TEM as the reference standard and lack of blinding, resulting in grading of evidence as low.

Genetics

In patients suspected of having PCD, should genotyping be used as a diagnostic tool?

There were no studies that fulfilled inclusion criteria to answer this question.

Explanation of the diagnostic test

PCD is a genetically heterogeneous disorder. As with autosomal recessive disorders in general, disease is more likely in offspring from consanguineous relationships, and has a 1:4 probability from any conception where both parents are healthy carriers. To date, mutations in more than 30 genes have been reported to cause PCD (Table 7). A more detailed explanation of the PCD-associated genes is presented in the supplementary file.

Gene	Locus	TEM finding	IF finding
DNAH5 [66]	5p15	ODA	Absent DNAH5 and DNAH9. [67–69]
DNAH11 [50]	7p15-21	Normal	DNAH11 is absent in patients with DNAH11 loss-of function mutations. DNAH5 and DNALI1 present [70, 71]
DNAI1 [72]	9p21-p13	ODA	DNAH5 staining may be present proximally but absent distally. DNAH9 absent within the ciliary axonemes. [67, 68]
DNAI2 [68]	17q25.1	ODA	DNAH5, DNAI2 and DNAH9 absent or aberrant [68]
NME8 (TXNDC3) [73]	7p14.1	ODA	Not reported
DNAL1 [74]	14q24.3	ODA	Not reported
CCDC151 [75]	19p13.2	ODA	DNAH5, CDC151, CCDC114 and ARMC4 absent; DNALI1 present. [75]
CCDC114 [76]	19q13.33	ODA	CCDC114 severely reduced, DNAH5 absent, DNALI1 undisturbed [76]
ARMC4 [77]	10p21	ODA	Reduced ARMC4 staining along cilia; complete distal loss of DNAH5; DNAH5 only on proximal ciliary end; DNALI1 present. [77, 78]
CCDC103 [79]	17q12	ODA+IDA	DNAH5, DNAH9 and DNALI1 are missing or reduced in a small number of patients. [79]
DYX1C1 (DNAAF4) [80]	15q21	ODA + IDA	DNAH5, DNAH9 and DNAI2 absent [80]
SPAG1 [81]	8q22	ODA + IDA	Absent DNAH5 and DNALI1 [81]
LRRC6 [82]	8q24	ODA + IDA	LRRC6, DNALI1 and DNAI2 absent or very reduced [82–84]
DNAAF2 (KTU) [85]	14q21.3	ODA + IDA	DNAH5 and DNAI2 absent distally with some residual staining. DNAH9 and DNALI1 absent.[85]
DNAAF1 (LRRC50) [86, 87]	16q24	ODA + IDA	DNAH5, DNAH9 and DNALI1, absent [87]
C21orf59 [88]	21q22.1	ODA + IDA	Absent DNAH5 and DNALI1[88]
DNAAF3 [89]	19q13	ODA + IDA	DNAH5, DNAH9 and DNALI1 absent [89]

ZMYND10 [84]	3p21.3	ODA + IDA	Absent DNAH5, DNAI2 and DNALI1.[84, 90]
DNAAF5 (HEATR2) [91]	7p22.3	ODA + IDA	DNAI1, DNAH5 and DNALI1 absent, HEATR2 reduced [91, 92]
HYDIN [93]	16q22	Normal/ subtle: increased frequency of transposition defects	Normal IDA (DNALI1) and ODA (DNAH5) [93]
RSPH1 [32]	21q22.3	Intermittent central pair/ transposition defects	RSPH1 and RSPH9 absent; RSPH4A present [32, 94, 95]
RSPH3 [96]	6q25.3	Intermittent central pair/ near absence of radial spokes	RSPH3 and RSPH11 absent; RSPH1, RSPH4A, and RSPH23 present (RSPH9 not reported). DNALI1 present. [96]
RSPH9 [94]	6p21	Intermittent central pair defect/ transposition	Absent RSPH9; RSPH1 and RSPH4A present. [94]
RSPH4A [94]	6q22	Intermittent central pair defect/ transposition	Absent RSPH4A, RSPH9 and RSPH1. [94]
DRC1 (CCDC164) [97]	2p23	Normal/ subtle: N-DRC links missing with occasional MT disorganisation	GAS8 and LRRC48 absent from ciliary axonemes. [97]
GAS8 (DRC4)[30]	16q24.3	Normal/ subtly abnormal: increased frequency of MT misalignment	DNALI1 and DNAH5 present; absent GAS8 [30]
CCDC65 (DRC2) [98]	12q13.12	Normal/ N-DRC links missing with occasional MT disorganisation	CCDC65 and GAS8 reduced[98]
CCDC39 [99]	3q26	MT disorganisation + IDA	Absent CCDC39 protein. ODA normal distribution (DNAH5, DNAI2, DNAH9); DNALI1 (IDA) absent; GAS8 in cytoplasm but absent from axoneme [99, 100]
CCDC40 [101]	17q25	MT disorganisation + IDA	Absent CCDC39 protein; RSPH4A and ROPN1L/RSP11 present in axonemes [100, 101]
RPGR[#][102]	Xp21.1	Variable	Normal, DNAH5 and DNALI1 present [103]
*OFD1** [104]	Xp22	Unknown	Not reported
CCNO [65]	5q11.2	Reduction of cilia number	DNAH5 present; rootletin mislocated in deeper regions of cytoplasm; CCNO not detectable [65]
MCIDAS [105]	5q11.2	Reduction of cilia number	MCIDAS, CCNO, DNAH5, CCDC39 and CCDC78 absent [105]

Table 7: Overview of PCD-causing genes, and their associated findings by TEM and IF analyses. ODA: outer dynein arm; IDA: inner dynein arm; n-DRC: nexin link- dynein regulatory complex; #: retinitis pigmentosa usually detected in adult patients; *: rare syndromic phenotype

To establish the genetic diagnosis, non-ambiguous biallelic mutations in autosomal recessive PCD and hemizygous mutations in X-linked PCD should be identified. The majority of reported mutations are nonsense, frameshift or splice mutations while missense mutations are identified in a minority of cases. Most of the mutations are private, but founder mutations (e.g. in *DNAI1*[106] and *DNAH5*[69])

and mutational hot spots (e.g. *CCNO*[107]) have been reported. The ranking of the effect of the mutations should follow international recommendations [108]: benign (class 1), likely benign (class 2), unknown significance (class 3), likely pathogenic (class 4), pathogenic (class 5).

The associations between genotype and structural defects documented by TEM and/or IF are well established, but much less is known about gene: HSVA associations. With studies based on small numbers of patients and often limited number of videos per patient[49], there is insufficient data to correlate mutations within a gene with dyskinesia phenotype; our knowledge to date suggests that disease-causing mutations in DNAH5 are always associated with predominantly static cilia whilst mutations in different regions of DNAH11 can lead to either static cilia or hyper-frequent, stiff cilia [49, 109]. Therefore, to confirm the genetic cause of PCD, the ultrastructural defect and gene should correlate; in the future, we may be able to use gene mutation: ciliary pattern correlations as further support.

In principle all DNA sequencing technologies can be applied for genetic testing in patients with a confirmed PCD or a high suspicion of PCD (further detailed in the supplementary file). However, due to the high number and the huge size of PCD genes high-throughput techniques are now widely used. The yield of allele-specific approaches is low in PCD given the high genetic and allelic heterogeneity. Detected mutations should be confirmed by Sanger sequencing and checked for segregation in the parents. Genetic laboratories have to be aware that large heterozygous genomic deletions have been reported in PCD individuals that might be missed by DNA sequencing technologies. Homozygous and heterozygous intragenic large duplications and deep intronic mutations are also missed by sequencing techniques. The detection of intragenic deletions and duplications will benefit from the fine set up of targeted next generation sequencing (NGS) panels; however, this approach requires specific development and sensitivity assessment. All techniques, especially the second line approaches, benefit from the knowledge of the ultrastructural defect of the patient in order to assess the relevance of the molecular findings. The ≈30 PCD genes implicated to date encompass more than 700 exons and thus it is not unusual to identify a heterozygous variant in a gene that is obviously not responsible for the disease of the patient based on ultrastructural data. Cell and whole organism models can be used to confirm that a gene is disease-causing.

Review of evidence directly addressing the question "in patients suspected of having PCD, should genotyping be used as a diagnostic tool?"

