European Respiratory Society and American Thoracic Society guidelines for the diagnosis of primary ciliary dyskinesia

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Abstract

Primary ciliary dyskinesia (PCD) is caused by pathogenetic variants in more than 55 genes. PCD is associated with early-onset chronic wet cough and rhinosinusitis, laterality defects, middle ear disease and reduced fertility. The clinical presentation is heterogeneous, and diagnosis often relies on multiple tests. The American Thoracic Society (ATS) and European Respiratory Society (ERS) have previously developed separate guidelines for diagnosis. Here, ERS and ATS members systematically reviewed the literature on diagnostic tools used in practice and developed unified evidence-based guidelines for PCD diagnosis using Grading of Recommendations, Assessment, Development and Evaluations methodology, and a transparent process of decision-making using evidence-to-decision frameworks. The Task Force panel formulated three PICO (Patients, Intervention, Comparison, Outcome) questions and three narrative questions. The accuracies of high-speed video microscopy, immunofluorescence and nasal nitric oxide were compared to a reference test of transmission electron microscopy and/or genetics. The panel gives a

strong recommendation for use of high-speed video microscopy, immunofluorescence and nasal nitric oxide as adjunct tests to transmission electron microscopy and/or genetics for PCD diagnosis. However, no adjunct test is suitable as a standalone test to diagnose PCD and no single adjunct or reference test is suitable to exclude PCD. Pursuing a genetic diagnosis is encouraged owing to the implications for management. The panel emphasises that tests should meet a minimum standard and proposes that patients are evaluated at a referral centre experienced in diagnosis. The pre-test probability based on symptoms should be considered when interpreting results.

Introduction

Primary ciliary dyskinesia (PCD) is a genetic disorder caused by pathogenic variants in one of more than 55 genes and is associated with dysfunction of motile cilia [1]. Motile cilia are microtubule-based cellular projections that have rhythmic movements important for moving fluid or propelling cells. Ciliated cells are important for the function of different organs, including the airways, sinuses, middle ears, brain, heart and reproductive organs. PCD is a multisystem disease resulting from dysfunction of motile cilia in these organs. PCD symptoms often start at birth, with most newborns developing neonatal respiratory distress. Individuals with PCD often have persistent wet cough, chronic rhinosinusitis, recurrent otitis media and recurrent respiratory infections beginning early in life and continuing throughout adulthood [2]. Many of these symptoms overlap with common paediatric diseases, which often delays the diagnosis. The disease course can be heterogeneous, but most people develop bronchiectasis and progressive lung disease that can lead to respiratory failure [3]. Individuals with PCD frequently have chronic middle ear disease and can have conductive and sensorineural hearing loss [4]. About half of individuals with PCD present with laterality defects owing to the role of motile cilia during embryonic development. This includes situs anomalies such as situs inversus totalis and dextrocardia, as well as heterotaxy syndromes [5]. Male individuals with PCD are often infertile owing to dysfunction of the sperm tail and cilia in the efferent duct, while female individuals are often subfertile owing to the involvement of cilia in the oviducts [6–8].

Recent prevalence estimates, based on population variant frequencies, predict that PCD affects at least one in 7500 of the population worldwide [9]. The prevalence of PCD is significantly higher among certain closed ethnic groups or those with high rates of consanguinity [10]. For instance, in the UK, the prevalence of PCD in British Asians from the Leeds and Bradford area is estimated to be as high as one in 2265 [11]. However, only 1236 people with genetically diagnosed PCD are known to the European Reference Network for respiratory diseases (ERN-LUNG) and 698 are included in the North American registry [12]. These figures suggest that most people with PCD remain unreported or undiagnosed.

While many people with PCD have characteristic clinical features from infancy, many individuals are diagnosed late in life or not at all, leading to irreversible lung damage [13]. One European study reported that 70% of people with PCD had seen medical professionals more than 50 times before the diagnosis was made at an average age of 10.9±14.4 years [14]. Late or missed diagnosis of PCD can be attributed to a lack of awareness and disease recognition, inaccessible specialised testing and nonadherence to professional guidelines to establish a PCD diagnosis. Moreover, milder or atypical forms of the disease are increasingly being recognised [15, 16].

PCD is a clinically and genetically heterogeneous condition. While PCD can be compatible with a normal lifespan, it is associated with increased morbidity, and some individuals have significant lung and heart disease, leading to an increased risk of premature death [13, 17]. People with PCD often have a progressive decline in lung function with age, and some may require lung transplantation. Older age at diagnosis can be associated with impaired baseline forced expiratory volume in 1 s [3]. There is evidence that stabilisation and improved lung function can be achieved after diagnosis and initiation of appropriate care [13, 18]. There is growing recognition that early multidisciplinary management of PCD can reduce morbidity.

To date, there are no approved treatments specific for PCD. Therapies to correct ciliary dysfunction at the cellular level are in preclinical or early-phase trials [19]. Clinical management is extrapolated from therapies used to treat other diseases with bronchiectasis. Only three PCD-specific randomised controlled trials have been published [20–22]. The emergence of newer PCD therapies highlights the importance of accurately diagnosing PCD to ensure that appropriate individuals are recruited and enrolled into future clinical trials, and that these patients have access to new therapies as they become available.

There are currently two separate European Respiratory Society (ERS) and American Thoracic Society (ATS) diagnostic guidelines for PCD. Despite similarities, there are significant differences in

recommendations, and both have become outdated because of advances in cilia genetics and newer evidence regarding test accuracy [23, 24].

The objective of this document is to provide a unified, updated joint ERS/ATS clinical practice guideline regarding how to diagnose or refute the diagnosis of PCD. Access and accuracy of the currently available diagnostic tests are considered in the recommendations.

Methods

Composition of Task Force panel

These joint ERS/ATS guidelines for the diagnosis of PCD were developed according to the ERS guidance for developing clinical practice guidelines, following the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach and a transparent process of decision-making using an evidence-to-decision (EtD) framework [25]. The Task Force (TF) was chaired by A. Shoemark and A. Horani (senior chairs), supported by B. Kinghorn and P. Kouis (junior chairs). M. Goutaki led the methodological work of the taskforce with overview from two ERS methodologists. The TF consisted of a multidisciplinary group with expertise in the diagnosis of PCD from different regions worldwide (supplementary table 1). The TF included two lay representatives, a person living with PCD and a parent of a child with PCD, who were full members and contributed to all recommendations. Conflicts of interest were disclosed by all members and were managed according to ERS policy. The TF conducted five in-person hybrid meetings, supplemented by additional virtual meetings, and discussed in detail all steps of the development of these guidelines.

Formulation of PICOs and narrative questions

Three questions were formulated pertaining to the diagnostic tests most commonly available for PCD. These were formulated in PICO (Population, Intervention, Comparator, Outcome) format. The whole TF reviewed and finalised the PICO questions, ensuring uniformity between PICOs. The TF also discussed and decided on three patient-important outcomes, with the advice of the patient-representative members. The "Population" comprised patients suspected for PCD (consecutive referrals for PCD testing). The "Intervention/index tests" were nasal nitric oxide (nNO) measurement (PICO1), high-speed video microscopy (HSVM) (PICO2) or immunofluorescence staining (IF) (PICO3). The "Comparator/reference test" was a definitive PCD diagnosis based on a hallmark defect identified by transmission electron microscopy (TEM) and/or a bi-allelic pathogenic genetic variant (described in further detail below). The "Outcome" was diagnostic accuracy, unclear test results or harm caused by the index test.

An ERS and ATS senior member, as well as a junior member, were assigned to work on each PICO question in a subgroup. The TF members agreed by consensus to split PICO1 and PICO2 into two sub-parts: PICO1a and PICO1b, and PICO2a and PICO2b, post data collection. This was done owing to the use of different techniques for the index test in the published literature, which the TF members deemed would be unsound to combine in the final analysis.

In addition to the three PICO questions pertaining to the index tests, the TF identified three complementary questions that are of high importance to PCD diagnosis, and which are addressed in narrative format. These questions were chosen by consensus.

Definition of reference tests

PCD is a genetic disease; thus, identifying pathogenic variants in disease-causing genes is accepted as sufficient to confirm a PCD diagnosis. TEM has been traditionally used for the diagnosis of PCD since the discovery of the association between motile cilia ultrastructure changes and PCD [26]. Owing to limited literature and gene discovery advances over the years, we did not limit the search to a specific genetic panel or number of genes tested. We used a pragmatic approach to include all papers reporting genetics and/or TEM and assessed the quality of those tests during the analysis phase. Owing to the lack of a universally agreed-on reference standard for the diagnosis of PCD, the panel chose to use a combined reference test of genetics and/or TEM for the literature search, which may have excluded cases and affected sensitivity.

