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Nasal nitric oxide for early diagnosis of primary ciliary dyskinesia: Practical issues in children

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Summary
Background: Primary ciliary dyskinesia (PCD) is a genetic disease characterized by abnormally beating cilia. In these patients, levels of nasal nitric oxide (nNO) are lower than those observed in healthy subjects.
Objectives: We identify the nNO levels in healthy pre-school uncooperative children and in PCD patients, in order the application of nNO measurement in the early identification of young children with PCD.
Methods: We measured nNO in 77 healthy children (50 uncooperative and 27 cooperative) and in 10 PCD patients. Fifteen cooperative healthy children were also asked to perform an uncooperative test.
Results: PCD patients presented low nNO levels (29.7 ± 5.7 ppb) compared to those observed in healthy children (358.8 ± 35.2 ppb; $p < 0.05$). nNO levels were increased in healthy cooperative children (650 ± 60.6 ppb; $p < 0.05$) as compared to those uncooperative aging more than 6 month (309.1 ± 45.9 ppb; $p < 0.05$) or less (128.1 ± 16.2 ppb; $p < 0.05$). Twenty-four uncooperative children with nNO values $\leqslant 200$ ppb performed a second evaluation at least 6 months later and mean levels increased from 104.7 ± 10.5 ppb to 169.9 ± 19.6 ppb ($p < 0.05$). In the 15 collaborative children nNO levels were higher during the breath holding manoeuvre (687.7 ± 96.9 ppb) than during the tidal breathing manoeuvre (335.9 ± 57.9 ppb; $p < 0.05$).
Conclusions: Healthy children have higher nNO levels than PCD patients. In 15% of uncooperative healthy children can be found low nNO levels, similar to PCD patients, but those values increased some months later, in successive evaluations. Nasal NO may be used for PCD screening even though repeated evaluations may be necessary in young children.

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Introduction

Primary ciliary dyskinesia (PCD) is a genetic disease characterized by defective motility of cilia, in most cases related to an ultrastructural defect,[1] which results in impaired mucociliary clearance of the upper and lower airways[2] associated with recurrent-chronic respiratory symptoms.[1,3]

Since respiratory symptoms are also common in healthy children and the disease is relatively rare (1 in 15–30,000 live births, in white population), the diagnosis of PCD is often missed for a long time.[4] Furthermore, diagnostic investigations such as the demonstration of ultrastructural defect at transmission electron microscopy and ciliary motion analysis require both expertise and laboratory facilities which are not widely available.

In order to prevent lung function deterioration due to inadequate treatment, an early identification of PCD patients is warranted.[5,6] Since nNO level in PCD patients has been observed to be 80–90% lower than in healthy controls,[7–15] including affected infants,[16,17] this parameter could provide useful first-line information in the diagnostic algorithm of PCD. Nevertheless, while normal values are available for school aged children,[18] reference values are lacking in pre-school aged children and infants who cannot perform the nNO test according to ERS/ATS guideline.[19]

The aim of this study was to identify the nNO levels in healthy infants and pre-school children devoid of signs and symptoms suggestive of ciliary dyskinesia (Table 1). A particular regard was dedicated to methodological issues in these uncooperative subjects, in order to better address the potential application of nNO measurement in the early identification of young children with PCD.

Table 1 Signs and symptoms suggestive of PCD.[2,3]

Neonatal period:

- respiratory distress or pneumonia in term neonates with no obvious predisposing cause
- rhinitis and/or nasal congestion that remain constant over time
- situs inversus
- moist sounding cough is unusual in this period but suggestive
- complex congenital heart disease, esophageal and biliary atresia, hydrocephalus
- positive family history of PCD

Infant and older child:

- chronic cough with sputum production
- rhinosinusitis
- chronic secretory otitis media with prolonged otorrhoea after tympanostomy
- pneumonia
- bronchiectasis
- repeated courses of antibiotics for chest infections
- atypical asthma refractory to treatment

Adults:

- as for older children with reduced importance of otitis media
- subfertility or infertility in male
- ectopic pregnancy

Patients and methods

Subjects

A total of 87 subjects participated to the study. Of these, 10 patients (7 males, mean 17 years) with PCD, two of whom were uncooperative children, diagnosed by electron microscopy[2,20] served as positive controls. Main clinical features for these patients are summarized in Table 2. Seventy-seven subjects were healthy children (46 males, 31 females), 50 of which were uncooperative infants aged less than 6 months ($n = 26$) or between 6 and 12 months ($n = 24$) and 27 were school-aged children (mean 7 years) able to perform the test procedures according to guidelines.[19]

The subjects were selected among those who had never received inhaled corticosteroids or nasal decongestant drugs and did not have adeno- or tonsillectomy.

The measurements were part of routine clinical evaluation.

Nasal NO measurements

Exhaled nasal nitric oxide (nNO) level was measured by inserting a nNO-inert olive in one nostril, completely occluding the nostril to avoid ambient air sampling.[18,19] The controlateral nostril was left open. The olive was connected *via* a Teflon® tube at the analyser and the nasal air was sampled continuously with a constant transnasal aspiration flow of 300 mL min^{-1}.[19,21] The nNO

Table 2 PCD patients' characteristics.

Patients no.	*Situs inversus (yes/no)*	Bronchiectasis (yes/no)	Sinusitis (yes/no)	Cilia ultrastructural defect
1; c	N	Y	Y	I-ODA
2; c	N	Y	N	I-ODA
3; u	Y	N	N	IDA
4; c	N	Y	N	I-ODA
5; c	Y	Y	N	I-ODA
6; c	N	Y	N	IDA
7; c	N	Y	N	ODA
8; c	N	Y	N	I-ODA
9; c	Y	Y	Y	I-ODA
10; u	Y	N	N	I-ODA

c, cooperatine; u, uncooperative; I-ODA, inner and/or outer dynein arms.