AMERICAN THORACIC SOCIETY DOCUMENTS

Diagnosis of Primary Ciliary Dyskinesia

An Official American Thoracic Society Clinical Practice Guideline: Executive Summary

Adam J. Shapiro, Stephanie D. Davis, Deepika Polineni, Michele Manion, Margaret Rosenfeld, Sharon D. Dell, Mark A. Chilvers, Thomas W. Ferkol, Maimoona A. Zariwala, Scott D. Sagel, Maureen Josephson, Lucy Morgan, Ozge Yilmaz, Kenneth N. Olivier, Carlos Milla, Jessica E. Pittman, M. Leigh Anne Daniels, Marcus Herbert Jones, Ibrahim A. Janahi, Stephanie M. Ware, Sam J. Daniel, Matthew L. Cooper, Lawrence M. Nogee, Billy Anton, Tori Eastvold, Lynn Ehrne, Elena Guadagno, Michael R. Knowles, Margaret W. Leigh, and Valery Lavergne; on behalf of the American Thoracic Society Assembly on Pediatrics

This official clinical practice guideline of the American Thoracic Society was approved May 2018

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

Supported by a pediatric assembly grant through the American Thoracic Society, as well as in part by the Division of Intramural Research, NHLBI, NIH (K.N.O.).

This Executive Summary is part of the full official ATS clinical practice guideline, which readers may access online at http://www.atsjournals.org/doi/abs/10.1164/rccm.201805-0819ST. Only the Executive Summary is appearing in the print edition of the *Journal*. The article of record, and the one that should be cited, is: Diagnosis of Primary Ciliary Dyskinesia: An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 2018;197:e24–e39. Available at http://www.atsjournals.org/doi/abs/10.1164/rccm.201805-0819ST.

ORCID IDs: 0000-0001-6066-6750 (A.J.S.); 0000-0002-9390-0651 (S. D. Davis); 0000-0003-2169-9407 (S. D. Dell); 0000-0003-1619-1393 (M.A.Z.); 0000-0001-6051-5020 (O.Y.); 0000-0001-5515-3053 (C.M.); 0000-0001-6979-2681 (M.L.A.D.); 0000-0002-8263-1265 (M.H.J.).

Correspondence and requests for reprints should be addressed to Adam J. Shapiro, M.D., Pediatrics, Montreal Children's Hospital, 1001 Boulevard Decarie, BRC.5016, Montreal, QC, H4A 3J1 Canada. E-mail: adam.shapiro@muhc.mcgill.ca.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 197, Iss 12, pp 1524–1533, Jun 15, 2018
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DOI: 10.1164/rccm.201805-0819ST
Internet address: www.atsjournals.org

Overview

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 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 policies.

Clinicians, patients, and other stakeholders should not view these recommendations as dictates. No guideline can account for all clinical circumstances. The implications of the strength of recommendations are described in Table 1.

This guideline applied 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 recommendation, including 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 evidence are different from those employed during regulatory agency reviews of applications seeking market approval.

Introduction

PCD, a genetically heterogeneous, mainly autosomal recessive disorder, is 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 with classic clinical features, 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

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.

ciliary axoneme using transmission electron microscopy (TEM) analysis, which can have serious drawbacks. Nonspecific ciliary changes, which can be induced by exposure to environmental pollutants or infection, may appear similar on TEM to findings seen in PCD. Also, the absence of axonemal defects does not exclude PCD, because 30% of affected individuals have normal ciliary ultrastructure (5). Other diagnostic tests have emerged, including nasal nitric oxide (nNO) measurement, genetic testing, digital high-speed videomicroscopy with ciliary beat pattern analysis (HSVM), and immunofluorescence imaging for specific axonemal proteins. However, there is no universally agreed-on "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 overlap with other conditions, such as cystic fibrosis (CF), immunodeficiency, chronic pulmonary aspiration, asthma, and recurrent respiratory viral 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 immediately 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 all four key clinical features are present, the sensitivity and specificity are 21% and 99%, respectively (7). Chronic otitis media with effusion is also common in children with PCD (8), but this feature does not distinguish children with PCD from those who do not have PCD. If two of these distinguishing features are present, the sensitivity and specificity for PCD are 80% and 72%, respectively. 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. Clinicians should consider diagnostic testing for PCD only in those patients who truly fit the clinical phenotype.