TEM

Historically considered the "gold standard" test, TEM was used as a reference test. The 2017 ERS guideline showed that TEM is 99% specific in confirming a PCD diagnosis if performed by centres with expertise in the analysis [23]. TEM is a time-consuming test, requires considerable technical skills and expertise to interpret results, and may not be readily available. It should be emphasised that lack of expertise in performing cilia TEM may result in false-positive and false-negative results. While there have

been many advances in genotyping, there are still individuals in whom no pathogenic variants in known genes have been discovered. In these cases, TEM remains the only way to confirm PCD. A recent international consensus statement defined diagnostic abnormalities on TEM and standardised terminology used to describe these changes [27]. There are two classes of defects: 1) Class 1 defects (outer dynein arm, inner and outer dynein arm, and inner dynein arm and microtubular disorganisation) and 2) Class 2 defects (outer dynein arm defects in <50% of cilia cross-sections, central pair defects, microtubule disorganisation and reduction in ciliation with mislocalisation of basal bodies). Class 1 defects confirm a diagnosis of PCD, while class 2 defects are suggestive of PCD and require supporting evidence from other diagnostic modalities. It should be emphasised that ultrastructure analysis can be normal in 20–30% of people with PCD, and these individuals will require additional tests to support the diagnosis [27, 28].

Genetic testing

Over 55 genes have been associated with PCD. Most forms of PCD follow a recessive inheritance pattern, although some X-linked and dominant forms have been reported. When performing a comprehensive genetic test, a bi-allelic variant in a known gene associated with PCD is identified in over 70% of people with PCD [29]. It is believed that this number will increase as new genes are identified or as more accurate genetic analysis tools become available. Where genetic confirmation is not achieved, additional tests are needed to support a diagnosis.

Currently known genes and their variants are graded and their evidence of pathogenicity is described in the ClinGen motile ciliopathy list (www.clinicalgenome.org/affiliation/40102, PCD gene list). Genes with enough evidence to be considered causative are designated as strong or definitive for PCD. The list is updated monthly by members of the panel and additional experts.

Often, studies describing genetic testing only include some of the known PCD genes, and this selection is influenced by publication date and testing method or panel used. Taking this into account, the TF defined a genetic reference test as any genetic test that provided a pathogenic or likely pathogenic variant (using American College of Medical Genetics and Genomics (ACMG) criteria) found in a known PCD-associated gene consistent with the known inheritance pattern for that gene (*e.g.* bi-allelic where autosomal recessive, hemizygous where X-linked, and mono-allelic where autosomal dominant) [30].

Systematic literature review

The literature search was designed and performed jointly for all questions by an independent information specialist, in collaboration with the methodologists and senior chairs of the TF. Searches were conducted on 10 August 2023 based on the guidance of the Cochrane Handbook for Diagnostic Test Accuracy and following the guidance of the Centre for Reviews and Dissemination, and updated on 11 February 2025 [31–33]. We searched nine online databases including MEDLINE, Embase and Cochrane Central (detailed search strategy available in the supplementary material). We used a date limit of 2008 to current, which was chosen by consensus to capture the last 15 years of PCD research because older methodology was considered by the TF to be less reliable. The search was deduplicated using the Bond deduplication tool [34]. Supplementary searches were performed at a later stage by checking the references of included studies and by asking TF members if they were aware of any relevant studies not identified.

Inclusion and exclusion criteria

Despite the large number of publications related to PCD, not all studies were eligible for inclusion in our analysis. According to the formulation of questions as described earlier, for all PICO and narrative questions, we included clinical cohorts, case series or randomised trials with consecutive patients (of any age) referred for PCD testing. This included cohorts of patients with bronchiectasis if they were all referred for PCD testing. For a study to be eligible, the study's population had to have all been tested for PCD using the index test(s) and reference tests (genetics and/or TEM), with an outcome of either diagnosing PCD or not diagnosing PCD. PCD cohort studies in which patients already had a clinical or confirmed diagnosis did not fit a "consecutive referral" definition and thus were not considered in the analysis. Moreover, case series studies with only PCD patients or case–control studies including PCD patients compared to healthy individuals or another disease group were excluded.

For a published study to be eligible for inclusion, the index test process had to be described in sufficient detail to discern how a test result was deemed positive or negative, and to be able to calculate test accuracy. A detailed list of the inclusion and exclusion criteria can be found in the supplementary material.

Screening of search results and data analysis

Screening at a title and abstract level was performed using a review management platform (Rayyan, Cambridge, MA) [35]. Title and abstract screening were performed independently by two panel members for each PICO question, using predefined inclusion and exclusion criteria. TF members were instructed to be inclusive at this stage; in case of uncertainty about eligibility, the study was considered as potentially eligible and the full text was screened. Screening of full texts was performed by a single TF member, using predesigned Microsoft Excel forms. In case of uncertainty, eligibility was decided after discussion with the chairs and methodologists. Studies that did not fulfil the inclusion criteria but were considered relevant for any of the questions were marked and used to inform the discussion and the EtD process.

Data extraction was performed separately for each question by a junior member using predesigned data extraction forms including information on study design, characteristics of study participants, details regarding the use of index and reference tests, and outcomes of interest. Data extraction forms were checked by question group members and methodologists. Junior members of PICO question groups assessed risk of bias using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies-2) [36, 37].

For PICO questions with more than three included studies, we synthesised the results quantitatively, by fitting a two-level mixed logistic regression model to account for the variability in pairs of sensitivity and specificity within each study, and a bivariate normal model to account for the difference in sensitivity and specificity between the included studies. This method allows the calculation of pooled sensitivity and specificity estimates, with the output expressed as a hierarchical summary receiver operator curve (HSROC). When meaningful, we performed sensitivity analyses, *e.g.* using a predefined cut-off value of 77 $nL \cdot min^{-1}$ for the index test nNO. All calculations were performed using STATA (version 18, StataCorp) with the commands metandi and metandiplot [38].

Assessing the certainty of evidence and strength of recommendations

We used the GRADE approach to assess the certainty of the evidence, to present the evidence to the panel (EtD) and to formulate the recommendations. For papers eligible for the narrative questions, grading was not performed, based on ERS rules.

Junior members of each PICO question group generated GRADE evidence profiles that were checked by the methodologists. We appraised the certainty of the body of evidence informing each outcome as very low, low, moderate or high certainty, based on the five GRADE domains of risk of bias, inconsistency, indirectness, imprecision and publication bias. For narrative questions, we described the identified studies using a narrative approach.

We developed EtD frameworks for all PICO questions, which were discussed by the whole panel as a basis to formulate recommendations and their strength. Recommendations were graded as strong ("we recommend") or conditional ("we suggest") according to GRADE terminology. The GRADE and EtD tables for all the PICO questions are shown in the supplementary material.

The strength of a recommendation (conditional or strong) was defined as the extent to which the panel was confident that the desirable consequences of an intervention outweigh its undesirable consequences. A strong recommendation was made when the panel was certain and it indicates that most experts and patients would choose to recommend the use of the test. A conditional recommendation was made with a lesser extent of confidence [39].

Assessing heterogeneity

We tried to minimise heterogeneity in terms of design and patient characteristics that might be associated with test accuracy by restricting the eligibility criteria for study design and population. We also split PICO1 and PICO2 into two sub-parts, because the differences in techniques used were considered an important source of heterogeneity. When possible and relevant, we performed sensitivity analyses to test whether different cut-offs had introduced further heterogeneity. We assessed inconsistency based on the pooled estimates from the meta-analyses and their 95% confidence intervals or, when no meta-analysis had been performed owing to the small number of studies, based on the individual study point estimates and the 95% confidence interval overlap. We acknowledge that using multiple reference tests could also be a source of heterogeneity; however, most identified studies used a combination of both tests as reference, so it was not possible to perform additional analyses to assess the diagnostic accuracy of each index test across each reference tests with the available data. Moreover, reference tests were also, in principle,

heterogeneous over the years, particularly genetic testing with different panels used and new genes discovered, so heterogeneity due to reference test characteristics would have remained an issue.

Development of recommendations and proposed diagnostic terminology

Recommendations were developed based on the EtD tables and GRADE framework. These were circulated within PICO groups and then to the whole panel for comment. Two online meetings were held during which panel members considered the evidence and discussed and voted on proposed recommendations for diagnosis. All voting panel members agreed to all statements.

Results

4842 manuscripts were identified from the initial search. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flowchart in figure 1 details the screening process and exclusions. In total, 33 studies were included in the final qualitative evidence synthesis and 14 studies in the quantitative evidence synthesis for PICO1 and PICO2.

PICO1: Should patients suspected for PCD be tested with nNO in addition to TEM and/or genetics?

PICO1a Should patients suspected for PCD be tested with nNO measurement during velum closure (breath hold; exhaling against resistance) in addition to TEM and/or genetics?

Recommendation

The panel recommends the use of measurement of nNO (during velum closure) in addition to genetics and/or TEM for the diagnosis of PCD among patients suspected to have PCD (strong recommendation for the intervention, moderate certainty of evidence).

PICO1b Should patients suspected for PCD be tested with nNO measurement during tidal breathing in addition to TEM and/or genetics?