Searches identified 462 studies, of which 95 met inclusion criteria for qualitative assessment (Supplementary Table 8). Most studies included patients with confirmed PCD with the aim of

identifying novel genes rather than diagnostic cohorts. There were no studies that fulfilled the inclusion criteria for quantitative assessment.

Narrative review of additional evidence

In populations with confirmed or highly suspected PCD diagnosis, it is possible to identify genetic causation in 50-75% of cases [110, 111]. The sensitivity of genetic testing as a first line diagnostic test for PCD is currently unknown but is likely to be low. With the identification of further PCD genes and high-through put sequencing technologies, PCD genetic testing as "stand alone" test might be considered in the future. Genotyping is useful in instances where confirmation of the diagnosis is difficult by other approaches (e.g. *DNAH11*, *CCNO*, *MCIDAS* and *RSPH* genes mutations). The detection of bi-allelic disease-causing mutations in autosomal recessive PCD or hemizygous mutations in X-linked PCD is highly specific.

Most studies to identify novel PCD gene defects used ultrastructural defects detected by routine TEM as the starting point for the genetics search, therefore the likelihood to identify mutations in PCD with ultrastructural defects is higher than in PCD devoid of ultrastructural defects. This underscores the need not to rely on TEM as the sole diagnostic test for PCD.

Reports of mutations in specific genes typically relate to small numbers of patients and are not necessarily ethnically representative; the contribution of each gene should therefore be interpreted with caution. Studies testing for *DNAH5* and *DNAI1* mutation suggest that these mutations account for ~50-70% of cases of ODA defects[67, 69, 112, 113]. Mutations in *CCDC39* or *CCDC40*[99–101, 114]account for almost all PCD individuals with microtubular disorganisation and absence of IDA. Of 58 unrelated PCD patients with normal ultrastructure 22% had biallelic mutations in *DNAH11*[70]. Mutations in the genes encoding radial spoke head and stalk proteins (RSPH1, RSPH3, RSPH4A, RSPH9), HYDIN and nDRC proteins (DRC1, CCDC65, GAS8) can cause PCD with normal or subtly abnormal ultrastructure (Table 7); to date, the contribution of these genes to the prevalence of PCD has not been determined.

A systematic population-based genetic Israeli study revealed that RGMC may be more frequent (6%) in their particular PCD populations than previously estimated [107].

Genetic analyses have shown that mutations in the RGMC genes *CCNO* and *MCIDAS* as well as the genes encoding radial spoke proteins (RSPH1, RSPH3, RSPH4A, RSPH9) and the CP associated protein HYDIN do not result in laterality defects. In addition so far all PCD individuals carrying biallelic

mutations in genes encoding nDRC proteins such as CCDC164, CCDC65 and GAS8 did not exhibit any situs abnormalities.

Given the large size of the regions sequenced in PCD patients, it is not unusual to identify one or several rare missense variants that are not linked to the disease. Great care should be taken to interpret those variants: it is important to perform segregation analysis for those variants and their interpretation can rely on expert labs. In some cases immunofluorescence microscopy (IF) has been proven to be a useful tool to determine pathogenicity of missense mutations in PCD individuals with mutations in RSPH4A and RSPH9 that encode radial spoke head proteins [94]. However, immunofluorescence analysis can be normal if the mutated protein is still expressed and correctly assembled within the axonemes such as reported for *DNAH11* missense mutations [71].

We did not find evidence to either confirm or refute genotyping as a diagnostic test for PCD. Whilst there is a need for evidence of the utility of genotyping in a diagnostic setting, Table 8 summarises the Task Force assessment of current published evidence in genetic testing in PCD.

Key unanswered questions and research needs

The role of genetic testing is not well defined in the PCD diagnostic pathway. We need studies to investigate the accuracy and limitations of genetics as a diagnostic tool for PCD. The standards for diagnostic testing for PCD need defining.

Summary

We were unable to determine the accuracy of genetics testing due to lack of suitable studies. Several studies have identified the genes responsible in patients with confirmed PCD, suggesting that genetic testing identifies the gene in approximately 65% of cases; this is likely to increase as more genes are identified. The question of diagnostic accuracy should be revisited as new data become available.

Task force statements on genetic testing for PCD

Whilst further evidence in a diagnostic setting is required, experts on the Task Force agreed:

1. Genetic testing to confirm diagnosis can be performed in PCD individuals diagnosed by other means (e.g. HSVA, TEM, IF) or in individuals with high clinical suspicion for PCD (typical clinical findings, low nasal NO) and no availability of other investigations such as HSVA, TEM or IF. A negative genetic test does not exclude PCD.

2. Genetic testing can also be performed to establish diagnosis in patients highly suspected of PCD and in whom HSVA, TEM or IF failed to confirm the diagnosis, as it can be the case for patients with *DNAH11*, *CCNO*, *MCIDAS* or *RSPH* gene mutations.

3. Genetic testing and interpretation of results should follow national and international best practice guidelines [115, 116].

4. Genetic diagnosis has to be consistent with the clinical and TEM/IF/HSV phenotype, or diagnosis reconsidered if the picture is inconsistent.

5. Allelic segregation analysis within the family (especially in both parents) is important to confirm the genotype in the probands (to differentiate between homozygosity and hemizygosity, and between compound heterozygosity and a complex allele).

6. Genetic testing in probands and in their relatives is helpful for genetic counselling to inform reproductive choices.

7. In the future genetic testing might be important for genotype specific therapy.

Table 8. Summary of the Task Force consensus on the published evidence on genetic testing in PCD diagnostics.

Immunofluorescence

In patients suspected of having PCD, should IF be used as a diagnostic tool?

There were no studies that fulfilled inclusion criteria to answer this question.

Explanation of the diagnostic test

Labelling of ciliary proteins was developed to improve understanding of the impact of disease-causing genes on ciliary proteins[117]. Specific antibodies with secondary fluorescent tags localise to proteins in human respiratory epithelial cells and are visualised by fluorescent or confocal microscopy. A number of antibodies against ciliary proteins are available including antibodies targeting the outer dynein arm, inner dynein arm, radial spoke head and dynein regulatory complex proteins. An example of this technique is shown in Figure 3.

Respiratory epithelial cells in suspension are placed onto glass slides, air-dried and fixed. The cells are incubated with antibodies to ciliary protein not implicated in PCD (e.g. acetylated tubulin) to label the axoneme, combined with antibodies of interest produced in a different species (e.g. anti-DNAH5 to identify outer dynein arm structures).

Figure 3. Representative immunofluorescence images of healthy controls and patients with GAS8, CCDC39 and RSPH9 mutations leading to absence of the protein in the cilia. Acetylated tubulin is used to stain the cilia and Hoechst 3342 to stain the nuclei. Scale bar is 10μm.

Review of evidence directly addressing the question "in patients suspected of having PCD, should IF be used as a diagnostic tool?"

Our search identified 276 studies (Supplementary Figure 1). No studies reported use of IF antibodies in a diagnostic setting and we were therefore unable to establish the accuracy of IF as a diagnostic test. Forty studies contributed to our understanding of the potential to use IF diagnostically, as summarized below (Supplementary Table 9).

Narrative review of additional evidence

Although the literature focuses on research to understand the downstream effects of mutations in PCD-related genes (Table 7), several centres now use IF to aid diagnosis[118]. This is likely to increase as more antibodies become available, and once data for the accuracy of the tests becomes

available. In the two largest patient-based cohort studies, DNAH5 IF was tested in 16 PCD patients and a further 17 families who had ODA defects observed by TEM; mislocalisation of the protein was reported in all cases. Furthermore DNAH5 protein was present in patients with cystic fibrosis and in healthy controls [67, 69]. A number of studies have used IF to examine protein mislocalisation related to genetic mutations (Table 7), providing indicators to the antibodies that might be used and findings expected when IF is used as a diagnostic test. IF can identify mislocalisation of proteins in PCD patients with a range of mutations, providing information on the pathogenicity of a mutation [94]. However most manuscripts report IF findings from small numbers of patients for each gene and mutation specific findings are not yet known.

With IF it is possible to identify almost all ultrastructural abnormalities detectable by TEM and also some cases where the TEM is apparently normal or subtly abnormal [30, 71, 94]. The sensitivity and specificity of IF is unknown but will reflect the combination and quality of antibodies; in the authors' experience, a number of antibodies do not work and validation including appropriate disease and healthy controls is required before they are used diagnostically. IF analysis can be normal if the mutated protein is still expressed within the axoneme [71].

We did not find evidence to either confirm or refute IF as a diagnostic test for PCD. Whilst further evidence in a diagnostic setting is required, the summary of Task Force findings from published evidence are shown in Table 9.

