AMERICAN THORACIC SOCIETY DOCUMENTS

Diagnosis of Primary Ciliary Dyskinesia

An Official American Thoracic Society Clinical Practice Guideline

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Background: This document presents the American Thoracic Society clinical practice guidelines for the diagnosis of primary ciliary dyskinesia (PCD).

Target Audience: Clinicians investigating adult and pediatric patients for possible PCD.

Methods: Systematic reviews and, when appropriate, meta-analyses were conducted to summarize all available evidence pertinent to our clinical questions. Evidence was assessed using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach for diagnosis and discussed by a multidisciplinary panel with expertise in PCD. Predetermined conflict-of-interest management strategies were applied, and recommendations were formulated, written, and graded exclusively by the nonconflicted panelists. Three conflicted individuals were also prohibited from writing, editing, or providing feedback on the relevant sections of the manuscript.

Results: After considering diagnostic test accuracy, confidence in the estimates for each diagnostic test, relative importance of test results studied, desirable and undesirable direct consequences of each diagnostic test, downstream consequences of each diagnostic test result, patient values and preferences, costs, feasibility, acceptability, and implications for health equity, the panel made recommendations for or against the use of specific diagnostic tests as compared with using the current reference standard (transmission electron microscopy and/or genetic testing) for the diagnosis of PCD.

Conclusions: The panel formulated and provided a rationale for the direction as well as for the strength of each recommendation to establish the diagnosis of PCD.

Keywords: primary ciliary dyskinesia; Kartagener syndrome; situs inversus; nitric oxide; diagnosis

Contents

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Goals of This Guideline

The purpose of this guideline is to analyze evidence and present diagnostic recommendations for primary ciliary dyskinesia (PCD). The guideline should empower clinicians to interpret these recommendations in the context of the individual patient and make appropriate clinical decisions about diagnostic tests. For each recommendation, it is important to consider both the summary of evidence reviewed and discussed by members of the committee, especially patient values and preferences, before applying these recommendations to specific clinical situations or policy decisions.

Clinicians, patients, and other stakeholders should never view these recommendations as dictates. No guideline can account for all clinical circumstances. The implications of the strength of the recommendations are described in Table 1.

This guideline applies the same reference standard for all clinical questions, but it does not necessarily provide recommendations for one diagnostic test over another or advocate for or against combinations or sequential tests. However, a suggested diagnostic algorithm for PCD is provided as part of this document. Strong or conditional ratings for each recommendation must be weighed individually (i.e., two recommendations with the same strong or conditional rating should not by default be considered equivalent recommendations), factoring in all components used to determine the grade of the recommendation, including the confidence in accuracy estimates of each diagnostic test; the relative importance of test results studied; desirable and undesirable consequences of each diagnostic test; and the cost, feasibility, acceptability, and implications of each diagnostic test. The methods used by guideline panels to appraise the evidence are different from those employed during regulatory agency reviews of applications seeking market approval.

From the outset of guideline development, the workgroup made certain assumptions. First, with PCD being a heterogeneous disease, no reference diagnostic standard is universally accepted. Thus, the workgroup proposed the combination of transmission electron microscopy (TEM) ultrastructural ciliary defect and/or genetic panel testing for mutations in known PCD genes as the most accurate "reference standard" for diagnosing PCD. Second, per the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach, the effects of diagnostic test results on patient-important clinical outcomes must be assessed to develop recommendations. With PCD being a rare disease, frequently misdiagnosed in past cohorts, and managed with a wide range of unproven therapies, the workgroup considered that long-term effects of appropriate/inappropriate diagnostic decisions would make modeling of diagnostic results imprecise. Thus, the workgroup decided to rank the importance of the test results, patient-important outcomes, and overall certainty in the evidence of effect of the test separately from the certainty in diagnostic test accuracy.

Introduction

PCD is a genetically heterogeneous, autosomal recessive disorder characterized by motile cilia dysfunction. Clinical manifestations of PCD include chronic upper and lower airway disease, left–right laterality defects, and infertility (1–4). The diagnosis is often delayed, even in children who have characteristic clinical features of PCD, in part related to limitations of available diagnostic tests. For over four decades, the diagnosis of PCD has been based on the presence of ultrastructural defects in the ciliary axoneme using TEM analysis, which can have serious drawbacks. Nonspecific ciliary changes, which can be induced by exposure to environmental pollutants or infection, may appear similar as visualized by TEM to findings seen in PCD. Also, the absence of axonemal defects does not exclude PCD, because 30% of all affected individuals have normal ciliary ultrastructure (5). Other diagnostic tests have emerged, including nasal nitric oxide (nNO) measurement, genetic testing, digital high-speed video microscopy with ciliary beat pattern analysis (HSVM), and immunofluorescence imaging for specific

Table 1. Interpretation of Strong and Conditional Recommendations for Stakeholders (Patients, Clinicians, and Healthcare Policy Makers)

Implications for	Strong Recommendation	Conditional Recommendation
Patients	Most individuals in this situation would want the recommended course of action and only a small proportion would not.	The majority of individuals in this situation would want the suggested course of action, but many would not.
Clinicians	Most individuals should receive the intervention. Adherence to this recommendation according to the guideline could be used as a quality criterion or performance indicator. Formal decision aids are not likely to be needed to help individuals make decisions consistent with their values and preferences.	Recognize that different choices will be appropriate for individual patients and that you must help each patient arrive at a management decision consistent with his or her values and preferences. Decision aids may be useful in helping individuals to make decisions consistent with their values and preferences.
Policy makers	The recommendation can be adopted as policy in most situations.	Policy making will require substantial debate and involvement of various stakeholders.

axonemal proteins. However, there is no universally agreed-upon "gold standard" for diagnosis, and no single modality has sufficient diagnostic sensitivity and specificity when applied to the general population (1, 6).

Some clinical features of PCD can overlap with other conditions, such as cystic fibrosis (CF), immunodeficiency, pulmonary aspiration, asthma, and recurrent viral respiratory infections. However, PCD is not a diagnosis of exclusion. Recently, investigators identified four key clinical features characteristic of PCD (7). *1*) Year-round, daily, productive (wet) cough and *2*) year-round, daily, nonseasonal rhinosinusitis begin in early childhood, often shortly after birth, and are almost universally present by 6 months of age. These respiratory symptoms may vary but never fully resolve, even after systemic antibiotic therapy (6). Approximately 80% of children with PCD have a history of *3*) neonatal respiratory distress syndrome as term newborns, defined as the need for supplemental oxygen or positive pressure ventilation support for more than 24 hours without clear explanation (6–9). Roughly 40–55% of patients with PCD have *4*) laterality defects (e.g., situs inversus totalis), whereas other situs anomalies (e.g., situs ambiguus), with or without congenital heart defects, are found in roughly 12% of affected individuals (4, 10). If two of these distinguishing features are present, the sensitivity and specificity for PCD are 80% and 72%, respectively. If all four are present, the sensitivity and specificity are 21% and 99%, respectively (7). Chronic otitis media with effusion is also common in children with PCD, and many require tympanostomy tube placement before 5 years of age (8), but this feature does not distinguish children with PCD from those who do not have PCD. In term newborns, the combination of situs inversus totalis and unexplained neonatal respiratory distress is highly suggestive of PCD, even in infants who have not yet developed chronic respiratory symptoms. Without at least two of these key features, patients are unlikely to have PCD, and further testing is usually unwarranted. Thus, clinicians should consider diagnostic testing for PCD only in those patients who truly fit the clinical phenotype.

Other diagnoses should be considered on the basis of a detailed clinical history. The otosinopulmonary features of PCD overlap with symptoms of CF, another genetic disorder of mucociliary clearance. Children with CF typically do not have neonatal respiratory distress or chronic otitis media, nor do they have daily cough until lung disease has significantly progressed. Nonetheless, children being evaluated for PCD should undergo sweat chloride testing at a laboratory certified to perform sweat chloride measurements. Similarly, various immunodeficiencies may also present with chronic upper and lower respiratory tract infections, though not typically with daily, year-round symptoms. Depending on the clinical manifestations, a complete blood count with leukocyte differential, serum quantitative immunoglobulin levels, serological assays for specific antibodies as a measure of vaccine response, and total serum complement levels should be measured. Although this testing does not fully exclude immune dysfunction, major deficiencies will usually be identified. Further evaluation by an immunologist may be required in some cases. Chronic aspiration may also manifest as chronic cough and recurrent pulmonary disease. Asthma and bronchial hyperreactivity may coexist with PCD, but they can be further explored with bronchial provocation testing or bronchodilator response testing.

