Primary Ciliary Dyskinesia: Updates on Diagnosis, Follow-Up and Treatment

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Abstract

Primary ciliary dyskinesia is a rare genetic disease caused by defects in the beating pattern and in the structure of cilia, resulting in chronic respiratory and otorhinolaryngologic disease in both children and adults. The diagnosis is complex and has recently been reviewed by different groups. It is reached through a panel of screening and diagnosis that includes nasal nitric oxide measurement, ciliary structure analysis with electron microscopy, ciliary beat analysis with high speed videomicroscopy, and genetic testing. There is no specific treatment and the standards of care are based on the management of patients with bronchiectasis. Due to its complexity and multidisciplinarity, this condition should be managed by experienced specialized centres. For these reasons, the authors believe it to be pertinent to review the current evidence and the international guidelines regarding diagnosis, follow-up and treatment of patients with primary ciliary dyskinesia.

Keywords: Child; Disease Management; Kartagener Syndrome/diagnosis; Kartagener Syndrome/treatment

Introduction

Primary ciliary dyskinesia (PCD) encompasses a heterogeneous set of genetic defects that cause changes in the beat pattern and often in the structure of the cilia, moving microscopic organelles located on the apical surface of respiratory epithelial cells.[1-5] In healthy individuals, cilia are responsible for mucus, bacteria and cellular debris clearance from the airways through their coordinated beating.[5] Patients with PCD have recurrent and chronic airway infections due to poor mucociliary clearance. Extra-respiratory symptoms are frequently observed, since the cilia participate in the determination of symmetry in the embryonic period, are also located in the fallopian tube epithelium and their structure is similar to the structure of the sperm flagella.[2] Currently, PCD continues to be misdiagnosed or have a delayed diagnosis,[2,6] so it is important to raise the level of clinical suspicion in patients with suggestive phenotype.[3] On the other hand, in the last decade there have been important advances in the diagnosis of PCD [1-4,7] underlining the importance of this review.

Epidemiology

The prevalence of PCD is not completely known,[8] but it is believed to be between 1:2,200 and 1:40,000.[6,9] This variability is due, in part, to underdiagnosis, given the heterogeneity of access to diagnostic techniques in different countries,[3,5,6] and the incidence is believed to be higher in certain groups with a higher degree of consanguinity.[5,10,11]

Clinical phenotype

PCD affects all airways, with symptoms predominantly occurring on a chronic and daily basis from the neonatal period.[1] Most newborns with PCD are clinically well in the first 12-24 hours of life,[1] with subsequent onset of respiratory symptoms in 65-87% of the cases.[9,12,13] This may include mild transient tachypnea, rhinitis or atelectasis of upper and middle lobes, often associated with poor feeding.[1-3] Although symptoms are transient in most cases, it may progress to respiratory failure requiring prolonged ventilatory support and supplemental oxygen therapy for days or weeks.[2,3]

Virtually all patients with DCP have daily persistent cough, with variable intensity, usually productive, from childhood to adulthood.[1,14] Although a complete resolution of symptoms is not frequent, there may be some improvement with antibiotic therapy (ABT), cough-suppressant therapy or suppression by the patient.[1-3] At preschool age, up to 80% of patients have recurrent

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pneumonia[1,14,15] and often bronchitis.[1] The onset of bronchiectasis is a marker of disease progression, and is present in 50% of children up to 8 years old and virtually all adults.[1,9,16]

The deficient mucociliary clearance causes mucus accumulation in the middle ear, nasal cavity and paranasal sinuses, leading to the high prevalence of otolaryngological symptomatology.[2] Chronic rhinitis and sinusitis (sometimes pansinusitis[14]) are common findings,[1,9,17,18] with at least 80% of patients reporting daily nasal obstruction throughout the year, often from birth,[7] with no seasonal pattern and no improvement between viral exacerbations.[1] Nasal polyposis may also occur,[19] although being less frequent in childhood.[2] Up to 80% of patients have recurrent otitis media with chronic ear discharge,[2,3,11] particularly in the first year of life,[11] and chronic serous otitis is also common.[2,3] Possible complications include conductive hearing loss and delayed language development.[2,3]