Key unanswered questions and research needs

We need validation studies to investigate the accuracy and limitations of IF as a diagnostic tool for PCD in diagnostic cohort studies. Each applied antibody needs validation in studies including appropriate PCD, disease and healthy controls.

Summary

We were unable to determine the accuracy of IF testing due to lack of suitable studies. Task force experts agree IF can be useful in clinical settings. IF is cheaper and easier than other diagnostic tests, providing a potential test for resource-limited settings.

Task force statements on IF testing for PCD
Whilst further evidence in a diagnostic setting is required, experts on the Task Force agreed: 1. IF is able to confirm pathogenesis of mutations (e.g. missense mutations in genes encoding

radial spoke proteins).

2. IF can detect PCD in some cases with normal ultrastructure or subtle ultrastructural defects.

3. IF can help establish the diagnosis of PCD in ODA, IDA, tubular disorganisation (*CCDC39*/*CCDC40* mutations), central pair (genes encoding radial spoke proteins) and nexin link defects.

Table 9. Summary of the Task Force consensus on the published evidence on immunofluorescence testing in PCD diagnostics.

Confirming or Excluding a Diagnosis of PCD

The Delphi Consensus Survey comprised four consecutive on-line surveys, each building on former rounds. The outcomes of each round are summarised in supplementary table 11. Experts from the ERS Task Force agreed (≥80% of respondents) on the following, which enabled us to propose a diagnostic algorithm (Figure 4):

Positive diagnosis: For patients with a supportive history of PCD, the following results are confirmatory of a positive diagnosis of PCD:

- Hallmark ciliary ultrastructure defects for PCD (absence of outer dynein arms, combined absence of inner and outer dynein arms, inner dynein arm absence combined with microtubular disarrangement), assessed by TEM.
- Non-ambiguous biallelic mutations in PCD causing genes.

The task force did not reach consensus (80%) that any other test in isolation nor in combinations could provide a conclusive positive diagnosis.

Highly likely diagnosis: In patients with a compatible history of PCD the following diagnostic test results make the diagnosis of PCD highly likely, but do not provide a definitive PCD diagnosis.

- Very low nNO plus HSVA findings consistently suggestive of PCD (e.g. static cilia, circling) on three occasions.
- Very low nNO plus HSVA findings consistent with PCD (e.g. static cilia, circling) following cell culture.

If the diagnosis is 'highly likely' but not conclusive, patients should be told that the diagnosis is likely but given the limitations of diagnostic tests, the diagnosis is not 100% certain and might need confirmation when better tests become available. Patients should have other causes for their symptoms excluded and should be treated as if they have PCD. As new diagnostic tests become available further investigations should be offered.

Excluding the diagnosis of PCD: The Task Force did not reach consensus (80%) that any single test nor combination of tests could exclude a diagnosis of PCD. However, based on the evidence reviewed they agreed that there are conditions under which the diagnosis is '**extremely unlikely'**. If the clinical suspicion is only modest and:

- nNO is high/ normal plus normal HSVA, or

- nNO is high/ normal plus normal HSVA following cell culture,

the patient can be counselled that the diagnosis is extremely unlikely and that further testing is not warranted. If the clinical suspicion is very high (e.g. Kartagener's syndrome, PICADAR score $\geq$10) current diagnostic tests are not sufficiently accurate to exclude a diagnosis.

General statements: Members of the Task Force suggest that diagnostic tests should only be conducted in laboratories with expertise in the field. The results should be interpreted by specialists with expertise in PCD and the results explained to the patient and their non-specialist carers. Diagnostic tests for PCD are currently imperfect. As our understanding and techniques for PCD advance, patients with a high clinical suspicion or inconclusive test results can be recalled and offered repeat testing.

A number of patients have diagnostic tests which do not satisfy the criteria for being labelled positive, 'highly likely' diagnosis or 'extremely unlikely'. These patients should be considered inconclusive; further investigation and management should be determined by a specialist with expertise in PCD.

Diagnostic Algorithm: Based on the culmination of evidence from the GRADE recommendations and the Delphi Consensus statement, the following step-wise approach to diagnostic testing can be used (Figure 4). Not all patients need to undergo all steps. For many patients, step 1 (nNO and HSVM) will provide a 'highly unlikely' diagnosis, and patients won't need further investigations. Some patients should proceed to step 2 (TEM or cell culture with repeat HSVM). Genetics testing (step 3) may help make a diagnosis in patients where other tests have failed to provide a definitive diagnostic outcome. Patients who remain 'inconclusive' could be recalled in the future as further tests become available. This approach will not be appropriate for all diagnostic services; local expertise and equipment should be taken into consideration.

Step 1: nNO + HSVA

If both are entirely normal, the diagnosis of PCD is very unlikely and further testing can be avoided unless the clinical suspicion is particularly high.

If nNO is low and/ or HSVA is abnormal: PCD is the likely/ possible diagnosis- repeat these step 1 tests and proceed to step 2.

Step 2: TEM

If TEM is normal: consider genetics testing for genes associated with normal or subtle TEM defects and repeat HSVA following cell culture.

If TEM shows 'hallmark' defects PCD is confirmed: consider genetics testing to further characterise the underlying defect.

Step 3: Genetics and repeat HSVA +/- cell culture

Further testing: In patients where the diagnosis is highly likely or remains inconclusive, further investigations such as IF or radioaerosol mucociliary clearance analysis might be used but the evidence is too limited for us to recommend them. PCD clinicians should consider recalling these patients for further testing in the future, as advances in PCD diagnostics are made.

Discussion

The ERS Task Force presents the first evidence based guideline for the diagnosis of PCD. This is timely, as new diagnostic tests (e.g. ciliary protein immunofluorescence and genetic testing), are increasingly deployed along with refinement of existing tests (e.g. EM tomography and computational averaging). As new evidence arises, the guideline will need revisiting. We have provided guidance on who should be referred for diagnostic testing. We have confirmed that no diagnostic test is perfect, and in the absence of a gold standard, access to a combination of tests is necessary. Using a modified Delphi approach we then used the evidence for individual tests to develop a diagnostic algorithm, providing the criteria to define patients as positive, 'highly unlikely 'extremely unlikely' and inconclusive.

The studies that contribute to the recommendations were all conducted in specialist PCD diagnostic centres. The tests are generally complex, requiring experienced scientists and clinicians to analyses and interpret results. Our findings therefore provide evidence for diagnostic centres with high throughput of samples, analysed by experienced technicians and with good quality control. New diagnostic centres will require support from experienced centres for training and ongoing quality control/ assessment. BEATPCD (http://www.beatpcd.org/ COST ACTION BM 1407) is coordinating a programme of research and training to improve the diagnosis and treatment of PCD. This includes provision of training schools, bursaries for short-term placements in specialist centres and networking for discussion of difficult and equivocal diagnostic decisions. Together with the

anticipated European Reference Network for PCD, the collaborative approach should drive up standards of diagnostic testing across Europe.

Results from diagnostic studies are an indirect measure of the downstream consequences of the application of a test, and therefore the confidence in the test accuracy measures was judged at best moderate to low. Evidence from patients does not alter the strength of published evidence but was considered when deciding the strength of recommendations. The Task Force conducted a questionnaire survey of 352 PCD patients from 25 countries, and 20 in-depth interviews [7]. Patients told us that an accurate diagnosis was an important outcome, leading to a better recognition of their problems by physicians and access to effective treatment, thus improving their health and quality of life.

Once the Task Force recommendations were agreed, a modified-Delphi survey was used to develop a consensus on diagnostic approach. Experts from the Task Force agreed that results from TEM and genetic testing can lead to a definite positive diagnosis. This provides a guide for future clinical trials as well as clinical care, with the caution that our definition will systematically exclude PCD patients with normal ciliary ultrastructure where the genetic mutations are not yet known. Furthermore, most of the Task Force (>50%) considered that the following combinations of tests could lead to a positive diagnosis, but we did not reach consensus (defined by $\geq$80%): Very low nNO PLUS hallmark HSVM consistently on two occasions; very low nNO PLUS hallmark HSVM following cell culture (supplementary table 11). We have also defined conditions where the diagnosis of PCD can be considered as 'extremely likely' and where diagnosis is 'extremely unlikely'. Given the current evidence, it is not possible to exclude a diagnosis with 100% confidence, but we have defined situations where further testing can reasonably stop. We anticipate that as our understanding of PCD grows, new phenotypes for this highly heterogeneous condition will be described that might not be detected by current diagnostic tests.

