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HOW TO USE NASAL NITRIC OXIDE IN A CHILD WITH SUSPECTED PRIMARY CILIARY DYSKINESIA

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ABSTRACT

Measuring nasal nitric oxide (nNO) is increasingly used as part of testing for Primary Ciliary Dyskinesia (PCD). The diagnosis of PCD is often delayed until after bronchiectasis is established in the lungs and auditory damage has occurred. It is important that all paediatricians are aware of clinical features that are suggestive of PCD that should prompt diagnostic testing. nNO levels are recognised to be low in patients with PCD and results generated by static chemiluminescence analysers using velum closure technique in older children have good sensitivity and specificity. However, to conclusively rule PCD in or out further tests of ciliary function are required and assessment of cilia ultrastructure, immunohistochemistry studies and genotyping may also be indicated. These tests are more complex, invasive and expensive than nNO. nNO is less well studied in younger children where tidal breathing measurements are required. Portable NO analysers are also increasingly used in practice. This paper discusses when to consider PCD as a possible diagnosis in a child along with the indications, physiological and technical background and clinical utility of nNO as a test for PCD in children.
INTRODUCTION - WHAT IS PCD?

Primary Ciliary Dyskinesia (PCD) refers to a varied group of genetic conditions caused by defective cilia function.[1] The specific defect in function, clinical phenotype and inheritance varies between different mutations. Motile cilia line the respiratory tract, ventricles of the brain and fallopian tubes and are responsible for the motility of spermatozoa. Normal cilia ultrastructure consists of 2 microtubules surrounded by 9 microtubules, known as the "9 plus 2 arrangement". The outer microtubules have inner and outer dynein arms, which generate ciliary movement.

In PCD cilia may be immobile, dyskinetic or aplastic.[2] There are already over 30 different mutations known to result in PCD, which is predominantly inherited in an autosomal recessive fashion.[3] Dysfunctional cilia cause reduced clearance of secretions in the respiratory tract and middle ear, problems with cerebrospinal fluid flow in the brain and defective transfer of sperm or ova for reproduction. Children with PCD are predisposed to recurrent lower respiratory tract infections, which often results in bronchiectasis and reduced lung function. Respiratory symptoms may appear early in life and there may be a history of unexplained neonatal respiratory distress. Other common clinical features include chronic rhinosinusitis and recurrent otitis media, causing conductive deafness, and reduced fertility.

In the fetus motile cilia present on the embryonic node are involved in left-right chest and abdominal organ arrangement.[1, 3] Situs inversus is found in about half of people with PCD.[2] Kartagener’s syndrome is a triad of situs inversus, chronic sinusitis and bronchiectasis. Children with heterotaxic congenital heart disease, where there is isomerism of the atrial appendage and often other organ involvement as well, for example polysplenia or asplenia, should also be investigated for PCD.[4] The estimated prevalence of PCD is 1 in
10 000, however this is recognised to be much higher in certain populations.[5] For example, a study found a prevalence of 1 in 2265 in a British Asian population and of these patients around half had consanguineous parents.[6]

It is important that clinicians are aware of features (see Table 1) that are suggestive of a potential diagnosis of PCD in order that children can be promptly referred for diagnostic testing. PICADAR (PrImary CiliARy DyskinesiA Rule) is a recently developed tool to help identify children that should be referred for testing (see Table 3).[7]

**Table 1: When should I investigate a child for Primary Ciliary Dyskinesia?**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1.</td>
<td>Several of the following: persistent wet cough, situs anomalies, congenital cardiac defects, persistent rhinitis, chronic middle ear disease with or without hearing loss, a term infant with neonatal upper and lower respiratory symptoms or neonatal intensive care admittance</td>
</tr>
<tr>
<td>2.</td>
<td>Patients with normal situs presenting with other features of Primary Ciliary Dyskinesia</td>
</tr>
<tr>
<td>3.</td>
<td>Siblings of children with Primary Ciliary Dyskinesia</td>
</tr>
<tr>
<td>4.</td>
<td>Symptoms of Primary Ciliary Dyskinesia and predictive tool such as PICADAR score &gt;5 (see Table 3, possible scores range from 0 to 14, questions are based on birth and neonatal history and presence of situs abnormalities, congenital heart disease, perennial rhinitis and hearing or ear problems)</td>
</tr>
</tbody>
</table>

Adapted from Lucas et al. (2016).[8]

