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Original article

Diagnosis of primary ciliary dyskinesia: When and how?

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ABSTRACT

Introduction: Primary ciliary dyskinesia (PCD) is a rare congenital disorder involving permanent ubiquitous structural and/or functional ciliary abnormalities.
Methods: A single-center retrospective study included 56 cases of PCD (respiratory form) out of a cohort of 280 patients with suspected PCD. The main features of history-taking and clinical examination were analyzed, to formulate a pragmatic diagnostic procedure, easy to implement in clinical practice.
Results: Chronic respiratory tract infectious symptoms are sensitive but non-specific for the diagnosis of PCD. Nasal brushing for phase-contrast microscopy study of ciliary morphology and activity proved to be a fast, easy, non-invasive, cost-effective and age-independent diagnostic method. In doubtful cases, depending on local availability, further tests are indicated: nasal nitric oxide level, electronic microscopy, genetic study and cell culture.
Conclusions: In suspected PCD, there being no gold standard method of screening and early diagnosis, nasal brushing with ciliary study is contributive, alongside numerous other complementary tests, on condition that the clinician is experienced and results are interpreted in the light of clinical examination and history-taking.

1. Introduction

Primary ciliary dyskinesia (PCD) refers to a group of genetic pathologies featuring permanent ubiquitous ultrastructural and/or functional abnormalities of mobile cilia. Symptomatology is polymorphous, mainly involving the pulmonary, ENT and genital regions. It is a rare condition, classically with recessive autosomal transmission. Prevalence is 1/10,000–20,000.

Diagnosis is hindered by lack of specific clinical signs; selection for ciliary study is based on a range of findings. Diagnostic procedure is complex, but has been formalized by expert teams [1,2]; even so, it remains varied in the literature, with no true single gold standard procedure universally agreed upon and applied internationally: the various complementary examinations are numerous [3–7]. Screening and/or diagnostic tests include nasal nitric oxide (NO) measurement, isotopic mucociliary clearance, optical microscopy with or without high-speed video-cinematography, electronic microscopy, ciliary cell culture, and genetic testing. Aside from questions of cost, complexity, availability and age-related feasibility for some of these tests, there is the question of the best-adapted diagnostic procedure given the large demand for assessment of suspected PCD, whatever the possible contribution of genetic testing guided by ultrastructural study of ciliary abnormalities, to improve phenotype-genotype analysis of congenital ciliopathy, both respiratory (i.e., PCD) and other (Bardet-Biedl and Alström syndromes) [8].

Based on our own experience and a review of the literature, the present study formulates a pragmatic diagnostic procedure that is fast, inexpensive, non-invasive, easy to implement in clinical practice, and age-independent, which is important for early diagnosis and treatment on which prognosis depends.

2. Material and methods

A single-center retrospective study over a 30-year period recruited 280 patients with suspected PCD, 56 of whom were finally diagnosed with respiratory PCD. A single specialist saw and selected all patients, and conducted phase-contrast optical microscopy (PCOM) study of ciliary morphology and activity. Over the years, patient selection for ciliary assessment and the microscopy technique improved with the clinician's learning curve, in terms of

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nasal mucosal brushing, staining, magnification level, slide scanning technique, double interpretation of ciliary morphology and function by the clinician and a biologist, and interpretation of results in the light of clinical and other complementary examinations. The technique could thus be better standardized, awaiting systematic use of high-speed video-microscopy and, above all, ever earlier diagnosis of PCD.

The cohort of 280 patients with suspected PCD comprised 110 female (39.3%) and 170 male subjects (60.7%). At assessment, 65% of patients were less than 20 years old, including 18% less than 1 year old, with a range of 1 month to 69 years.

Referral was by pediatricians and pediatric pneumologists (57%), ENT specialists (19%), adult pneumologists (12%), fertility specialists (8.3%), geneticists (2%) and other specialists (1.7%). Presenting symptoms basically consisted in chronic respiratory infection.

Epidemiological data (age, gender, referring physician's specialty), individual and familial history (situs inversus, parental consanguinity, familial history of PCD, respiratory infection, sterility) and data on ENT and pulmonary symptomatology were systematically collected. Differential diagnoses comprised cystic fibrosis, alpha-1 antitrypsin deficiency, allergic bronchopulmonary aspergillosis, bronchiectasis related to systemic disease, immune deficiency, and atopic pathology, with analysis of the clinical relevance of allergologic work-up to symptomatology in case of associated atopy and PCD.

