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*Towards a global assessment of pediatric non-cystic fibrosis
chronic pulmonary disorders:
new insights in disease diagnosis and monitoring*

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Chapter 1

-Background and Aim of the Study Project-

Classification of chronic lung disease (CLD) in children has traditionally been characterized by a clear distinction between cystic fibrosis (CF), the most common lethal inherited genetic disorder affecting 1 in every 2500-3500 live births worldwide (1), and less common “non-CF” disorders. The latter represent a heterogeneous group of several conditions with different etiologies, but with overlapping clinical features (2). Despite complete agreement on definitions has not been achieved, non-CF CLD generally describes a clinical syndrome characterized by symptoms of chronic endobronchial suppuration with or without radiological evidence of bronchiectasis at chest high-resolution computed tomography (HRCT) (3). Prevalence of non-CF CLD widely varies among countries, with peaks of one in 68 children in central Australia (4), and significantly lower prevalence in Europe, where approximately one in 7'440 children aged less than 15 years is reported to be affected by one of these conditions (5).

Despite neglected and often still considered orphan diseases, pediatric non-CF chronic lung disorders are responsible of relevant burden for both families and health professionals. Indeed, these conditions still represent an important cause of respiratory morbidity in both developed (6) and developing (7) countries, with some affluent countries even reporting childhood fatalities (8). As a consequence, considerable stress, anxiety and depression have been reported in primary caregivers who look after these patients (9), and economic costs due to the frequent need for healthcare resources utilization are increasingly appreciated (10).

In the assessment of a child with chronic symptoms of endobronchial suppuration, i.e. wet cough and recurrent respiratory exacerbations with evidence of bacterial infection or colonization, a conclusive diagnosis may be cumbersome to achieve, as a number of different etiologies may underlie similar clinical presentations. Apart from CF, bronchiectasis and CLD may derive from a variety of conditions including recurrent aspiration, mucociliary dysfunction (mainly primary

ciliary dyskinesia, PCD) and primary or secondary immune deficiencies (3). The assessment of respiratory impairment in these conditions has traditionally relied on few tools which allow the evaluation of both lung function and structure and that are used for monitoring disease evolution and for modulating treatment (11). Despite the traditional dichotomy in assessing respiratory disease has been represented by the evaluation of lung function impairment on one side and by the visualization of lung structural damage on the other, an active search has been performed in order to find new and more sensitive biomarkers aimed at overcoming the limits of currently used diagnostic tools. This search has provided several promising results, particularly the metabolomic profiling of exhaled breath condensate, which has proven useful in differentiating respiratory phenotypes through the recognition of specific markers (12). These findings will hopefully lead towards diagnostic tools able to characterize, at a molecular level, different respiratory conditions and to provide a detailed definition of borderline phenotypes.

Although justified in order to better monitor progression of lung impairment, these efforts aim to improve the management of only one, even though essential, aspect of pediatric CLD: the respiratory involvement. Nevertheless, an often unmet need in non-CF CLD children is a more global approach, taking into account other relevant issues such as growth, nutrition and psychological aspects, sometimes underestimated by pediatric respiratory physicians. Indeed, while in CF the involvement of the gastrointestinal tract and other organs entails a traditionally systemic approach to patients' care, in non-CF respiratory conditions this issue is often neglected and also research in the field results to be relatively sparse. However, an increasing awareness is emerging regarding the need to adequately address nutritional status and growth in non-CF chronic respiratory disease of childhood, due to the strong relationship with lung impairment and to the evidence that these two elements may deeply influence each other. Furthermore, unlike other chronic conditions, in non-CF CLD and bronchiectasis poor attention has been dedicated to the impact of the disease on patients' life. Quality of life (QoL), particularly health-related QoL (HRQoL), represents a crucial parameter to assess disease severity, efficacy of treatments and to

highlight areas of possible improvement in patients' care. Indeed, despite the clinical manifestations of different chronic respiratory disorders in children may sometimes overlap (e.g. wet cough, dyspnea, fatigue), their impact on patients may vary significantly. Hence, specific evaluation tools are needed in order to standardize QoL assessment for both clinical and research purposes.

Given these premises the main aims of this PhD thesis are:

1. To evaluate currently used and emerging tools in the diagnosis and monitoring of pediatric non-CF CLD;
2. To assess the role of nutritional status assessment in the management of non-CF CLD children.

Chapter 2

-Chronic lung disease diagnosis and monitoring: current limits and potential new tools-

2.1 Lung disease monitoring: pulmonary function versus pulmonary structure

In comparison to other organs which lend themselves to easy monitoring, e.g. serum creatinine in renal disease or liver function tests and imaging in liver disorders, lung is far more elusive, particularly in children. Therefore, to meet the clinicians' need to assess and monitor lung function and structure, the development of reliable tools able to non-invasively evaluate these crucial components represents a research priority and a still open challenge (13).

Pulmonary function tests (PFTs), namely spirometry, represent the most commonly used tool for diagnosis and monitoring of respiratory diseases both in children and adults. Nevertheless, PFTs are relatively insensitive markers of early disease and fail to detect changes in the peripheral airways (14). Spirometry is further limited by the high dependence on patient's cooperation and by the lack of robust normal values in the infant population. Finally, there is increasing recognition that there may be structural lung damage on HRCT in children with normal spirometry, both in CF (15) and non-CF CLD (14). In this setting, the search for more sensitive measures of lung function impairment has led to an increasing interest in the lung clearance index (LCI) derived from multibreath washout tests (MBW). LCI is defined as the number of volume turnovers of the lungs required to reduce an inert gas to 1/40th of its starting concentration and is increased in many conditions, such as CF and asthma (16,17). It is non-effort dependent, and therefore it is applicable also to younger age groups who are typically unable to perform spirometry. Moreover, a growing body of literature supports the role of LCI as an earlier marker of lung impairment than spirometry in several chronic respiratory conditions, particularly with regard to distal airway disease. Of course, most of the available evidence derives from CF studies that showed that LCI becomes abnormal earlier than spirometry, and correlates significantly well with forced expiratory volume at 1 second (FEV₁) in later-stage disease (16,18). Studies in non-CF CLD are less conclusive. A

British study showed that, in adults with non-CF bronchiectasis, LCI is associated with spirometric airflow obstruction and may discriminate between patients and healthy subjects (19). Similarly, when assessed in PCD, LCI was altered even in presence of normal FEV₁ (20), and was more sensitive than FEV₁ in detecting structural abnormalities (21). Nevertheless, a comparative study of LCI, HRCT and spirometry in CF and PCD found that, unlike CF, LCI does not correlate with HRCT parameters in PCD, suggesting that it is not a sensitive test of airway disease in advanced PCD lung disease (22).

If lung function assessment still relies on spirometry, a non-invasive tool limited by low sensitivity in evaluating distal airways and early disease, structural evaluation is based on a highly sensitive technique that provides detailed imaging of lung anatomy, i.e. computed tomography (CT). Chest CT, particularly HRCT, has become the imaging technique of choice for the evaluation of lung abnormalities at any age (23,24). However, despite its high efficacy in providing detailed images of pulmonary structure, CT has been criticized for its ionizing radiation burden and the possible consequences of cumulative doses, particularly deriving from frequent follow-up examinations in patients with chronic disorders, in pregnancy, and during childhood (25). Several surveys on the effects of repeated CT examinations have demonstrated that lifetime cancer risks are cumulative and not negligible, even though the consequences of low levels of exposure have not been clearly elucidated (26,27). In this setting, chest magnetic resonance imaging (MRI) is being increasingly regarded as a reliable radiation-free technique for the assessment and follow-up of several chest disorders (28-30). Application of MRI in lung imaging has long been limited by technical problems, namely a low signal-to-noise ratio because of the low proton density of the lung, and artifacts deriving from cardiac and breathing motion (31). Over the last decade, a growing body of literature has been produced to compare the efficacy of chest MRI, mainly 1.5-T MRI, with other traditional imaging techniques in children or adults with respiratory conditions (30,32-35). These studies mostly agreed in the conclusion that MRI is comparable to conventional chest x-ray and CT, and could represent a reliable radiation-free option in lung disorders.

In the last years, we focused our attention on the possibility to adopt high-field 3.0-T MRI, which shows better temporal and/or spatial resolution and faster acquisition times when compared to 1.5-T MRI (36), in the assessment of lung structural impairment in patients with non-CF CLD. Particularly, we demonstrated that chest MRI is as effective as HRCT in assessing the extent and severity of lung abnormalities in children with PCD, primary immunodeficiencies and recurrent pneumonia (37). We also extended the use of this non-invasive technique to a particular category of patients, namely subjects with ataxia telangiectasia (AT), a rare autosomal recessive disease characterized by heightened sensitivity to ionizing radiations, increased risk of developing lymphoid malignancies, and the progressive development of CLD as a major cause of morbidity and mortality (38). An imaging tool able to assess lung structural damage is virtually lacking in AT, as HRCT is not applicable to patients due to the ionizing radiation burden (25). Therefore, in fifteen patients with AT, we assessed lung structural abnormalities by means of chest high-field MRI, and found abnormalities such as bronchiectasis, mucous plugging or consolidations in all subjects either they were symptomatic or not. Hence, given that AT patients should avoid imaging techniques entailing radiation exposure, we concluded that chest MRI should be proposed in the diagnostic pathway for AT pulmonary disease assessment (39).

The increasingly accepted reliability of chest MRI in visualizing lung structural abnormalities both in CF and non-CF CLD has raised the possibility of using such tool in comparison studies. Among non-CF chronic respiratory conditions, PCD is a rare autosomal recessive disease whose management is widely mutated from CF care, due to phenotypical similarities between the conditions (40). Therefore, we performed a comparative assessment of clinical, functional, microbiological and MRI findings in PCD and CF patients in order to evaluate different expression of lung disease.


