Accuracy of diagnostic testing in primary ciliary dyskinesia: are we there yet?

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Diagnostic testing in PCD: an appraisal of current and future diagnostic techniques
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Although primary ciliary dyskinesia (PCD) is a rare disease, symptoms like recurrent upper and lower respiratory tract infections are extremely common, especially in the paediatric population. Therefore, an important but complicated task for clinicians is to identify patients suffering from this autosomal-recessive condition among the large cohort of individuals with recurrent respiratory symptoms. However, diagnosing PCD is difficult, as there is no single gold standard and it relies on a combination of complex techniques, only available in few specialised centres worldwide [1–4]. There are no evidence-based guidelines and consequently, diagnostic algorithms vary among centres depending on local expertise.

The article by JACKSON et al. [5] is the first to report on the diagnostic accuracy of the most commonly used diagnostic techniques in PCD (high-speed video microscopy (HSVM), nasal nitric oxide and transmission electron microscopy (TEM)), both when used as single tests and when used in different combinations, in a very large cohort of patients specifically assessed at a national PCD centre (Southampton, UK), which also included “inconclusive” patients. Does this mean that we can finally prepare an evidence-based guideline for diagnostic testing of PCD and that we can provide clinicians in various countries with a solid diagnostic algorithm for PCD? Although the data in the article provide an important first step, some issues remain to be addressed.

Similar to the sweat test in cystic fibrosis, HSVM remains an important cornerstone in the diagnosis of PCD, as shown by JACKSON et al. [5] and others [1, 3, 6–8]. This test has both excellent sensitivity and specificity. However, as all who are involved in evaluating ciliary biopsies know, ciliary beat pattern can vary considerably within a single biopsy. In addition, beat patterns are heavily influenced by respiratory tract infections. Even in patients without underlying PCD, beat patterns can remain abnormal for up to 6 weeks after an infection (secondary dyskinesia). Extensive investigator training can help to learn how to discriminate primary from secondary dyskinesia to some extent. However, repeated biopsies are often necessary and even then, contradictory findings are sometimes observed. These problems can be resolved by routine use of cell cultures [9–11], but these tests are available in even fewer centres. In addition, reported success rates of cultures vary considerably (from 54% to over 79%) [9, 12], the tests are time-consuming (up to 8 weeks) and costs are relatively high.

In addition, more recently PCD variants have been described with very subtle motility defects, such as those associated with mutations in GAS8 [13]. Likewise, novel mucociliary clearance disorders associated with a reduced number of normal functioning cilia caused by mutations in CCNO [14] and MCIDAS [15] genes producing a clinical respiratory phenotype similar to PCD may be missed by HSVM, even if cell
culture is performed, as lack of cilia is often wrongly considered secondary to infections or a culture artefact.

Many have advocated the use of nasal nitric oxide as a screening test for PCD [16–19]. Nasal nitric oxide is generally low in PCD patients and can thus be used to discriminate this condition from other diseases leading to a similar respiratory phenotype. The test is widely available, easy to perform and relatively cheap [16, 20] However, as is shown by the data from JACKSON et al. [5], up to 10% of the patients have nasal nitric oxide levels within the normal range. Excluding patients with normal nasal nitric oxide levels from further diagnostic testing would thus lead to an unacceptably high percentage of patients being missed.

Similarly, it has been clear for many years now that TEM can show a (nearly) normal ciliary ultrastructure in specific PCD subtypes [13, 21, 22]. The current study confirms this by showing that in approximately one fifth of PCD patients seen in their clinic TEM appears to be normal. This means that TEM is also an unreliable tool to exclude PCD if used in isolation. However, if used by adequately trained personal, it appears to have 100% specificity. Recently, electron tomography has evolved as a research tool enabling three-dimensional visualisation of the ultrastructure of cilia. This technique has demonstrated ultrastructural defects in PCD patients with HYDIN mutations, who did not appear to have defects on classic TEM [21]. Although there are still some limitations to this technique, such as microscopic resolution, limited penetration depth and the speed of data processing, with the current progress in both hardware and software development, we can hope that sensitivity of TEM will further increase in the future and make PCD diagnosis for more difficult cases possible as well.

Jackson and co-workers did not include all diagnostic techniques that are available to date. Recently, immunofluorescence labelling has become available [23]. For immunofluorescence labelling diagnosis, specific antibodies are used to identify proteins that are normally part of the ultrastructure of the cillum in human respiratory epithelial cells and can be absent, mislocalised or present in reduced amounts in PCD patients. Although immunofluorescence labelling has predominantly been used in PCD research in the past, an increasing number of centres are using it as part of their diagnostic work-up. This technique holds great promise, as it is relatively cheap, fast and easy to use, and provides the possibility of identifying abnormal ciliary protein composition or even the underlying molecular defect at a protein level [24]. Immunofluorescence labelling also offers the possibility to identify patients with apparently normal TEM. Therefore, it would be interesting to establish the sensitivity and specificity for this technique as well, both when used alone and when used in combination with the other diagnostic tools available.

Genetic testing was not included in the diagnostic algorithm. So far, >30 disease causing genes have been described for PCD and mucociliary clearance disorders. Thanks to the development of high throughput next-generation sequencing technologies in the past few years, mutations can now be identified in up to 70% of cases. However, the specificity of genetic testing is very high and genetic testing can identify patients missed, for example, by TEM such as patients with mutations in DNAH11 [22], HYDIN [21] and GAS8 [13]. As in ~30% of cases no mutations can be identified, genetic testing cannot be used to exclude the diagnosis of PCD at the moment.

In summary, the article by JACKSON et al. [5] is an important step towards evidence-based guidelines for PCD diagnostics, including the most commonly used techniques like nasal nitric oxide, HSVM and TEM. But we are not there yet. The work emphasises the importance of HSVM as a technique with relatively high sensitivity and specificity. However, more recently described defects with minor abnormalities in ciliary beat pattern or reduced generation of normal cilia, can easily be missed. Techniques like nasal nitric oxide and TEM are valuable tools for confirming the diagnosis of PCD, but one should be cautious to exclude the diagnosis in the case of normal findings, which contrasts with current practice in many centres. Newer techniques, like genetics and immunofluorescence labelling, hold great promise for the future. As with all clinical dilemma’s, the clinician should remain critical in cases of high clinical suspicion and be willing to re-evaluate all diagnostic steps.

References