**DIAGNOSIS OF PCD**

The diagnosis of PCD is often delayed and children may unfortunately have established bronchiectasis and hearing impairment by the time of diagnosis.[2, 9, 10] It follows that early diagnosis is essential to implement specialist multidisciplinary follow up and appropriate
treatment so that lung function and hearing can be preserved and quality of life improved as far as possible.[2] A recent European survey showed median age of diagnosis to be 5.8 years in children with PCD and 3.5 years for those with PCD and situs inversus.[9] Children frequently attend several clinicians on multiple occasions before a diagnosis of PCD is established.[11]

Children suspected of having PCD are usually assessed by tertiary respiratory paediatricians. Diagnosis is established by a combination of clinical history and tests including nasal nitric oxide (nNO), analysis of ciliary beat frequency and pattern by high-speed video microscopy analysis (HSV), transmission electron microscopy (TEM), genotyping and immunofluorescence.[1] Importantly, no single diagnostic test will identify all cases of PCD and there is no "gold standard" test as such. In November 2016 evidence-based guidelines were published by the European Respiratory Society that include a diagnostic algorithm for the diagnosis of PCD.[8] The first step of the suggested algorithm involves nNO and use of HSV where if both are normal further diagnostic tests can be avoided unless the clinical suspicion is very high.[8]

**USE OF NASAL NITRIC OXIDE**

HSV and TEM demand specialist laboratory expertise and are time consuming and expensive. Importantly they also require either a nasal or tracheal brushing sample to harvest airway epithelial cells, which can be painful and distressing for the paediatric patient or may require a bronchoscopy under general anaesthesia.[1, 2] Furthermore, samples may be insufficient or ex vivo cell culture may be technically unsuccessful. Measuring nNO is both a less invasive and less technically demanding procedure.
NO is produced by enzymatic action of nitric oxide synthetases and biologically is a key signalling molecule involved in multiple processes; most notably causing smooth muscle relaxation and vasodilatation.\[12\] It is important to make the distinction between nNO, which is often expressed in terms of flow as nL/minute, and fractional exhaled (whole breath) NO that is commonly used as a biomarker of eosinophilic inflammation in asthma and reported as a concentration in parts per billion.\[13, 14\]

Several studies have identified significantly low levels of nNO in patients with PCD compared with healthy volunteers and other respiratory disease comparators, most notably cystic fibrosis.\[15-20\] The exact mechanism by which nNO levels are low in PCD is currently unknown.\[2, 19, 20\] Divergent hypotheses have been proposed ranging from increased metabolism or reduced production of NO by airway epithelial cells in PCD to abnormalities in the paranasal sinuses leading to obstruction of NO or reduced production at this site.\[20\]

nNO concentration is detected by sampling nasal gas. The "gold standard" method of measuring nNO concentration is by using a static chemiluminescence analyser.\[1, 21\] The patient is seated and a nasal sampling tube is placed into one nostril while air is entrained into the other nostril. The patient is then asked to hold their breath whilst performing a velum closure manoeuvre for 20 seconds.\[21\] Velum closure is when the patient exhales through their mouth against resistance. This ensures that the soft palate is closed and prevents any contamination from the pharynx.\[21\] The analyser displays nNO concentration in real time enabling a measurement from the peak plateau to be taken. A concentration in parts per billion is then converted to nL/minute to reflect the sampling rate. Medications the patient is taking are recorded, drugs such as nasal decongestents or steroids or antihistamines may alter nNO levels.\[21\]
The sensitivity and specificity of nNO as a test for PCD varies between different studies depending on the method of sampling, analyser used and age of the individual patients.[8, 19] There is no universally agreed diagnostic threshold although 77nL/minute is most commonly used for children of school age and above using the velum closure technique.[19] The 2016 ERS guidelines conclude that sensitivity and specificity for diagnosing PCD by static chemiluminescence analyser measurements using velum closure technique in children over 6 years are favourable, at between 0.9-1 and 0.75-0.97 respectively.[8]

Stationary chemiluminescent analysers are expensive and best suited to large tertiary centres. Portable electrochemical devices have also been developed, which are cheaper and easier to use. There is limited data comparing stationary and portable nNO devices but studies have shown them to be reliable in assessing nNO levels.[22, 23] Portable devices require the patient to hold their breath for longer and do not display the sampling in real time preventing sampling acceptability to be measured.