In several subgroups of patients, the cilia of the embryonic node responsible for establishing the symmetry of the visceral organs are also affected.[3] Thus, there is a spectrum of organ laterality defects, including *situs inversus totalis* (mirror image) in 50% of patients and *situs ambiguus* (partial asymmetry) in at least 12%.[1,20-22] *Situs ambiguus* may be associated with complex congenital heart disease and minor septal defects.[1,20] Classically, the triad of *situs inversus totalis*, bronchiectasis and sinusitis is known as Kartagener syndrome.[23]

Finally, the structure of the flagellum of sperm and cilia of fallopian tubes is similar to that of respiratory cilia.[1,3] For this reason, there is infertility in the vast majority of men with PCD and subfertility and risk of ectopic pregnancy due to abnormal oocyte transport in women.[24-26] Patients with PCD may also have hydrocephalus and polycystic kidney disease.[27,28]

Although it is a rare entity, the most common manifestations of PCD are frequent in the general population, particularly in the paediatric age.[7] Additionally, some of the symptoms overlap with other chronic lung diseases such as asthma, respiratory manifestations of some immunodeficiencies, bronchiectasis or cystic fibrosis.[7] In many patients, some manifestations may be mild or even absent.[14] For these reasons, a high degree of suspicion is necessary to consider PCD and perform diagnositic evaluation (Table 1).[1,2,7]

Given the low specificity of PCD symptoms, some groups have developed predictive diagnostic tools to aid in the selection of patients who must undergo the diagnostic procedures listed below.[29,30] An European group[30] has developed the PICADAR (PrImary CiliAry DyskinesiA Rule) tool and has already validated it externally in a second

diagnostic center.[30] This applies to patients with persistent, early-onset cough and consists of seven predictive parameters: neonatal respiratory symptoms in full term babies, neonatal unit admission, chronic rhinitis, middle ear symptoms, *situs inversus*, and congenital heart defect (Table 2). Its sensitivity and specificity were 0.90 and 0.75 respectively for a five-point cut-off score. These systematically defined early clinical features may help identify children with probable PCD and refer them for diagnosis.

Table 1. Who should be referred for diagnostic testing of primary ciliary dyskinesia?
1. *Situs inversus*, especially if accompanied by respiratory or nasal symptoms
2. Neonatal respiratory distress of unknown cause
3. Sibling with primary ciliary dyskinesia (PCD), particularly if symptomatic
4. Daily lifelong wet cough (note: maybe suppressed by child and under-recognised by parents)
5. If considering testing for cystic fibrosis, also consider testing for PCD particularly if rhinitis, sinusitis or glue ear are present
6. Unexplained bronchiectasis
7. Serous otitis media in association with lower and upper airway symptoms
8. Cardiac disease associated with heterotaxy if there is suspicion of respiratory, nasal or ear problems
Note: A lower threshold for referral should be considered if the patient is from a consanguineous background or is of an ethnic origin known to have increased prevalence of PCD.

Adapted from: Lucas JS, et al. Arch Dis Child 2014;99:850-6; Werner C, et al. Cilia 2015;4:2.[2,3]

Table 2. The PICADAR score is a screening tool with seven simple questions to be applied when there is persistent productive cough starting in childhood
• Was the patient born full term? Yes = 2 points
• Did the patient experience chest symptoms in the neonatal period? Yes = 2 points
• Was the patient admitted to a neonatal unit? Yes = 2 points
• Does the patient have a *situs* abnormality (*situs inversus* or heterotaxy)? Yes = 4 points
• Does the patient have a congenital heart defect? Yes = 2 points
• Does the patient have persistent perennial rhinitis? Yes = 1 point
• Does the patient experience chronic ear or hearing symptoms (e.g. glue ear, serous otitis media, hearing loss, ear perforation)? Yes = 1 point