Methods

Committee Composition

This guideline committee consisted of 2 cochairs (A.J.S. and V.L.), 2 co–vice-chairs (S. D. Davis and M.M.), 12 additional pediatric pulmonologists with PCD expertise, 5 adult pulmonologists with PCD expertise, and 1 of each of the following experts: PCD genetics, cardiology/genetics, pediatric radiology, pediatric otolaryngology, and neonatology. There were four representatives from PCD advocacy groups, including two adult patients with PCD and two parents of pediatric patients with PCD. The committee worked with a health research methodologist (V.L.) who has expertise in evidence synthesis and the guideline development process. This methodologist, who is a clinician and also has expertise in clinical diagnostic laboratory tests, conducted systematic reviews and prepared the evidence summaries following the GRADE approach as described previously (11).

Confidentiality Agreement and Conflict-of-Interest Management

Committee members signed a confidentiality agreement and disclosed potential conflicts of interest according to American Thoracic Society (ATS) policy. All conflicts were successfully managed. At least 50% of the committee chairs, vice-chairs, and

members were free from industry ties. Twelve of 30 members with recognized expertise in PCD (A.J.S., S. D. Davis, S. D. Dell, M.R., T.W.F., D.P., M.J., M.R.K., C.M., S.D.S., M.W.L., and L.M.) reported ties to industry-sponsored research as primary investigators in PCD therapeutic trials; however, these relationships were easily managed for this diagnostic guideline, which does not evaluate PCD therapies. Three members (M.W.L., S. D. Davis, and T.W.F.) reported involvement in clinical trials with a novel nitric oxide measurement device, and these members participated in the discussion of the evidence with the rest of the committee but were recused from discussions related to the evidence-to-decision framework as well as from formulating, writing, and grading recommendations related to nNO testing. The conflicted members were allowed to stay in the same room to provide expert input while discussions among nonconflicted members took place; however, the members could do so only when specifically requested by nonconflicted members. Adherence to the rules was strict, with one of the cochairs (A.J.S.) responsible for monitoring the discussions for adherence to these rules. The methodologist also participated in discussions but was a nonvoting participant.

Meetings and Conference Calls

Face-to-face committee meetings were held at the ATS annual conferences in Denver, Colorado (May 2015), and in San Francisco, California (May 2016), and at the PCD Foundation conference in Minneapolis, Minnesota (August 2017). Members who could not attend were invited to participate via teleconference. Additional planning meetings were held regularly over the telephone between A.J.S. and S. D. Davis. Conference calls and e-mail correspondence were used to discuss specific issues requiring input from others.

At the Denver meeting, a group of committee members ($n = 18$) discussed the scope and objectives of the project and formulated clinically relevant questions, each dealing with a different diagnostic test. This process was monitored and approved by the lead ATS methodologist (J. Brozek). For each of the four proposed clinical questions, a subcommittee was responsible for all remaining steps of the process. After various editing done via e-mail communications, the four questions were finalized during a conference call in October 2015. At the San Francisco meeting, committee members ($n = 19$) met, evidence summaries were presented and discussed, and the recommendations were formulated for three of the four clinical questions. All meetings were attended by staff from the ATS documents committee. One member (D.P.) took detailed notes on all conversations and decision making conducted at the meetings. Members who could not attend were invited to participate via teleconference (M.L.C. in 2016, five members in 2017). The first clinical question, regarding genetic testing, was revised under the guidance of the committee methodologist and the lead ATS methodologist (*see* Formulating Clinical Questions subsection below). A follow-up conference call was held in January 2017, and e-mail communications were finished by July 2017 to complete the guideline development for this remaining question. The final committee meeting in Minneapolis ($n = 19$) was held to discuss the strength of final recommendations, and greater than 70% agreement through anonymous online voting was required to make a strong recommendation. The ATS provided financial and logistical support for meetings and conference calls. The views and interests of the ATS had no influence on the topics discussed and recommendations made.

Formulating Clinical Questions

The committee created four questions with direct relevance to clinical challenges commonly faced by physicians and patients surrounding PCD diagnosis. Specific attention was paid to creating questions that were relevant to centers without expertise in PCD, which commonly encounter diagnostic difficulties with PCD. The committee identified possible results for each test evaluated with each question and explicitly rated their relative importance (from the perspective of a patient suspected of having PCD) from not important to critical, following the approach suggested by the GRADE working group for diagnosis (11–14). Despite providing an indirect link to clinical outcomes, ranking test results by their relative importance helps focus attention on those that are most relevant to patients and helps resolve or clarify potential disagreements in decision making. Critical test results uniformly selected for all four questions include maximizing true-positive results as well as limiting false-negative results, thus favoring tests with high sensitivity. To this end, the committee reasoned that delays in diagnosis and treatment of PCD may be harmful but that starting nontoxic therapies (such as daily airway clearance and aggressive antibiotics) in patients who may not have PCD (i.e., false-positive results) would be beneficial (and not harmful) in any chronic suppurative lung disease, regardless of the underlying cause. Rankings of all outcomes were agreed on through consensus of the committee.

The clinical question regarding genetic testing for diagnosis of PCD was initially created with three separate subquestions. These questions were formulated when commercial genetic testing was not comprehensive, with panels investigating only 12 to 19 PCD genes, providing PCD mutation detection rates estimated at only 50%. However, midway through the first year of this guideline process, access to extended genetic panel PCD testing became commercially available when one company introduced a 32–PCD gene next-generation sequencing (NGS) panel, including deletion/duplication analysis. The new 32-gene panel was estimated to detect at least 70% of PCD mutations, which is comparable to properly performed TEM studies. With the increased access to extended genetic panel testing, the committee decided that the question on genetic diagnosis of PCD should be reformatted to address the possible benefits of extended genetic PCD panels because this would be most useful to clinicians attempting to diagnose PCD. In January 2017, this genetic testing question was slightly reformatted to reflect this new goal, and subquestions were removed. Initially, this question included scenarios in which genetic testing would be pursued depending on nNO testing results. Ultimately, the subcommittee believed that this pathway was not clinically relevant, because access to nNO testing is often not available to clinicians investigating PCD, and the stand-alone diagnostic accuracy for PCD genetic testing is much more relevant to clinicians.

Literature Search

A senior medical librarian (E.G.) designed a search strategy using medical subject headings and text words from the title, abstract, and keyword fields, limited to human studies and articles in any language. The following databases were searched from inception forward: MEDLINE (PubMed), PubMed (National Library of Medicine), Embase (Ovid), BIOSIS (Ovid), Web of

Science (Thomson Reuters), Scopus (Elsevier), Cochrane (Wiley), Africa-Wide Information (EBSCO), AMED (Ovid), and Global Health (Ovid) (*see* the online supplement). Two updates were made, in September 2015 and in July 2016. Additional publications not included in the search were added individually from committee members' personal libraries. Reviewers evaluated previous meta-analyses for additional articles. Two reviewers (A.J.S. and D.P.) screened titles and abstracts to identify articles for full review and evaluated the full text of articles deemed potentially relevant by either reviewer using predefined inclusion and exclusion criteria. Articles identified for full-text review were regrouped by topicality and further evaluated by each subcommittee according to inclusion/exclusion criteria specific to their question. These specific criteria were agreed on *a priori* by each subcommittee and varied between questions. Disagreement was resolved by group discussion with the chairs and cochairs.

Evidence Review and Development of Clinical Recommendations

First, data abstraction occurred independently and in duplicate (by one subcommittee member and one chair) for all included studies for each diagnostic question using predesigned data abstraction forms that had been piloted before being used. Disagreements were resolved through discussion with a third member. In addition to accuracy, individual study risk of bias and applicability were assessed independently by two reviewers (V.L. and A.J.S.), using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (15). During the data extraction phase, authors of accepted articles were contacted via e-mail if inconsistencies in the article text or data were found. Author contact was established to investigate methods that were unclear; to quantify reference diagnostic data when not included; and to clarify contentious issues in PCD diagnosis, such as inclusion of isolated inner dynein arm (IDA) defects in the reference standard of diagnosed PCD. If authors did not respond to several e-mail attempts or the imprecision was not resolved, their articles were excluded from further analysis.