The results of this analysis were included in a study that has been recently accepted for publication in the *Italian Journal of Pediatrics*.

RESEARCH

Open Access



Lung structure and function similarities between primary ciliary dyskinesia and mild cystic fibrosis: a pilot study

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Abstract

Background: Primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) are increasingly compared. There are no chest magnetic resonance imaging (MRI) comparative studies of PCD and CF. We assessed clinical, functional, microbiological and MRI findings in PCD and mild CF patients in order to evaluate different expression of lung disease.

Methods: Twenty PCD (15.1 years) and 20 CF subjects with mild respiratory impairment (16 years, 70% with pancreatic insufficiency) underwent MRI, spirometry, and sputum cultures when clinically stable. MRI was scored using the modified Helbich system.

Results: PCD was diagnosed later than CF (9.9 versus 0.6 years, $p = 0.03$), despite earlier symptoms (0.1 versus 0.6 years, $p = 0.02$). In the year preceding the study, patients from both groups underwent two systemic antibiotic courses ($p = 0.48$). MRI total scores were 11.6 ± 0.7 and 9.1 ± 1 in PCD and CF, respectively. FEV₁ and FVC Z-scores were -1.75 (range, -4.6 – -0.7) and -0.6 (-3.9 – -1.8) in PCD, and -0.9 (range, -5.4 – -2.3) and -0.3 (-3.4 – -2.5) in CF, respectively. No difference was found between lung function or structure, despite a higher MRI subscore of collapse/consolidation in PCD versus CF (1.6 ± 0.1 and 0.6 ± 0.2 , $p < 0.001$). These findings were confirmed after data-control for diagnostic delay. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were more frequent in CF than in PCD ($p = 0.05$ and $p = 0.003$, respectively).

Conclusions: MRI is a valuable radiation-free tool for comparative PCD and CF lung disease assessment. Patients with PCD may exhibit similar MRI and lung function changes as CF subjects with mild pulmonary disease. Delay in PCD diagnosis is unlikely the only determinant of similarities.

Keywords: Computed tomography, Cystic fibrosis, Lung function, Magnetic resonance imaging, Primary ciliary dyskinesia

Background

Primary ciliary dyskinesia (PCD) is characterized by altered ciliary beat frequency and/or pattern resulting in impaired airways clearance [1]. Clinical manifestations include neonatal respiratory distress, recurrent upper airway infections and chronic suppurative lung disease. Due to phenotypical similarities with cystic fibrosis (CF) but the relative

infrequency of PCD, management of the latter is borrowed from CF protocols for many therapeutic strategies, such as clearance techniques [2]. Unlike for CF, however, the efficacy of such treatments for PCD is less obvious [3, 4]. The explanation likely resides in the different structure and pathophysiology of these entities [5]. Hence, studies to better define the inherent differences between the two conditions might lead to clarify the underlying mechanisms of PCD progression [6].

Assessment of CF and PCD lung disease traditionally includes spirometry and chest computed tomography (CT). Despite the availability of several functional parameters, FEV₁ still remains a convenient and widely used outcome for both clinical and research purposes.

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Nevertheless, FEV₁ deteriorates long after structural damage occurs [7–10], making CT essential in monitoring early and late lung changes in both conditions [1, 11]. However, the perceived risk of ionizing radiation, particularly in the young, limits frequent CT scans. Thus, chest magnetic resonance imaging (MRI) might be a valuable radiation-free alternative [12–14]. Even though its application in pulmonary imaging has long been limited by technical problems such as the low proton density of the lung, increasing evidence supports the reliability of MRI in assessing lung structural damage [12, 13]. Nevertheless, limited access to technology, poor experience in image interpretation, long acquisition times and high costs have prevented chest MRI from being routinely adopted in CF and PCD management.

A recent comparative CT study assessed lung structure in PCD and CF [15] but to our knowledge, no such comparative study using chest MRI has been undertaken to date. The primary aim of the present study was to comparatively assess lung structure in patients with PCD or mild CF by means of chest MRI. The secondary aim was to compare clinical, functional, and microbiological findings in the two cohorts, in order to evaluate different expressions of lung disease.

Methods

Study design and patients

This was a prospective, single-center comparative study of PCD and CF lung disease. Between January 2014 and May 2015 we enrolled all mild CF patients, selected on the basis of previously published functional criteria [16], and PCD patients consecutively seen at the Department of Translational Medical Sciences, Federico II University, Naples, who fulfilled the following inclusion criteria: stable lung disease, without acute dyspnea or cough, no pulmonary function changes and no requirements for intravenous antibiotics in the previous 4 weeks [10, 17]. Subjects with acute respiratory infection, developmental delay, or other conditions that could compromise compliance to MRI or spirometry, e.g., age < 6 years and/or claustrophobia, were excluded. No CF patient had undergone neonatal screening, and CF had been diagnosed according to published criteria [18]. Pancreatic insufficiency was defined as stool elastase < 100 µg/g. Abnormal motility and ultrastructural analysis of nasal cilia on transmission electron microscopy confirmed PCD diagnosis [19]. Diagnostic criteria of the enrolled PCD patients were also reviewed according to the recently published international guidelines which require typical ciliary ultrastructure or mutations in PCD causing genes for positive diagnosis, and very low nasal nitric oxide combined with consistent findings at high-speed video microscopy analysis for highly likely diagnosis [20].

At our center, CF patients routinely undergo chest high-resolution CT (HRCT) about every two years [21], whereas the time-interval between consecutive CT scans

changes on a case by case basis for PCD. Therefore, when a HRCT was scheduled for routine assessment during follow-up, we presented the study to patients and/or legal guardians, and asked them to undergo chest MRI, spirometry and sputum culture on the same day. We used this approach to verify the reliability of chest MRI scans by real time comparison to CT, the gold standard for lung abnormalities. Clinical data and sputum culture results from the preceding 12 months were collected.

All patients routinely undergo 3-monthly visits. PCD treatment strategy derives from CF care, and includes twice daily chest physiotherapy preceded by nebulized hypertonic saline, and oral and/or intravenous antibiotics based on sputum culture in case of exacerbations. Inhaled antibiotics and/or dornase alpha in PCD are not used in Italy, since they are approved for CF only. Written informed consent was obtained from patients and/or legal guardians. The study was approved by the local Ethical Committee (Comitato Etico per le Attività Biomediche, Federico II University; approval number 184/2014).

Spirometry

Spirometry was performed according to published criteria [22]. We expressed FVC, FEV₁, and FEF_{25–75} as Z-scores [23]. We considered FEV₁ as the primary outcome parameter to assess differences between groups, and a Z-score < -1.64 as abnormal [23].

Microbiology data

Sputum cultures obtained during the 12 months preceding enrolment were collected. Chronic airway infection was defined when the same pathogen was detected in at least three consecutive cultures within 6 months and after adequate antibiotic therapy.

MR scanning

MRI was performed with a 3.0-T MR scanner (Magnetom Trio, Siemens Erlangen, Germany), a maximum gradient strength of 40 mT/m, a slew rate of 200 mT/m/ms, and 32 radiofrequency channels. We used a dedicated 12-element integrated matrix coil system that covered the whole thorax for signal reception. It consisted of 1 anterior and 1 posterior flexible phased-array coil, each containing a set of 6 receiver elements. The applied sequence was a T2-weighted half-Fourier single-shot turbo spin-echo (HASTE) sequence, performed using an electrocardiograph-gating to reduce cardiac motion artifacts, and respiratory-gating by a navigator signal that monitored the diaphragm position. The field of view was patient-adapted. Sequence parameters were: repetition time/echo time/flip angle, infinite/92 milliseconds/150 degrees; 25 to 30 slices; slice thickness, 5 mm; distance factor, 20%; transversal

orientation (matrix, 380 256); acquisition time, approximately 90 s. Parallel imaging was used for all measurements using the GRAPPA (Generalized Autocalibrating Partially Parallel Acquisition) algorithm with an acceleration factor of 2 and 24 reference lines. No patient required sedation. Door-to-door time was 5.5 min (range, 5–8). All MR studies were of diagnostic quality and were well tolerated.

HRCT scanning

For all patients CT was part of the routine assessment and did not represent a study procedure. The HRCT scan was performed with a 4-slice CT scanner (Aquilion, Toshiba, Japan) and a bodyweight adapted protocol (adolescents: 120 kV, 140 mAs; children over 45 kg: 120 kV, 65 mAs; children over 35 kg: 120 kV, 45 mAs; children below 35 kg: 120 kV, 30 mAs), with 1x4 mm collimation, 10 mm gap, 0.5 s rotation time, automatic exposure control, multiple inspiratory breath holds of 3 s each, with the patient in a supine position. Scanning extended from the lung apices to below the costophrenic angles. The field of view of each sequence was patient adapted. Images were reconstructed using a high-resolution algorithm. The total time for acquisition of the images was approximately 5 min, including positioning of the patient. Contrast medium was not administered. For documentation of radiation exposure, the dose length product was recorded, and the effective dose (E) and the weighted CT dose index were calculated. A lung window setting (+1500/-500 Hounsfield unit) was used for image analysis. Images were reviewed on a workstation (iMac MacOS 10.4/OsiriX v.2.7.5 32 bit).

Image evaluation

After removal of identifying information, MRI and CT images, in a randomized patient order, were evaluated to reach consensus between a radiologist and a pediatric pulmonologist with more than 10 years of experience in chest imaging interpretation. In case of disagreement between the two observers, the debated abnormality was scored by the most trained rater. The observers were not directly involved in the patients' care, and, with the exception of subjects with *situs viscerum inversus* who were easily identifiable as PCD, they were blinded to any clinical and previous radiological data that could bias interpretation. To avoid recall bias, and to prevent raters from being influenced by a previously scored CT while evaluating MR images, CT scans were scored at least 6 weeks after MR images. Further details on image evaluation criteria are reported in the Additional file 1. The scoring system used is detailed in the Additional file 2.