Methods

From the outset of guideline development, the workgroup made certain assumptions. First, because PCD is a heterogeneous disease, no reference diagnostic standard is universally accepted. Thus, the workgroup proposed the combination of classic TEM ultrastructural ciliary defect and/or genetic panel testing for mutations in known PCD genes as the most accurate "reference standard" to diagnose 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.

The guideline development panel consisted of 2 cochairs (A.J.S. and V.L.), 2 co–vice-chairs (S. D. Davis and M.M.), and 26 panelists. The committee worked with a health research methodologist (V.L.) who has expertise in evidence synthesis and guideline development. After a systematic review of the literature, selection of relevant studies, data extraction and pooling (when appropriate), and assessment of the certainty of the evidence, results were presented at three face-to-face meetings and during several teleconferences. After discussing the evidence-to-decision tables, recommendations were developed and graded using the GRADE approach for diagnosis (11). The committee encountered challenges in adopting strengths of recommendation, notably for the PICO questions (i.e., patient, problem, or population; intervention; comparison, control, or comparator; outcome) 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 (including all PCD stakeholders) opinion that diagnostic test accuracy should be of primary importance in the decision-making process of a diagnostic guideline.

All participants disclosed conflicts of interest during panel composition, throughout the process, and until completion of the guideline. Twelve members (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 investigators in PCD therapeutic trials and were allowed to participate without restrictions. Three members (M.L., S. D. Davis, and T.W.F.) reported involvement in a clinical trial with a nitric oxide measurement device. These members participated in the discussion of the evidence with the committee but were recused from discussions concerning evidence-to-decision framework and from formulating, writing, grading, and voting on recommendations regarding nNO measurements. The American Thoracic Society provided financial and logistical support for meetings and conference calls. The views and interests of the American Thoracic Society had no influence on the topics discussed or the recommendations.

Recommendations for Specific Diagnostic Questions

Please refer to the full-length online document and supplemental materials,

which include supporting evidence profiles for recommendations.

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 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, inherited disease caused by biallelic pathogenic mutations in one of many identified causative genes. To date, 39 genes are linked to PCD. The limitations of TEM have made molecular genetic testing an attractive alternative, because more than 50% of patients with PCD possess two pathogenic mutations in a known disease-causing gene (12). Because comprehensive genetic testing for PCD is increasingly available in Clinical Laboratory Improvement Amendment–certified laboratories and costs have fallen, the impetus to consider molecular genetic testing as a first-line diagnostic test for PCD has increased.

Summary of evidence. Five articles were included in the meta-analysis, but only one cohort-type study could be fully analyzed for diagnostic accuracy. The other four case-series studies included only patients with PCD. In the cohort-type study, 534 pediatric subjects referred to a North American multicenter consortium were prospectively evaluated 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 next-generation sequencing genetic testing for 26 known PCD-causing genes. Two hundred five participants were diagnosed as "definite PCD," of whom 164 subjects carried two pathogenic variants in a PCD gene (138 detected with the standard genetic panel, 26 additional subjects with PCD detected with extended genetic panel testing), whereas 41 patients showed classic TEM defects with negative extended genetic panel test results. One hundred eighty-seven participants were categorized as "other diagnosis or undefined" on the basis of normal TEM and negative 26-gene panel analysis results. The remaining 142 participants with a compatible PCD clinical phenotype and low nNO measurements, but without TEM defects or disease-associated gene anomalies, were labeled as "probable/possible PCD."

The sensitivity for PCD diagnosis by an extended genetic panel (>12 genes) in this study was 80%, indicating that 20% of patients were diagnosed by TEM alone without biallelic, pathogenic variants in known PCD genes. Although the 142 patients with "possible/probable PCD" could have been considered to have true-negative results per 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 analysis, and the risk of bias was increased. In a worst-case scenario, if all of these patients were included as having false-negative results, the sensitivity of the extended genetic panel would 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 but not detected by TEM or a standard panel of less than or equal to 12 genes. Nearly all cases of PCD detected with the extended gene panel, but missed with the standard panel, had ultrastructural defects (Section E1 in the online supplement).