All studies that contribute to our recommendations were hampered by the lack of a gold standard to investigate the accuracy of individual tests. We therefore accepted manuscripts that constructed a reference standard from a number of tests ('composite diagnostic outcome') or used an imperfect test e.g. TEM as a surrogate [119]. Studies using TEM or genetics as the reference standard will systematically exclude PCD patients with normal ultrastructure or where the genetic mutation is not known. Additionally, some of the evidence was based on studies where the index test was included in the composite diagnostic decision. These important limitations present a strong risk of bias and may have over inflated or deflated the sensitivity and specificity that we report for each test. None of the diagnostic tests had internationally agreed standards for conduct or reporting. This resulted in

disparity of methods between the studies that we reviewed. The Task Force suggests that the following need to be taken into consideration to advance our understanding of diagnostic tests for PCD:

1. Methodologists should be involved in the design of future studies to investigate diagnostic accuracy of tests. Consideration is needed for the lack of a perfect reference standard.

2. To allow comparisons between studies, international standards for conduct of diagnostic tests and reporting of results is needed. The standards should be evidence based.

3. Reporting of the clinical phenotype of patients included in diagnostic studies should be improved and standardized.

4. Impact of a diagnosis on patient outcome and quality of life should be investigated.

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Figure Legends

Figure 1: Diagram of normal ultrastructure of the ciliary axoneme in transverse section

Figure 2: Electron microscopy images of PCD defects. A. Inner and outer dynein arm defect, B. Outer dynein arm defect, C. Inner dynein arm and microtubular disarrangement, D. central pair and transposition defect

Figure 3. Immunofluorescence microscopy can be used to identify structural defects of motile cilia and to aid diagnosis of PCD. Antibodies directed against the outer dynein arm heavy chain DNAH5 (red, A) can be used to detect outer dynein arm defects caused by various genetic defects. Antibodies against DNAH11 (green, B) can detect *DNAH11* loss-of function mutations that cause PCD with normal ultrastructure. The antibodies directed against GAS8 (red, C) can identify isolated defects of the nexin-dynein regulatory complex (C). Antibodies against CCDC39 (red, D) are used to detect defects of the 96nm axonemal ruler (D) caused by CCDC39 or CCDC40 mutations. Anti-RSPH9 antibodies (red, E) can be used to identify various defects of the radial spoke head complex (E). Normal localisation of ciliary components is shown by co-localisation (yellow color) with ciliary axonemal markers such as acetylated tubulin (green in A,D,E), alpha/beta tubulin (red in B) or unaffected ciliary components (i.e. DNAH5, green in C). In contrast, absence of structural components involved in ciliary motility is shown by absence of the protein in mutant cells (lower panels in A-E). Nuclei are shown in blue. Scale bars represent 10µm.

Figure 4. Following development of recommendations using the GRADE approach, a Delphi survey allowed us to propose a diagnostic algorithm for PCD. Not all patients need to go through all steps. Please see the text for details of the implications of each diagnostic outcome (positive, highly likely and highly unlikely), as well as the consequences for the many patients who will continue to have an inconclusive outcome using currently available diagnostic tests. Patients with uncertain outcomes should be reconsidered for further testing as advances in diagnostic tests are made.

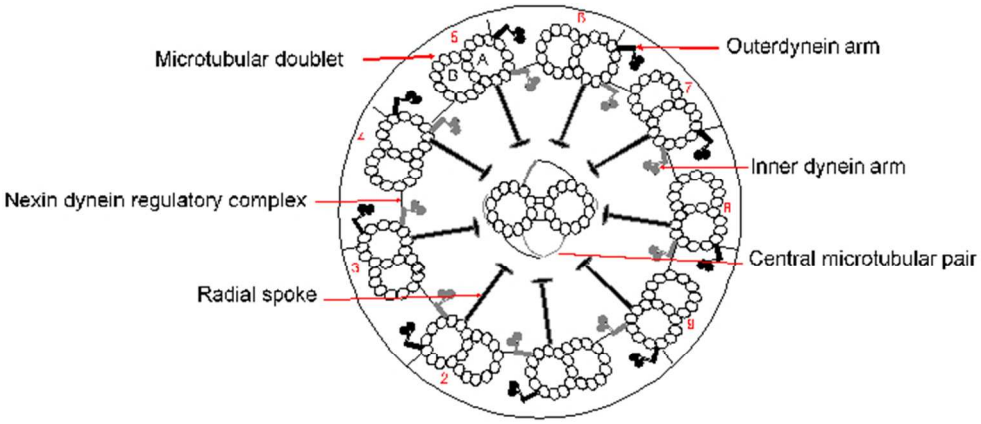
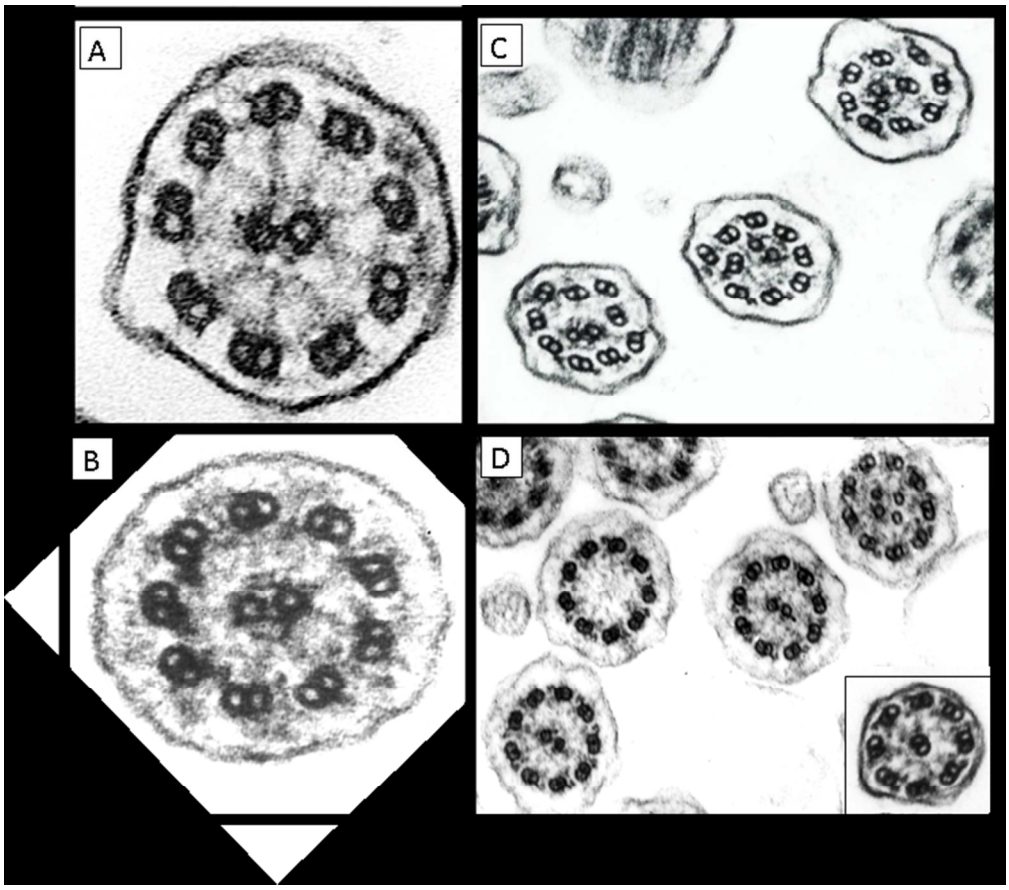
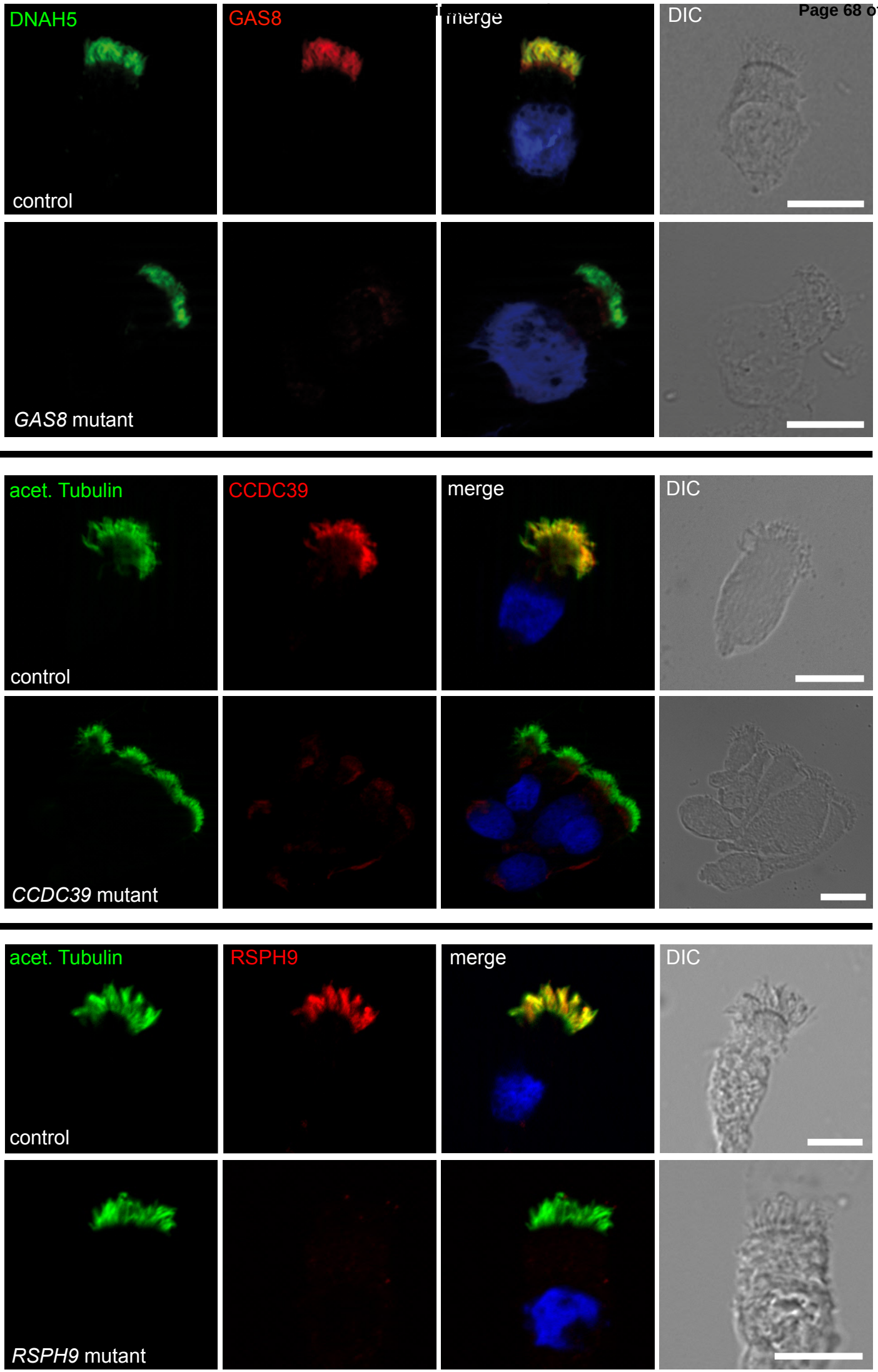