Subsequently, diagnostic test accuracy estimates were pooled and meta-analyses performed for each test using Review Manager version 5.3 (The Cochrane Collaboration) and STATA version IC 14 (StataCorp) software. For this guideline, we liberally used the term "diagnostic test accuracy" when referring to the different measures used to evaluate the ability of a test to discriminate between the target condition and health (such as sensitivity and specificity or negative and positive predictive values) rather than the formal statistical meaning. Pooling and meta-analyses of study data were performed by the methodologist (V.L.) when appropriate. Of note, pooled analysis presented in this document may at times differ from other published meta-analyses, owing to differences in inclusion and exclusion criteria. The meta-analysis for the clinical question on the diagnostic accuracy of nNO was published before finalization of the related recommendation provided in this guideline (16).

Evidence tables for each question were prepared by the methodologist, following the GRADE approach for diagnosis (11, 13, 17) and using the GRADEpro Guideline Development Tool online software (18). The certainty in test accuracy (also known as confidence in accuracy estimates) was first assessed as per the GRADE recommendation for diagnosis—that is, after evaluating the five domains (risk of bias, precision, consistency, directness of the evidence, and other considerations)—and graded into one of four levels: high, moderate, low, or very low. All committee members reviewed the evidence profile tables, and modifications and additions were made when appropriate.

Development of recommendations for each question was based on the GRADE evidence-to-decision frameworks for diagnosis using the GRADEpro Guideline Development Tool online software (18). This framework helped organize discussion around each recommendation and ensured that each of the following factors was considered: the test accuracy with its associated certainty, the balance of desirable and undesirable consequences of compared diagnostic options, the link between test results and management decisions, the effects of the management guided by the tests results, the overall certainty of the evidence of effects of the test, the patients' values and preferences, the implications for resource use and health equity, the acceptability of the test to stakeholders, and the feasibility of implementation (19). The overall certainty of evidence for each recommendation (i.e., the certainty of the effect of testing and subsequent management decisions on patient-important outcomes) was assessed following the GRADE approach for diagnosis and categorized into one of four levels: high, moderate, low, or very low (19). Recommendations and their strength were decided by consensus through anonymous online voting by committee members. The committee agreed on the final wording of recommendations and remarks with further qualifications for each recommendation (e.g., subgroup considerations, justification, implementation considerations), and a unified diagnostic algorithm was created and accepted by the committee. The recommendations were either "strong" or "conditional" according to the GRADE approach (20, 21). As suggested by GRADE, we used the phrasing "we recommend" for strong recommendations and "we suggest" for conditional recommendations. Table 1 provides suggested interpretation of these recommendations by intended stakeholders, including patients, clinicians, and health policy makers.

The committee encountered challenges in adopting strengths of recommendation, notably for the questions on nNO and extended genetic panel testing as replacements of the reference standard. The analysis for these tests provided moderate certainty of evidence in diagnostic test accuracy, and over 70% of committee members initially voted for "strong" recommendations in this diagnostic guideline. However, long-term, patient-important outcome data are lacking in PCD, and there is major uncertainty regarding the impact of PCD diagnosis on long-term patient health (i.e., very low certainty in the overall evidence). This led to the final strengths of recommendation for nNO and extended genetic panel testing being downgraded to "conditional" recommendations, despite the committee's (including all PCD stakeholders) opinion that diagnostic test accuracy should be of primary importance in the decision-making process of a diagnostic guideline. This discrepancy highlights the challenges of making strong diagnostic recommendations for rare diseases, because even perfect diagnostic accuracy will often result in a conditional recommendation without long-term outcome data, which does not exist for many rare diseases. The committee believed that novel approaches

to this challenge should be investigated by the GRADE consortium and other regulatory bodies overseeing diagnostic clinical practice guidelines.

Manuscript Preparation

The writing committee (A.J.S., S. D. Davis, D.P., V.L, J.E.P., and T.W.F.) drafted the guideline document. The manuscript was then reviewed by the entire committee. Feedback was provided primarily through electronic communication and telephone conference calls, which included some of the committee members. The entire committee (both conflicted and nonconflicted members) had the opportunity to correct factual errors, clarify the presentation of background information or evidence summaries, and suggest changes to the rationale sections if they improperly captured the discussion from the face-to-face meetings. The wording of recommendations (including strength and direction) was not altered once recommendations were finalized during the face-to-face meetings and teleconferences. The chairs (A.J.S., S. D. Davis, M.M., and V.L.) confirmed that the written version of the guideline reflected the recommendations made by the committee members. The same process was followed for each version of the document. The final approved version was submitted to the ATS for peer review.

Recommendations for Specific Diagnostic Questions

Question 1: Should an Extended Genetic Panel (Testing >12 Genes) Be Used as a Diagnostic Test in Adult and Pediatric Patients with a High Probability (At Least Two of Four Key Clinical Features) of Having PCD (as a Replacement of Reference Standards of Classic TEM Structural Ciliary Defect and/or Standard Genetic Panel Testing for Mutations in ⩽12 Genes Associated with PCD)?

Background. PCD is a genetically heterogeneous and predominantly autosomal recessive disorder caused by biallelic pathogenic mutations in one of the many identified PCD causative genes (39 to date). Each PCD diagnostic test carries limitations, and those tests dependent on respiratory mucosal (ciliary) biopsy (TEM, ciliary beat frequency [CBF], and HSVM) are encumbered by the need for on-site high-quality specimen sampling, processing, and analysis. The widespread lack of local expertise and resources in ciliary biopsy testing has made molecular genetic testing an attractive alternative. Genetic testing for a Mendelian disease has the added value of procuring inherently high specificity; however, sensitivity may be expected to be lacking in a genetically heterogeneous disease such as PCD. In a comprehensive review of the PCD literature in 2015, Zariwala and colleagues demonstrated that more than 50% of patients with PCD possess two pathogenic mutations *in trans* in a known PCD causative gene (22). However, the sensitivity of genetic testing is anticipated to increase as commercial diagnostic panels incorporate novel identified PCD genes. Because genetic testing for PCD is already available in Clinical Laboratory Improvement Amendment–certified laboratories and costs have been decreasing, the impetus to consider molecular genetic testing as a first-line diagnostic test for PCD is increasing.

Specific methodology. Studies using multigene panels, with sample sizes of 10 or more subjects, for diagnostic assessment of PCD were evaluated. After the initial review of titles and abstracts (*see* the online supplement), 91 records were identified for full-text review to determine their eligibility for inclusion in the analysis. The genetic testing subcommittee members (S. D. Dell, D.P., M.A.Z., and S.D.S.) agreed on inclusion and exclusion criteria for full-text review of all 91 articles (*see* Figure E1.1 in the online supplement). The committee excluded 86 articles from the analysis on the basis of lack of multigene analysis, sample size of less than 10 patients, article addressing only disease carrier frequency, or lack of genetic testing information (*see* Section E1 of the online supplement). Five articles were eligible for evidence synthesis (7, 23–26); however, two of these articles used the same patient cohort (25, 26). Four studies included only PCD cases and thus could not provide complete diagnostic accuracy information (23–26). One cohort study that evaluated genetic and TEM testing in a population of patients referred for suspected PCD was included in the data analysis (7). When necessary, we obtained additional data from authors to compare genetic testing results with our prespecified reference standard for this question.

Summary of evidence. In the single analyzed article, Leigh and colleagues prospectively evaluated 534 pediatric subjects referred to a multicenter consortium for high clinical suspicion of PCD (7). Subjects invariably had chronic otosinopulmonary disease symptoms, with CF already ruled out in most cases. All subjects underwent TEM and NGS genetic testing of 26 known PCD-causing genes. Two hundred five participants were diagnosed as "definite PCD" per our reference standard of classic TEM structural ciliary defect and/or standard genetic panel testing for mutations in up to 12 genes associated with PCD. Among this cohort of 205 patients with definite PCD, 164 patients carried two pathogenic variants in a PCD gene (138 detected with the standard genetic panel, 26 additional ones detected with extended genetic panel testing), and 41 patients showed classic TEM defects with negative extended genetic panel testing. One hundred eighty-seven were categorized as "other diagnosis or undefined" (i.e., absence of classic TEM structural ciliary defect and/or absence of standard genetic panel testing for mutations in ⩽12 genes associated with PCD), among whom 186 participants had a negative 26-gene panel. The remaining 142 participants with a compatible PCD clinical phenotype and low nNO measurements, but no identified TEM defect or disease-causing gene, were labeled as "probable/possible PCD."