Statistical analysis

Data are presented as median and ranges, unless otherwise stated. The Mann–Whitney *U* test assessed differences in clinical, functional and structural parameters between groups. Comparisons of functional and structural parameters were reassessed by one-way analysis of covariance to control for diagnostic delay, which was used as covariate. This adjustment allowed to compare lung imaging and lung function data from the two groups undoing the influence of diagnostic delay, which was significantly higher in PCD than in CF. Statistical significance of intragroup comparisons was not determined due to low statistical power deriving from small sample size. Fisher's exact test was used for categorical variables. Spearman correlation coefficient assessed the relationships among variables. Statistical significance was set at a *p*-value of ≤ 0.05 . Data were analyzed with a statistical software package (SPSS-PC, version 13.0; SPSS; Chicago, IL).

Results

Thirty-two CF and 28 PCD subjects were eligible. Due to acute respiratory infection 13 subjects (7 CF; 6 PCD) were excluded. Of the remaining, 4 CF and 2 PCD subjects refused MRI due to claustrophobia. One CF patient underwent MRI, but was excluded due to poor compliance resulting in low-quality images. Table 1 summarizes clinical, anthropometric, lung function and microbiological findings from the forty patients ultimately enrolled (20 with PCD, 20 with CF). Cilia ultrastructure of patients with PCD is reported in the Additional file 3. According to the recently published guidelines on PCD diagnosis [20], 16 out of 20 patients met the criteria for a positive PCD diagnosis, due to hallmark ciliary ultrastructure defects. The four remaining patients had a combination of non-typical ciliary defects, very low nasal nitric oxide, and static or circling cilia at the motility study, thus meeting the definition of highly likely PCD diagnosis [20].

No differences in age, gender and anthropometric parameters were found between the groups. Pancreatic insufficiency was detected in 70% of CF patients. Despite earlier onset of respiratory symptoms ($p = 0.02$), PCD was diagnosed later than CF, with a delay of approximately 9 years ($p = 0.03$). Duration of follow-up at a tertiary center was significantly longer in CF than PCD ($p = 0.009$). The number of systemic antibiotic courses and hospital admissions during the previous 12 months was comparable in the groups. Similarly, no difference emerged from the comparison between functional parameters. No difference was found between PCD and CF in the proportion of subjects with a FEV₁ Z-score < -1.64 (50 and 35%, respectively, $p = 0.5$).

Table 1 Characteristics of patients with PCD and CF

	PCD	CF	<i>p</i>
N	20	20	
Male : Female	12:8	13:7	1
Age (yrs)	15.1 (8.7–29.4)	16 (8–26)	0.60
Age at diagnosis (yrs)	9.9 (0.1–20.5)	0.6 (0–16)	0.03
Age at onset of respiratory symptoms (yrs)	0.1 (0.1–4)	0.6 (0–13)	0.02
Duration of follow-up at tertiary center (yrs)	6.9 (0.1–27.2)	14.5 (0.1–25.9)	0.009
Height (Z-score)	−0.71 (−2.55–1.81)	−0.47 (−2.41–1.5)	0.88
Weight (Z-score)	0.34 (−3.71–2.72)	−0.12 (−3.74–1.43)	0.22
BMI (Z-score)	0.77 (−2.84–2.7)	0.39 (−2.53–1.88)	0.13
Pancreatic insufficiency, n (%)	NA	14 (70)	–
Nasal nitric oxide (ppb)	14 (5–54)	NA	–
<i>Situs viscerum inversus</i> , n (%)	12 (60)	NA	–
Systemic antibiotic courses (previous 12 months)	2 (0–7)	2 (0–6)	0.48
Hospital admissions (previous 12 months)	0 (0–1)	0 (0–5)	0.38
FEV ₁ (Z-score)	−1.75 (−4.6–0.7)	−0.9 (−5.4–2.3)	0.24
FVC (Z-score)	−0.6 (−3.9–1.8)	−0.3 (−3.4–2.5)	0.37
FEV ₁ /FVC (Z-score)	−1.6 (−3.5–0.1)	−1.1 (−4.2–1.1)	0.24
FEF _{25–75} (Z-score)	−2 (−4.4–0.1)	−1.2 (−5.5–1.1)	0.24
Sputum Microbiology			
Chronic infection by <i>P. aeruginosa</i>	1/20	3/20	0.60
Chronic infection by <i>H. influenzae</i>	6/20	2/20	0.23
<i>P. aeruginosa</i> (≥1 sample)	4/20	11/20	0.05
<i>H. influenzae</i> (≥1 sample)	15/20	11/20	0.32
<i>S. aureus</i> (≥1 sample)	3/20	13/20	0.003
Genetic analysis			
ΔF508/ΔF508	–	6/20	
ΔF508/other	–	10/20	
other/other	–	4/20	

Data are presented as median and ranges (in parenthesis)

NA not applicable

Nine PCD (45%) and 15 CF patients (75%) had complete microbiological data, whereas 11 PCD and 5 CF subjects had sputum cultures performed every 4–6 months during the preceding year. Compared to PCD, CF subjects showed a significantly higher prevalence of *Pseudomonas aeruginosa* isolation in at least one sputum sample during the previous year ($p = 0.05$), despite no difference in the prevalence of chronic infection. *Staphylococcus aureus* was more frequently isolated in CF than PCD ($p = 0.003$), whereas no difference was found in the prevalence of both *Haemophilus influenzae* isolation and chronic infection between the groups.

Table 2 summarizes median MRI and CT scores from PCD and CF. For both techniques, total and specific scores were not different, although, both at MRI and

CT, severity of collapse/consolidation subscore was higher in PCD than CF ($p < 0.001$). Total scores were slightly lower in pancreatic sufficient *versus* pancreatic insufficient CF at both MRI and CT [8.5 (0–13) *versus* 11.5 (1–15) and 9 (0–13) *versus* 12 (1–15), respectively]. Total MRI and CT scores were not different in PCD *versus* CF ($p = 0.23$ and $p = 0.21$, respectively).

In PCD and CF, total MRI score was in the mild range (0–9) in 15 and 45%, and in the moderate range (10–18) in 85 and 55% of cases, respectively ($p > 0.05$ for both comparisons).

Image evaluation in PCD and CF showed excellent agreement between the techniques for all scores ($r > 0.9$). We could not compute agreement between CT and MRI scores for extent of CF sacculations/abscesses, severity

Table 2 Chest MRI and CT scores of patients with PCD and CF

	MRI			CT		
	PCD	CF	<i>p</i>	PCD	CF	<i>p</i>
Severity of bronchiectasis	1.6 ± 0.1	1.5 ± 0.2	0.70	1.7 ± 0.2	1.5 ± 0.2	0.48
Severity of peribronchial wall thickening	1.7 ± 0.1	1.4 ± 0.2	0.09	1.8 ± 0.09	1.5 ± 0.1	0.14
Extent of bronchiectasis	1.9 ± 0.2	2 ± 0.2	0.49	1.9 ± 0.2	2 ± 0.2	0.58
Extent of mucous plugging	1.6 ± 0.2	1.2 ± 0.2	0.28	1.7 ± 0.2	1.3 ± 0.2	0.36
Extent of sacculations or abscesses	0.05 ± 0.05	0	0.33	0.05 ± 0.05	0	0.33
Generation of bronchial divisions involved (bronchiectasis or plugging)	2.9 ± 0.1	2.3 ± 0.2	0.07	2.9 ± 0.1	2.3 ± 0.2	0.07
Severity of bullae	0	0	–	0.2 ± 0.2	0	0.16
Severity of emphysema	0.1 ± 0.07	0	0.16	0.1 ± 0.07	0	0.16
Severity of collapse or consolidations	1.6 ± 0.1	0.6 ± 0.2	<0.001	1.6 ± 0.1	0.6 ± 0.2	<0.001
Total score	11.6 ± 0.7	9.1 ± 1	0.23	12 ± 0.8	9.3 ± 1	0.21

Data are presented as mean and standard error of the mean

of bullae and severity of emphysema, because of the constant value of these categories at CT and MRI in all subjects. Similarly, no agreement was calculated for MRI severity of PCD bullae due to the constant value of this category. Only severity of emphysema showed poor agreement ($r = 0.44$) between PCD CT and MRI score. Figs. 1a and b are examples of chest MRI from a PCD and a CF patient, respectively.

Total MRI score was significantly related to FEV₁ Z-score in PCD ($r = -0.45$, $p = 0.04$) and CF ($r = -0.43$, $p = 0.05$).

Due to relevant difference in age at diagnosis between PCD and CF, we comparatively re-assessed lung function and structure after control for diagnostic delay. We confirmed our original findings, as no differences emerged between PCD and CF for spirometry and MRI or CT scores (Table 3). The higher score for severity of collapse/consolidation in PCD was confirmed after correction for diagnostic delay.