In the four case-series studies, we calculated sensitivities of each extended genetic panel compared with our reference standard. In general, sensitivity improved with the increasing number of PCD genes tested. Sensitivities were 71.9% when testing 12 genes (13), 73.3% when testing 19 genes (14), 54.8% when testing 24 genes (15), 80% when testing 26 genes (7), and 93.9% when testing 32 genes with deletion/duplication analysis (16). The lower sensitivity of 54.8% with the 24-gene panel (15) may be related to differences in population stratification, because PCD genes included in this panel were similar to those in other studies. Two studies conducted genetic testing in the same patient population ($n = 45$ families) and directly demonstrated increasing sensitivity as the number of analyzed genes increased (13, 16).

Recommendation. In patients presenting with a strong clinical phenotype for PCD, we suggest using an extended genetic panel as a diagnostic test over TEM analysis 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. 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 (17). Another academic care center reported only 63% feasibility in clinical TEM cases (18), which is congruent with reports from international PCD centers of excellence (5, 19–21). Even experienced centers require repeat biopsies for interpretable TEM in 11–22% of patients (19, 20, 22). Thus, the potential for broad variability in the preparation and interpretation of TEM specimens raises concerns that many of the patients with PCD diagnosed by TEM defects alone in the cohort-type study would be missed at other clinical centers. Thus, the actual sensitivity of extended panel genetic testing is likely higher at clinical centers.

Exome sequencing of PCD populations has revealed many PCD-causing genes (*CCNO*, *MCIDAS*, *DNAH11*, *CCDC65*, *CCDC164*, *GAS8*, *HYDIN*, *RPGR*, and *RSPH1*) that are associated with normal or nondiagnostic TEM (23–30). However, the 26-gene panel used in the cohort-type study did not include many of these newly discovered PCD-associated genes, nor did it include deletion/duplication analysis of PCD genes tested. Evidence

indicates that an additional 8% of cases could be diagnosed by reflex deletion/duplication analysis on a 32-gene panel (16). Thus, the sensitivity of the extended panel used in this study would be considerably higher with inclusion of newly discovered PCD genes and deletion/duplication analysis.

Extended genetic panel testing does have clinical limitations. Negative extended-panel results do not exclude PCD, because some additional PCD genes are likely yet to be discovered. 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 test results may result in false-positive or false-negative diagnoses. Consultation with geneticists and genetic counselors may be required with extended genetic panel testing. Last, North American, European, and international health plans will need to adopt payment policies for PCD genetic testing on their populations.

Question 2: Should a Low nNO Level (Using 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 a Replacement for 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 (31), and these measurements have been used as a screening tool before proceeding to confirmatory diagnostic testing, such as TEM and genetic testing, which can be costly and may yield nondiagnostic results (12, 18, 32).

Summary of evidence. In 12 study populations (1,432 patients comprising 524 PCD, 908 non-PCD), using a reference standard of TEM with or without genetic testing, summary sensitivity of nNO in affected individuals aged 5 years and older was 97.5% (95% confidence interval [CI], 92.8–99.2%), and specificity was 96.4% (95% CI, 88.6–98.9%). Excluding studies that applied TEM alone as the reference standard, nNO measurements had sensitivity of 96.4% (95% CI, 89.4–98.8%) and specificity of 96.2% (95% CI, 84.2–99.2%). Successful nNO measurements were obtained in more than 90% of subjects in this meta-analysis, making this test highly feasible. However, diagnostic accuracy of nNO in children under 5 years of age has not been established. Also, some patients with CF have reduced nNO and must be excluded from consideration as part of the diagnostic evaluation for PCD (Section E2 in the online supplement).