The sensitivity for the diagnosis of "definite PCD" by an extended genetic panel (>12 genes) in this study was 80%, indicating that 20% of patients were diagnosed by TEM alone (without a causative PCD gene found). Despite the fact that 142 patients with "possible/probable PCD" could have been considered to have true negative results according to our reference standard, the panel members believed that these patients probably had PCD and thus had potential false-negative results. However, without a clear reference standard diagnosis, these patients were excluded from our analysis, and the risk of bias was increased according to QUADAS-2 (Figures E1.2 and E1.3 and Table E1.1). In a worst-case scenario, if we were to assume that all of these patients had false-negative results, the sensitivity of the extended genetic panel would considerably decrease to 47%.

The specificity for PCD diagnosis was 99.5% in the analyzed study, indicating that

0.5% of patients were identified by the extended panel alone without being detected by TEM and/or a standard panel of up to 12 genes (our reference standard). Specifically, one case was considered a "false-positive result" owing to a positive *SPAG1* gene result on the extended gene panel when the TEM result was nondiagnostic. Importantly, nearly all cases of PCD detected by the extended gene panel, but missed on the standard panel, were already detected by TEM defects.

Although we were unable to analyze the specificity of PCD diagnosis in the four case-series studies, we were able to calculate sensitivities of each published extended genetic panel compared with our prespecified reference standard. Largely, the sensitivity of the genetic panel test improved with the increasing number of genes tested for PCD. Sensitivities were 71.9% when testing 12 genes (25), 73.3% when testing 19 genes (23), 54.8% when testing 24 genes (24), 80% when testing 26 genes (7), and 93.9% when testing 32 genes with deletion/duplication analysis (26). The lower sensitivity of 54.8% with the 24-gene panel (24) may be due to differences in population stratification, because PCD genes included in this panel were similar to those in other studies. Importantly, two studies conducted genetic testing in the same patient population ($n = 45$ families) and directly demonstrated increasing sensitivity as the number of analyzed PCD genes increased (sensitivity of 71.9% with 12-gene panel increased to sensitivity of 93.9% with 32-gene panel including deletion/duplication analysis) (25, 26).

Recommendation. In patients presenting with a strong clinical phenotype for PCD, we suggest using an extended genetic panel as a diagnostic test over TEM ciliary testing and/or standard (⩽12 genes) genetic panel testing (**conditional recommendation, moderate certainty of evidence in test accuracy but very low certainty in the overall evidence**). A majority of committee members initially endorsed a strong recommendation for extended genetic panel testing, based on its diagnostic accuracy, the benefit of genetic family planning, and the potential to identify more rapid pulmonary function decline and poorer clinical outcomes in certain genotypes (8). However, without robust, long-term, patient-important outcome data, the committee felt compelled to limit this recommendation to a conditional recommendation to adhere to the GRADE approach.

Justification and implementation considerations. This recommendation encourages the use of extended genetic panel testing for diagnosis of PCD (Table E1.2 [evidence-to-decision table]) as a replacement for standard genetic panels (⩽12 genes) and/or TEM ciliary testing. With this recommendation, it is noteworthy that TEM analyses in the cohort-type study were processed by one expert technician and reviewed by blinded investigators at a specialized PCD research center, where TEM specimens were suitable for interpretation in 88% of cases (27). Conversely, one tertiary academic care center reported only 63% feasibility in clinical TEM testing for PCD (28), with 37% of clinical cases failing to have biopsy specimens adequate for TEM analysis. This report is congruent with other publications showing poor feasibility for ciliary TEM testing (ranges of 60–80% feasibility) at international PCD centers of excellence (5, 29–31). In addition, these expert PCD centers require repeat ciliary biopsies for successful TEM analysis in 11–22% of patients, providing additional travel and medical costs to tested patients (29, 30, 32). Furthermore, the potential for broad variability in the handling, preparation, and interpretation of even those specimens that are adequate for TEM analysis raises concerns that many of the patients with PCD diagnosed by TEM defects alone in Leigh and colleagues' study (7) would be missed in other clinical centers. This has been confirmed through TEM testing in the same multicenter consortium as Leigh and colleagues, where approximately 20% of patients diagnosed by TEM defects at their local clinical centers lacked the same diagnostic finding upon repeat TEM testing in the expert consortium (33). Thus, the actual sensitivity of extended panel genetic testing is likely higher than 80% in clinical centers, where TEM testing for PCD is often fraught with false-positive, false-negative, and nondiagnostic specimens. In contrast, routine phlebotomy for genetic testing is highly feasible in all clinical centers, does not require patient travel over long distances, and should not require repeat sample acquisition.

In the past several years, exome sequencing of well-characterized PCD populations has revealed many PCD-causing genes (*CCNO*, *MCIDAS*, *DNAH11*, *CCDC65*, *CCDC164*, *GAS8*, *HYDIN*, *RPGR*, and *RSPH1*) resulting in normal, near-normal, or nondiagnostic TEM studies of respiratory cilia (34–41). However, the 26-gene panel used by Leigh and colleagues (7) did not include most of these newly discovered PCD-causing genes associated with normal TEM studies. The inclusion of these genes, which are now routinely found on most commercial PCD genetic panels, would further increase the sensitivity of PCD genetic testing over that seen by Leigh and colleagues. Last, Leigh and colleagues' study did not include deletion/duplication analysis of the 26 PCD genes tested. One study of PCD diagnosis by molecular genetic testing indicates that 8% of cases may be diagnosed by reflex deletion/duplication analysis on a 32-gene panel (26). Thus, the sensitivity of the extended panel used by Leigh and colleagues should be considerably higher with deletion/duplication analysis included. Currently, some commercially available NGS genetic panels for PCD diagnosis include deletion/duplication analysis.

This recommendation to use extended genetic panel testing rather than TEM ciliary testing and/or standard (⩽12 genes) genetic panel testing was voted by consensus, based on a very low certainty in the overall evidence for improved long-term, patient-important outcomes. The committee considered the aforementioned variables, resulting in higher test sensitivity in actual clinical practice (increasing sensitivity with newer panels and overestimation of TEM performance in research), as critically important for this recommendation. In addition, PCD stakeholders believed that this recommendation for extended genetic panel testing will directly benefit patients through improved diagnostic success. PCD stakeholders also appreciated the benefits that early and accurate genetic PCD diagnosis may have for long-term clinical and psychosocial outcomes.

Extended genetic panel testing does have clinical limitations. First, a negative panel does not rule out PCD, because some additional PCD genes are likely yet to be discovered. Next, only biallelic mutations in the same PCD gene are disease causing, and parental gene carrier testing may be required to verify that mutations arise *in trans*. Variants of unknown significance can provide nondiagnostic results, and incorrect interpretation of genetic variants may result in false-positive or false-negative

diagnoses. Some regions of PCD-causing genes are not screened by standard genetic testing and may result in false-negative results. Thus, consultation with local genetic specialists may be required for interpretation of extended genetic panel testing. Last, North American, European, and international health plans will need to adopt payment policies for PCD genetic testing in their populations.

Future research opportunities. Further investigation of possible/probable PCD cases through genetic sequencing is essential to finding new PCD-causing genes and new pathogenic variants in known genes (including possible intronic or regulatory region mutations). With the increasing number of PCD-causing genes included in commercially available genetic testing panels, the sensitivity of the accessible genetic testing will continue to improve. However, genetic panels must be routinely and frequently updated to include all newly discovered PCD-causative genes. Last, databases listing and explaining the presenting phenotypes associated with variants of unknown significance in PCD genes will be necessary to further elucidate genotype–phenotype relationships in people with PCD.

Question 2: Should a Low nNO Level (Detected with Chemiluminescence Technology), after Excluding CF, Be Used as a Diagnostic Test for PCD in Adult and Pediatric Patients 5 Years of Age or Older with a High Probability (At Least Two of Four Key Clinical Features) of Having PCD (as Replacement of Reference Standards of Classic TEM Structural Ciliary Defect and/or Biallelic Causative Mutations in PCD Genes)?