Discussion

Recently CF and PCD have been increasingly compared [15, 24–29]. To our knowledge, this is the first study that comparatively assessed PCD and CF lung disease using MRI. Our main finding is the absence of striking differences in lung function and structure between the two cohorts. Actually, we found a significant difference between patients' age at PCD or CF diagnosis, hence follow-up was longer in CF. We initially guessed that PCD deteriorated as CF because diagnosis occurred late and patients were referred to a tertiary care center after longer periods of inadequate management. Surprisingly, once controlled for diagnostic delay, the absence of any difference between the groups was confirmed, with the only exception of the collapse/consolidation subscore

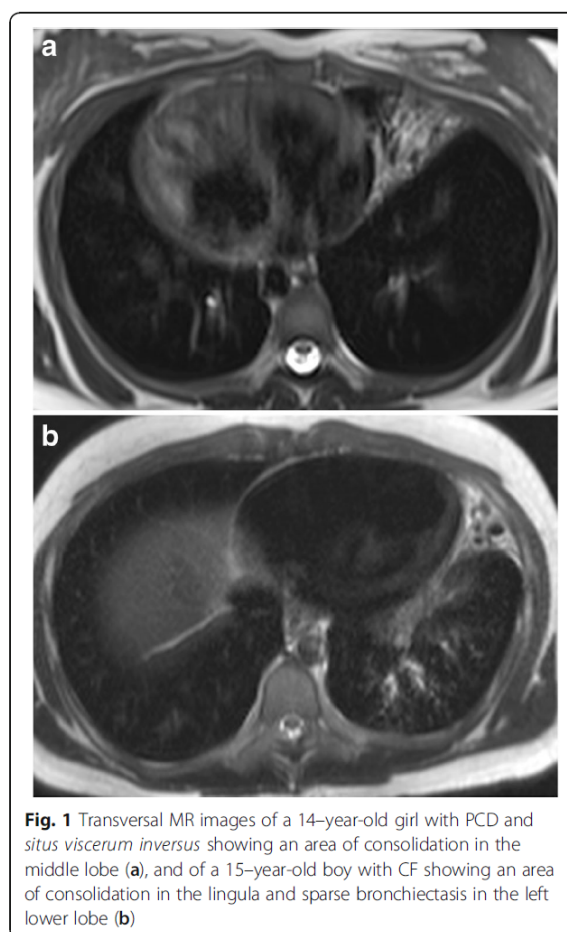


Fig. 1 Transversal MR images of a 14-year-old girl with PCD and *situs viscerum inversus* showing an area of consolidation in the middle lobe (a), and of a 15-year-old boy with CF showing an area of consolidation in the lingula and sparse bronchiectasis in the left lower lobe (b)

Table 3 Lung imaging and lung function of PCD and CF patients controlled for diagnostic delay

	MRI			CT		
	PCD	CF	<i>p</i>	PCD	CF	<i>p</i>
Severity of bronchiectasis	1.6 ± 0.2	1.6 ± 0.2	0.9	1.7 ± 0.2	1.6 ± 0.2	0.9
Severity of peribronchial wall thickening	1.7 ± 0.2	1.5 ± 0.2	0.4	1.8 ± 0.1	1.7 ± 0.1	0.4
Extent of bronchiectasis	1.8 ± 0.2	2.2 ± 0.2	0.3	1.8 ± 0.2	2.3 ± 0.2	0.3
Extent of mucous plugging	1.6 ± 0.2	1.3 ± 0.3	0.4	1.6 ± 0.3	1.5 ± 0.3	0.5
Extent of sacculations or abscesses	0.02 ± 0.04	0.03 ± 0.04	0.2	0.02 ± 0.04	0.03 ± 0.04	0.2
Generation of bronchial divisions involved (bronchiectasis or plugging)	2.8 ± 0.2	2.7 ± 0.2	0.1	2.8 ± 0.2	2.6 ± 0.2	0.1
Severity of bullae	0	0	–	0.1 ± 0.1	0.1 ± 0.1	0.1
Severity of emphysema	0.08 ± 0.05	0.03 ± 0.06	0.2	0.06 ± 0.05	0.04 ± 0.06	0.1
Severity of collapse or consolidations	1.5 ± 0.2	0.8 ± 0.2	<0.001	1.5 ± 0.2	0.8 ± 0.2	<0.001
Total score	11.0 ± 0.9	10.1 ± 0.9	0.1	11.4 ± 0.9	10.4 ± 0.9	0.1
Lung function	PCD		CF	<i>p</i>		
FVC (Z-score)	–0.3 ± 0.3		–0.5 ± 0.3	0.08		
FEV ₁ (Z-score)	–1.4 ± 0.4		–1.4 ± 0.4	0.1		
FEF _{25–75} (Z-score)	–2.0 ± 0.4		–1.8 ± 0.4	0.4		
FEV ₁ /FVC (Z-score)	–1.7 ± 0.3		–1.4 ± 0.3	0.5		

Data are presented as mean and standard error of the mean

that was persistently more severe in PCD. Overall, these data add further evidence to the widespread concept that PCD is not the mild disease believed.

The relevant delay in the ultimate diagnosis is universally recognized in PCD [1, 30], and we confirm it. What additionally emerges from current data is that a delayed diagnosis is not the sole responsible for the similarities found, hence further explanation should be provided. Despite counterintuitive, the lack of association between early PCD diagnosis and better pulmonary outcomes, strongly suggested by our results, has been highlighted previously [31, 32]. Furthermore, a recent longitudinal study of adult PCD subjects has confirmed that, even though negatively associated with baseline FEV₁, age at diagnosis does not correlate with longitudinal functional measurements, thus raising doubts on the real impact of optimal management at an early stage on the subsequent disease progression [33]. These studies did not correlate chest CT scores with age at diagnosis, but it is reasonable to hypothesize that, likewise functional impairment, also structural abnormalities are poorly affected by it. Nevertheless, the controversy surrounding the association between age at PCD diagnosis and lung function impairment clearly brings into focus the need for future larger studies.

It is well known that in CF chronic depletion of periciliary layer volume results in hyperviscous mucus layer adhesion to cells, thus inhibiting cough clearance [34]. Conversely, the PCD periciliary layer volume is apparently normal and airway surface well hydrated, hence cough-

dependent mucus clearance is preserved [35]. However, despite distinct basic defects [19, 36], CF and PCD share a common hallmark, i.e., retention of airways mucus and growth of biofilms [5, 37]. Although we do not provide data on therapeutic adherence, the poorer PCD patients' compliance to treatment in our practice might explain the similarities we found, despite comparable time interval between visits. In PCD poor compliance to treatment likely derives from unawareness of potential threats of the disease, especially if inadequately treated [38]. Conversely, CF patients, particularly those with good nutritional status like ours, generally show adequate prescriptions adherence [39]. Inhaled antibiotics and dornase alpha, which in Italy are authorized only for CF, are not routinely used [40], and this might help interpreting our findings. Future comparative studies might realize novel PCD management strategies, and the efficacy of drugs not routinely used in PCD should be further investigated. A final, critical point is that the genetic background may influence PCD lung disease expression [41]. As we cannot provide PCD genotype, a selection bias is not excluded.

Few original studies have compared PCD and CF lung disease [15, 25]. A comparative analysis of chest CT, also including PCD patients from our center, reported less lung changes in PCD than CF [25]. In that study, PCD patients were compared to a previously published CF cohort undergoing chest imaging for clinical reasons, unlike current CF subjects [42]. The difference between imaging scoring systems makes any comparison between the two studies unreliable. A recent study found that

lung disease severity at CT was similar between PCD and pancreatic sufficient CF, but significantly higher in pancreatic insufficient CF [15]. Conversely, in the current study we did not find differences between the two diseases in terms of lung function and structure, except collapse/consolidation. Comparing that study population with ours, it is worth noting that age at enrolment was similar in PCD and CF. Nevertheless, our CF patients had milder pulmonary involvement, with fewer exacerbations and less chronic *Pseudomonas aeruginosa* infection rate (15% versus 51%, respectively) than the comparison cohort. More importantly, in the current study lung damage at MRI was in the moderate range in approximately half of CF versus two thirds of PCD, with the remaining subjects from both groups showing mild impairment. Actually, FEV₁ Z-score was normal in our CF and abnormal in our PCD subjects (-0.9 versus -1.75, respectively), although the difference was not significant. Overall, these data suggest that the CF patients from the two studies differ in disease severity, despite comparable lung function, with milder pulmonary disease in our cohort [15]. Unfortunately, the small number of our CF patients with or without pancreatic insufficiency precluded the comparison between these CF subgroups. Furthermore, the limited sample size may have biased our findings.

Our study has both strengths and limitations. Undoubtedly the use of a non-invasive tool, namely MRI, is an element of novelty in the comparison of CF and PCD. Chest MRI has been used in both diseases separately, and its reliability has been widely demonstrated through comparisons with traditional techniques [12, 13]. We found an excellent agreement between MRI and CT for most abnormality parameters in both PCD and CF. However, although CT is a useful staging test, it is impractical for monitoring lung disease because of radiation burden [43]. Conversely, spirometry is an insensitive marker of lung disease progression [10, 42]. In this setting, MRI is attractive and reliable as radiation-free option [13, 44, 45]. In addition, the re-analysis of data after control for diagnostic delay highlighted that the lack of differences between PCD and CF is unlikely due to prolonged untreated PCD.

Our study has also limitations. First, as shown by chest imaging scores and spirometry, our analysis compared mild CF subjects and PCD patients mostly presenting mild-to-moderate functional and structural impairment, with virtually no patients showing more severe disease. Moreover, the modified Helbich score does not take into account the non-comparable involvement of different lobes. However, we opted for this scoring system as it was previously used in CF and PCD, and because the observers were already trained in it. In addition, as previously stated, enrolled CF patients presented mild lung disease, unlike PCD subjects whose pulmonary impairment was likely more severe. Indeed, despite CF and

PCD recruitment was timed to coincide with a routine CT, the lack of a shared protocol defining the time interval between scans in PCD might have determined the enrolment of more severe PCD. Similarly, it could also be speculated that the enrolled PCD patients have more severe disease entailing referral compared to those with a late or totally missed diagnosis, and this could further bias results. Indeed, the majority of our PCD patients fully met the stringent definition for positive PCD diagnosis recently formalized by international guidelines and only in four of them findings from several diagnostic tests made PCD diagnosis “highly likely”. For these subjects genetic testing is certainly warranted, but given the highly suggestive clinical picture, the very low nasal nitric oxide levels, the abnormal cilia motility and the ultrastructural defects found – even though not hallmark of PCD – we felt they could be included in our PCD cohort. Of course, we deeply commend the ERS Task Force for the effort in standardizing the diagnostic pathway in PCD. Their guidelines, requiring an interaction between more diagnostic tools to achieve a definite diagnosis, will certainly help in the characterization of patients and in correlating disease severity with cilia ultrastructure and motility, and with the genetic background. This will also strengthen data from multicenter PCD studies enrolling patients whose diagnosis will no longer be questioned.