An important further caveat is that in children under 6 years nNO measurement is particularly challenging and furthermore differences between children with PCD and healthy controls are less marked in the youngest age groups.[24] Pre-school children often struggle to perform velum closure manoeuvres therefore tidal breathing measurements have to be used that are less well validated and appear to be less accurate.[25]

**CLINICAL BOTTOM LINE**
All paediatricians should be aware of clinical features suggestive of PCD that should trigger referral for diagnostic assessment. nNO has good sensitivity and specificity when measured using a static analyser involving a velum closure manoeuvre in older children. It is also a fairly easy and inexpensive test to perform and does not require invasive sampling of epithelial cells from the nose or lower airway. Importantly the 2016 ERS guidelines do not consider the accuracy of nNO in isolation to be adequate to rule PCD out or in. The current clinical bottom line is such that even if nNO is normal children should undergo further testing if there is a strong clinical history. Table 2 summarises the advantages and disadvantages of nNO. A key area for further research and development in order to expand the utility of nNO in the diagnosis of PCD in younger children includes the validation of techniques and reference ranges for younger children - in whom there is arguably the greatest need to expedite and improve diagnostic techniques.

Table 2. Advantages and disadvantages of nNO as a test for PCD in children

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>May prevent invasive, complex and expensive ciliary investigations</td>
<td>Velum closure difficult in young children</td>
</tr>
<tr>
<td>Rapid test to perform</td>
<td>Stationary chemiluminescence equipment can be expensive</td>
</tr>
<tr>
<td>Standard protocols and good sensitivity and specificity in older children using velum closure technique (for example a cut-off level of 77 nL/minute in this context has been found to have 98% sensitivity)</td>
<td>Small percentage of people with PCD have a normal nNO level, therefore false negatives are possible; equally if used inappropriately at a population screening level could lead to false positives and flooding of tertiary services. Current bottom line is that nNO is not considered accurate enough to rule PCD in or out in isolation where there is clinical suspicion. Data on nNO levels and measurement techniques in children &lt;6 years are limited</td>
</tr>
</tbody>
</table>
CLINICAL VIGNETTES ILLUSTRATING INDICATIONS AND LIMITATIONS OF nNO TESTING

1. *What clinical history should make you consider screening for PCD in a child?*

PCD is suggested with early onset respiratory symptoms and any of:

1. Situs inversus totalis or heterotaxic syndrome
2. Neonatal nasal congestion and/or unexplained neonatal respiratory distress
3. Positive family history of PCD
4. Males with dysmotile sperm
5. Persistent productive cough, bronchiectasis and severe upper airway disease after common causes excluded
6. Early onset of the combination of both severe upper and lower respiratory tract infections
7. Persistent or frequent serous otitis media (glue ear) associated with respiratory symptoms

The ERS guidelines also suggest use of a recently developed predictive tool, PICADAR, see Table 3.[7, 8] This uses seven simple questions to predict the likelihood of a child having PCD. A score greater than 5 is considered significant, and has a sensitivity of 0.9 and specificity of 0.75, that should prompt referral for testing.

**Table 3. Primary CiliAry DyskinesiA Rule (PICADAR tool)[7]**

| Does the patient have a daily wet cough that started in early childhood? | YES – complete PICADAR  
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>No – stop PICADAR is not designed for patients without a wet cough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was the patient born pre-term or term?</td>
<td>Term</td>
<td>2</td>
</tr>
<tr>
<td>Did the patient experience chest symptoms in the</td>
<td>Yes</td>
<td>2</td>
</tr>
</tbody>
</table>
2. **In a child presenting with recurrent otitis media, a chronic wet cough since birth and sinusitis: does a low nNO level rule in a diagnosis of PCD?**

nNO should only be considered a screening test for PCD. Given the clinical history the pre-test probability of PCD is high, the child has a PICADAR score of 6, and therefore they have been appropriately screened and a diagnosis of PCD is highly likely. The nNO test does however, have limitations. Low nNO levels can also occur in cystic fibrosis, sinusitis, nasal polyps, nasal obstruction and during an acute respiratory tract infection.[1] Further testing, for example HSVA, is also indicated for a definitive diagnosis of PCD to be reached.[8] Conversely it is also worth noting that there have been rare cases of PCD reported where nNO levels are within the normal range, for example some people with PCD associated with RSPH1 mutations have been found to have nNO levels >77 nL/min.[26] Therefore false negative results are also possible. This reiterates that if nNO levels are normal despite a classical history, referral for diagnostic testing is warranted.[2]

Collins et al. published model predictive values for nNO levels using 282 consecutive referrals to their PCD service.[27] Using a screening threshold of 77 nL/min to exclude PCD they found that their data produced a sensitivity of 93.6% (95% CI 78.6-99.2) and specificity of 84.1% (78.9-88.4) with a positive predictive value of 42% (30.2 -54.5) and negative predictive value of 99.1% (96.7 to 99.9).[27] This suggests that a screening threshold of 77 nL/min is generally effective in excluding PCD (whilst being mindful of the aforementioned