Recommendation

The panel suggests the use of measurement of nNO (during tidal breathing) in addition to genetics and/or TEM for the diagnosis of PCD among patients suspected to have PCD (conditional recommendation for the intervention, very low certainty of evidence).

Justification

The strong recommendation for nNO using vellum closure is based on high test accuracy for nNO measurements performed and the importance of accurate diagnosis (benefits) outweighing any potential undesirable effects. The conditional recommendation for tidal breathing is owing to the lower diagnostic accuracy of the identified studies.

Having evaluated the evidence, the TF also concluded the following (recommendation remarks):

1) A normal nNO result does not exclude PCD and therefore the test *should not* be used as a standalone diagnostic test.
2) A positive nNO test result can *support* a diagnosis of PCD if in concordance with other tests.
3) For younger patients (2–5 years old) unable to achieve velum closure, measurement during tidal breathing can be informative but has lower accuracy and more variability.
4) Equipment, measurement, reporting and interpretation should follow ATS and ERS technical standard guidance [40, 41].
5) Some patients with PCD have nNO levels above the recommended threshold for diagnosis, and other tests should be considered if the clinical suspicion of PCD remains.

Low nNO levels are described in most individuals with PCD compared to healthy and disease controls, although reasons for the low nNO remain uncertain. Measurement is noninvasive and relatively quick; thus, nNO measurement has been widely incorporated as part of the diagnostic pathway of PCD [42]. Measurement requires aspiration of gas from one nostril with gas entrained *via* the other nostril, following standardised methods, using a stationary chemiluminescence analyser during a velum closure manoeuvre (breath hold or oral exhalation against a resistance) [40, 41]. Chemiluminescence analysers are the standard device used to measure nNO levels and have been recommended by past ATS and ERS guidelines [23, 24]. These devices produce a real-time display of the NO signal, which is important for quality assurance. However, most preschool children are unable to co-operate with velum closure; therefore, tidal breathing measurements are often used instead. More recently, electrochemical analysers have been developed, and while discriminative nNO values are possible with electrochemical handheld NO devices,

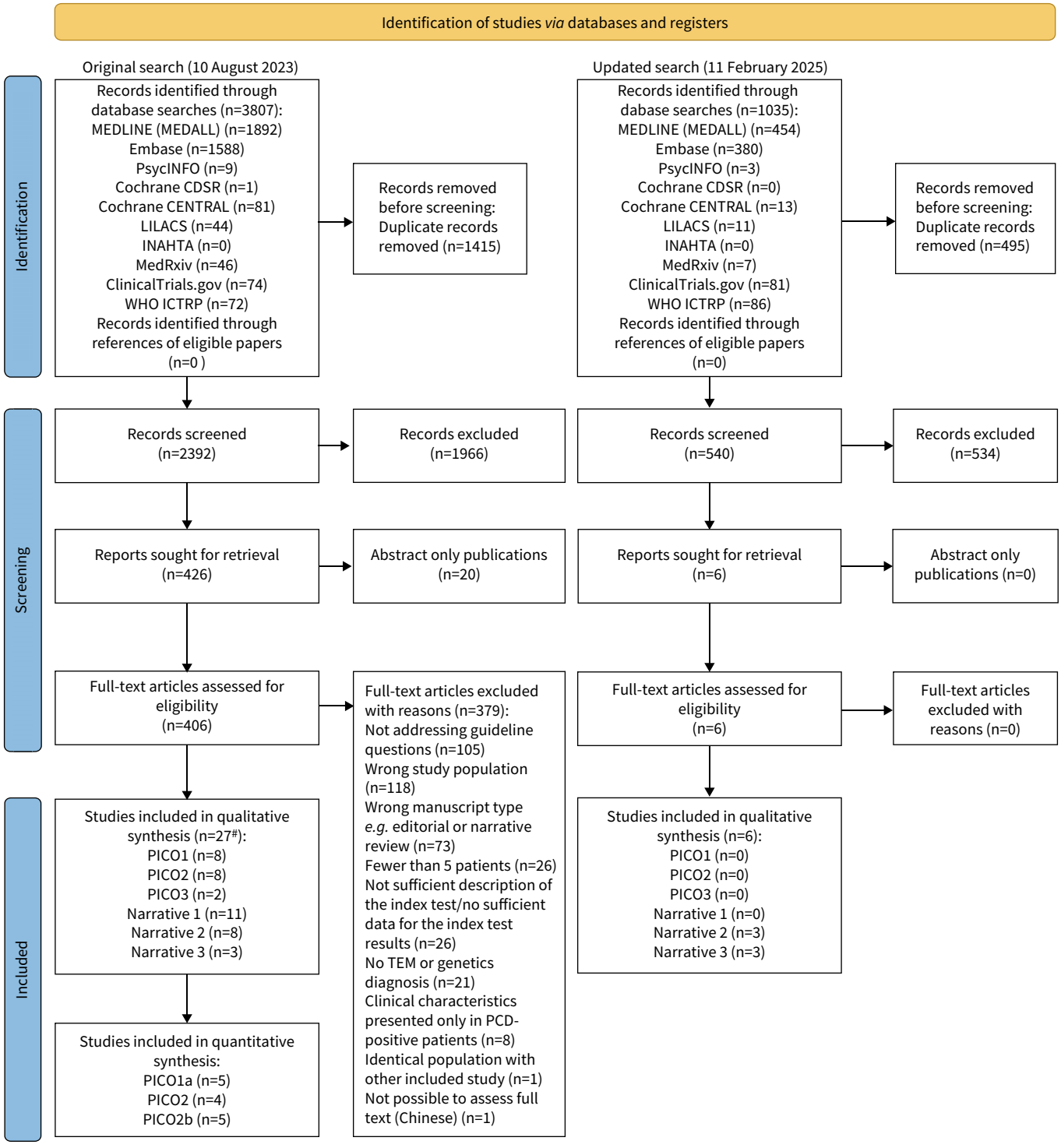

FIGURE 1 Literature screening and inclusion. The flow chart describes the screening process for published literature and included studies for all questions. CDSR: Cochrane Database of Systematic Reviews; CENTRAL: Cochrane Central Register of Controlled Trials; LILACS: Latin American and Caribbean Health Sciences Literature; INAHTA: International Network of Agencies for Health Technology Assessment; PCD: primary ciliary dyskinesia; TEM: transmission electron microscopy; WHO ICTRP: World Health Organization International Clinical Trials Registry Platform. #: each study might have been included in more than one question.

they do not have fully standardised operating procedures. Indeed, studies used to address this PICO question tested only chemiluminescence analysers. A recent ERS technical standard provides guidance for measuring nNO [40].

TABLE 1 Sensitivity and specificity of included studies in PICO1a (vellum closure studies) and pooled estimates of diagnostic accuracy

Study	Threshold ($nL \cdot min^{-1}$)	Breathing manoeuvre	Sensitivity (95% CI)	Specificity (95% CI)	PCD prevalence	Sample (n)
Leigh 2013 [43]	77	ER	0.986 (0.924–0.999)	0.75 (0.644–0.838)	0.46	155
Boon 2014 [44]	90	ER	0.895 (0.752–0.971)	0.835 (0.762–0.892)	0.214	177
Jackson 2016 [45]	30	BH	0.91 (0.760–0.980)	0.960 (0.930–0.980)	0.11	301
Coles 2020 [46]	77	ER or BH	0.8 (0.440–0.965)	0.862 (0.683–0.961)	0.147	34
Raidt 2022 [47]	77	ER	0.922 (0.873–0.957)	0.865 (0.785–0.924)	0.6	301
	Diagnostic OR (95% CI)		**Sensitivity (95% CI)**	**Specificity (95% CI)**	**LR+ (95% CI)**	**LR− (95% CI)**
Pooled estimates of diagnostic accuracy	88.63 (49.15–159.80)		0.926 (0.881–0.955)	0.876 (0.786–0.932)	7.49 (4.29–13.05)	0.008 (0.05–0.13)

PCD: primary ciliary dyskinesia; ER: exhalation against resistance; BH: breath hold; OR: odds ratio; LR+: positive likelihood ratio; LR−: negative likelihood ratio.

Review of evidence directly addressing PICO1

Measurements during tidal breathing are usually conducted in younger children and inherently provide lower levels of nNO with greater within-subject variability than measurements during velum closure. When reviewing the evidence, the TF considered measurement during velum closure (breath hold; exhaling against a resistance) (PICO1a) separately from measurement during tidal breathing (PICO1b). Eight studies met the inclusion criteria. Supplementary table 2 summarises the studies considered for PICO1a and 1b, and supplementary figures 1 and 2 present forest plots of the sensitivity and specificity of nNO of the included studies.