The mentioned drawbacks, together with the limited sample size, make it difficult to generalize the data. Further research on larger populations from multicenter sites, possibly including all ranges of severity is needed to verify whether our results are replicable. Finally, longitudinal studies comparing PCD and CF from early life to adolescence/adulthood would likely improve our knowledge on differences in the speed of lung disease progression between the two entities.

Conclusions

This comparative study of PCD and CF suggests that the two conditions may share similar lung function and MRI changes and confirms that chest MRI is a valuable radiation-free tool. Comparative studies of PCD and CF lung disease may hopefully also help to develop PCD-specific protocols not derived from CF.

Additional files

Additional file 1: Image evaluation. (DOC 24 kb)

Additional file 2: Modified Helbich scoring system for HRCT and MRI. (DOC 39 kb)

Additional file 3: *Situs viscerum inversus* and cilia ultrastructure of patients with PCD. (DOC 31 kb)

Abbreviations

CF: Cystic fibrosis; CT: Computed tomography; FEV₂₅₋₇₅: Forced expiratory flow between 25% and 75% of FVC; FEV₁: Forced expiratory volume in the

first second; FVC: Forced vital capacity; HRCT: High-resolution computed tomography; MRI: Magnetic resonance imaging; PCD: Primary ciliary dyskinesia

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the risk of individual privacy violation, but are available from the corresponding author on reasonable request.

Authors' contributions

MM participated in the design of the study, collected the data and drafted the manuscript. SM participated in the design of the study, performed the statistical analysis, and drafted the manuscript. CM carried out the imaging studies, scored the HRCT and MR images, and helped to draft the manuscript. VC and PI participated in the collection of data and helped to draft the manuscript. FDG and AT substantially contributed to patient enrollment and to the collection of data. MC performed the electron microscopy analysis of cilia ultrastructure in PCD patients and helped to draft the manuscript. VR and FS conceived the study and participated in its design and coordination. FS also scored HRCT and MR images. All authors read and approved the final manuscript.

Authors' information

Francesca Santamaria Participant in BEAT-PCD (COST Action 1407).

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Written informed consent was obtained from patients and/or legal guardians. The study was approved by the local Ethical Committee (Comitato Etico per le Attività Biomediche, Federico II University; approval number 184/2014).

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2.2 *Old and new biomarkers*

The broad spectrum of clinical presentation and the poor correlation between routinely used functional and structural parameters and the pathogenic mechanisms underlying non-CF CLD has made the identification of objective biomarkers an urgent research priority. The ideal biomarker which could potentially revolutionize clinical management of children with chronic respiratory disorders should be cheap, easy, non-invasive, reproducible and sufficiently informative in order to be helpful in diagnosis, phenotyping, management or treatment of respiratory diseases.

The gold standard method to obtain airway samples and quantify inflammatory markers both in CF and non-CF lung disease is still represented by broncho-alveolar lavage (BAL) (41,42). Nevertheless, its invasivity and cost, the need for general sedation, and the potential risks of the procedure strongly limit the use of such tool, particularly in pediatric settings where chronic patients are followed-up and inflammatory markers are periodically monitored. Therefore, the need for less invasive means of sampling airway surface liquid has oriented research towards the development of new technologies, among which an increasing interest has been raised by the technique of exhaled breath condensate (EBC).

Collection of EBC is generally performed by asking patients to breathe quietly through a chilled tube connected to a condenser with a saliva trap to reduce salivary contamination, in order to obtain a liquid mostly consisting of water vapor with a small fraction of secretions. This condensate is then promptly frozen in liquid nitrogen to suppress metabolism and preserve metabolite concentrations (43). The analysis of such samples relies on highly sensitive methods, particularly metabolomics, whose application has proven feasible and promising in several fields of medicine (44). Metabolomics basically aims at identifying specific metabolic profiles through the identification of numerous low-molecular-weight endogenous metabolites by means of mass spectroscopy or nuclear magnetic resonance (44). Application of metabolomics to pediatric respiratory disorders has produced some evidence that supports its usefulness in characterizing airway biochemical fingerprints, and potentially providing elements for the identification of new pathways, particularly

in asthma (45). Unfortunately, despite the great interest raised, inadequate diffusion of both metabolomics and EBC in pediatric respiratory centers has limited the research in this field, and, till now, pediatric CLD, including CF, has been poorly investigated with these tools.

Nevertheless, moving from the observation that metabolomics recognizes markers separating children with stable or unstable CF (46), our group recently showed that nuclear magnetic resonance discriminates PCD from CF subjects, suggesting that distinct inflammatory and metabolic processes likely generate different metabolites that may be found in the EBC from patients with the two conditions (12). Confirming the extraordinary potential of metabolomics in the characterization of chronic respiratory diseases, this finding further supports the role of noninvasive assessment of EBC for identifying different CLD phenotypes and for tailoring of treatment.

In the field of non-invasive biomarkers in respiratory medicine a relevant role has been gained, over the last two decades, by nitric oxide (NO), a biological mediator that was first described as a vascular smooth muscle relaxant and was subsequently found to be present in the expired breath of animals and humans (47). Nitric oxide has several effects in the respiratory tract, including smooth muscle relaxation, blood flow regulation and modifications of ciliary beat frequency, mucus secretion and cell-mediated immune processes (48). Lower respiratory tract typically shows decreased NO levels in comparison to the upper airways, where its concentrations achieve the maximum in the paranasal sinuses. The possibility of measuring NO in children, combined with the availability of relatively cheap portable devices, has progressively made NO assessment a crucial part in the diagnosis and management of several pediatric respiratory disorders. Nitric oxide measurement includes fractional exhaled NO (FeNO) and nasal NO (nNO), which have proven useful for different purposes in different conditions. FeNO analysis is well validated and far more widespread than nNO measurement due to its main clinical application, represented by the evaluation, both at diagnosis and during the follow-up, of children with bronchial asthma (49). Indeed, as a marker of airway inflammation, FeNO is valuable in monitoring patients' response and adherence to maintenance therapy and has been shown to be helpful in predicting both onset and

exacerbations of asthma (49). Application of FeNO to other respiratory conditions is substantially limited and available evidence is scarce, especially in children. Despite some studies have been conducted in order to evaluate the utility of FeNO in community acquired pneumonia, bronchiectasis, bronchiolitis and diffuse lung disease, data are far too limited to support conclusive recommendations or to allow translation of this tool to the routine management of these conditions (49).

On the other hand, nNO has been proposed as an additional tool in diagnosis, treatment, and management of several airways diseases. Nevertheless, we recently reviewed the available literature regarding nNO measurement application to pediatric respiratory diseases, particularly rhinosinusitis, allergic rhinitis, adenoidal hypertrophy and more severe chronic conditions such as CF and PCD. What we concluded is that, despite a growing body of evidence pointing towards the utility of nNO in the diagnostic work-up of several disorders, this biomarker has currently one major application that is represented by the screening of PCD (50). Indeed, the markedly reduced nNO concentrations typically found in PCD is a well-known feature of the condition, and is often useful in clinical practice to orientate further diagnostic testing and to support diagnosis of patients with atypical PCD phenotype and normal ciliary ultrastructure (51,52). Conversely, nNO measurement is of limited value for other respiratory disorders, and further research is needed to recommend the inclusion of such biomarker in the management protocols of other pediatric respiratory conditions.

Findings from this analysis of current and potential nNO applications were published in *Pediatric Pulmonology*.

Clinical Application of Nasal Nitric Oxide Measurement in Pediatric Airway Diseases

Angelo Manna, MD,¹ Silvia Montella, MD,¹ Mauro Maniscalco, MD,² Marco Maglione, MD,^{1*} and Francesca Santamaria, MD^{1*}

Summary. Nitric oxide plays an important role in several physiological and pathophysiological processes in the respiratory tract. Different ways to measure nasal nitric oxide levels in children are currently available. The possibility of obtaining nasal nitric oxide measurement from relatively young children, combined with the availability of portable devices that can be used even in the office setting, opens new perspectives for nasal nitric oxide analysis in the pediatric daily practice. This review presents a synopsis about the current clinical applications of nasal nitric oxide measurement in the pediatric clinical practice. A total of 3,775 articles on the topic were identified, of which 883 duplicates were removed, and 2,803 were excluded based on review of titles and abstracts. Eighty-nine full text articles were assessed for eligibility and 32 additional articles were obtained from the reference lists of the retrieved studies. Since very low nasal nitric oxide levels are found in the majority of patients with primary ciliary dyskinesia, most publications support a central role for nasal nitric oxide to screen the disease, and indicate that it is a very helpful first-line tool in the real-life work-up in all age groups. Decreased nasal nitric oxide concentration is also typical of cystic fibrosis, even though nasal nitric oxide is not as low as in primary ciliary dyskinesia. In other upper airway disorders such as allergic rhinitis, rhinosinusitis, nasal polyposis, and adenoidal hypertrophy, clinical utility of nasal nitric oxide is still critically questioned and remains to be established. Since nNO determination is flow dependent, a general consensus from the major investigators in this area is highly desirable so that future studies will be performed with the same flow rate. A shared nNO methodology will enable to overcome the challenges that lie ahead in incorporating nNO measurement into the mainstream clinical setting of pediatric airway diseases. *Pediatr Pulmonol.* 2015;50:85–99. © 2014 Wiley Periodicals, Inc.