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. A recently published meta-analysis showed excellent diagnostic accuracy for nNO as a PCD diagnostic test, in comparison with an extended reference standard of TEM and/or genetic testing (33). Both TEM and genetic analysis are imperfect reference standard PCD tests, with currently estimated sensitivities at 70% (5, 12, 34), and each of these tests can identify PCD that may be missed by the other. Thus, these reference standard tests can sometimes lead to nondiagnostic results (15, 18–20). Conversely, nNO measurement is a highly feasible test in cooperative patients 5 years of age or older. A recent multicenter cohort study of PCD diagnostic referrals further suggests that nNO testing is possibly more accurate than both TEM and/or genetic testing when using established, standardized protocols for NO measurement, because nearly 25% of the referred population had compatible PCD phenotypes and reduced nNO levels (after excluding CF) but negative extended genetic panel test results and nondiagnostic TEM (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 the accepted confirmatory PCD tests of TEM and/or genetic analysis when used in a population with a high probability of having PCD (at least two key clinical PCD features) (*see* Table E2.4 [evidence-to-decision table] in the online supplement). 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 outweighs 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, provided that measurements were made using established, standardized protocols with chemiluminescence devices.

Therefore, in individuals 5 years of age or older, with an appropriate clinical phenotype for PCD (at least two of four key clinical features), and in whom CF is excluded, the diagnostic accuracy of nNO measurement (performed with chemiluminescence devices using standardized protocols) is comparable to that of TEM and/or genetic testing. Because nNO values may be decreased with acute viral respiratory infection 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 electron microscopic studies and negative genetic test results, repeatedly low nNO values may be the only positive PCD diagnostic test result and should be verified on at least two separate occasions.

nNO testing is noninvasive, relatively inexpensive to patients (after institutions purchase costly analyzers), 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 the lack of test standards for children under 5 years old.

Even with low nNO measures, patients should progress to further corroborative PCD diagnostic studies, including genetic and/or TEM testing, which may provide long-term prognostic information (8, 30); improve understanding of PCD; and account for other respiratory tract illnesses, including acute sinusitis or viral infection, that may lead to reduced nNO values (35–37). Patients with biallelic disease-causing mutations in some genes (e.g., *RSPH1*) can have nondiagnostic nNO results. Genetic testing may also permit family planning. Finally, defining the PCD genotype may allow for future mutation-specific therapies, as occurred in CF (38).

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 for Reference Standards of Classic TEM Structural Ciliary Defect and/or Biallelic Causative Mutations in PCD Genes)?

Background. HSVM is used in specialized laboratories to diagnose PCD (39–42). With this technique, beating ciliated edges of airway epithelia are recorded at frame rates between 120 and 500 frames per second and replayed at slower rates to assess ciliary beat frequency (CBF) and ciliary beat patterns (CBPs).

Summary of evidence. We analyzed four cross-sectional cohort-type studies that consecutively recruited participants and evaluated HSVM as a diagnostic tool for PCD (39–42). These studies reported data for two qualitative parameters of CBP analysis by describing the percentage of dyskinetic beating cilia (41, 42) or a ciliary dyskinesia "score" (39, 40). None of the studies used genetic testing as a reference standard. 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 diagnostic accuracy results was large, signifying great variation in the certainty of results (Section E3 in the online supplement).

Authors of one report (39) evaluated CBPs from nasal biopsies of 371 participants, using TEM as the reference standard for PCD diagnosis; 70 were diagnosed with PCD. A ciliary dyskinesia score greater than 2 was the most accurate HSVM measure, resulting in sensitivity of 92.5%, specificity of 97.6%, positive predictive value of 91.2%, and negative predictive value of 98%. A second study (40) compared various qualitative and quantitative HSVM assays in subjects with suspected PCD, using reference diagnostic standards of nNO measurement and TEM. Of the 34 participants, 15 were deemed non-PCD, 10 were diagnosed with definite PCD by the reference standard, and 9 had an inconclusive diagnosis. Quantitative CBP analyses diagnosed 9 of the 10 subjects with confirmed PCD, whereas qualitative measures identified only 7 patients with PCD. In the inconclusive TEM group, quantitative and qualitative ciliary analyses determined that six additional subjects had PCD on the basis of low nNO levels.