Background. nNO levels are reproducibly reduced (<77 nl/min) in PCD (42), and given that nNO results are immediately available at certain centers, these measurements are often used as a screening tool for PCD before proceeding to TEM and/or genetic analysis for confirmatory diagnostic testing. These latter tests are expensive, can take weeks to months to complete, and frequently yield nondiagnostic results (22, 28). Inexperience in obtaining biopsy samples can lead to insufficient cilia for TEM analysis, and inexperience in processing and interpretation can lead to false-positive or false-negative TEM results (28, 43). Finally, current genetic testing cannot detect biallelic mutations in all cases of PCD (22).

Specific methodology. Studies were included if they evaluated, in cooperative patients (generally ⩾5 yr old) who were deemed to have a high probability of having PCD (based on a compatible clinical phenotype), the accuracy of nNO testing (index test) compared with the reference standards of classic TEM ultrastructural ciliary defect (outer dynein arm defect, outer dynein arm plus IDA defect, IDA defect with microtubule disorganization, radial spoke or central apparatus defect) and/or biallelic mutations in known PCD genes. Studies were excluded if any of the following were present:

1. Fewer than 10 patients with PCD were included in the recruited population.
2. The index test was inadequate: nNO measurement used electrochemical technology (NIOX MINO; Circassia Pharmaceuticals), only nonvelum closure techniques were used (tidal breathing), and/or nasal sampling flow rates outside the ATS/European Respiratory Society recommended range were used (44).
3. The reference standard relied on only a single HSVM for PCD confirmation (without a second positive PCD diagnostic test result or without HSVM after cellular regrowth in culture) or greater than or equal to 30% of subjects had nonstandard TEM defects (unrepeated, isolated IDA defects without MTD) (43).
4. Diagnostic testing accuracy was either not provided, not accurate, or not calculable.
5. Index testing was incorporated into the reference standard.

nNO data from patients with CF were excluded from the analysis because nearly one-third of patients with CF can have nNO levels below the PCD diagnostic cutoff of 77 nl/min (45).

After initial review of 6,204 references by title and abstract, 76 full-text articles were assessed for eligibility, of which 65 were excluded (Figure E2.1). Twelve study populations derived from 11 articles were included in the quantitative synthesis (4, 42, 46–54) and underwent full-text review by the subcommittee (M.J., M.R., O.Y., A.J.S., and V.L.) (Tables E2.1 and E2.2). A meta-analysis provided a summary estimate for sensitivity and specificity and a hierarchical summary receiver operating characteristic curve. The QUADAS-2 tool was used to assess study quality, and the GRADE approach was used to assess the diagnostic test accuracy of studies to evaluate the certainty of evidence (Figures E2.2 and E2.5). Further details on methodology can be found elsewhere (16).

Summary of evidence. In 12 study populations (1,432 patients comprising 524 PCD, 908 non-PCD), with use of a reference standard of TEM alone or TEM and/or genetic testing, summary sensitivity was 97.5% (95% confidence interval [CI], 92.8–99.2%), and specificity was 96.4% (95% CI, 88.6–98.9%) (Figures E2.3 and E2.4). Excluding studies using TEM alone as the reference standard, the seven studies using an extended reference standard of TEM and/or genetic testing showed a summary sensitivity of 96.4% (95% CI, 89.4–98.8%) and specificity of 96.2% (95% CI, 84.2–99.2%) (Figure E2.6 and Table E2.3). Successful measurements were obtained in more than 90% of subjects in this meta-analysis, making this test highly feasible.

Recommendation. In cooperative patients 5 years of age or older with a clinical phenotype consistent with PCD and with CF excluded, we suggest using nNO testing for the diagnosis of PCD over TEM and/or genetic testing (**conditional recommendation, moderate certainty in test accuracy but very low certainty in the overall evidence**). A majority of committee members initially endorsed a strong recommendation for nNO testing, based on its excellent diagnostic accuracy. However, without long-term, patient-important outcome data, the committee revised this recommendation to a conditional recommendation.

Comment. Because nNO values may be transiently decreased with acute viral respiratory infections or sinusitis, establishing a low nNO on two separate occasions is indicated. In patients with a compatible clinical phenotype and low nNO on two occasions, a presumptive diagnosis of PCD may be established; TEM and/or genetic testing are indicated for clinical prognosis and to enhance understanding of PCD.

Justification and implementation considerations. Our recently published meta-analysis of 12 study populations shows excellent diagnostic accuracy for nNO as a PCD diagnostic test, in comparison with the extended reference standard of TEM and/or

genetic testing (16). Two prior meta-analyses have come to similar conclusions (55, 56). Both TEM and genetic analysis are imperfect reference standard PCD tests, with currently estimated sensitivities at 70% (5, 22, 57); each of these tests detects PCD cases that can be missed by the other test. In addition, these reference standard tests can frequently provide nondiagnostic results, with up to 40% of clinical biopsies showing inadequate cilia for TEM analysis (28–30) and up to 43% of genetic testing detecting no mutations, monoallelic mutations, or variants of unknown significance (24). Conversely, nNO measurement is a highly feasible test in cooperative patients (generally $\geqslant$5 yr old), with successful measurements accomplished in more than 90% of patients in this meta-analysis. Failure to obtain reliable nNO values were secondary to nasal obstruction, equipment malfunction, high ambient nitric oxide values, or lack of patient cooperation. A recent multicenter cohort study of PCD diagnostic referrals further suggested that nNO testing is more accurate than both TEM and/or genetic testing for a PCD diagnosis because nearly one-fourth of the referred population had compatible PCD clinical phenotypes and low nNO values (after ruling out CF) but negative extended genetic panel testing and normal or nondiagnostic TEM studies (7).

Although nNO testing has been largely considered a PCD screening test, these analyses show that nNO has diagnostic accuracy similar to (and possibly better than) that of the accepted confirmatory PCD tests of TEM and/or genetic analysis (Table E2.4 [evidence-to-decision table]) when used in a population with a high probability of having PCD (at least two key clinical PCD features). The use of nNO as a PCD screening test in general populations without key clinical PCD features will result in reduced positive predictive value and is strongly discouraged. The direct desirable consequences of using nNO testing instead of TEM and/or genetic testing outweigh the undesirable consequences, and the overall impact of avoiding direct costs and complications justifies using nNO testing as a replacement for the reference standards. The overall rates of false-negative results (which were considered critical) and false positive results were small (when using established, standardized protocols with chemiluminescence devices), and thus the downstream consequences were considered similar between nNO, TEM, and genetic testing. Nevertheless, despite the reported high accuracy of nNO in comparison with the reference standards, nNO might be even more sensitive than TEM and/or current genetic testing, thus potentially reducing false-negative results and their downstream consequences. Because nNO values may be decreased with acute viral respiratory infections or sinusitis, verification of low nNO values on at least two separate occasions seems prudent when using this as a PCD diagnostic test. In cases of strongly suspected PCD with normal TEM studies and negative genetic testing, repeatedly low nNO values may be the only positive PCD diagnostic test result and should be verified on at least two occasions.

Therefore, in individuals 5 years of age or older, with an appropriate clinical phenotype for PCD, and when CF is excluded, the diagnostic accuracy of nNO measurement (performed with chemiluminescence devices using established, standardized protocols) is comparable to that of TEM and/or genetic testing. nNO testing is noninvasive, relatively inexpensive for patients (after institutions purchase a costly chemiluminescence analyzer), and provides immediate results. However, there are limitations, including the need to travel to specialized centers that perform the testing, training of device operators, lack of U.S. Food and Drug Administration approval for devices in the United States (and thus the inability of institutions to gain reimbursement for clinical testing), and the lack of test standards for children under 5 years old.

Even with low nNO measures, patients should still progress to further corroborative PCD diagnostic studies, including genetic and/or TEM testing, which may provide long-term prognostic information (8, 41); improve the general understanding of PCD; and account for other respiratory tract illnesses, including acute sinusitis or viral infection, which may lead to reduced nNO values (58–60). PCD stakeholders agreed on the critical importance of confirming genetic and/or TEM defects after a diagnosis is made with nNO measurements. Patients with biallelic disease-causing mutations in some genes (e.g., *RSPH1*) can have nondiagnostic nNO results. Genetic testing may also inform family planning. Finally, defining the PCD genotype may allow development future mutation-specific therapies, as occurred in CF (61).