Nasal ciliary brushing was performed excluding any local nasal treatment, without local anesthesia, using a synthetic brush (100–1215/50, LTA Médical, Montreuil, France) at the mid-third of the inferior turbinates and septum. Samples were examined within the hour, mounted between slide and coverslip after trypan-blue staining, on PCOM, using a Zeiss microscope at ×16 magnification to locate cell areas and ×30 for ciliary analysis. The ENT physician who took the sample and a medical biologist assessed cellularity (levels of live ciliary cells, leukocytes, puss, dead cells), ciliary morphology (ballooning, short cilium, megacilium, deciliation) and ciliary function: semi-quantitative visual assessment of ciliary beat frequency (normal, slow or absent) and of ciliary function (amplitude, synchronization, coordination). Normal ciliary beat frequency was 9–12 Hz under the present observation conditions; in pathologic cases, it was slower ($\leq$ 1–3 Hz) or, more often, the cilia were immobile or displayed only incipient and clearly ineffective activity. Examination focused on individual ciliary cells and especially nasal cavity epithelial plaques, with at least 2 slides par patient and per sampling. Examination was repeated as necessary at 1 or 6 months or following antibiotic therapy (Fig. 1).

Ciliary function assessment on PCOM coupled to high-speed video-cinematography in non-respiratory congenital ciliopathy such as Bardet-Biedl or Alström syndrome (study awaiting publication) validated the PCOM technique, demonstrating excellent correlation between visual assessment on PCOM and video-cinematographic measurement of ciliary beat frequency and kinetics in 39 out of 40 patients (data not shown here).

In the same session, samples were taken for electron microscopy, usually reserved in our center for cases in which PCOM results are inconclusive, consisting in glutaraldehyde fixation, semi-fine (4 μm) sections to locate cilia then ultrafine (0.05–0.5 μm) sections for qualitative and quantitative ultrastructural ciliary analysis.

In 6 cases, genetic study (*DNAI1, DNAH5*, etc.), guided by the ultrastructural study, was performed.

In 10 cases, ciliary function was assessed in vivo on nasal isotopic mucociliary clearance, which is more accurate than the saccharine test but not feasible in very young children as it requires strict immobility. Isotopic mucociliary clearance studied the displacement speed and migration phenomenology of the tracer (iodine^{131} and/or technetium^{99m}) coupled to albumin aggregate, using a gamma-camera and computerized data processing.

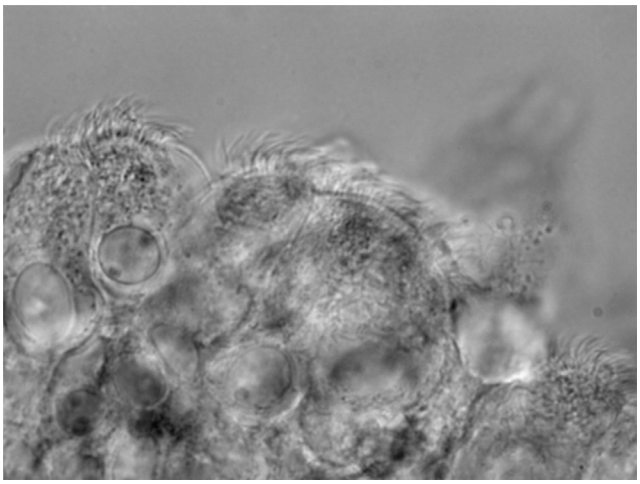

Fig. 1. Normal cilia from nasal brushing on phase-contrast optical microscopy after trypan-blue staining.

In our center, analysis of exhaled NO and cell culture to study ciliogenesis were not available.

3. Results

The cohort of 280 patients with suspected PCD was analyzed, and the PCD (n = 56) and non-PCD (n = 224) subgroups were compared at end of assessment.

3.1. Cohort of 280 patients with suspected PCD

In the 280 patients of the cohort, initial nasal brushing was indicative of PCD in 14% of cases, normal in 61%, indicative of a post-infection aspect in 17% and inconclusive in 8%. In 34 cases (12%) a second brushing was indicative of PCD in 38% of cases, normal in 44%, and indicative of a post-infection aspect in 18%. PCD was diagnosed on a third brushing in 3 cases and in a fourth in 1.

Thus, for 56 patients, nasal brushing in a suspicious clinical context indicated a diagnosis of PCD.

Electron microscopy was performed in 49 patients (17.5%), and:

- confirmed diagnosis of PCD in 19 patients (6.8% of the overall cohort and 34% of the PCD subgroup), with absence of outer/inner dynein arms, and axoneme structure defect on qualitative and quantitative ciliary analysis on transverse sections (30–50 transverse sections per patient);
- ruled out diagnosis of PCD in 15 patients;
- revealed abnormalities suggestive of infectious pathology (atypic, polymorphous, heterogeneous abnormalities) in 5 patients;
- was inconclusive in 10 patients.

Electron microscopy was repeated in 4 patients, diagnosing PCD in 1 and non-PCD in 1 and, remaining inconclusive in 2.

Thus, PCD diagnosis was confirmed on electron microscopy in 20 of the 56 PCD patients (36%).

3.2. Subgroups of 56 PCD and 224 non-PCD patients

The PCD and non-PCD subgroups were comparable for epidemiological characteristics of age and referral; mean age at diagnosis was 17.2 years in the PCD subgroup and mean age at assessment

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