For PICO1a, five studies met the inclusion criteria (n=968 patients). All studies had no serious risk of bias. A meta-analysis confirmed that nNO measurement during velum closure is accurate as part of the diagnostic pathway for PCD, with a pooled sensitivity of 0.93 (95% CI 0.88–0.95) and specificity of 0.88 (95% CI 0.79–0.93) (table 1, figure 2). A sensitivity analysis using the standard cut-off value of 77 $nL \cdot min^{-1}$ resulting in similar pooled sensitivity (0.94, 95% CI 0.87–0.97) and specificity (0.83, 95% CI 0.77–0.87) estimates (supplementary table 3 and supplementary figure 3).

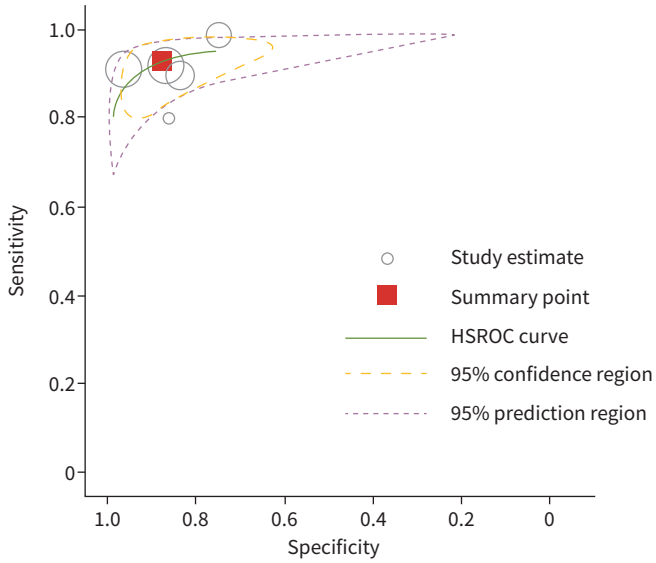

FIGURE 2 Meta-analysis of diagnostic accuracy for PICO1a. Figure shows the hierarchical summary receiver operating characteristic curve (HSROC) curve for the included vellum closure studies, with the 95% prediction and confidence regions.

For tidal breathing manoeuvres (PICO1b), three studies that met the inclusion criteria were identified (n=421 patients) without serious risk of bias. Measurement of nNO had a sensitivity of 0.77 to 1.00, suggesting that NO measurement using tidal breathing manoeuvres has a reduced ability to detect PCD when compared with measurement using velum closure manoeuvres, while specificity ranged considerably between 0.57 to 0.90. Therefore, NO measurement using tidal breathing manoeuvres risks a larger number of non-PCD patients being misclassified as having PCD (supplementary table 1, supplementary figure 2).

The studies that were reviewed were performed in consecutive patients referred for PCD diagnostic testing, and patients were likely to have a strong clinical history (good pre-test probability). False-positive diagnoses will be unacceptably high if the pre-test probability is lower than the study population described, therefore nNO measurement should only be performed in individuals with a compatible clinical history of PCD [48].

PICO2: Should patients suspected for PCD be tested with ciliary beat pattern assessment using HSVM in addition to TEM and/or genetics?

PICO2a: Should patients suspected for PCD be tested with ciliary beat pattern assessment of post-cell culture using HSVM in addition to TEM and/or genetics?

PICO2b: Should patients suspected for PCD be tested with ciliary beat pattern assessment of pre-cell culture using HSVM in addition to TEM and/or genetics?

Recommendation

The panel recommends ciliary beat pattern assessment using HSVM in addition to TEM and/or genetic testing to diagnose PCD (strong recommendation for the intervention, very low certainty of evidence).

Although the analyses for PICO2a and PICO2b were done separately, the panel decided on a joint recommendation.

Justification

The panel formulated a strong recommendation for HSVM in addition to TEM and/or genetics for patients referred for PCD diagnosis. The recommendation is based on the test performance and the importance of accurate diagnosis (benefits) outweighing any potential undesirable effects.

Despite the very low certainty of evidence about the test performance, HSVM is the only diagnostic test in which ciliary dyskinesia can be directly visualised and the primary nature determined by reproducibility of an abnormal finding after culture *in vitro*. In the absence of HSVM as part of the PCD diagnostic algorithm, PCD diagnoses will be missed, such as in patients with PCD who have normal nNO or in whom nNO cannot be performed accurately, *e.g.* patients under 5 years of age in conjunction with normal TEM (~30%) and/or incomplete genetic testing (~30%). This scenario has been described repeatedly in the literature during the previous years of gene discovery (dynein axonemal heavy chain 11 (*DNAH11*), HYDIN Axonemal Central Pair Apparatus Protein (*HYDIN*), radial spoke head component 1 (*RSPH1*)). Owing to 60% private mutations and many variants of unknown significance (VUS) on genetics testing, concordance between adjunct tests is needed to enable a diagnosis. HSVM shows strong genotype–phenotype correlations that are grounded in biological evidence for gene function (*e.g.* loss of dynein motor proteins results in ciliostasis). HSVM does not cause any known or expected decrease in longevity, nor are there reports of immediate serious complications or long-term rare serious adverse events. Samples are taken by brushing the inside of the nose and used for three different tests to assess ciliary structure and function to aid in the diagnosis of PCD. Other tests take many months to provide results, but HSVM provides real-time data. Any short-term minor side effects or inconvenience for the patient would be incurred by sampling for the reference and other adjunct tests because the same sample is used. Importantly, HSVM is used in combination with other diagnostic tests and is not a standalone test. Increased resource use is balanced by an accurate and early diagnosis, which reduces the need for additional testing. A patient with an unconfirmed diagnosis who has not undergone HSVM is likely to have to repeat the reference or other adjunct test at another time, or remain with a less certain diagnosis.

Having evaluated the evidence, the TF also concludes the following (recommendation remarks):

1) A normal HSVM result does not exclude PCD and therefore the test *should not* be used as a standalone diagnostic test.
2) A positive HSVM test result can *support* a diagnosis of PCD if in concordance with other tests.
3) Post-culture analysis should be used whenever possible, because pre-culture HSVM testing has lower specificity compared to post-culture analysis.

TABLE 2 Sensitivity and specificity of included studies in PICO2a (post-culture HSVM studies) and pooled estimates of diagnostic accuracy

Study	Sensitivity (95% CI)	Specificity (95% CI)	PCD prevalence	Sample (n)
Coles 2020 [46]	1 (0.478–1.00)	0.982 (0.901–1.00)	0.08	59
Jackson 2016 [45]	1 (0.923–1.00)	0.895 (0.857–0.926)	0.25	370
Papon 2012 [49]	0.700 (0.348–0.933)	1 (0.782–1)	0.40	25
Stannard 2010 [50]	0.925 (0.841–0.976)	0.976 (0.952–0.992)	0.21	340

	Diagnostic OR (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR− (95% CI)
Pooled estimates of diagnostic accuracy	625.81 (200.03–1957.84)	0.925 (0.709–0.984)	0.981 (0.912–0.9960)	47.98 (10.96–210.07)	0.08 (0.02–0.34)

HSVM: high-speed video microscopy; PCD: primary ciliary dyskinesia; OR: odds ratio; LR+: positive likelihood ratio; LR−: negative likelihood ratio

4) HSVM requires a high throughput of samples to gain and maintain the requisite expertise but is a very useful supportive diagnostic test in which phenotypical beat patterns can be attributed to genotype and a diagnosis. The panel *suggests limiting* the use of HSVM to centres with expertise in performing these tests and referring patients in need of HSVM testing to those specialist centres.

HSVM is the process of analysing, using light microscopy, the cilia beat pattern and frequency in airway epithelial cells immediately after taking nasal or bronchial brush biopsies or after regeneration of ciliated airway epithelia in cell culture. Specific features, including beat pattern (such as an effective forward and recovery ciliary beat or areas of stasis or rotation), amplitude, mucociliary clearance and frequency, are recorded. HSVM should not be limited to beat frequency.

Review of evidence directly addressing PICO2

Eight studies met the inclusion criteria. An analysis of four studies using post-culture data (PICO2a) demonstrated a sensitivity of 0.92 (95% CI 0.71–0.98) and specificity of 0.98 (95% CI 0.91–1.00) (table 2, figure 3a). An analysis of five studies using pre-culture data (PICO2b) demonstrated higher sensitivity of 0.98 (95% CI 0.94–0.99) and considerably lower specificity of 0.80 (95% CI 0.55–0.93) (table 3, figure 3b).