Diagram of normal ultrastructure of the ciliary axoneme in transverse section
Figure 1



Electron microscopy images of PCD defects. A. Inner and outer dynein arm defect, B. Outer dynein arm defect, C. Inner dynein arm and microtubular disarrangement, D. central pair and transposition defect.
Figure 2


DNAH5
GAS8
merge
DIC
control
GAS8 mutant
acet. Tubulin
CCDC39
merge
DIC
control
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acet. Tubulin
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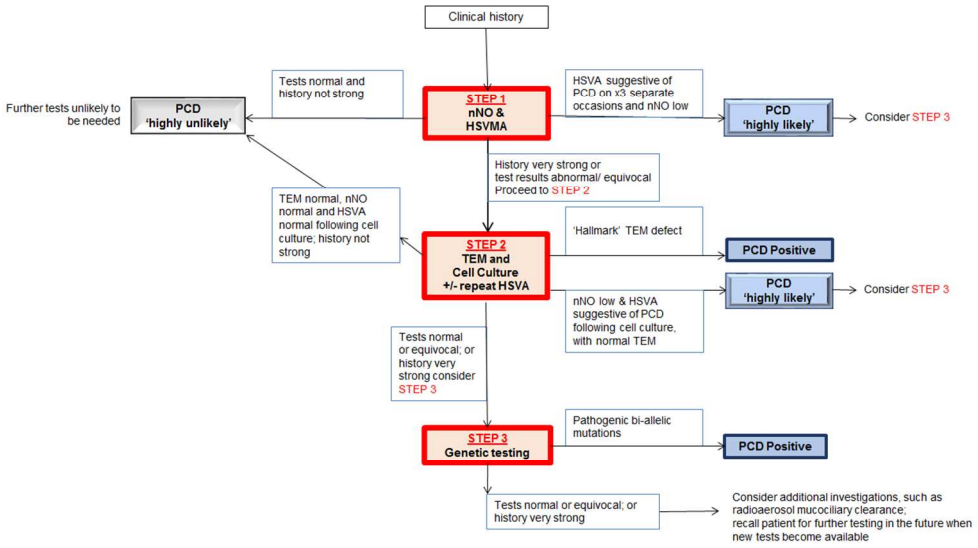
control
RSPH9 mutant



Following the GRADE recommendations, a Delphi survey allowed us to propose a diagnostic algorithm for PCD. Not all patients need to go through all steps. Please see the text for details of the implications of each diagnostic outcome (positive, highly likely and highly unlikely), as well as the consequences for the many patients who will continue to have an inconclusive outcome using currently available diagnostic tests. Patients with uncertain outcomes should be reconsidered for further testing as advances in diagnostic tests are made

Figure 4

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) ****Titieni**, A (Germany)	Paediatric pulmonology. Junior scientist in PCD/ Resident in Pediatrics	Systematic reviewer: IF and genetics 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.