Future research opportunities. Further research is needed on nNO measurements in children younger than 5 years of age, who cannot cooperate with velum closure maneuvers and therefore perform this technique through tidal breathing. Additional research needs include determination of age-specific distributions of nNO levels in disease control populations and determination of appropriate diagnostic cutoffs for tidal breathing nNO measurements. A major limitation of nNO measurement is the high cost of chemiluminescence devices, because this is the only technology currently recommended for nNO measurement in PCD diagnosis (44). Further research examining the diagnostic accuracy of portable electrochemical nNO devices could potentially validate less expensive alternatives for PCD diagnosis. Last, further standardization of nNO device measurement software is required, because significant differences can occur with automated online measurement programs compared with offline operator-driven protocols (62).

Question 3: Should HSVM Alone Be Used as a PCD Diagnostic Test in Adult and Pediatric Patients with a High Probability (At Least Two of Four Key Clinical Features) of Having PCD (as a Replacement of Reference Standards of Classic TEM Structural Ciliary Defect and/or Biallelic Causative Mutations in PCD Genes)?

Background. HSVM is used in a number of specialized laboratories to diagnose PCD (50, 63–65). With use of a digital high-speed video camera attached to a microscope, beating ciliated epithelial edges are recorded at frame rates of between 120 and 500 frames per second and are then replayed at slower rates to view ciliary motion. Samples can then be evaluated to assess ciliary function by measuring CBF and/or ciliary beat pattern (CBP). Recent expert consensus recommended HSVM ciliary functional assessment of both CBF and CBP coupled with TEM as a means of diagnosing PCD (66). However, conducting HSVM proves challenging, requiring significant expertise and training. Furthermore, this expertise is limited to a few laboratories in Europe and

Canada; therefore, clinical applicability is restricted.

Specific methodology. For this analysis, studies using HSVM (⩾120 frames per second recording) were reviewed. After the initial appraisal of titles and abstracts, 35 articles were identified for full-text review by the subcommittee (S. D. Davis, C.M., and M.A.C.) (Section 3 of the online supplement). Agreed-upon exclusion criteria used for full-text review were *1*) fewer than 10 patients with PCD in the studied population, *2*) index test of HSVM included in the reference diagnostic standard, *3*) HSVM with CBP analysis technique not used, and *4*) unable to calculate sensitivity or specificity (Figure E3.1). Authors were contacted via e-mail if exclusion criteria were unclear. Upon further review by the committee cochairs (A.J.S. and V.L.), four articles are included in the quantitative meta-analysis (50, 63–65).

Summary of evidence. Four cross-sectional, cohort-type studies consecutively recruited participants and evaluated the ability of HSVM to diagnose PCD by CBP analysis. Two studies were prospective, with sample sizes of 371 (63) and 34 (50) participants with suspected PCD. Two were retrospective, with sample sizes of 231 (65) and 158 (64) participants. None of the included studies examined PCD diagnosis by genetic testing in the reference standard.

Stannard and colleagues (63) evaluated CBP using nasal ciliated epithelial samples in 371 participants, with TEM as the reference standard for PCD diagnosis. Of the 371 participants, 70 were diagnosed with PCD on the basis of electron microscopy. The Stannard group evaluated CBF, ciliary dyskinesia score, percentage of dyskinetic edges, and immotility index to determine abnormal CBP. A ciliary dyskinesia score greater than 2 was the most accurate of these HSVM measures, resulting in sensitivity of 92.5%, specificity of 97.6%, positive predictive value of 91.2%, and negative predictive value of 98%.

Papon and colleagues (50) evaluated HSVM in 34 participants with suspected PCD, using a reference diagnostic standard of nNO measurement coupled with TEM for PCD diagnosis. This group conducted both qualitative and quantitative analysis using 12 different HSVM measures. Of the 34 participants, 15 were deemed non-PCD, 10 were diagnosed with definite PCD by reference standard, and 9 received an inconclusive diagnosis. On the basis of this small sample size, the investigators reported that the use of quantitative HSVM CBP analysis diagnosed 9 of the 10 patients with PCD and improved diagnostic accuracy, whereas qualitative measurements identified only 7 patients with PCD. In the inconclusive group of nine patients, quantitative ciliary analysis identified a further four and qualitative analysis a further two with abnormal HSVM who had low nNO levels. However, on reviewing the discordant nNO and TEM findings within individual subjects in this group, genetic testing would be required to accurately identify PCD in this group of patients. Of note, the methodology required to perform this quantitative analysis is complex and may not be feasible in a clinical setting.

Using a retrospective cohort of 231 patients referred for PCD in Leicester, England, Hirst and colleagues (65) evaluated the utility of HSVM in improving the diagnosis of PCD after epithelial biopsy samples were grown under air–liquid interface tissue culture conditions. This group used TEM as the diagnostic reference standard with qualitative HSVM analysis as the index test. HSVM was performed in both fresh ciliated samples and later after cellular regrowth to allow for assessment of any ciliary functional gain after cell culture. The results revealed that 28 participants had definite PCD with diagnostic TEM defects, and all showed 100% dyskinesia within biopsy samples on HSVM in the fresh specimens. However, only 12 of 28 (43%) PCD biopsy samples successfully regrew in culture, but postculture HSVM and TEM studies were consistent with the preculture results. In a separate study, Hirst and colleagues (64) retrospectively analyzed HSVM after culture regrowth in 158 participants referred for PCD diagnosis, using a reference diagnostic standard of TEM defects, clinical history, or abnormal ciliary function. However, 73 participants from one site were excluded from our analysis because the index test of HSVM was also incorporated into the reference diagnostic standard. The investigators reported that the CBP analyses postculture confirmed the ciliary phenotype in 100% of PCD cases, and in some cases, they were better at identifying abnormal CBP versus preculture fresh analyses.

The selected studies reported data (Figure E3.3) for two qualitative parameters of CBP analysis: either by describing the percentage of dyskinetic beating cilia on the epithelial edge (64, 65) or by reporting a ciliary dyskinesia score for the edge (50, 63). Overall, the pooled sensitivity and specificity for all four studies were 97.3% (95% CI, 59.8–99.9%) and 96.5% (95% CI, 63.7–99.8%), respectively. However, the 95% CI of these diagnostic accuracy results was extremely large, signifying great variation in the certainty of these results. This is illustrated by the summary receiver operating characteristic curve and 95% CIs (Figure E3.4)

Because genetic PCD testing was not performed in any of the studies, it is possible that the reported accuracy may be overestimated (Figures E3.2–E3.4 and Table E3.1). Three of the four studies were conducted by the same research group in the United Kingdom; these investigators are expert at using HSVM as well as the air–liquid interface tissue culture technique. The single analyzed study done outside of the United Kingdom was conducted in a small sample of patients and demonstrated much worse diagnostic accuracy. Of note in this study, the accuracy of the reference standard used for diagnosis appeared to vary in PCD and inconclusive cases.

Recommendation. We suggest *not* using CBP analysis by HSVM as a replacement diagnostic test in patients with a high probability of having PCD (**conditional recommendation, low certainty in the diagnostic accuracy of the test but very low certainty in the overall evidence**).

Justification and implementation considerations. Ciliary functional assessment (CBF and CBP) by HSVM is often used as a primary PCD diagnostic tool in some countries. Experts now recommend HSVM analysis after cellular regrowth of tissue samples, but CBP may still be affected by the manipulation of fresh tissue, leading to a different functional phenotype after culture (66–68). Significant technical expertise and equipment are required to successfully grow ciliated epithelial cultures, which often fail to regrow, even at expert centers (65). If cellular regrowth cannot be achieved, families must travel repeatedly to centers of expertise for multiple biopsies and repeat HSVM analyses.