Key words: children; nasal nitric oxide; allergic rhinitis; cystic fibrosis; primary ciliary dyskinesia; nitric oxide (NO); cystic fibrosis (CF); allergy.

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INTRODUCTION

Nitric oxide (NO) is a gas phase molecule generated by three isoenzymes of NO synthase (NOS) that are differentially regulated and expressed within the airways.^{1,2} All NOS isoenzymes convert L-arginine to L-citrulline, and thus contribute to NO production. Neuronal NOS (NOS1) and endothelial NOS (NOS3) are expressed constitutively, and are activated by calcium ions to produce little amounts of NO that generally acts as intracellular messenger and neurotransmitter. Inducible NOS (NOS2) is not constitutively expressed, but is dependent on transcription factors (such as nuclear factor κ B), that are activated by pro-inflammatory cytokines (Fig. 1).³

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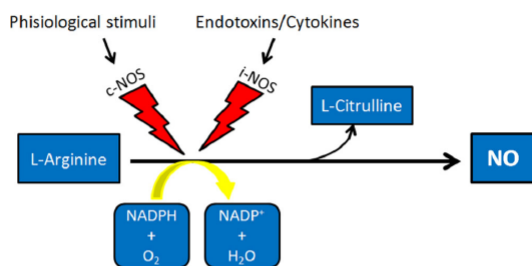


Fig. 1. Schematic representation of nitric oxide (NO) biosynthesis by the conversion of L-arginine to L-citrulline via nitric oxide synthase (NOS) isoenzymes.

At present, several effects of NO in humans have been demonstrated. Direct effects occur at low NO concentrations and allow NO to react directly with a biological target molecule (like a metal-containing protein or DNA). Indirect effects imply that NO reacts with oxygen or superoxide anion to generate reactive nitrogen species, which in turn react with the biological targets.⁴ Nitric oxide significantly contributes to non-specific host defenses against airway bacterial, viral, and fungal agents,⁵ and is implicated in the modulation of cilia beating.⁶ Since early infancy, the levels of NO appear significantly higher in the upper compared to the lower airways,⁷ with maximal concentration found in the paranasal sinuses.⁸

Nasal NO (nNO) measured during quiet nasal exhalation likely derives from NO produced in the sinuses and in the nasal mucosa, where the expression of NOS has been demonstrated.^{8,9} The assessment of nNO requires a limited cooperation by the patient, and is rapid and easy to perform. Moreover, the recent availability of hand-held analyzers equipped for nNO measurement, combined with low cost and simple use of the devices, has made nNO determination widespread in primary practice. While fractional exhaled nitric oxide (FeNO) analysis is well validated at any age for measuring airways inflammation, namely in bronchial asthma,¹⁰⁻¹⁸ nNO has by far less application in the assessment of pediatric upper airways disorders. Data from studies of nNO measurement in children are relatively scarce except for those supporting its use as screening tool for primary ciliary dyskinesia (PCD).^{19,20} Nonetheless, an official guideline and several original studies pointed out that nNO analysis may play a role in a number of pediatric airway disorders.²¹⁻⁵² In particular, nNO was used to monitor upper airways inflammation and to evaluate the effect of treatment in allergic rhinitis (AR).²⁶ While in rhinosinusitis the role of nNO as a diagnostic or prognostic marker is still unclear,⁵³ nNO levels measurement has been recently used to identify obstructive adenoids in children with adenoidal hypertrophy.⁵⁴ Finally, children with cystic fibrosis (CF) exhibit lower nNO than healthy subjects, and levels appear significantly reduced in cases with more severe genotypes.⁵⁵

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This integrative review will describe the various nNO measurement techniques currently used in pediatric clinical practice, and will focus on pros and cons of each technique. Moreover, current clinical applications of nNO in children with airway diseases, including congenital and acquired conditions, will also be discussed, highlighting what is consolidated knowledge and which are the areas of uncertainty.

Search of the Relevant Literature

We carried out an electronic keyword literature search for English articles published on this topic up to February 2014 in the Scopus, Highwire Press, Web of Science, PubMed, and Medline databases. The terms "nasal nitric oxide" were used as keywords in combination, and the studies obtained were evaluated for selecting relevant literature. This search strategy yielded 3,775 articles, of which 883 duplicates were removed. From 2,892 abstracts screened, 2,803 were excluded because they were not relevant to the topic (editorials, adult studies, meeting abstracts). A total of 89 full text articles were assessed for eligibility. Reference lists from the retrieved articles were manually searched as well, providing 32 additional articles eligible for our topic. Figure 2 shows the flow-chart of the study selection process that eventually included 121 articles in the reference list.

nNO in the Pediatric Clinical Practice

nNO Measurement Techniques

The American Thoracic Society (ATS) and the European Respiratory Society (ERS) currently recommend two methods of nNO assessment: nasal aspiration via one nostril during breath-hold and velum closure, and nasal exhalation through a tight facemask with fixed flow (likewise oral FeNO).³⁷ As far as nasal aspiration, the closure of the pharynx by the velum is strongly recommended for preventing the air entry from the lower respiratory tract into the nasal cavity, and the leak of nNO via the posterior velopharyngeal aperture. This can be obtained through two maneuvers. The first is a slow oral exhalation against a resistance of at least 10 cm H₂O. A nasal olive is gently placed against one nostril to achieve an airtight seal, thus ensuring air could only leave the nostril via the central sampling channel within the olive. A suction pump aspirates air at a constant flow rate while the subject exhales from the mouth against an expiratory resistance after a full inspiration to total lung capacity. The second maneuver is nasal aspiration by breath holding with the velum elevated. In this case, a suction pump aspirates air through a nasal olive placed in one nostril with the subject holding his/her breath after inspiration to total lung capacity. When a transnasal flow is used, the nasal exhalation through a tight facemask with a stable fixed flow is the method of choice.³⁷ A tightly

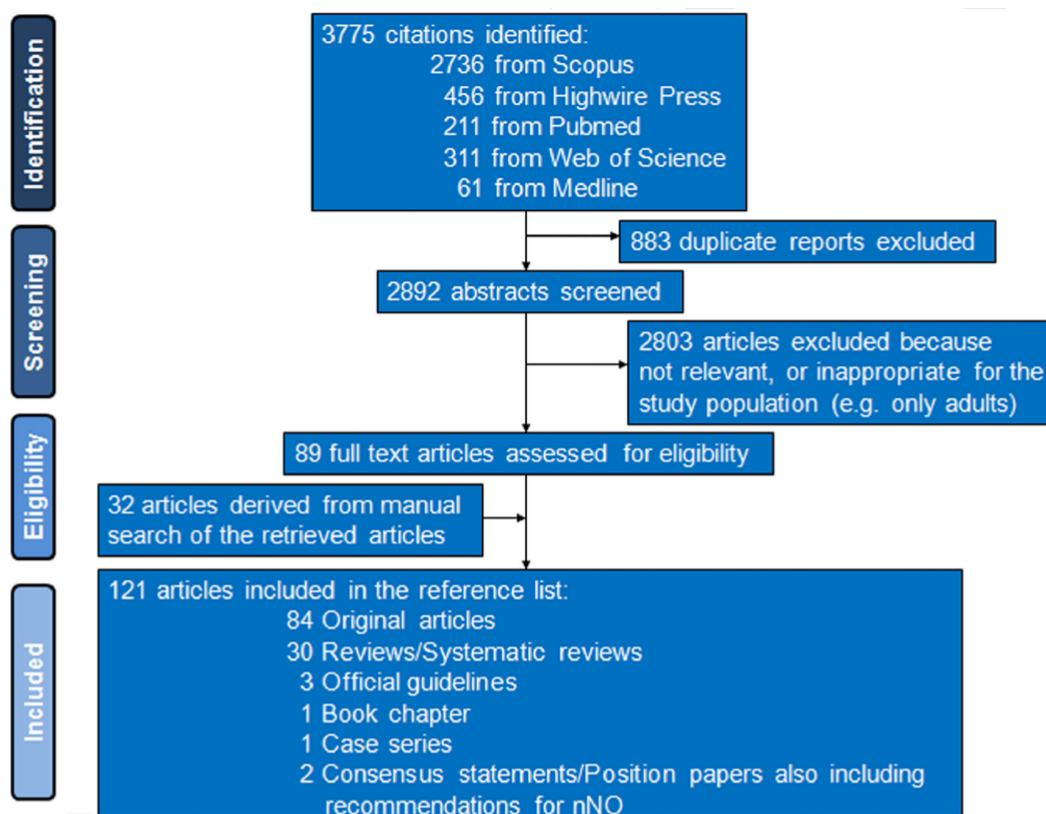


Fig. 2. Flow-chart of the study selection process.