Adapted from: Behan L, et al. Eur Respir J 2016;47:1103-12.[3]

Diagnosis

Although several tests can support the diagnosis of PCD, there is no gold standard method that allows its confirmation.[4,5] Aethiology may be related to defects in ciliary biogenesis, structure, function or organization, so no single test allows for its diagnosis.[1,4] Thus, international recommendations suggest the use of a complementary panel with the inclusion of the tests described below,[1,4,5,14] following the recent guidelines of the European Respiratory Society.[4]

Measurement of nasal nitric oxide

Nasal nitric oxide (nNO) measurement is recommended as part of the diagnostic panel of PCD in adults and children.[1,4,5] It is a sensitive, fast and non-invasive test with immediately available results.[4,31,32] nNO levels are almost universally low in patients with PCD, for unclear reasons.[33-35] Measurement of nNO in adults and children over 6 years is performed with a probe placed in one of the nostrils, attached to a chemiluminescence analyser (preferably) or electrochemical device. The patient should perform maneuvers that lead to velum closure while the measurement is performed (breath holding or oral exhalation against resistance).[3,4] Children under 6 years of age may have difficulty performing these maneuvers,[1,2] and alternative methods, without palatal closure, can be used. An example is measurement during open mouth tidal breathing, however, this technique is less sensitive for the diagnosis of PCD.[4,31,36,37] Standardisation of nNO analysis and reporting is still required, with particular need for reference cut-off values for different analysers and respiratory manoeuvres.[38]

Assessment of ciliary beat frequency (CBF) and ciliary beat pattern (CBP), by high-speed video microscopy

The evaluation of the frequency and pattern of respiratory ciliary beat by HSVA is recommended as one of the diagnostic tests and should be performed exclusively in highly experienced centers.[4,5] This technique allows the observation of cilia in high-definition, high-speed and with the possibility of repetition in slow motion.[39,40] Respiratory epithelial cells can be obtained by brushing with a cytology brush along the inferior nasal concha in a procedure lasting only a few seconds[2,41] or, alternatively, during bronchoscopy.[41] Identifiable abnormalities include nonmotile cilia or cilia with minimal movement, stiff cilia with reduced beat amplitude, circular motion or hyperkinetic cilia.[3] Sample collection should be performed at the patient's baseline health state; repeat biopsies may be necessary to ensure that abnormalities are not due to viral infections, tobacco, environmental exposure, poor sample quality or processing.[1]

Analysis of ciliary ultrastructure by electron microscopy

Analysis of the ultrastructure of ciliary axonemes of respiratory epithelial cells by electron microscopy is another recommended diagnostic test.[1,4,5] Ultrastructural defects associated with PCD include, but are not limited to, the absence of the outer dynein arms from the outer doublets, missing central pairs and disarrangement of the microtubules.[2] The type of defect found is related to the genetic defect, ciliary beat pattern and clinical manifestations.[2,3,42] The sample must be obtained at the patient's baseline health state, since respiratory exacerbations can cause secondary changes in the ciliary ultrastructure.[43] For this reason, the harvest should be postponed for at least two weeks after an infectious exacerbation.[1] *In vitro* epithelial tissue culture allows for posterior reanalysis with a lower risk of occurrence of secondary lesions.[7] In addition, 3–30% of patients with PCD are reported to have uncertain or normal cilia ultrastructure, thus TEM cannot rule out PCD.[1,2]