Turnaround time for HSVM on primary brush biopsy samples (without culture) in these studies was <8 h, while for cell culture samples, the time ranged from a few weeks to 2–3 months. The cost of HSVM using pre-culture samples is highly dependent on the facility performing such analysis and can range from a few

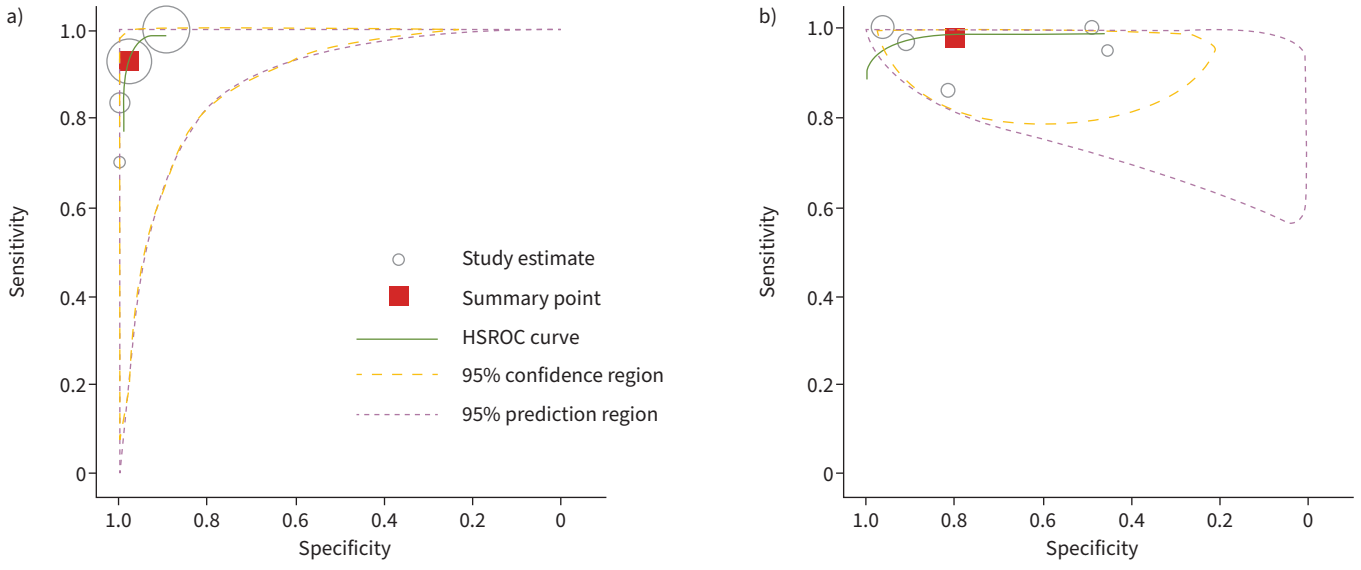

FIGURE 3 Meta-analysis of diagnostic accuracy for PICO2. Figure shows the hierarchical summary receiver operating characteristic curve (HSROC) curve for a) the included post-culture studies (PICO2a) and b) pre-culture studies (PICO2b), with the 95% prediction and confidence regions.

TABLE 3 Sensitivity and specificity of included studies in PICO2b (pre-culture HSVM studies) and pooled estimated of diagnostic accuracy

Study		Sensitivity (95% CI)	Specificity (95% CI)	PCD prevalence	Sample (n)
Baz-Redón 2020 [51]		1 (0.863–1)	0.490 (0.344–0.637)	0.34	74
Coles 2020 [46]		0.857 (0.421–0.996)	0.815 (0.686–0.908)	0.11	61
Guo 2020 [52]		0.951 (0.835–0.994)	0.456 (0.168–0.766)	0.79	52
Pifferi 2013 [53]		1 (0.877–1)	0.910 (0.831–0.960)	0.24	117
Rubbo 2019 [54]		1 (0.962–1)	0.964 (0.875–0.996)	0.63	360
	Diagnostic OR (95% CI)	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**LR+ (95% CI)**	**LR− (95% CI)**
Pooled estimates of diagnostic accuracy	189.57 (26.30–1366.15)	0.979 (0.937–0.993)	0.800 (0.547–0.931)	4.92 (1.86–13.01)	0.026 (0.01–0.09)

HSVM: high-speed video microscopy; PCD: primary ciliary dyskinesia; OR: odds ratio; LR+: positive likelihood ratio; LR−: negative likelihood ratio.

USD to several hundred USD per sample. Analysis of cultured cells has the added costs of culture media and the technical time to care for cells and can range from several hundred to a thousand USD per sample. There is also an added risk of culture failure, requiring re-growing cells, because this test requires considerable technical expertise.

HSVM currently does not have well-established guidelines, which may result in a degree of subjectivity in assessing results.

PICO 3: Should patients suspected for PCD be tested with IF in addition to TEM and/or genetics?

Recommendation

The panel recommends IF in addition to TEM and/or genetic testing to diagnose PCD (strong recommendation for the intervention, high certainty of evidence).

Justification

The strong recommendation is based on the high diagnostic accuracy of the test and the importance of accurate diagnosis (benefits) outweighing any potential undesirable effects.

Having evaluated the evidence, the TF also concludes the following (recommendation remarks):

1) A normal IF result does not exclude PCD and therefore the test *should not* be used as a standalone diagnostic test.
2) A positive test result can *support* a diagnosis of PCD if in concordance with other tests.
3) To improve diagnostic accuracy of IF, and avoid a high proportion of inconclusive results, sample collection, processing and analysis should be performed in a laboratory with experience performing this test. Moreover, the accuracy of IF is dependent on the antibody panel chosen and the quality of the used antibodies. Furthermore, batch-to-batch variability between antibodies is common and may affect the accuracy of the results if performed by an inexperienced laboratory.
4) IF may provide additional evidence for the diagnosis in cases associated with genetic VUS.

IF refers to a test whereby fluorescently tagged antibody staining is used to identify absent or mis-localised protein expression in airway cilia, typically obtained from nasal brushing samples of patients. A panel of antibodies may be employed to directly or indirectly target ciliary structural defects caused by most known PCD-associated genes [55].

Review of evidence directly addressing PICO3

Two studies met the inclusion criteria, and both were deemed to have a low risk of bias [51, 56]. Shoemark *et al.* [56] used a panel of six antibodies in 277 patients evaluated for PCD in the UK and reported a sensitivity of 0.88. Baz-Redón *et al.* [51] reported a sensitivity of 0.84 in 47 patients referred for evaluation of PCD (including only patients with reference standard testing) in Spain (table 4). The results of the studies could not be pooled. Neither study included an antibody against DNAH11 on the panel, which could explain many of the false-negative PCD cases. Both studies had a specificity of 1.00, while insufficient samples and inconclusive results ranged from 8% to 27% [51, 56]. One report calculated a median turnaround time for IF of 14 days (range 1–40 days), compared to 27 days (range 9–61 days) for TEM. The cost of IF was 187 USD per sample and was substantially lower than 1452 USD for TEM [56].

TABLE 4 Sensitivity and specificity of included studies in PICO3 (immunofluorescence)

Study	Sensitivity (95% CI)	Specificity (95% CI)	PCD prevalence	Sample (n)
Baz-Redón 2020 [51]	0.84 (0.639–0.955)	1.00 (0.846–1.00)	0.28	74
Shoemark 2017 [56]	0.88 (0.688–0.975)	1.00 (0.986–1.00)	not described	386

PCD: primary ciliary dyskinesia.

IF provided timely and accurate diagnostic results for many patients evaluated for PCD and the included studies did not report adverse events or test burden. Targeted IF may have utility in resolving genetic uncertainty associated with VUS, particularly for genes associated with normal or near normal TEM (*e.g.* pathogenic variants in *DNAH11* or *HYDIN*) [57, 58]. In the case of *HYDIN*, TEM may appear near normal while genetic analysis is often confounded by the pseudogene *HYDIN2*. IF can show absent localisation of sperm flagellar 2 (SPEF2) and aid in the diagnosis of PCD caused by pathogenic variants in *HYDIN* [58]. Confirming the specific genetic cause of PCD may establish patient eligibility for future precision therapies.

Summary of results

Box 1 shows a summary of the recommendations from the three PICO questions.

The panel voted and reached a 100% agreement that taken together the results can be summarised as follows:

- nNO, HSVM and IF staining are all valuable in PCD testing and could all be included in a PCD diagnostic testing algorithm.
- No single test has 100% specificity and sensitivity, which necessitates the use of multiple tests in a diagnostic approach.
- There is no evidence for using any single test in a specific order; however, there may be practical considerations to start with one test compared to others.
- When diagnosis cannot be confirmed using genetics or TEM, additional tests are needed. Not all tests are required in all circumstances. However, limiting the number of tests conducted may impact the diagnostic accuracy.
- The quality and technique of the test conducted are critical. Tests should be performed to meet a minimum standard (for guidance see PICO text). More extensive tests will be more accurate (*e.g.* a 10-antibody *versus* 2-antibody panel for IF).
- Referral to an expert centre for diagnosis should be conducted whenever possible.

Narrative questions

The TF members identified three areas that are of high importance for PCD diagnosis, including clinical symptoms associated with PCD, access to testing in resource-limited areas and emerging diagnostic tools. These questions were chosen by consensus. Published papers pertaining to these questions were identified during the screening process [59]. Following ERS methodology, no risk of bias assessment or GRADE approach is required for narrative questions [59].

Narrative 1: What are the clinical manifestations in the newborn period, childhood and adulthood driving a decision to refer a patient for PCD diagnostic testing?