Using a retrospective cohort of 231 patients referred for PCD, authors of a third article (42) reported on HSVM improving PCD diagnosis after collected epithelial cells were grown in primary culture at an air–liquid interface. Using TEM as the diagnostic reference standard, qualitative HSVM was performed on both fresh cells and after cellular regrowth to allow for assessment of any ciliary functional gain after culture. Twenty-eight participants had definite PCD with characteristic TEM defects, and all of the fresh specimens had dyskinetic cilia. However, only 43% of the isolated epithelial cells successfully regrew in culture; postculture HSVM and TEM studies were consistent with the preculture results. In a separate study (41), investigators retrospectively analyzed HSVM after culture regrowth in 158 participants referred for PCD diagnosis, using a reference diagnostic standard of clinical history, TEM, 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 beat pattern analyses postculture confirmed the ciliary phenotype in 100% of PCD cases, and in some cases, it was better at identifying abnormal CBP than in freshly isolated cells. It is important to note that the same investigators, who are highly skilled in epithelial cell culture techniques and HSVM, conducted three of the reviewed studies. The single study outside this group had fewer subjects and lower diagnostic accuracy. Because genetic testing was not performed in any of the studies, it is possible that reported accuracy is overestimated.

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, and very low certainty in the overall evidence**).

Justification and implementation considerations. Ciliary functional assessment by HSVM is used as a solitary PCD diagnostic tool in some countries. HSVM requires substantial experience and is best performed in centers that specialize in PCD. However, this approach can have limitations. Slow or abnormal beating, as occurs after airway injury or infection, can be an acquired defect and may lead to a false-positive conclusion. Ciliary beat also may be affected by manipulation of fresh tissue samples and can lead to a different functional phenotype after the retrieved airway cells are allowed to recover in culture (43–45). Experts now recommend HSVM after cellular regrowth, but isolated cells often fail to regrow, even at expert centers. Significant technical expertise and equipment are required to successfully conduct ciliated epithelial cultures (42). Consequently, only a few international centers have the requisite expertise to conduct ciliary functional analysis using HSVM.

Interpretation of HSVM has not been standardized, with some centers using different quantitative functional CBP analysis based on qualitative assessments, such as immotility index, percentage of dyskinetic edges, and distance of ciliary tip traveled. Other sites apply qualitative CBP

descriptions, including "stiffness" and "failure of bending" of cilia. With this lack of standardization in both sample preparation and interpretation, the HSVM technique itself is not easily transferred to other centers, and its applicability across centers remains problematic. Interrater agreement of HSVM beat pattern analysis is quite poor, even in samples from healthy control subjects (46). 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. However, given the overall limitations of HSVM and the potential for false-positive and false-negative results with HSVM, we cannot recommend using HSVM as the sole diagnostic tool for PCD. Currently, HSVM is more appropriate for PCD diagnosis in expert research settings until investigators offer significant clinical advancements in HSVM feasibility and test standardization.

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. CBF and waveform analysis without high-speed recording have been used as a PCD diagnostic method that can be employed with inexpensive light microscopy and straightforward recording technology (47–51). Some investigators even suggest these tests as first-line screening, and if results are normal, further diagnostic testing may not be necessary (52, 53).

Summary of evidence. Three cross-sectional studies address this question. Each uses TEM defects as the reference standard, and none include PCD diagnosed by genetics. The only prospective cohort study was a single-center analysis that examined the diagnostic testing accuracy of CBF in 371 consecutively referred patients with symptoms of PCD (39). Using CBF alone, with a prespecified cutoff of 11 Hz, 13% of PCD cases confirmed by TEM studies were missed, with sensitivity and specificity of 87% and 77%, respectively. Other waveform analysis techniques using HSVM were superior to CBF measurement alone; however, they were considered not relevant to answering this specific question that focuses only on standard-speed video recording. The other cohort study was a retrospective multicenter analysis of 73 patients referred for suspicion of PCD (41). Only subjects recruited from one site (Leicester, UK) were included in our data analysis, because the other recruiting center incorporated the index test (CBF and ciliary motility assessment) within the reference standard and possibly did not perform TEM if ciliary motility was normal. Analysis of these patients showed that CBF values, at a prespecified cutoff of 10 Hz, provided 68% and 78% sensitivity and specificity, respectively, compared with TEM studies. The third article described a smaller population that was evaluated retrospectively (54), showing a low specificity of CBF compared with TEM as a PCD diagnostic test. Because genetic testing was not performed in any of these studies, it is possible (if not likely) that the reported accuracy of CBF was overestimated. Unfortunately, there were no eligible studies on ciliary waveform analysis using standard-speed light microscopy (Section E4 in the online supplement).