Genetic studies

Genetic studies for the identification of disease-causing mutations is an integral part of most PCD diagnostic protocols.[1,4] In parallel to its clinical variability, PCD is genetically heterogeneous, with 33 genes associated with the disease and with new genes being identified at a rapid pace.[2,5,44-46] There is a correlation between the identified mutations and the changes of the ciliary ultrastructure in TEM and of the HSVA phenotype.[2,3] Most of these mutations are of autosomal recessive inheritance, with the exception of two genes in the X chromosome (*RPGR* and *OFD1*).[47-49] Genetic studies can identify gene variants of unknown significance,[1] so the genetic diagnosis is established in only 65% of patients, and therefore, it does not exclude PCD.[3,4,14] Improvement of genetic diagnosis and stratification of patient populations according to the mutations identified will constitute the first step in the evaluation of future gene therapy strategies.[2]

Evaluation of ciliary proteins by immunofluorescence

The use of antibodies to identify proteins of the dynein arms of the ciliary axonemes by immunofluorescence allows the identification of defects in these structures.[50,51] This technique has shown promising results[1] and appears to be unaffected by secondary insults.[52] Although currently restricted to some research centers, it may be particularly useful in resource constrained situations, given its simplicity of execution and low cost.[4]

Diagnostic criteria

Because of the heterogeneity of possible outcomes, there is currently no uniform international approach.[3] Despite the combination of techniques available, the results may remain inconclusive and/or retesting may be needed.[2]

The recent guidelines of the European Respiratory Society establish criteria for definitive diagnosis and very probable diagnosis of PCD.[4] Diagnosis is considered definitive when a patient with a clinical history suggestive of PCD presents: (i) defects that cause abnormalities in the ciliary ultrastructure evaluated by electron

microscopy, or (ii) non-ambiguous biallelic mutations in PCD causing genes in the genetic studies. Diagnosis is considered highly likely in patients with a history suggestive of PCD that present: (i) very low nNO plus HVM findings suggestive of PCD on three successive occasions, or (ii) very low nNO plus HVM findings suggestive of PCD after *in vitro* cell culture (Table 3). It is believed that future advances in diagnostic tests and a better understanding of the pathophysiology of PCD will allow clinical clarification of a significant proportion of these patients.[7]

The availability and combination of diagnostic tests varies between countries and between centres,[6] and there is currently no uniform international consensus on the results that define the diagnosis of PCD as definitive, possible or excluded.[3,7] Likewise, there is no overall uniformity for the execution and interpretation of results of any of the tests used.[7] It is possible that in the future research will continue to modify the understanding of the different phenotypes of PCD and, consequently, its diagnostic criteria.[3]

Table 3. Diagnosis criteria of primary ciliary dyskinesia
1. Clinical presentation suggestive of primary ciliary dyskinesia
2. Confirmation of diagnosis by at least two of the following tests:
a. Very low nNO values
b. Unequivocally abnormal findings in electron microscopy
c. Unequivocally abnormal findings in high-speed video microscopy
d. Biallelic mutations in PCD causing genes in the genetic studies
Note: if only nasal nitric oxide and high-speed video microscopy analyses are altered, the tests should be repeated prior to diagnosis

Adapted from: Lucas JS, et al. Arch Dis Child 2014;99:850-6; Werner C, et al. Cilia 2015;4:2; Barbato A, et al. Eur Respir J 2009;34:1264-76; Lucas JS, et al. Eur Respir J 2014;44:1418-22.[2,3,8,71]

Follow-up

There are no randomized clinical trials on the follow-up and treatment of patients with PCD,[1-3] although European studies on therapeutic outcomes are currently under way.[53] Therefore, care provided is based on international consensus and recommendations used for other chronic respiratory diseases, such as cystic fibrosis and non-cystic fibrosis bronchiectasis.[1] However, differences in several phenotypic parameters between these diseases suggest that the extrapolation of disease management may not be appropriate in all circumstances of PCD.[2,54-56] Nonetheless, these entities share a similar pattern of regular evaluations in specialised centres and multisystemic targeted treatment, with special attention to airway clearance and infection control.[2] In addition, it should be noted that family genetic counseling at the time of diagnosis should be part of the multidisciplinary approach of PCD patients.[5]