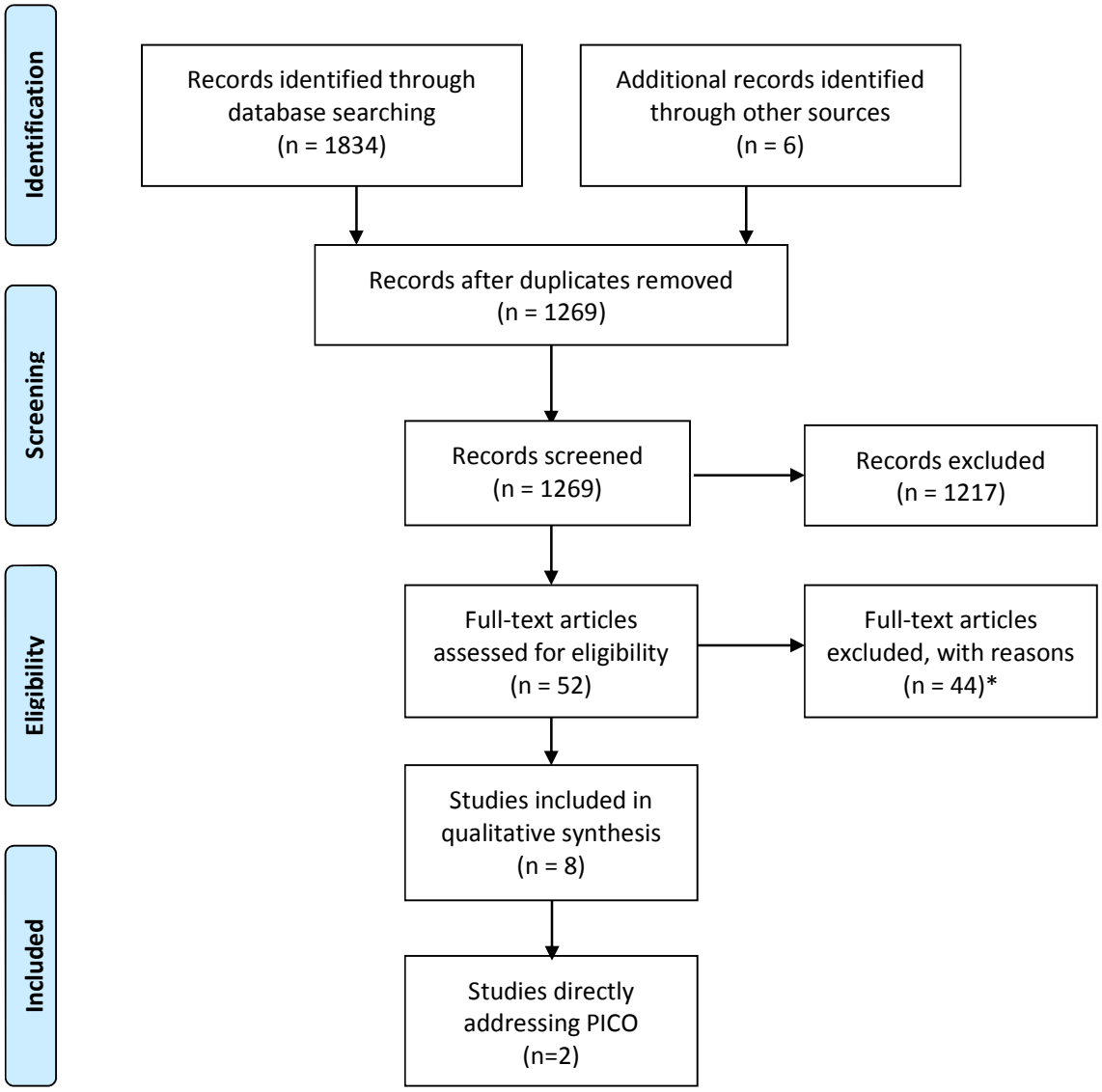
Results

Literature search

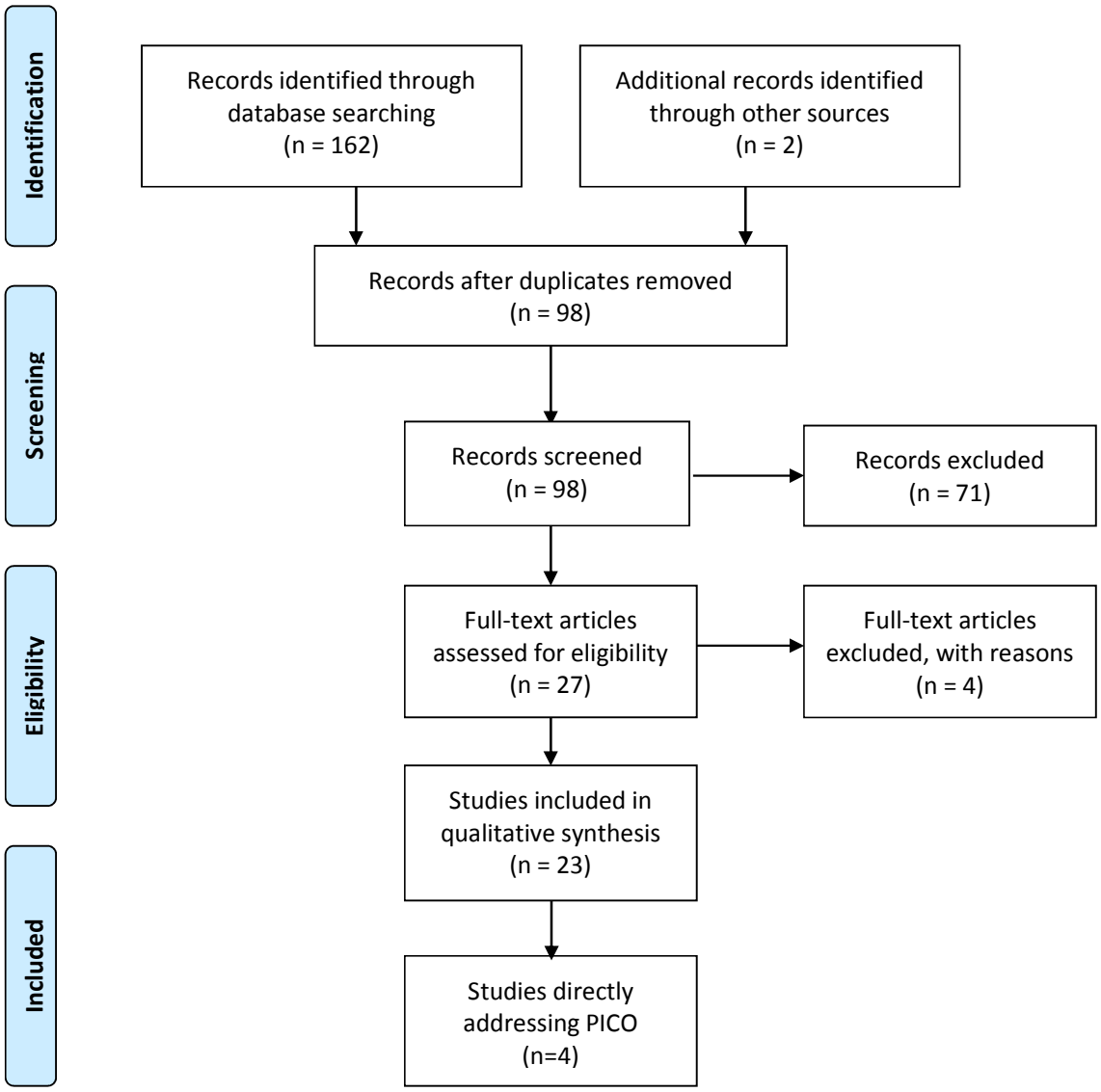
The outcomes of the literature searches for each work group are summarised by PRISMA flowcharts (supplementary figure 1a-f)

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.