Recommendation. We suggest *not* using CBF measurement as a diagnostic test in patients with a high probability of having PCD (conditional recommendation, low certainty in the diagnostic accuracy of the test, and 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 recognized reference standards were identified through our systematic review.

Justification and implementation considerations. This analysis shows that the diagnostic accuracy of CBF is poor compared with the reference standard of TEM testing. The majority of studies and recommendations supporting ciliary motion analysis using CBF or bright-field microscopy were published over 15 years ago (47–50, 55), and since then, no prospective validation studies have proven this technique as diagnostic of PCD. Although not meeting inclusion criteria for this analysis, another study using genetic testing as the diagnostic reference standard revealed overlapping CBF values between patients with PCD, healthy control subjects, and disease control subjects (56). Furthermore, there are no significant differences in direct or indirect benefits when using CBF as a diagnostic test (because CBF is often calculated with detailed functional analysis using HSVM).

PCD stakeholders expressed strong agreement with this recommendation. The committee realized that light microscopy with CBF measurement is a feasible and inexpensive test often used in centers lacking experience in PCD. The committee also recognized that advising against this testing will likely require referral of patients with potential PCD to more specialized PCD centers for definitive diagnosis using more specialized and expensive investigations such as TEM and genetic testing. However, with the high rate of false-negative results of CBF measurements using only bright-field microscopy without HSVM, the potential for misdiagnosis is great, should these practices continue.

Conclusions: Proposed Diagnostic Algorithm

On the basis of our evidence review, 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, although nNO measurements (when measured correctly) may have diagnostic accuracy equivalent to 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 PCD tests, specifically nNO measurement with genetics or TEM.

