EDITORIAL

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Nasal nitric oxide measurement for primary ciliary dyskinesia diagnosis: The impact of underlying genetic defects on diagnostic accuracy

Primary ciliary dyskinesia (PCD) is an inherited, genetically heterogeneous disorder characterized by the dysfunction of motile cilia that are found in a number of human tissues including the upper and lower airways epithelium (respiratory cilia). Respiratory cilia project from the apical membrane of epithelial cells that line up the airways and through their active and coordinated beating, drive the process of mucociliary clearance, which is responsible for the removal of inhaled pathogens and other hazardous substances from the respiratory system. In PCD, mucociliary clearance is impaired and as a result patients suffer from recurrent respiratory infections which lead to chronic destructive airway disease.[1] Other common manifestations of the disease include sinus and ear infections, situs abnormalities and male infertility.[2]

Mutations in more than 40 genes have been found to date to be causative for PCD and more than 70% of patients with a confirmed PCD diagnosis, harbor biallelic mutations in one of these genes.[3] Known PCD genes encode outer and/or inner dynein arm proteins as well as proteins that make-up the central pair and radial spoke apparatus. Other known PCD genes encode proteins in the nexin-dynein regulatory complex and cytoplasmic proteins involved in intraflagellar transport and preassembly of dynein arms.[3] Not surprisingly, the large number of the involved genes translates in diverse ultrastructural and motility defects, which make PCD diagnosis difficult.[4] The available diagnostic tests for PCD include measurement of nasal nitric oxide (nNO), assessment of ciliary ultrastructure using Transmission Electron Microscopy (TEM) and assessment of ciliary motility pattern using High Speed Video Microscopy (HSVM) as well as genetic and immunofluorescence (IF) analyses. Nevertheless, no single test has been found to have sensitivity and specificity close to 100% for PCD, and as result a combination of tests is usually employed as part the diagnostic work-up in PCD referral centers.[5] Current European Respiratory Society guidelines propose a diagnostic algorithm which includes the performance of both nNO and HSVM as a first step followed up by TEM,[5] while the clinical practice guidelines adopted by the American Thoracic Society recommend the employment of nNO or genetic testing coupled with TEM in the case that nNO is unavailable or patient is less than 5 years old.[6]

In contrast to the other two main tests (TEM and HSVM) for PCD diagnosis, measurement of nNO has the advantage of yielding immediate results, can be performed in centers with limited experience, does not require expensive equipment like HSVM and TEM analysis and is less invasive as it does not require nasal brushing. Furthermore, two independently conducted systematic reviews, reported excellent summary estimates of diagnostic accuracy for nNO in PCD (sensitivity > 95% and specificity > 94%).[7,8] On the other hand, TEM is characterised by a low detection rate (misses approximately 26% of PCD cases)[9]; requires expensive equipment along with specialised personnel while in many occasions; a repeat nasal brushing is needed in order to obtain a sample of adequate quality for ultrastructural evaluation.[10] HSVM has also been reported to be a highly sensitive (96%) and highly specific (91%) test for PCD,[11] which can provide same-day results to the patient, although variation of ciliary beat pattern within the sample and subjectivity in interpretation of the results may compromise diagnosis.[12]

The use of nNO for PCD diagnosis is a widely common test across PCD specialised centres for more than two decades. Nevertheless, almost all studies examining the diagnostic accuracy of this test in PCD have been conducted in European or North American settings[7,8] and data from other parts of the world are missing. Furthermore, the current, widely accepted nNO cut-off which is considered to be diagnostic for PCD (< 77 nL/min) is based on a North American study[13] and differences within other populations may exist. In this issue of *Pediatric Investigation*, Zhang et al have evaluated the ability of nNO, to discriminate among PCD, Cystic

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Fibrosis (CF), asthma, non-CF-non-PCD bronchiectasis and post-infectious bronchiolitis obliterans (BO) patients in China. In this study, the authors demonstrated that nNO can effectively discriminate PCD from other diseases in their population. Levels of nNO among PCD patients were significantly lower compared to nNO levels in each of the other groups, with the exception of the CF group where some overlap was observed. The overall sensitivity and specificity reported was 86.1% and 93.9% respectively for an optimal cut-off line of 76 nL/min.[14] Using the same cut-off, specificity rose to almost 100% when CF patients were excluded from the analysis. The optimal cut-off suggested by this study for the Chinese population (76 nL/min), is similar to the previously suggested 77 nL/min, however the sensitivity estimate associated with this cut-off is much lower (86.1%) compared to the one reported by Leigh et al (98%).[13]

A possible explanation for the differences in the nNO sensitivity for PCD across different studies may lie in the underlying distribution of genetic defects of PCD patients in each population. According to the authors, biallelic mutations in *DNAH5* and *DNAI1* genes were identified in only 8.3% of the Chinese patients while in the western cohorts this percentage may be as high as 30%.[15] In contrast, mutations in genes encoding proteins involved in the central and radial spoke apparatus (*HYDIN*, *RSPH4A* and *RSPH9*) accounted for almost one third of Chinese patients. Interestingly, out of the five confirmed PCD patients with nNO value > 76 nL/min, three were found to harbour biallelic mutations in *HYDIN* gene and one in *RSPH4A* gene, while 4 patients were characterised by central cilia microtubular disorganization in TEM (one patient with *HYDIN* mutations had insufficient sample for TEM).[13] In previous studies, some *RSPH4A* patients were reported to have nNO above the cut-off value,[16] although the same was not true for patients with a *HYDIN* genetic defect.[17] Nevertheless, there is evidence that gene defects affecting other radial spoke proteins such *RSPH1* and *RSPH9* as well as proteins of the nexin-dynein regulatory complex (*GAS8*) may be associated with nNO above the established cut-offs in some PCD patients.[18-20]

In summary, the study by Zhang et al confirms that nNO is characterised by high diagnostic accuracy for PCD and can be efficiently used to discriminate PCD from other respiratory diseases in previously understudied populations such as Chinese children. At the same time, the study findings highlight some of the challenges associated with the performance of nNO measurements for PCD diagnosis, such as the overlap between nNO values of CF and PCD children as well as the possibility of low diagnostic performance of nNO in PCD patients with specific genetic defects. Adoption of existing guidelines that advocate for nNO testing to be performed following the exclusion of CF diagnosis is expected to limit the risk of misdiagnosis while future studies are needed to provide additional evidence on the diagnostic performance of nNO in PCD patients with central apparatus, radial spoke and nexin-dynein regulatory complex defects.

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CONFLICT OF INTEREST

None.

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