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
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<tr>
<td>Was the patient admitted to the neonatal unit?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Does the patient have a situs abnormality?</td>
<td>Yes</td>
<td>4</td>
</tr>
<tr>
<td>Does the patient have a congenital heart defect?</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Does the patient have persistent perennial rhinitis?</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Does the patient experience chronic ear or hearing symptoms?</td>
<td>Yes</td>
<td>1</td>
</tr>
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caveat of potential false negative results) but that targeted screening of children with a raised pre-test probability based on their clinical history is likely to be most useful and effective in order to avoid overwhelming specialist services with children with false positive screening results in the absence of classical symptoms.[27]

In summary therefore the history and screening test in this clinical scenario is suggestive of PCD and the patient requires prompt referral to a tertiary paediatric respiratory service for diagnostic testing.

3. **In a neonate born at term with unexplained respiratory distress and areas of collapse on chest radiograph, who required admission to a neonatal unit: does a normal nNO level rule out a diagnosis of PCD?**

Given the clinical history of a neonate with unexplained respiratory distress there is a possibility of PCD if the child goes on to develop other classical problems. The use of nNO for screening of neonates and infants is associated with difficulties. The para-nasal sinuses are underdeveloped in infants and healthy infants have been found to have lower nNO levels.[19] Furthermore, neonates and infants are unable to perform velum closure. Threshold screening levels for PCD are unknown and sampling techniques are not well established in infants. Indeed nNO testing is not well validated in children under 6 years of age.[2] Different respiratory manoeuvres have been suggested for use by younger children to measure nNO including performing measurements during breath holding, humming or tidal breathing.[19] Nonetheless the European Respiratory Society guidelines advise as a weak recommendation to measure nNO during tidal breathing in children under 6 years of age as part of PCD diagnostic workup.[8]
4. **Should all children be screened for PCD using nNO?**

nNO is of maximum value when used as a targeted test in patients who have an increased likelihood of having PCD, see Table 1 and the PICADAR tool in Table 3. nNO testing can produce false positive results and if nNO screening were to be used at a population level the number of children potentially referred on for further diagnostic PCD tests would cause unnecessary worry for families plus major expense and swamping of specialist testing services. At present nNO is not considered sufficiently accurate to rule PCD in or out definitively.
MULTIPLE CHOICE QUESTIONS

1. Which of the following statements are true?
   A. PCD is often diagnosed under 3 years of age
   B. Diagnostic testing for PCD is currently cheap and easy to perform
   C. Diagnostic testing for PCD always involves genetic testing
   D. If nNO and HSVA are normal the diagnosis of PCD is very unlikely
   E. Fractional exhaled nitric oxide is a screening test for PCD

2. Which of the following clinical histories would warrant testing for PCD?
   A. A child with recurrent lower respiratory tract infections, grommets and history of unexplained neonatal respiratory distress
   B. A child with a chronic productive cough and steatorrhoea
   C. A child with dextrocardia
   D. A child with nocturnal cough and exercise-induced wheeze
   E. A child with recurrent upper respiratory tract infections, sinusitis and family history of PCD

3. Which of the following statements are true?
   A. The recommended technique to perform nNO screening uses a velum closure respiratory technique
   B. Threshold screening values for nNO in infants are well established
   C. nNO measurement in older children using velum closure technique has good sensitivity and specificity for PCD
   D. Chemiluminescence analysis of nNO levels is possible using a portable machine
E. Alternative potential respiratory manoeuvres for nNO screening in preschool children include tidal breathing, humming and breath holding

4. Limitations of nNO screening for PCD include:

A. Lower values of nNO can also occur in CF, sinusitis, nasal polyps, nasal obstruction and with an acute respiratory tract infection

B. It is a screening test with poor sensitivity

C. A negative test cannot rule out a diagnosis of PCD when clinical history is highly suggestive

D. Some patients with PCD may have normal nNO levels

E. It cannot be used accurately in children less than 5 years of age

5. Further research regarding screening for PCD is needed to:

A. Ascertain threshold screening values for PCD

B. Standardise acceptable respiratory manoeuvres for pre-school children to perform nNO screening

C. Establish reasons for low nNO levels in patients with PCD

D. Ascertain threshold screening values for PCD in neonates and infants

**Answers**

1. D
2. A,C,E
3. A,C,E
4. A,C,D,E
5. B,C,D
COMPETING INTERESTS
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