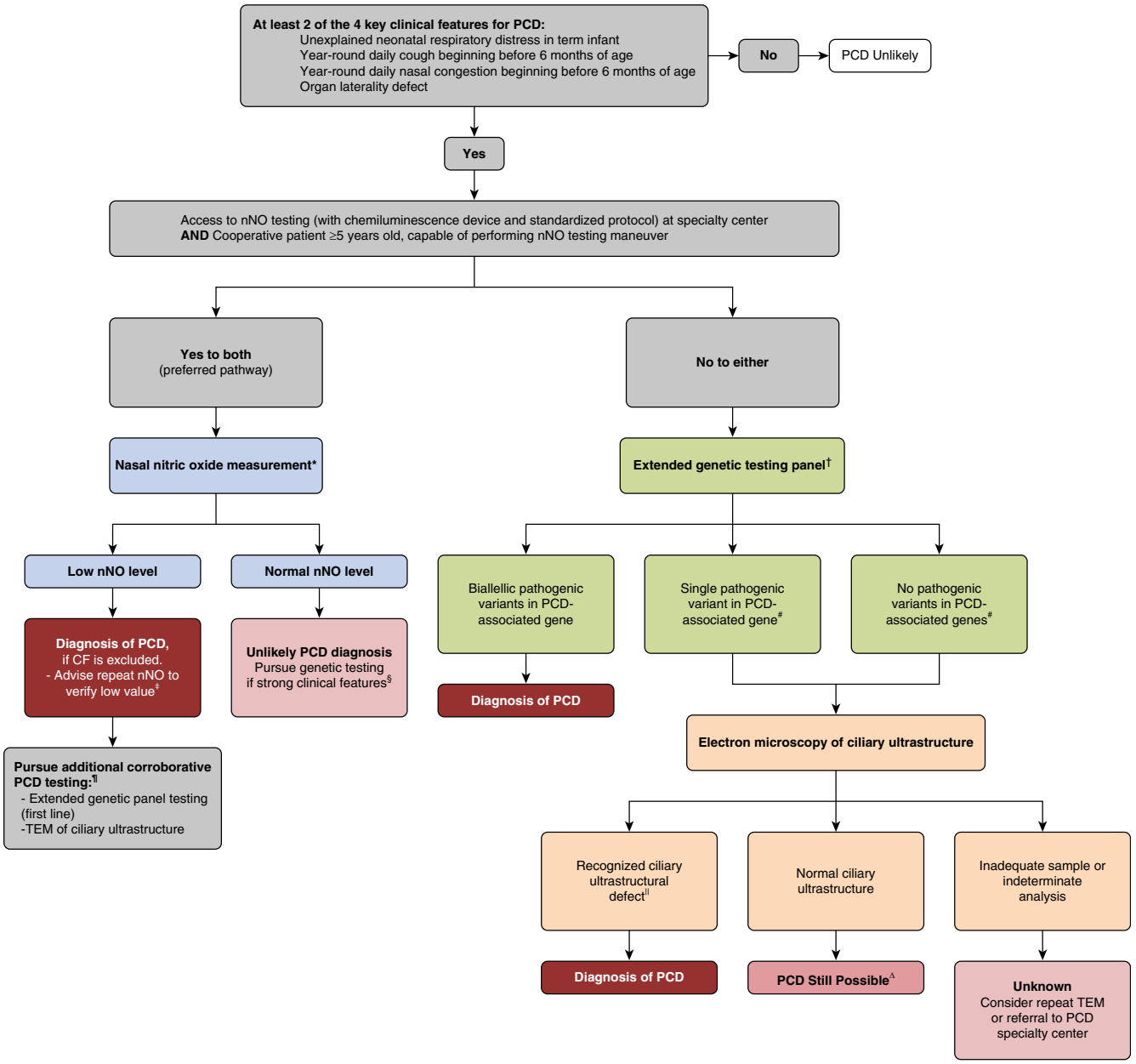

Figure 1. Suggested diagnostic algorithm for evaluating the patient with suspected primary ciliary dyskinesia. *Cystic fibrosis should be ruled out before performing nNO measurement, as roughly one-third of CF patients can have nNO values below PCD diagnostic cutoffs. nNO measurements should only be 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.

The proposed algorithm represents an idealized setting where 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 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:

Adam J. Shapiro, M.D. (*Co-Chair*)
Valery Lavergne, M.D. (*Co-Chair, Methodologist*)
Stephanie D. Davis, M.D. (*Co–Vice Chair*)
Michele Manion (*Co–Vice Chair*)
Billy Anton
Mark A. Chilvers, M.D.
Matthew L. Cooper, M.D.
Sam J. Daniel, M.D.
M. Leigh Anne Daniels, M.D.
Sharon D. Dell, M.D.
Tori Eastvold
Lynn Ehrne
Thomas W. Ferkol, M.D.
Elena Guadagno, M.L.I.S.
Ibrahim A. Janahi, M.D.
Marcus Herbert Jones, M.D.
Maureen Josephson, M.D.
Michael R. Knowles, M.D.
Margaret W. Leigh, M.D.
Carlos Milla, M.D.
Lucy Morgan, M.D.
Lawrence M. Nogee, M.D.
Kenneth N. Olivier, M.D.
Jessica E. Pittman, M.D.
Deepika Polineni, M.D.
Margaret Rosenfeld, M.D.
Scott D. Sagel, M.D.
Stephanie M. Ware, M.D., Ph.D.
Ozge Yilmaz, M.D.
Maimoona A. Zariwala, Ph.D.

Author Disclosures: A.J.S. served as site principal investigator for a Parion/Vertex clinical trial for VX-371: Clearing Lungs with ENaC Inhibition in Primary Ciliary Dyskinesia (CLEAN-PCD), registration number NCT02871778. S. D. Davis served as a consultant for Eli Lilly and Vertex; received research support from Circassia (formerly Aerocrine), Parion/Vertex, and the Cystic Fibrosis Foundation; served as a speaker for ABcomm Inc. and Parion/Vertex; received support from an educational grant from Gilead; and served on an advisory committee for Parion/Vertex. 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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