There is also a lack of standardization in HSVM interpretation techniques, with some centers using various quantitative functional measures based on qualitative

assessments, such as immotility index, percentage of dyskinetic edges, and distance of ciliary tip traveled, whereas other centers use mainly qualitative descriptions of beat pattern, including "stiffness" of cilia and "failure of bending" along the entire axoneme. With this lack of standardization in both sample preparation and CBP interpretation, the HSVM technique itself is not easily transferred to other centers (poor feasibility), and the applicability of the technique across centers remains poor (Table E3.2 [evidence-to-decision table]). Only a few international centers have the necessary expertise to conduct ciliary functional analysis with HSVM. Last, interrater agreement of HSVM beat pattern analysis is quite poor, even in samples from healthy control subjects (69). Given these limitations and the potential for false-positive and false-negative results for PCD diagnosis, we suggest not using CBP assessment with HSVM as a routine diagnostic tool (Table E3.2). Despite this, CBP analysis may still have a role in the assessment of patients with PCD, because currently there is no "gold standard" PCD test, and both TEM and genetic testing have significant diagnostic limitations. Currently, HSVM is more appropriate for PCD diagnosis in expert research settings until investigators offer significant clinical advancements in HSVM feasibility and test standardization. PCD stakeholders strongly support this recommendation until such standardization is achieved.

Future research opportunities. Conducting genotype–phenotype relationships using HSVM may help investigators elucidate the underlying mechanisms leading to progressive lung disease in PCD. However, this will require standardization of protocols (including tissue culture conditions) and development of robust, validated CBP measurements. Studies comparing HSVM analysis with TEM defects and PCD-causing gene mutations may delineate disease mechanisms and aid PCD diagnosis; however, this would require participation by multiple centers to achieve adequate sample size. To improve general applicability of HSVM, further research is indicated which demonstrates that multiple centers can successfully use this tool when following validated standard operating protocols.

Question 4: Should CBF or Ciliary Waveform Analysis Using Light Microscopy without High-Speed Recording Be Used as a PCD Diagnostic Test in Adult and Pediatric Patients with a High Probability (At Least Two of Four Key Clinical Features) of Having PCD (as Replacement of Reference Standards of Classic TEM Structural Ciliary Defect and/or Biallelic Causative Mutations in PCD Genes)?

Background. Calculation of CBF has historically been suggested as a PCD diagnostic method that can be used with inexpensive bright-field microscopy and straightforward recording technology (70, 71). In addition, some clinicians employ ciliary waveform analysis without high-speed recording to diagnose PCD (72–74). Some academic centers even suggest these tests as first-line screening, and if results are normal, further PCD diagnostic testing (such as TEM or genetic testing) may not be necessary (75, 76). However, most expert North American PCD centers avoid CBF measurement or waveform analysis without high-speed video recording in PCD, because several recently discovered genetic forms of PCD result in normal CBF with only subtle changes in CBP (77). In addition, most PCD researchers have migrated from standard-speed video recording to HSVM because this method provides more detailed ciliary waveform information for analysis (*see* Question 3 above).

Specific methodology. Studies using standard-speed video microscopy and HSVM were evaluated in this analysis, but when HSVM was used, the analyzed data were limited to only CBF values, and ciliary waveform analysis was excluded altogether if HSVM was used. After the initial review of titles and abstracts, 51 articles were identified for full-text review by the question subcommittee (A.J.S., M.W.L., L.M.). After agreeing on specific inclusion and exclusion criteria for the full-text review, the subcommittee completed a full-text review of all 51 articles (Figure E4.1). Upon further review by the committee cochairs (A.J.S., V.L.), three articles examining CBF for PCD diagnosis were included for further analysis (63, 64, 78). One article addressing ciliary waveform analysis with standard-speed video recording was considered for further examination; yet, this additional article did not employ currently recognized PCD reference diagnostic standards and did not use the reliable methods to perform ciliary waveform analysis (73). Thus, no articles addressing ciliary waveform analysis with standard-speed video recording were included in the final analysis.

Summary of evidence. Three cross-sectional studies addressed this question. All three used TEM defects as the reference standard, and none examined patients with PCD diagnosed by genetics. Two of these were cohort-type studies, but the only prospective cohort study, by Stannard and colleagues, was a single-center study that examined diagnostic testing accuracy of CBF in 371 consecutively referred patients with symptoms of PCD (63). With use of CBF alone, with a prespecified cutoff value of 11 Hz to diagnose PCD, approximately 13% of PCD cases were missed on the basis of TEM studies confirming the diagnosis, with sensitivity and specificity of 87% and 77%, respectively. The authors offered other waveform analysis techniques and scoring systems that perform superior to CBF measurement alone, but these are all performed with HSVM and thus were not considered to answer this specific question on standard-speed video recording. The other cohort study, by Hirst and colleagues, was a retrospective, multicenter analysis of 73 patients referred for suspicion of PCD (64). Only patients recruited at the Leicester site were included in our data analysis, because the other recruiting center commonly incorporated the index test (CBF and ciliary motility assessment) within the reference standard and possibly did not perform TEM testing if ciliary motility was normal. Analysis of the Leicester patients revealed that CBF values, at a prespecified cutoff of 10 Hz, provided 68% and 78% sensitivity and specificity, respectively, compared with TEM studies. The third study included a smaller population and was conducted retrospectively (78). This showed a low specificity of CBF compared with TEM diagnosis of PCD. Because genetic PCD testing was not performed in any of the previous studies, it is possible (if not likely) that reported accuracy was overestimated (Figures E4.2 and E4.3 and Table E4.1).

Recommendation. We suggest *not* using CBF measurement as a diagnostic test in patients with a high probability of having PCD (conditional/weak recommendation,