fitting mask covering the nose connected to the analyzer through a mouthpiece with sterile filter is used for nasal measurements. Subjects start each maneuver by inhaling NO-free air from the analyzer through the nose during a full inspiration to total lung capacity, and then exhaling at a fixed flow rate.^{37,48} Nasal NO can also be measured with nasal aspiration, or with nasal exhalation in a tight face mask, during humming, i.e., exhalation while phonating “m” without opening the lips or forming words.⁵⁶ As for nasal exhalation, also in this case, a tightly fitting mask covering the nose is used. For each maneuver, during inspiration to total lung capacity, subjects inhale through the nose NO-free air from the analyzer, and then phonate “m”, without opening the lips or forming words, as loud as possible for 10 sec at a constant flow rate; nNO during humming is generally calculated as the mean concentration throughout the last 80% of exhalation.^{48,56} The maneuver induces a vibrating or oscillating exhalation flow through the nose, leading initially to high NO peak levels that in healthy subjects gradually decrease into a plateau. This effect is related to the fast gas exchange

between highly NO-rich sinus and nose. The gas exchange is induced by the sounding airflow which in turn makes the sinus similar to a Helmholtz resonator.⁹ Humming has been proposed as a diagnostic tool in sinus disease, with most research being made in chronic sinusitis, in AR, in CF, and in PCD.^{9,40,48,57,58} At present, all techniques used for nNO measurement in children have pros and cons. The aspiration technique needs cooperation because of breath holding.³⁷ Nasal exhalation through a tight facemask requires less cooperation, but since it does not necessitate velum closure, air may enter from the lower to the upper airways.³⁷ Finally, although the humming maneuver is the sole technique that evaluates paranasal ventilation and osteomeatal complex abnormalities, it requires cooperation and therefore cannot be obtained from young children.¹⁵

With all the above-mentioned techniques, a constant transnasal flow rate produces a washout phase followed by the establishment of a steady NO plateau, during which nNO levels are recorded. Nasal NO concentration appears inversely related to the transnasal airflow rate.^{37,59} In

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particular, nNO concentration measured at different flow rates exhibits a hyperbolic relationship with the sampling flow rate used, likely because at higher flow rates the wash-out effect might be greater than that observed with lower flows, thus inducing lower NO values.^{37,60} A target airflow rate of 0.25 to 3 L/min is currently recommended in the measurement of nNO output, because this flow rate provides a steady plateau level of NO concentration within 20 to 30 sec.³⁷ With decreased airflow rates, higher absolute NO values are found, but the relative influence of environmental NO is lower.³⁷

Using the aspiration technique, intra-subject reproducibility is achieved when the intra-individual coefficient of variation within three measurements is below a certain limit, usually in the range of 4–8%. However, published studies on the evaluation of the intra-individual coefficient of variation report inconsistent values, ranging from 7.7% (95% CI 6.2–10.5%) in adults to 18.0% (95% CI 13.4–27.4%) in children.³⁹

Compared to the large number of nNO studies in school-aged children and adolescents, the literature on nNO measurement in young children is less extensive. Several studies demonstrated that nNO measurement can be obtained from uncooperative children during tidal breathing with nasal aspiration technique.^{10,23–25,27,33,41,43,45–47,50,61} In this case, the child is seated either on a chair, or on parent's knees. A nasal aspiration by a suction pump through a nasal olive placed against the nostril to achieve an airtight seal is performed, while the child breathes tidally. Older children are encouraged to breathe through their mouth. A good reliability of this nNO measurement technique has been shown, with a high within-subject repeatability demonstrated by a mean coefficient of variation <12%,^{10,50} a mean nNO difference of 9.6 ppb (limits of agreement ranging from 127 to 146),⁴² and an intraclass correlation coefficient of ≥ 0.8 .⁴⁷ A definition list of all the above mentioned techniques is reported in Table 1.

Stationary devices are the most commonly used equipments in nNO studies. Unfortunately, these systems have some limitations, including high cost (US \$ 32,000–52,000) and need for frequent calibrations and technical maintenance. An hand-held analyzer simple to use and more cost effective (US \$ 4,500–8,000) has been developed to overcome these drawbacks.⁶² Some recent studies demonstrated that nNO measurement through the hand-held device has acceptable agreement with the measurement obtained from the stationary unit during nasal exhalation, tidal breath, and humming (Fig. 3).^{48,50,62} A portable device equipped also for nNO analysis through the aspiration method is currently available (Fig. 4) and can be used to distinguish patients with ciliary defects from healthy subjects.^{50,63}

nNO Reference Values

In humans, nNO levels are very high compared to NO measured in the expired breath as FeNO.^{8,64–66,67} The

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TABLE 1— Definition List of nNO Measurement Techniques

Technique	Description	Velum closure
Oral exhalation against resistance	Nasal aspiration by a suction pump through a nasal olive placed in one nare at a constant flow rate, while the subject exhales from the mouth against an expiratory resistance after a full inspiration to total lung capacity.	Yes
Breath hold	Nasal aspiration by a suction pump through a nasal olive placed in one nare at a constant flow rate, while the subject holds his breath after a full inspiration to total lung capacity.	Yes/No ¹
Nasal exhalation	Nasal exhalation through a tightly fitting mask covering the nose connected to the analyzer through a mouthpiece. Subjects start each maneuver by inhaling NO-free air from the analyzer through the nose during a full inspiration to total lung capacity, and then exhaling at a fixed flow rate.	No
Humming	Nasal aspiration by a suction pump through a nasal olive placed in one nare at a constant flow rate or nasal exhalation through a tightly fitting mask covering the nose connected the analyzer through to a mouthpiece. Subjects start each maneuver by inhaling NO-free air from the analyzer through the nose during a full inspiration to total lung capacity, and then phonating the “m” without opening the lips or forming words as loud as possible for 10 sec at a constant flow rate.	No
Tidal breath	Nasal aspiration by a suction pump through a nasal olive placed in one nare at a constant flow rate, while the child breathes tidally. Older children are encouraged to breathe through their mouth.	Yes/No ¹

¹Depending on the cooperation of the children.

supravelar airways can generate NO concentrations several fold greater than the lower respiratory tract, and nNO is particularly high in the paranasal sinuses. Struben *et al.* found that nNO levels are normally distributed in healthy children aged 6 to 17 years (mean 449 ppb;



Fig. 3. Example of nasal nitric oxide measurement during the humming maneuver through a tightly fitting mask covering the nose in a 15-year-old healthy girl.

standard deviation 115 ppb).³⁸ These authors also demonstrated that nNO concentration depends on the environmental NO and, in children younger than 12 years, on patient's age, while sex, passive smoking, body mass index, weight, and height do not seem to influence it. On the other hand, a recent study of a cohort of adolescents demonstrated lower, albeit not significantly, nNO values in active smokers compared to non-smokers.⁶⁸ Table 2 summarizes individual and environmental factors that may affect nNO values.^{12,37,38,69–75} Compared to velum closure techniques, tidal breathing results in significantly reduced nNO levels.^{23–25,27,33, 41,43,45–47,50} In a study of preschool Italian children, Piacentini and colleagues reported that nNO was significantly lower in uncooperative subjects



Fig. 4. Example of nasal nitric oxide measurement by aspirating air from one nostril during tidal breath in a 7-year-old healthy boy.

TABLE 2—Factors Affecting Nasal Nitric Oxide (nNO) Values

Factor	Type of influence	Refs.
Ambient air	Ambient NO could reduce the gradient for NO diffusion from nasal epithelium to lumen	37,75
Age	In children younger than 11 years, age may affect NO output. In adults, nNO is not age-dependent.	12,38,69
Circadian change	Lower nNO levels in the morning compared to the afternoon values of the same day.	70
Physical exercise	Nasal NO concentration falls during heavy physical exercise	71
Measurement techniques	The subject's cooperation influences results. Nasal NO concentration is inversely related to the transnasal airflow rate used	37,59
Medications		
Nasal decongestants	Decrease nNO levels	72
Nasal corticosteroids	Controversial results	73,74

aging less than 12 months than in cooperative school-aged children, with some values overlapping those reported in PCD.⁴¹ The likely explanations are that younger children have only partially pneumatized paranasal sinuses, with relatively reduced mucosal surface, or also that during tidal breathing nNO concentration may be diluted by the air originating from the lower airways that contain much less NO.⁷⁶ A recent study provided reference values in healthy preschool children, and confirmed that in the absence of active cooperation, nNO levels are reduced.⁴⁵ These data lead us to recommend a prudential interpretation of low nNO levels in preschool children, particularly in infants younger than 6–12 months, in whom reduced nNO could be a false abnormal finding.

Table 3 summarizes nNO values obtained in healthy children according to the measurement technique used.^{10,21–27,29–36,38–43,45–48,50–52} All the measurement techniques provide different nNO values whose variability can be explained, at least in part, by the transnasal airflow used. Since lower nNO levels are obtained with higher aspiration flows,^{37,77} a standardization of sampling flow is warranted in order to address a threshold for normal values.

nNO in Allergic Rhinitis

AR is characterized by clinical symptoms such as rhinorrhea, nasal obstruction, nasal itching, and sneezing that follow exposure to relevant allergens.⁷⁸ Likewise to asthma, a high expression of the NOS2 isoform has been

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TABLE 3—Nasal NO Values According to the Measurement Technique in Healthy Children and Adolescents