Respiratory monitoring

After diagnosis, the aim of initial respiratory management is to optimise respiratory function and limit disease progression.[2] Patients may be clinically well, with no significant complaints or changes in physical exam, despite a history of chronic productive cough. For this reason, patients should be followed up in specialized centers using lung disease assessment methods.[2] Paediatric pulmonology visits should be performed in centres with experience, two to four times a year, with functional respiratory studies, microbiological tests and respiratory physiotherapy review.[1-3]

Cultural studies of sputum or respiratory secretions should be performed two to four times a year.[8,57] Although most common pathogens in children with PCD are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*,[58,59] *Pseudomonas aeruginosa* and other Gram negative agents as well as non-tuberculous mycobacteria should also be investigated.[14,57] The results of these tests will allow for antimicrobial susceptibility-guided therapy during respiratory exacerbations.[1] When there is clinical deterioration and no response to ABT guided by the standard cultures, infection by non-tuberculous mycobacteria, fungi or bronchopulmonary aspergillosis should be investigated, which may include bronchoalveolar lavage cultures.[1] In addition, hospital infection control policies, including signaling of patients infected with resistant agents, should be performed, although cases of cross-infection have not been described in patients with PCD.[1,38]

Spirometry should be performed at least two to four times a year to monitor disease progression,[1,3] although it has not been shown to be a sensitive marker of declining respiratory function, particularly in younger patients.[3,38] Determination of lung clearance index by multiple-breath washout techniques may play a role in this regard.[3,38]

A chest radiograph should be taken at the time of diagnosis and every two to four years in stable patients to monitor disease progression.[1] The use of sequential thoracic CT scans for follow-up should be decided on an individual basis and always with the lowest possible radiation dose.[38] At least one thoracic CT scan is recommended after diagnosis, ideally when the child is old enough to cooperate (and thus avoid sedation) to identify bronchiectasis, which may be anticipated according to the clinical course.[1,16,60] Some centers recommend thoracic CT every five years, although there is no evi-

dence showing improved outcomes[61] and there is a cumulative radiation risk.[1] Magnetic resonance imaging has similar results to high-resolution CT in determining respiratory disease severity and extension in non-cystic fibrosis bronchiectasis,[62] and may be an important and radiation-free tool for the longitudinal follow-up of these patients.[3,38]

Finally, the investigation of sleep-disordered breathing should also be part of the follow-up,[2,3] since their incidence is higher in these patients, particularly of obstructive sleep apnea.[63,64]

Ear, nose and throat monitoring

Children should be evaluated by an otorhinolaryngologist once or twice a year, while adults should be evaluated only if clinically justified.[1] In this context, conductive hearing loss secondary to chronic serous otitis is the main concern.[1] There is often some improvement during adolescence, although some patients maintain this condition until adulthood.[1] An audiological examination should be performed at the time of diagnosis and some groups advocate biannual and annual evaluation in children and adults, respectively.[1,3]

In addition, the occurrence of chronic rhinosinusitis,[1] which can substantially affect patients' quality of life, should also be investigated.[65] Depending of the patient's age, nasal endoscopy may allow for the identification of nasal polyps.[1] Nasal function tests (rhinomanometry and olfactory tests) may be performed (especially in the presence of nasal polyps) in order to assess nasal congestion and its effect on olfactory function.[38]

Treatment

Treatment of respiratory disease in patients with PCD is mainly based on the promotion of mucociliary clearance of the respiratory tract and selective ABT.[2] Airway clearance by respiratory physiotherapy techniques should be performed daily and techniques should be reviewed at all visits.[1-3,38] Adjunct use of bronchodilators, mucolytics or physical exercise may be considered, although there is no evidence to support their use.[2,38] Daily cardiovascular exercise is also recommended,[1-3] since reduced physical capacity is associated with deterioration of lung function.[66] In addition, exercise allows optimization of mucus clearance.[2]