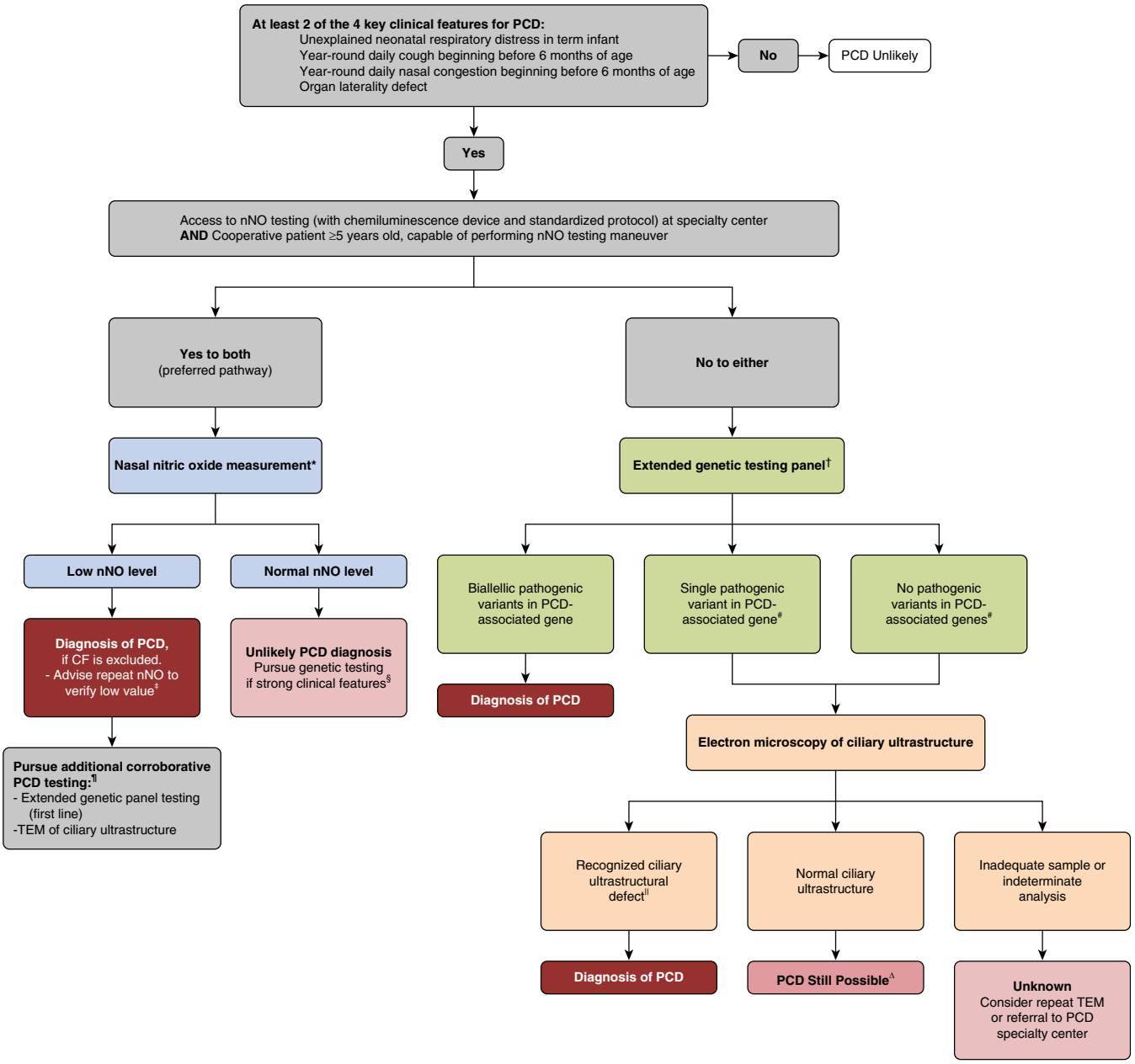

Figure 1. Suggested diagnostic algorithm for evaluating the patient with suspected primary ciliary dyskinesia. *Cystic fibrosis should be ruled out before performing nNO measurement, as roughly one-third of CF patients can have nNO values below PCD diagnostic cutoffs. nNO measurements should only be performed with chemiluminescence analyzers using standardized protocols at centers with specific expertise in nNO measurements. Some nNO analyzers have not received approval from federal agencies worldwide (U.S. Food and Drug Administration and Health Canada have not approved all chemiluminescence devices for clinical use), which may have implications for clinical implementation. †Genetic panels testing for mutations in more than 12 disease-associated PCD genes, including deletion/duplication analysis. ‡As nNO levels can be significantly decreased by viral respiratory tract infections, a repeat nNO measurement, at least 2 weeks after the initial low value (expert opinion), is recommended to ensure that the initial low value is not secondary to a viral process. A normal nNO value upon repeat testing suggests that the patient does not have PCD, as nNO values remain consistently low in PCD. §Most forms of PCD resulting in normal nNO levels have normal or nondiagnostic electron microscopy studies. Thus, genetic testing is recommended in these cases. #Or presence of variants of unknown significance. For the purposes of this algorithm, "likely pathogenic" variants and "pathogenic" variants are grouped together as pathogenic. ¶Additional corroborative testing may provide information on clinical prognosis, further understanding of the disease, and suggest potential future therapeutic considerations. ||Known disease-associated TEM ultrastructural defects include outer dynein arm defects, outer dynein arm plus inner dynein arm (IDA) defects, IDA defects with microtubular disorganization, and absent central pair, identified using established criteria (1, 6, 13). Of note, the presence of IDA defects alone is rarely diagnostic for PCD. △Up to 30% of PCD cases can have normal ciliary ultrastructure of electron microscopy (EM). Consider referral to PCD specialty center if there is a strong clinical phenotype but all EM and genetic testing are negative. CF = cystic fibrosis; nNO = nasal nitric oxide; PCD = primary ciliary dyskinesia; TEM = transmission electron microscopy.

low certainty in the diagnostic accuracy of the test but very low certainty in the overall evidence). No recommendation could be made regarding the use of ciliary waveform analysis without HSVM as a diagnostic test for PCD, because no studies using currently recognized reference standards were identified by our systematic review.

Justification and implementation considerations. This analysis shows that the diagnostic accuracy of CBF calculation is poor in comparison with the reference standard of TEM testing. Although not meeting inclusion criteria for this analysis, another study of PCD cases using genetic testing as the diagnostic reference standard demonstrated overlapping CBF values between patients with PCD, healthy control subjects, and disease control subjects (77). Furthermore, there are no significant differences in cost (compared with the reference standard, when CBF is performed as part of a larger ciliary motility assessment with HSVM), direct benefits, or indirect benefits when using CBF as a diagnostic test (Table E4.2 [evidence-to-decision table]). The majority of studies and recommendations supporting ciliary motion analysis via CBF or standard-speed video microscopy were published over 15 years ago (70–73, 79), and since then, no prospective validation studies have proven this technique as diagnostic of PCD.

PCD stakeholders expressed very strong agreement with this recommendation because they appreciate the benefits of early and accurate PCD diagnosis may have for long-term clinical and psychosocial outcomes. Stakeholders also believe it is critically important to properly diagnose patients with PCD on the basis of genetics and/or TEM defects in order to identify criteria causing a continued decline in this subgroup of patients with PCD, which may lead to targeted, novel therapies for this subgroup.

The committee realizes that bright-field microscopy with CBF measurement is a feasible and inexpensive test that is sometimes used in centers lacking experience in PCD. The committee also realizes that prohibiting this testing will require referral of potential patients with PCD to more specialized PCD centers for definitive diagnosis using more expensive investigations, such as TEM and genetic testing. However, with the high rate of false-negative results of CBF and light microscopy without HSVM, potential patients with PCD will continue to receive incorrect diagnoses if these practices continue. Thus, centers relying on CBF measurement as their sole PCD diagnostic tool should refer all potential patients with PCD to specialized PCD centers for more reliable diagnostic testing (Table 4.2).

Future research opportunities. Further investigation of real-time ciliary waveform analysis without HSVM, accompanied by automated waveform and CBF interpretation software, may provide a role for real-time light microscopy in the future. However, with the increasing use of HSVM recording for ciliary waveform analysis, it seems doubtful that further research into non-HSVM waveform analysis will occur.

Conclusions: Proposed Diagnostic Algorithm

On the basis of our review of available evidence, we propose a diagnostic algorithm for patients who have a clinical phenotype consistent with PCD (Figure 1). The committee was unable to strongly recommend a single PCD diagnostic test and recommends that a panel of diagnostic tests be applied to diagnose PCD, which may require referral to a PCD specialty center to provide comprehensive evaluation and testing. In addition, whereas nNO measurements (when measured correctly) may have diagnostic accuracy equivalent to that of TEM and genetic testing, it should not completely replace these tests in all cases. Rather, clinicians should appreciate the added diagnostic value of multiple positive tests, specifically nNO measurement with genetics or TEM.

The proposed algorithm represents an idealized setting in which all diagnostic tests are accessible to a provider. The authors recognize, however, that there may be international differences, and providers must consider diagnostic options based on availability. Obviously, the algorithm will need to be modified with the emergence of newer tests. ■

This official clinical practice guideline was prepared by an *ad hoc* primary ciliary dyskinesia subcommittee of the ATS Assembly on Pediatrics.

Members of the subcommittee are as follows:

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S. D. Dell served as site principal investigator for a Parion/Vertex clinical trial for VX-371 (*see above*); served on an advisory committee and as a consultant for Vertex; and served as a consultant for Novartis. T.W.F. served as site principal investigator for a Parion/Vertex clinical trial for VX-371 (*see above*); served as site principal investigator for a device trial for Circassia (formerly Aerocrine); holds five United States and international patents; and received research support from the Children's Discovery Institute and the Cystic Fibrosis Foundation. M.R.K. served on an advisory committee for Corus Pharma and Proteostasis Therapeutics; and served as a consultant for Gilead and Vertex. M.W.L. served as site principal investigator for the Parion/Vertex clinical trial for VX-371 (*see above*); served as site principal investigator for a device trial for Circassia (formerly Aerocrine); and served on an advisory committee for Vertex. C.M. served as a consultant for AbbVie, Gilead, Proteostasis, and Vertex; served as a site principal investigator for a Proteostasis clinical trial for PTI-428 (an investigational oral treatment for cystic fibrosis); and served as site principal investigator for a Parion/Vertex clinical trial for VX-371 (*see above*). L.M.N. received royalties from UpToDate for coauthoring a chapter on genetic surfactant dysfunction disorders. K.N.O. received research support from Insmed and Matinas BioPharma; and served as a speaker for Bayer HealthCare. D.P. served as consultant for Vertex. S.D.S. received research support from the Cystic Fibrosis Foundation. O.Y. received travel support for conferences from Abdi Ibrahim, Allergopharma–Merck Turkey, Bilim, and GlaxoSmithKline. M.A.Z. served as a consultant for a Parion/Vertex clinical trial for VX-371 (*see above*). V.L., M.M., B.A., M.A.C., M.L.C., S.J.D., M.L.A.D., T.E., L.E., E.G., I.A.J., M.H.J., M.J., L.M., J.E.P., M.R., and S.M.W. reported no relationships with relevant commercial interests.

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