Commonly reported characteristics include neonatal respiratory distress in a term infant, a persistent wet cough from early childhood, year-round rhinosinusitis, serous otitis media and hearing loss, bronchiectasis, infertility/subfertility and laterality/heterotaxy defects. A systematic review and meta-analysis confirmed that clinical symptoms of individuals with PCD are highly variable, reflecting true heterogenicity as well as methodological biases [2].

A total of 11 studies met the inclusion criteria and were considered to address narrative question 1. Supplementary table 12 shows a summary of the main findings of these studies.

PCD symptoms are nonspecific, and recognising the pattern of symptoms is key. Two symptom-based tools can be used to support non-expert identification of individuals likely to have the condition. Behan *et al.* [60] analysed the symptoms of consecutive paediatric and adult referrals to a PCD reference centre and compared these to the diagnostic outcome. The resulting algorithm led to a seven-point questionnaire-based

BOX 1 Summary of recommendations from PICO questions

Summary of recommendations from PICO questions
Nasal nitric oxide (nNO)
Having evaluated the evidence, the Task Force recommends:
1) nNO during velum closure can be used in addition to transmission electron microscopy (TEM) and/or genetics to diagnose primary ciliary dyskinesia (PCD) (strong recommendation, moderate certainty of evidence).
2) nNO during tidal breathing can be used in addition to TEM and/or genetics to diagnose PCD (strong recommendation, moderate certainty of evidence).
The Task Force also concluded:
1) A normal nNO result does not exclude PCD and therefore the test *should not* be used as a standalone diagnostic test.
2) A positive nNO test result can *support* a diagnosis of PCD if in concordance with other tests.
3) For younger patients (2–5 years old) unable to achieve velum closure, measurement during tidal breathing can be informative but has lower accuracy and more variability.
4) Equipment, measurement, reporting and interpretation should follow American Thoracic Society and European Respiratory Society technical standard guidance [40, 41].
5) Some patients with PCD have nNO levels above the recommended threshold for diagnosis, and other tests should be considered if a clinical suspicion of PCD remains.
High-speed video microscopy (HSVM)
Having evaluated the evidence, the Task Force recommends:
1) HSVM can be used in addition to TEM and/or genetics to diagnose PCD (strong recommendation, very low certainty of evidence).
The Task Force also concluded:
1) A positive HSVM test result can *support* a diagnosis of PCD if in concordance with other tests.
2) Post-culture analysis should be used whenever possible, because pre-culture HSVM testing has lower specificity compared to post-culture analysis.
3) HSVM requires a high throughput of samples to gain and maintain the requisite expertise but is a very useful supportive diagnostic test where phenotypical beat patterns can be attributed to genotype and a diagnosis. The panel *suggests limiting* the use of HSVM to centres with expertise in performing these tests and referring patients in need of HSVM testing to those specialist centres.
Immunofluorescence (IF)
Having evaluated the evidence, the Task Force recommends:
1) IF can be used in addition to TEM and/or genetics to diagnose PCD (strong recommendation, high certainty of evidence).
The Task Force also concluded:
1) A normal IF result does not exclude PCD and therefore the test *should not* be used as a standalone diagnostic test.
2) A positive test result can *support* a diagnosis of PCD if in concordance with other tests.
3) To improve diagnostic accuracy of IF, and avoid a high proportion of inconclusive results, sample collection, processing and analysis should be performed in a laboratory with experience performing this test. Moreover, the accuracy of IF is dependent on the antibody panel chosen and the quality of the used antibodies. Furthermore, batch-to-batch variability between antibodies is common and may affect the accuracy of the results if performed by an inexperienced laboratory.
4) IF may provide additional evidence for the diagnosis in cases associated with genetic variants of unknown significance.
Other conclusions
1) nNO, HSVM and IF staining are all valuable in PCD testing and could all be included in a PCD diagnostic testing algorithm.
2) No single test has 100% specificity and sensitivity, which necessitates the use of multiple tests in a diagnostic approach.
3) There is no evidence for using any single test in a specific order; however, there may be practical considerations to start with one test compared to others.
4) Not all tests are required in all circumstances. However, limiting the number of tests conducted may impact the diagnostic accuracy.
5) The quality and technique of the test conducted are critical. Tests should be performed to meet a minimum standard (for guidance see PICO text). More extensive tests will be more accurate (*e.g.* a 10-antibody *versus* 2-antibody panel for IF).
6) Referral to an expert centre for diagnosis should be conducted whenever possible.

tool, the PrImary CiliARy DyskinesiA Rule (PICADAR), which can statistically predict those most likely to have a positive diagnosis (the score can be found in supplementary table 15). For individuals with a persistent cough that started in early childhood, PICADAR provides a score based on neonatal chest symptoms at term, admission to a neonatal intensive care unit, situs anomalies, congenital heart defect, persistent perennial rhinitis and chronic ear or hearing problems. Leigh *et al.* [61] used a combination of symptoms to develop a score based on expert-predetermined questions. They found that the combination of unexplained neonatal respiratory distress, early-onset year-round wet cough, early-onset year-round nasal congestion and laterality defects was most useful to distinguish PCD patients from others. Scores that are modified based on the age of patients are needed, but age-stratified studies are still lacking.

Patients with situs inversus, which is rare in the general population, are diagnosed earlier than symptomatic individuals with normal situs. The two symptom-based tools are more likely to identify those with laterality defects, yet an increasing number of genes are not associated with situs abnormalities [62]. Furthermore, abnormal motile cilia function is increasingly recognised in people with airway symptoms

who have variants in genes previously known to cause syndromic ciliopathies. These individuals may have additional clinical characteristics such as retinitis pigmentosa and skeletal abnormalities.

In summary, healthcare professionals should have a high suspicion for individuals with symptom patterns typical of PCD. Increased awareness of PCD symptoms has the potential to facilitate earlier diagnosis in most cases. Tools such as PICADAR and the ATS clinical criteria can assist physicians looking for these patterns. However, the clinical spectrum of PCD is broadening and physicians need to be aware that patients may present with atypical symptoms.

Narrative 2: What additional diagnostic tests could be useful for diagnosing PCD?

Additional diagnostic tools can be considered to further improve diagnostic accuracy in PCD, and these tests can be broadly categorised into genetic and clinical tests.

A total of 11 studies met the inclusion criteria and were considered to address narrative question 2. Supplementary table 13 shows a summary of the main findings of these studies.

In the clinical setting, genetic testing typically relies on commercial gene panels that consist of known PCD disease-associated genes or custom next-generation sequencing panels. To improve variant identification, additional techniques can be used, such as long-read sequencing [63]. Addition of whole exome sequencing or whole genome sequencing (WGS) can identify PCD genes that are not yet included in these commercial panels [64–68]. The main differences between these two techniques are that WGS is better suited for detecting structural and noncoding variants but is costlier and can have reduced read depth, thus data quality in the exons can be reduced. Both techniques can detect splicing-related defects, including deep intronic variants in WGS, but the determination of pathogenicity relies on prediction tools. Additionally, identifying potential deletions and duplications is challenging given the number of such events in WGS. Expanded genetics may identify non-PCD variants. Pre-test and post-test genetic counselling may be required.

RNA-sequencing in blood or epithelial cells is another technique that, when used in conjunction with DNA testing, can detect deep intronic splice mutations, validate the effect of splicing from a potential splice defect and indirectly identify a promoter deletion, or nonsense-mediated mRNA decay, by showing loss of heterozygosity or loss of expression (>50%) [69, 70]. This approach requires considerable expertise and a dedicated bioinformatics pipeline.

Another test that could have clinical applications is pulmonary radio-aerosol mucociliary clearance (PRMC) [71, 72]. This functional imaging test assesses pulmonary mucociliary clearance efficiency by measuring the rate of inhaled radiolabelled aerosol clearance from the tracheobronchial tree. Recent PRMC studies found this test to be highly sensitive and specific for diagnosing PCD, but it requires patient cooperation, skilled technicians and standardised protocols.

Finally, several studies found during the literature review described variations of the saccharin test as a diagnostic tool in PCD. However, these studies were excluded. Past research has shown that the saccharin test is an unreliable diagnostic test with low sensitivity and specificity, due to technical challenges in children, non-standardised test protocols and inconsistent result interpretation [73]. The TF members never use saccharin testing as part of a PCD diagnosis approach. Future evolving tools may include artificial intelligence for data collection and analysis for HSVM, IF and TEM in combination with clinical data, to remove some of the subjectivity of these tests.

Narrative 3: Overcoming PCD diagnostic challenges in resource-limited settings: what strategies work?

Given the complexity and multifaceted approach to PCD diagnosis, resource-limited settings present additional challenges and obstacles in making the diagnosis, because expertise in PCD and specific PCD diagnostic tests vary depending on country and region. We defined resource-limited settings as those characterised by a lack of funds to cover healthcare costs, on an individual or societal basis, leading to limited access to medication, equipment, supplies, devices and/or expertise for specific diseases (*i.e.* PCD).