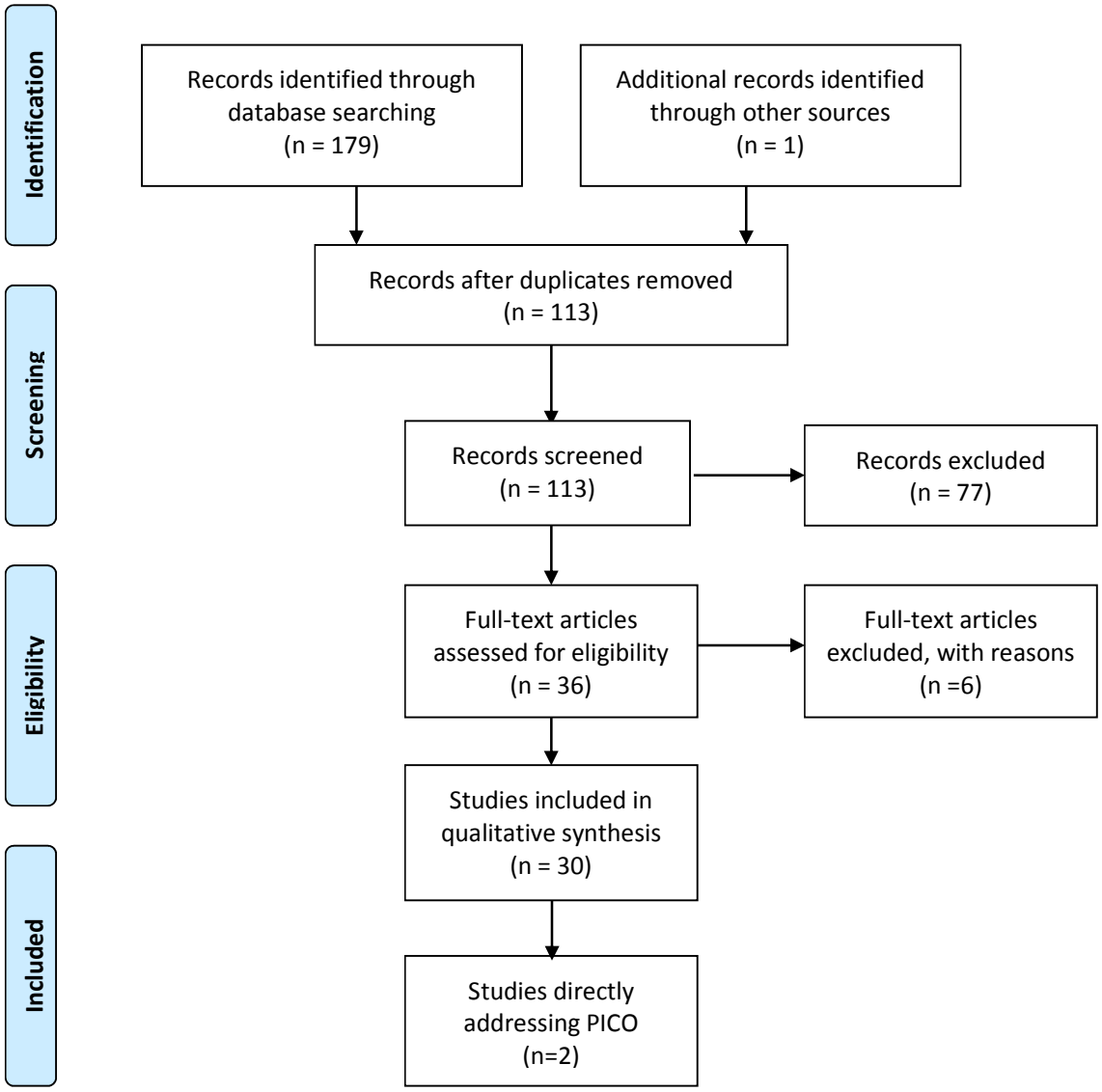
a) Clinical Features



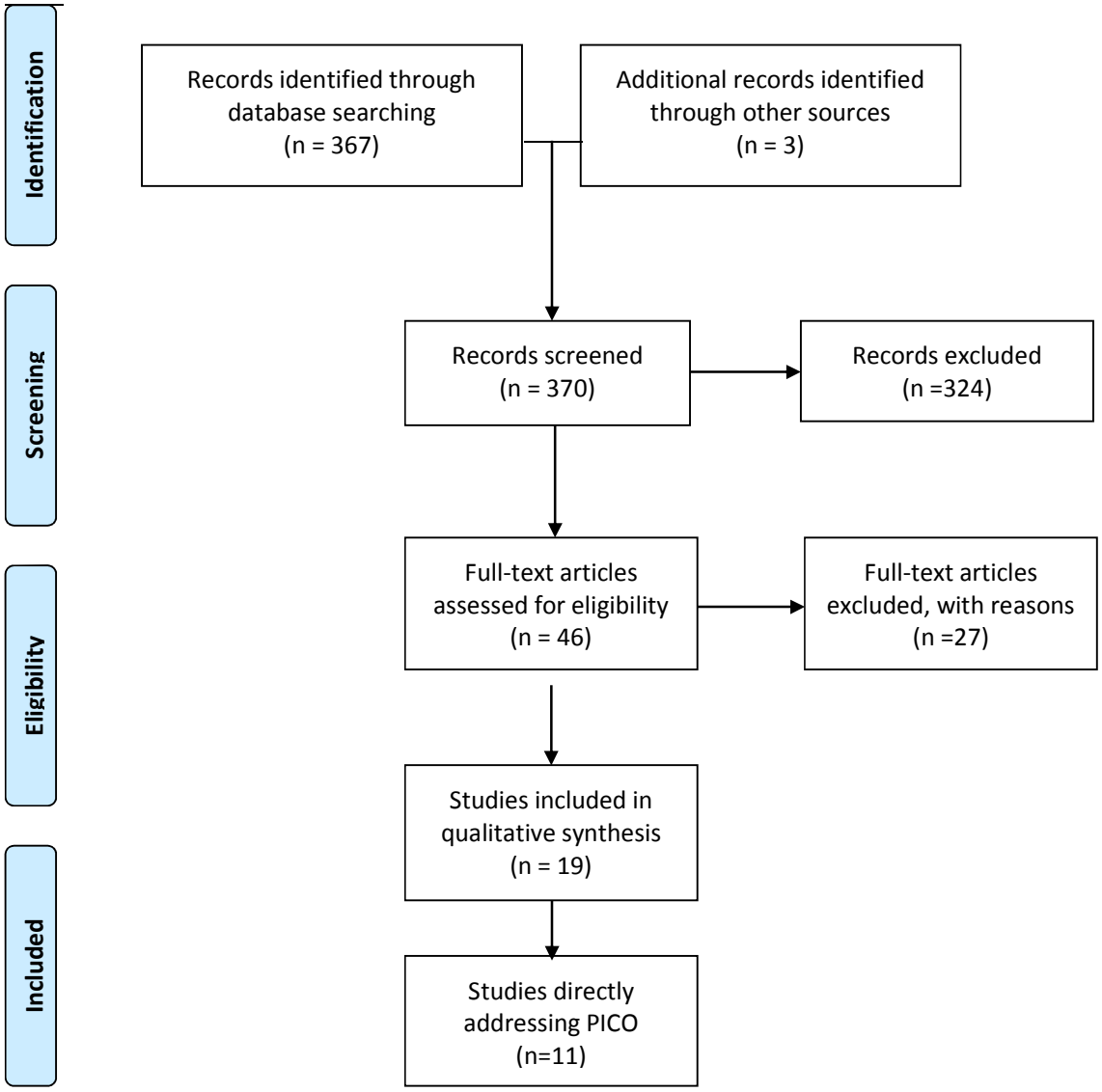
b) Nasal Nitric Oxide



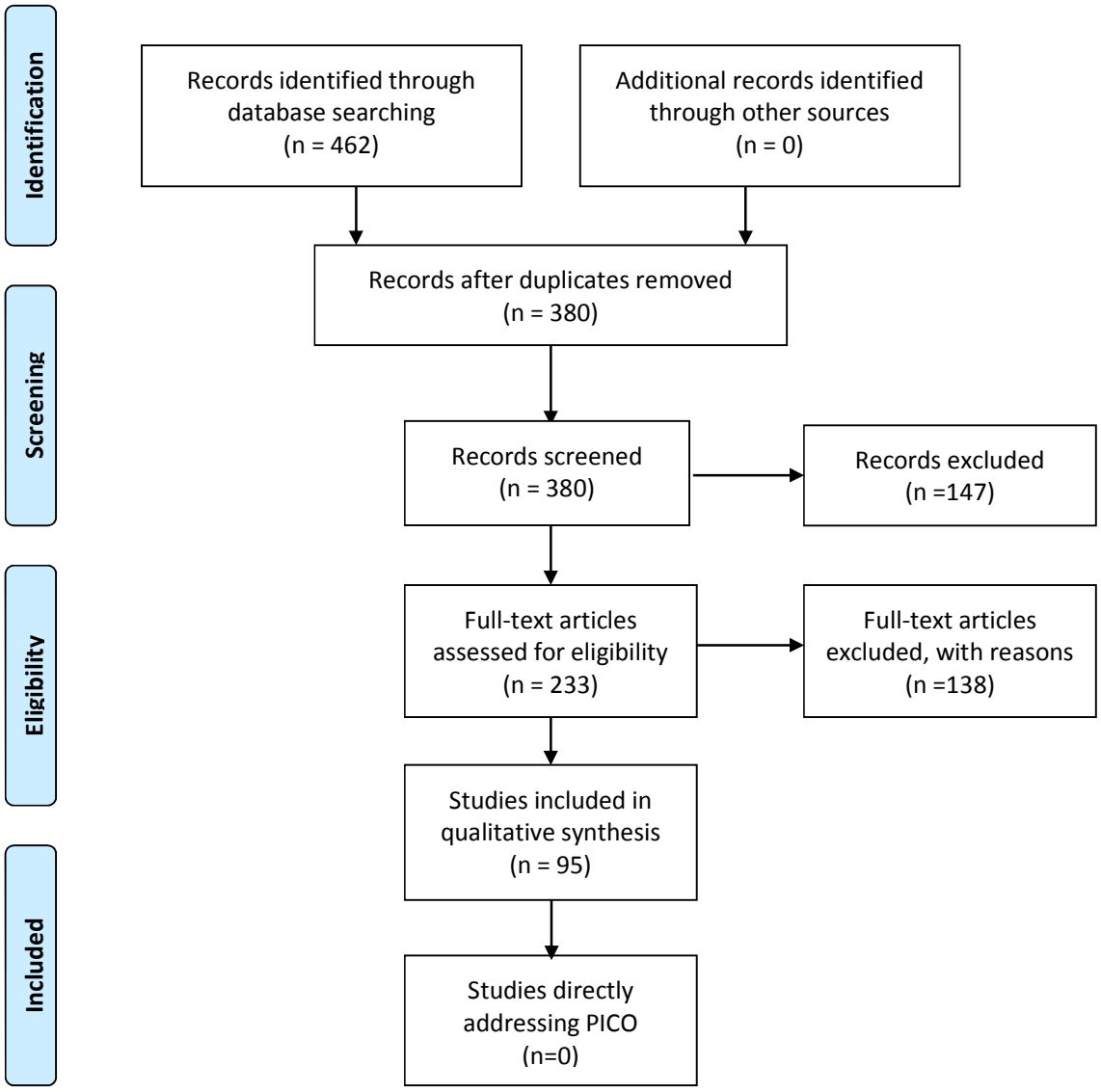
c) High Speed Video



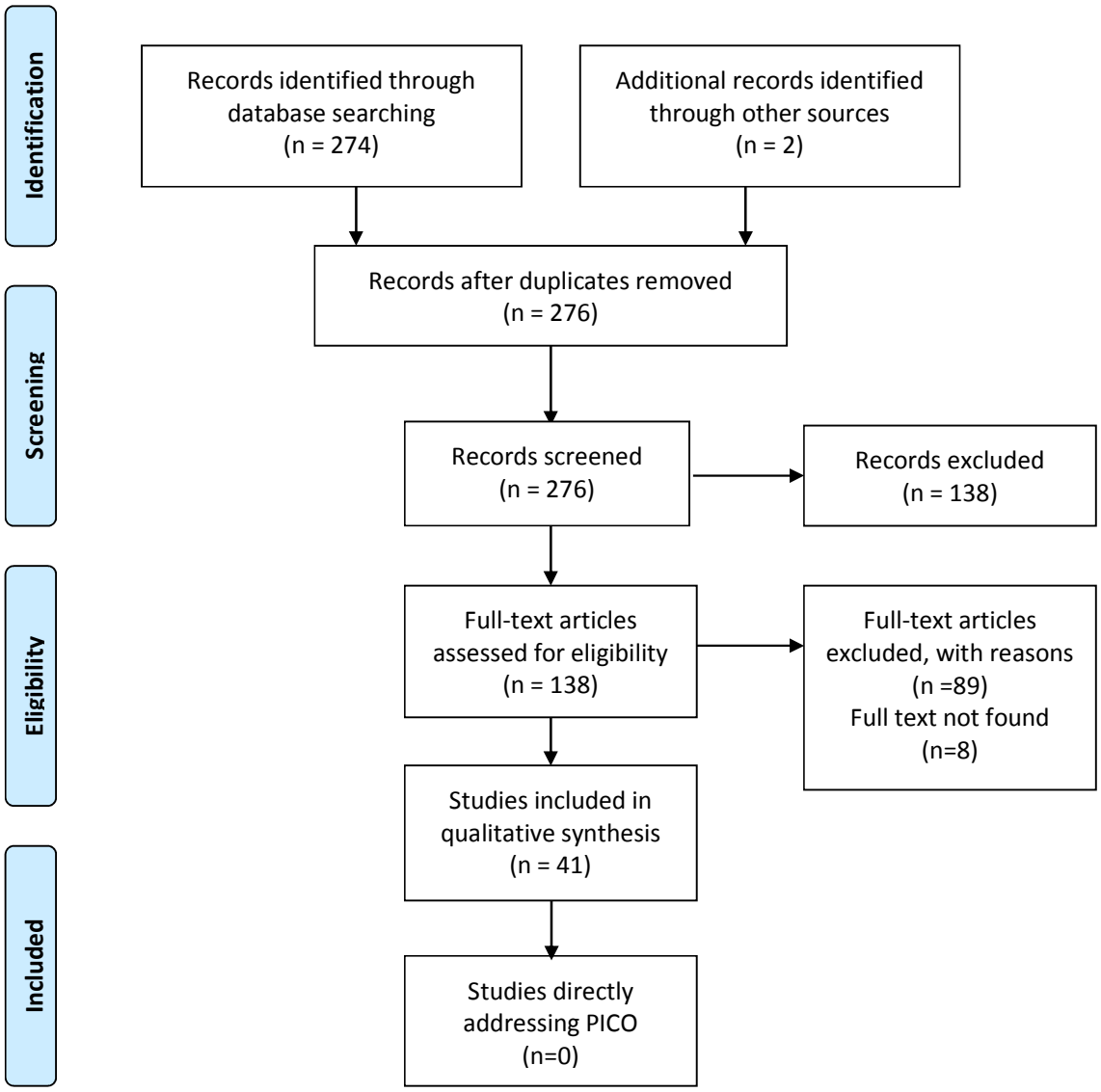
d) TEM


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 (n, thr
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, 52.5) Oral exhalation against r Tidal breathing (n=97, 47
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, velum cl (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=74, 82. Tidal breathing – 5 peaks
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 (breath ho (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	
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)	Strongly Agree %	A
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	4
Bi-allelic mutations in PCD causing gene	52	3
Nasal nitric oxide (persistently abnormal x3)5	14	5
High speed video analysis (pattern and frequency) once	5	5
High speed video analysis (pattern and frequency) (consistently abnormal x3)	29	2
High speed video analysis (pattern and frequency)	5	3
Immunofluorescence (hallmark; PCD once)	5	1
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	0
Immunofluorescence normal	0	5
No bi-allelic mutations in PCD causing gene	5	5
Nasal nitric oxide normal or high	0	9
High speed video analysis (entirely normal CBF and CBP)	18	2
High speed video analysis entirely normal following culture (suspension or ALI) if original sample was equivocal	27	2

b) Survey 2 (respondents n=17)	Strongly Agree %	A
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	5

Very low nNO PLUS hallmark HSVM consistently on three occasions	47	2
Very low nNO PLUS hallmark HSVM following cell culture	35	2
Very low nNO PLUS hallmark IF	6	2
HSVM consistently hallmark abnormal on three occasions	0	4
HSVM hallmark abnormal following cell culture	6	5
Where there is only modest clinical suspicion of a diagnosis of PCD and diagnosis can be EXCLUDED:		
High/ normal nNO AND HSVMA normal	18	5
High/ normal nNO AND HSVM normal following cell culture	24	5
High/ normal nNO AND TEM normal	6	1
High/ normal nNO AND IF normal	0	1
High/ normal nNO AND genetics normal	6	1
Genetics and TEM normal	6	1
Entirely normal HSVM following culture	12	4
Entirely normal HSVM	6	3
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	2
High/ normal nNO AND HSVM normal following cell culture	0	2
High/ normal nNO AND TEM normal	0	6
High/ normal nNO AND IF normal	0	6
High/ normal nNO AND genetics normal	0	6
Genetics and TEM normal	0	0
Entirely normal HSVM following culture	0	2
Entirely normal HSVM	0	1

c) Survey 3 (respondents n=15)	Strongly Agree %	A