Infectious exacerbations should be selectively treated with ABT, ideally guided by prior cultures.[1-3] Fever is not a reliable sign in many patients, and ABT should be initiated after gradual and persistent increase in the volume or purulence of secretions or sputum.[3]

Some authors recommend the initiation of ABT in the presence of worsening respiratory symptoms, a deterioration of lung function or positive cultures (even in the absence of symptoms).[1,2] Pathogen-oriented broad-spectrum antibiotics (amoxicillin plus clavulanic acid or a cephalosporin equivalent) for a period of two to three weeks are often recommended.[1] More severe exacerbations or intolerance to oral therapy may justify intravenous therapy.[1,2] In some patients with severe lung disease, ABT may be considered every three months or therapy with azithromycin three times a week to limit disease progression.[2] A randomised clinical trial to measure the efficacy of azithromycin maintenance therapy for six months in the prevention of respiratory exacerbations in PCD patients is under way.[53] Infection with *Pseudomonas aeruginosa* (especially in adults) is treated with eradication protocols based on cystic fibrosis protocols and chronic infection is usually treated with inhaled suppressive ABT.[1-3]

In selected patients, inhaled therapy with hypertonic agents may be of some benefit as they optimise mucus clearance.[1-3] Other options include use of inhaled bronchodilators and DNAse, although prospective studies are needed to show their efficacy.[1-3,38]

The insertion of tympanostomy tubes in patients with conductive hearing loss is currently controversial.[2,3,38] Although some studies show positive results,[67,68] poor mucociliary clearance may lead to the occurrence of chronic ear discharge after their placement.[69] For this reason, hearing loss is usually treated conservatively with hearing aids, educational support or, where appropriate, speech therapy.[2,3] If tympanic membrane perforation occurs (particularly after repeated TT placements), tympanostomy is recommended.[2]

In patients with chronic rhinosinusitis, daily nasal irrigation with isotonic[1,2,38] or hypertonic saline is recommended.[3] In exacerbations, ABT and, according to some groups, topical corticosteroids are recommended, although evidence supporting this practice is lacking.[1,3] Nasal surgery may be warranted in selected cases with significant chronic obstruction[3] or, rarely, with nasal polyposis.[2] There is no evidence to support prophylactic adenotonsillectomy.[2]

PCD patients should be vaccinated according to national protocols, and pneumococcal and annual influenza vaccines should be added.[1,2,4,8] During the first year of life, seasonal immunoprophylaxis against respiratory syncytial virus should be considered, particularly in infants with severe respiratory and cardiac disease, according to local recommendations.[1,70] Eviction of tobacco smoke exposure is recommended and smoking cessation support should be provided to the family.[2]

PCD patients who present congenital heart defect with surgical indication should undergo corrective or palliative procedures.[3]

There is currently no evidence to support the routine use of inhaled corticosteroids,[1-3] inhaled mucolytics,[2] n-acetylcysteine[8] or intravenous immunoglobulin.[1]

Conclusion

In the last decade, international efforts have enabled a revolution in the understanding of the pathophysiology and diagnostic approach of PCD. Recently, Better Experimental Screening and Treatment for Primary Ciliary Dyskinesia (Bestcilia),[72] consortium founded by the European Commission with European and US researchers between 2012 and 2015, allowed for important advances in the characterisation of clinical course and optimisation of diagnostic and treatment programmes in different countries. Nevertheless, PCD remains underdiagnosed, since its symptoms alone are non-specific and there is no gold standard diagnostic test. Clinicians of different specialties should be familiar with the clinical manifestations that suggest PCD and referral flowcharts should be created and adapted to the local settings.

Finally, it should be noted that, due to the incidence of the disease and the complexity and specificities of the diagnostic methods, diagnosis must be carried out by a dedicated and qualified multidisciplinary group with adequate infrastructures and communication with international reference centres.

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