Our search identified 15 studies, of which six studies met inclusion criteria and addressed diagnostic strategies in resource-limited settings [51, 60, 74–77]. Supplementary table 14 shows a summary of the main findings of these studies. Symptom-based tools such as PICADAR (supplementary table S15) or the scoring tool by Leigh *et al*. [61] described above offer inexpensive, suitable tools for use in a low-resource setting to guide referral practices for PCD diagnostic testing for those patients with a high likelihood of having PCD. The PICADAR score was used in a study in a resource-limited setting in Egypt alongside other clinical prediction scores (North America Criteria Defined Clinical Features (NA-CDCF), the Clinical

Index Score (CI)). The PICADAR showed similar predictive values as the original study but requires validation in other settings outside the UK [77].

Two studies highlight the importance of national/international collaboration for PCD diagnosis [51, 74]. Baz-Redón *et al.* [51] evaluated a Spanish population of 74 people with suspected PCD using a panel of four fluorescently labelled antibodies (DNAH5, dynein axonemal light intermediate chain 1 (DNALI1), growth arrest-specific 8 (GAS8) and RSPH4A or RSPH9). While the majority of patients were evaluated at one central hospital (Hospital Universitari Vall d'Hebron), patients and samples from 17 other hospitals or research institutes in Spain were evaluated at the reference centre, exemplifying a quick, accessible and low-cost strategy for PCD diagnosis. This test, however, as described in PICO3, is not suitable as a standalone test. Birkhead *et al.* [76] described reliance on TEM alone, highlighting 60% of referred patients who did not receive a definitive positive or negative diagnosis with that strategy.

Rumman *et al.* [74] collaborated with TEM and genetics experts at the University of Southampton and University College London to investigate data from 464 Palestinian children and adults with a compatible PCD clinical phenotype, performing ultrastructural analysis and genetic testing, and diagnosed 68 individuals with PCD. The authors described use of nNO measured by an electrochemical device along with clinical history to help prioritise further testing. They described several barriers to PCD diagnostic testing, including inadequate funding for genetic testing, an insufficient healthcare system infrastructure, and political restrictions and/or blockades limiting patient access to care.

Gatt *et al.* [75] showed the effectiveness of familial testing for variants in a consanguineous Bedouin population, before widening to a panel and whole exome sequencing approach. In populations with common genes or founder effects, identifying the most common genes/mutations and performing local targeted mutation testing for relatives or people from the same geographical region as the reference cases may be cost effective [75, 78].

Strategies to circumvent barriers to PCD diagnosis in resource-limited settings include collaboration with established PCD centres for procedural training (nasal ciliary biopsy, nNO testing), diagnostic expertise (TEM, HSVM and IF) and access to funding mechanisms for expanded genetic testing. Additionally, networks such as the BEAT-PCD, ERN-LUNG and the Genetic Disorders of Mucociliary Clearance Consortium, and patient foundations including the North American PCD Foundation and PCD Support UK, can help with the diagnosis and management of PCD by supporting local physicians. There is a knowledge gap for PCD testing in resource-limited areas; epidemiological studies/surveys are needed to evaluate the barriers and knowledge gaps among physicians in low-resource countries, and existing tools and innovative diagnostic solutions need validation.

Proposed implementation of the guideline into clinical practice

On review of the evidence and conclusions from the PICO questions, narrative questions and clinical experience, and over a series of in-person and online meetings, the TF has agreed on the proposed algorithm (figure 4). Findings from the PICO questions are summarised in box 1.

Patients suspected of PCD should be referred to a diagnostic centre whenever possible. If this is not feasible, practitioners should consult with a regional or referral PCD centre for cases that cannot be confirmed using a reference test.

Confirming a diagnosis of PCD

To confirm a diagnosis of PCD, a clinical history or symptoms consistent with PCD should first exist (see narrative question 1 for details). The TF recommends the use of ATS clinical criteria, PICADAR or clinical criteria from the ERS adult bronchiectasis guidelines [24, 60, 61, 79]. In addition to compatible symptoms, and in line with the reference tests for this guideline, either 1) a positive genetic test or 2) a TEM class 1 defect should be identified [27].

A positive genetic test is defined as pathogenic or likely pathogenic variants (ACMG criteria) found in a known PCD gene following the known inheritance pattern (*e.g.* bi-allelic where autosomal recessive, hemizygous where X-linked, and mono-allelic where autosomal dominant). A known PCD-associated gene is defined as one with definitive or strong evidence for disease according to the ClinGen motile ciliopathy list. A genetic diagnosis is strongly encouraged whenever possible because of genotype–phenotype differences in prognosis and potential future gene-specific therapeutic options.

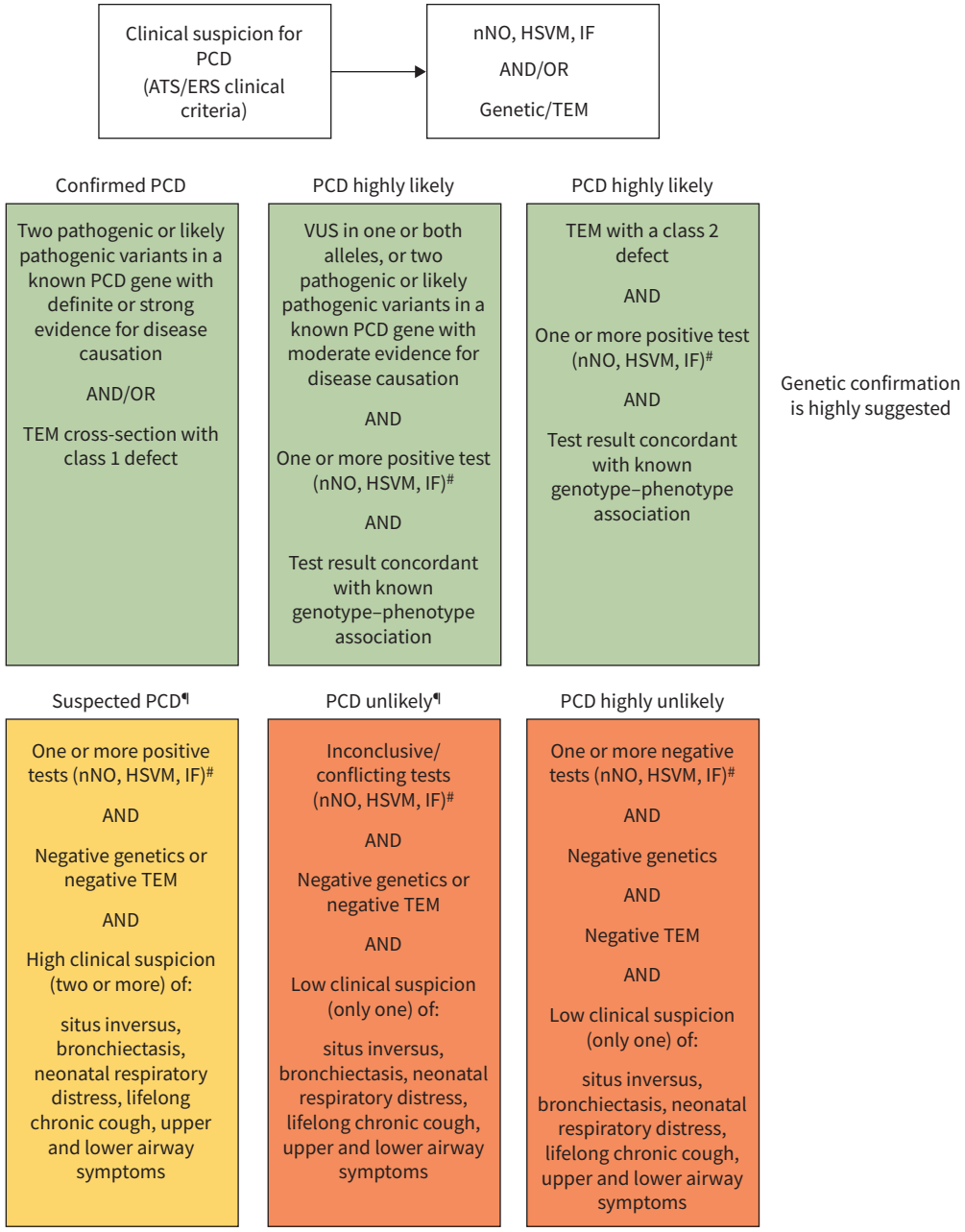

FIGURE 4 Graphical presentation of proposed algorithm for the diagnosis of primary ciliary dyskinesia (PCD). Based on assimilation on evidence from PICO/narrative questions and clinical expertise of the panel. Individuals should have supporting clinical symptoms to pursue additional diagnostic testing (reference or adjunct). Designations of "confirmed PCD" (green), "PCD highly likely" (green), "suspected PCD" (yellow), "PCD unlikely" (orange) and "PCD highly unlikely" (orange) are proposed. ATS: American Thoracic Society; ERS: European Respiratory Society; nNO: nasal nitric oxide; HSVM: high-speed video microscopy; IF: immunofluorescence; TEM: transmission electron microscopy; VUS: variants of unknown significance. #: HSVM, nNO and IF were assessed in the PICO questions of this guideline; ¶: patients should be re-rested in future visits and as updated tests become available.