Patients with a clinical history compatible with PCD should have access to a range of diagnostic tests which should include		
nNo	87	1
HSVM	87	0
TEM	93	7
Genetics	40	4
IF	7	2
Regarding the diagnosis of PCD		
Tests should be conducted in laboratories with expertise in PCD diagnostics	87	1
Test results should be interpreted by specialists with expertise in PCD diagnostics	87	1
Test results should be reported to patients and their non-specialist carers by a PCD specialist clinician	73	1
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	2
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	4
Very low nNO PLUS abnormal HSVM consistently on three occasions	53	3
Very low nNO PLUS abnormal HSVM following cell culture	53	2
Very low nNO PLUS abnormal IF	13	2
HSVM consistently abnormal on three occasions	13	6
HSVM abnormal following cell culture	20	4
Abnormal HSVM twice	0	4
Abnormal IF	7	2
Very low nNO	0	2
Very strong clinical history e.g. Kartagener's syndrome, PICADAR >10 but no access to diagnostic tests	7	4
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	2
Patients should be managed as if they have PCD until the diagnosis can be definitively confirmed or excluded.	53	4
Patients should be told that the diagnosis is likely but not definite	73	2
Patients should be invited to have further tests as new tests become available or refinements to existing tests occur.	93	7
Diagnosis extremely unlikely; the following statements are true:		
Current diagnostic tests for PCD are imperfect	67	3
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	5
All patients where the diagnosis is considered "highly unlikely" should be counselled that current diagnostic testing is imperfect	33	6
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	4
High/ normal nNO AND HSVM normal following cell culture	47	4
High/ normal nNO AND TEM normal	13	4
High/ normal nNO AND IF normal	7	1
High/ normal nNO AND genetics normal	7	2
Genetics and TEM normal	13	2
High/ normal nNO AND HSVMA normal	13	4
High/ normal nNO AND HSVM normal following cell culture	20	4
High/ normal nNO AND TEM	7	2
High/ normal nNO AND IF normal	7	7
High/ normal nNO AND genetics normal	7	1
Genetics and TEM normal	0	2

d) Survey 4 (respondents n=19)	Strongly Agree %	A
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	4
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	1
Once all tests are conducted, if still inconclusive the patient should be considered 'possible PCD'	11	6
Possible PCD patients should have other causes for their symptoms excluded	72	2
Possible PCD patients should be treated as if they have PCD until the diagnosis is confirmed or excluded.	39	6

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