Excluding a diagnosis of PCD

Based on the evidence-based recommendations presented in the guideline, the TF considers the following when excluding a diagnosis of PCD:

1) A multiple test approach is required in the evaluation and exclusion of PCD, because no single test has sufficient sensitivity or specificity to be a standalone test, as this guideline has shown. Therefore, no single test should be used to exclude a diagnosis of PCD (including index or reference tests).
2) In individuals with symptoms suggestive of PCD, a combination of negative tests makes PCD less likely, but the diagnosis cannot be eliminated. A negative test is defined as: nNO >77 $nL \cdot min^{-1}$, normal forward and recovery stroke of cilia on HSVM, normal IF panel, a negative genetic panel of known PCD genes and normal TEM. Diagnostic accuracies are shown in tables 1–4. Those patients that have multiple negative tests can be designated as "highly unlikely PCD".
3) It is important to emphasise that it is not possible to rule out PCD in a patient with very high clinical suspicion (*e.g.* situs inversus totalis with bronchiectasis and neonatal respiratory distress with lifelong chronic upper and lower airway symptoms) compared to those with a low clinical suspicion. Patients with a high clinical suspicion of PCD yet negative tests (index and reference) should be designated as "suspected PCD" and re-evaluated during future visits.
4) The quality and technique of the test conducted are critical. Tests should be performed to a minimum standard (for guidance refer to the corresponding text for each PICO question). More extensive tests will be more accurate in evaluating individuals for PCD. For instance, an extensive genetic test such as whole exome sequencing is more accurate than a 30-gene panel, which in turn is more accurate than a 10-gene panel.
5) More extensive testing for alternative diagnoses (*e.g.* cystic fibrosis and primary immunodeficiency) should be considered during the evaluation of individuals for PCD.
6) In a patient with a low clinical suspicion for PCD, the sensitivity and specificity of testing is lower, and the value of testing should be considered in the context of the overall evaluation. Not all tests (reference or index) are required to rule out the diagnosis in someone with low clinical suspicion, and the clinician may choose to use one or no tests when clinical suspicion is very low.

Inconclusive test results

Inconclusive tests for PCD include: 1) VUS in one or both alleles of a known PCD-associated gene, 2) pathogenic or likely pathogenic variants in genes designated as moderate evidence on ClinGen, 3) TEM results with class 2 defects.

In patients with a clinical suspicion for PCD, with VUS in one or both alleles, or pathogenic or likely pathogenic variants in genes with moderate evidence, a diagnosis of *PCD highly likely* can be made if there is a positive adjunct test (TEM class 2 defect, positive HSVM post-culture or positive IF test) which is concordant with the clinical history and known genotype and phenotype associations.

In patients with clinical suspicion for PCD who have class 2 TEM defects, a diagnosis of *PCD highly likely* can be made if there is a positive adjunct test (IF or HSVM) which is concordant with the clinical history and known genotype and phenotype associations.

Patients with a strong clinical history of PCD and abnormal adjunct tests, but for whom no reference tests are available or the results are inconclusive or normal, should be designated as *suspected PCD*, *e.g.* someone who has characteristic clinical features of PCD with low NO and persistent HSVM abnormalities when assessed post-cell culture, despite negative genetic testing and TEM. The TF suggests that these individuals be followed in a PCD expert setting and treated accordingly. The diagnosis of PCD should be reviewed regularly as tests become available or accessible, as diagnostic tests improve over time or as new gene variants are associated with PCD. Additionally, the TF encourages referral to research centres involved in PCD gene discovery.

Concordance between tests is important. If there is a lack of concordance between tests or tests are inconclusive, these tests can be repeated during future visits.

Discussion

In summary, this joint ERS/ATS TF recommends that, in a person with a supporting clinical suspicion, a PCD diagnosis can be *confirmed* by identifying a class I defect using TEM or pathogenic or likely pathogenic variants in a genetic test of a gene associated with PCD. nNO, HSVM and IF staining are all valuable in PCD diagnostic testing and could all be included in a PCD diagnostic testing algorithm. No single test has 100% specificity and sensitivity, which necessitates the use of multiple tests in the diagnostic approach. There is no evidence for using any single test in a specific order; however, there may be practical considerations to start with one test compared to others. Not all tests are required in all circumstances. Limiting the number of tests conducted may affect the diagnostic accuracy. No adjunct test is suitable as a standalone test to diagnose PCD and no single adjunct or reference test is suitable to

exclude PCD when symptoms are highly suggestive of PCD. The quality and technique of the tests are critical. Tests must be performed to a minimum standard (for guidance see PICO text). More extensive tests will be more accurate (*e.g.* a 10-antibody *versus* a 2-antibody panel for IF). Referral to an expert centre for diagnosis should be conducted whenever possible.

Patients receiving an early diagnosis have better lung function and lower rates of pulmonary exacerbation [80]. Early and accurate diagnosis is also a priority for patients and was considered highly important by patient and parent panel members as well as the broader patient community.

The ERS/ATS TF proposes using the term *suspected PCD* for individuals who have clinical features of PCD and positive adjunct tests (post-culture HSVM, nNO, IF) if genetic or TEM confirmation cannot be made using current approaches.

A genetic diagnosis is highly encouraged due to genotype–phenotype differences in prognosis and potential future gene-specific therapeutic options. Genetic panels include a variable number of genes. Although the TF did not examine the specificity and sensitivity of the number of genes included on genetic panels, the TF proposes using the most comprehensive tests available whenever feasible.

The TF used a stringent methodological approach to put forth an international guideline which will allow a united diagnosis of PCD wherever in the world the patient presents. This will aid access to care and inclusion for future clinical trials. The TF included a balanced number of representatives from the American and European professional respiratory societies, representation of people with PCD or families affected by PCD, methodologists, clinicians, diagnostic scientists, geneticists from established centres and representation from countries with different economic models of PCD testing. Consideration was given to different clinical situations and improvement in genomics.

There are some weaknesses to the guidelines. Because PCD is a rare disease, the evidence remains limited for most tests. It is also a heterogenous condition with many genes. Diagnostic testing likely performs differently for different genotypes and therefore results may not be accurate for rare genes or some gene variants (some genes only affect a small number of people).

The sensitivity and specificity of all tests depends on the quality and expertise of the laboratories interpreting these tests. Therefore, the accuracy of well-established tests may be reduced if performed by laboratories that do not perform them routinely (such as TEM, IF, HSVM and genotyping) or in clinical settings that do not have expertise in interpreting results. Furthermore, although we assessed adjunct tests individually, the power of combined index tests was not addressed. Owing to incomplete disease understanding, the reference standard is an imperfect combination of TEM and genetics with varying numbers of genes, thus estimates used to inform the recommendations may have been biased by the reference standard.

PCD research is dynamic, with new findings including novel genes associated with PCD continuously discovered. Understanding the role of new findings is critical in interpreting test results, especially in suspected PCD cases and when index tests or reference tests are inconclusive.

Areas for future research

With the improvements in genetic testing, large-scale genetic projects will be needed to understand the true prevalence of PCD. Indeed, at the time of writing of these guidelines, an estimated 30% of patients who could be classed as “PCD highly likely” do not have a definitive genetic diagnosis despite testing [29, 80]. Research is needed to identify PCD-associated genes and improve the accuracy of genetic tests. Novel genetic tools, like WGS, long-read DNA sequencing and RNA-sequencing analyses, have high promise in diagnosis but they need to be examined in prospective cohorts to determine their specificity, sensitivity and applicability in clinical settings. The TF also identifies the role of using next-generation sequencing in place of panel-based tests to improve specificity, sensitivity and accuracy as an area of needed research.

Access to testing remains limited in many areas due to barriers imposed by insurers, limited access to expert clinical settings or limited access to technology. Overcoming these barriers is important to improve diagnosis and, ultimately, care for patients. We propose epidemiological studies and international networks to further understand these barriers so they may be overcome.

PCD is a lifelong condition that presents early in life. Delayed diagnosis has implications on the health of affected individuals, which can lead to significant morbidity. The role of newborn screening using genetic tools to diagnose PCD is identified by the TF as an area of needed research.

While many tests are in clinical use, there is inter-test variability and lack of standardisation in some tests. Despite widespread use, there are still many gaps in our current knowledge regarding the use of electrochemical analysers in the diagnostic work-up of PCD. Standardised procedures and reference data are needed. The TF concludes that larger, well-conducted diagnostic studies for all tests are required and that standardisation of HSVM and IF are areas of needed research.

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