Technique	Subjects, n (age range)	Transnasal airflow	Values (ppb) ¹	Equipment ²	Refs.	
Breath hold	14 (7–27 yrs)	280 ml/min	322 (270.6–510.6)	NIOX [®] , Aerocrine AB	40	
	11 (15–36 yrs)	66 ml/min	1195.5 ± 618.2	280 Sievers Instruments [®]	28	
	6 (5–10 yrs)	1200 ml/min	401 ± 145 nl/min	280 Sievers Instruments [®]	31	
	289 (11.6 yrs)	700 ml/min	449 ± 115	NIOX [®] , Aerocrine AB	38	
	43 (3–7.2 yrs)	300 ml/min	397 ± 163.8	NIOX [®] , Aerocrine AB	45	
	19 (11 ± 3.7 yrs)	330 ml/min	1110 ± 399.1	CLD88 [®] EcoPhysics	47	
	8 (12.2 ± 3 yrs)	300 ml/min	843 ± 152	NIOX [®] , Aerocrine AB	39	
	27 (7 yrs)	300 ml/min	650 ± 60.6	NIOX [®] , Aerocrine AB	41	
	20 (10.8 ± 3.5 yrs)	250 ml/min	553 (116–1437)	LR 2000 [®] , Logan Research Ltd	30	
	24 (12.4 ± 1 yrs)	1200 ml/min	224 (175.5–285.2)	Exhalyzer D [®] , Eco Medics	35	
	49 (4–63.6 yrs)	300 ml/min	908 ± 33	NIOX [®] , Aerocrine AB	46	
	14 (11.5 ± 0.4 yrs)	250 ml/min	505 ± 66.8	LR 2000 [®] , Logan Research Ltd	34	
	18 (13.5 ± 3.5 yrs)	300 ml/min	772 (690–886)	NIOX [®] , Aerocrine AB	51	
	21 (15.6–58.4 yrs)	300 ml/min	890 ± 62	NIOX [®] , Aerocrine AB	50	
	21 (5–15 yrs)	Not reported	186 ± 15	Rotork 447 [®]	26	
	12 (16–19 yrs)	250 ml/min	1014 (490–1632)	LR 2000 [®] , Logan Research Ltd	29	
	54 (6–17 yrs)	250 ml/min	1024 (158–2502)	LR 2000 [®] , Logan Research Ltd	21	
	Exhalation against resistance	17 Caucasians, 7 Orientals, 6 Negroids (3–17 yrs)	3000 ml/min	481 ± 283 nl/min	280 Sievers Instruments [®]	32
		139 Pacific Islands (5–17 yrs)	500 ml/min	403 (34–1120)	LR 2000 [®] , Logan Research Ltd	36
		19 (11 ± 3.7 yrs)	330 ml/min	1193 ± 374.3	CLD88 [®] EcoPhysics	47
30 (4–63.6 yrs)		300 ml/min	788 ± 41	NIOX [®] , Aerocrine AB	46	
78 (5–73.6 yrs)		300–500 ml/min	304.6 ± 118.8	280i NOA Sievers [®] CLD88 [®] EcoPhysics NIOX [®] , Aerocrine AB	52	
13 (7–27 yrs)		3000 ml/min	165 (123.5–221.9)	NIOX [®] , Aerocrine AB	48	
Humming	14 (7–27 yrs)	3000 ml/min	212 (158.7–244.8)	NIOX [®] , Aerocrine AB	40	
	19 (11 ± 3.7 yrs)	330 ml/min	3035 ± 1525.2	CLD88 [®] EcoPhysics	47	
Tidal breath	22 (3.6 ± 2.6 mths)	200 ml/min	169.4 ± 56.1	280 Sievers Instruments [®]	10	
	250 (1.5–7.2 yrs)	300 ml/min	288 ± 118.5	NIOX [®] , Aerocrine AB	45	
	19 (11 ± 3.7 yrs)	330 ml/min	852 ± 313	CLD88 [®] EcoPhysics	47	
	16 (8.4 ± 0.7 yrs)	700 ml/min	245 ± 15	CLD 700 AL Med [®] EcoPhysics	25	
	66 (<5 yrs)	300 ml/min	230 ± 112	NIOX [®] , Aerocrine AB	42	
	24 (6–12 months)	300 ml/min	309 ± 45.9	NIOX [®] , Aerocrine AB	41	
	26 (<6 months)	300 ml/min	128 ± 16.2	NIOX [®] , Aerocrine AB	41	
	52 (4–63.6 yrs)	300 ml/min	534 ± 30	NIOX [®] , Aerocrine AB	46	
	21 (15.6–58.4 yrs)	300 ml/min	486 ± 34	NIOX [®] , Aerocrine AB	50	
	66 (3–68 yrs)	660 ml/min	233 ± 66.8	CLD 700 AL Med [®] EcoPhysics	24	
	68 (4–34 yrs)	700 ml/min	96 ± 47	CLD 700 AL Med [®] EcoPhysics	23	
	91 (<1 month)	200 ml/min	59.65 ± 19.72	280 Sievers Instruments [®]	43	
	14 (<1 month)	100 ml/min	23.7 (5.7– 112.0)	280 Sievers Instruments [®]	33	
	Nasal exhalation	133 (6–15 yrs)	Not reported	216 (204–228)	CLD 700 AL Med [®] EcoPhysics	27
		13 (7–27 yrs)	3000 ml/min	46 (31.9–65.1)	NIOX [®] , Aerocrine AB	48
		19 (5–15 yrs)	700 ml/min	21 ± 9.1	CLD 700 AL Med [®] EcoPhysics	22
14 (7–27 yrs)		3000 ml/min	32 (26.6–47.2)	NIOX [®] , Aerocrine AB	40	
19 (11 ± 3.7 yrs)		330 ml/min	701 ± 295.2	CLD88 [®] EcoPhysics	47	

Unless specified, all subjects are Caucasians.

¹Expressed as mean values ± Standard Deviation, or as mean or median values with range or CIs in parenthesis.

²All studies used chemiluminescence stationary devices.

reported in patients with AR.⁷⁹ While FeNO is consistently high in AR,⁸⁰ data on nNO are controversial as some studies found increased nNO levels,^{26,81–85} while others did not.^{86,87} These findings may be explained by the fact that high nNO concentrations derive from the induction of NOS in the inflamed mucosa provided osteomeatal patency.⁸⁸ However, in the presence of nasal retained mucus and/or mucosal swelling and/or local obstruction due to nasal polyposis, measurement of nNO by the aspiration technique will result in a vacuum, and nNO may be reduced.⁸⁹

Several studies evaluated the effect of treatment of AR on nNO levels.^{26,81,83,85,86,87} Montelukast given for 2 months did not modify nNO in children with perennial AR.⁸⁶ Systemic levocetirizine administered for 12 weeks significantly reduced nNO levels after allergen specific nasal challenge with house dust mites,⁸³ but shorter periods of treatment did not.^{81,86} Data about nasal steroids are controversial as two studies showed a significant nNO decrease after short or long course with nasal beclomethasone,^{26,86} while 4-weeks treatment with nasal mometasone furoate or beclomethasone did not modify nNO levels.^{85,87} Nasal steroids are reported to down-regulate the transcription of NOS2.⁹⁰ However, nNO levels are likely affected by NO diffusion from the sinuses, thus influencing the ultimate nNO levels found in the presence of mucosal obstruction.⁸ Nevertheless, all the above mentioned studies used different measurement techniques and different transnasal airflows, and this might explain the controversial findings. Finally, so far, the effect of immunotherapy on nNO in AR has not been investigated.

These data prompt us to conclude that, pending a standardization of nNO measurement techniques, the role of nNO in the management of pediatric AR is still ambiguous.⁹¹

nNO in Rhinosinusitis

Rhinosinusitis derives from inflammation of the nose and the paranasal sinuses, and is characterized by two or more of the following features: nasal blockage, anterior or postnasal drip, facial pain or pressure, and reduction or loss of smell.⁹² In children, rhinosinusitis is a common complication of upper respiratory tract infections. In a study of 1-to-5 years old children with persistent upper respiratory symptoms, chronic rhinosinusitis (CRS) with symptoms lasting more than 12 weeks was demonstrated in 16% of the subjects.⁹³

Sinus NO is found in the thousands range of parts per billion (ppb), and decreases to approximately half in the nose and by a factor of 100 in the lungs.⁸ Given the proximity of the nasal and oral cavities, the elevated amounts of NO in the sinuses should limit bacterial colonization of these structures.⁸ Pediatric studies found lower nNO levels in children with acute or chronic disease compared to healthy controls.^{24,25,94} A likely explanation is the decreased NO passage from paranasal sinuses into

the nasal lumen because of the mechanical obstruction caused by sinuses edema, congestion, and mucus accumulation. In this setting, a negative pressure within the sinuses would also occur, thus favoring aspiration of bacteria-laden material from the nose into the sinuses and altering the NO passage.⁹² Indeed, NO metabolites might damage sinus epithelium and impair local nNO production.⁹⁵ Excess secretions and thick aqueous epithelial lining typically occurring in sinusitis would then inhibit the diffusion of gaseous NO into the air-filled sinus cavity, this leading to further nNO decrease. Unfortunately, the application of nNO measurement in rhinosinusitis, particularly its use as a diagnostic or prognostic marker, is not sufficiently clear.⁹⁶ Conversely, measurement of nNO may be practicable as an effect indicator of treatment. In children with acute maxillary sinusitis baseline low nNO levels significantly increased after 2 weeks course with amoxicillin/clavulanate.²⁵ Whether the increase of nNO after antibiotics is due to restored patency of the osteomeatal complex or to bacterial killing is still unknown. However, nNO determination has been proposed as an outcome measure after treatment of rhinosinusitis (Evidence Level IIa).⁹² Indeed, a study conducted on rat alveolar macrophages found that nasal decongestants xylometazoline and oxymetazoline decrease NOS, and supported the observation that administration of nasal decongestants affects nNO concentration.⁷² Unfortunately, there are no *in vivo* data that support the same beneficial effects in humans.

nNO in Adenoidal Hypertrophy

Hypertrophy of the pharyngeal tonsils or adenoids, a single aggregation of lymphoid tissue placed between the nasal septum and the pharyngeal wall, is extremely common in the pediatric population. It causes obstruction of the nasal passages and of the Eustachian tubes, and eventually results in reduced nasal mucus clearance.⁹⁷ Recent evidences point towards a role for NO in the development of pharyngeal tonsils or adenoids hypertrophy. Serum arginase and iNOS activities measured in the hemolyzed supernatant fraction of blood erythrocytes were found significantly higher in children with adenotonsillar hypertrophy than in the post-operative state and in healthy controls.⁹⁸ Torretta and colleagues showed that nNO levels from forty-five children with chronic adenoidal hypertrophy were above the range of normality (721.2 ppb), with 70% of the values superior to 450 ppb.⁵⁴ These findings support the hypothesis that in the presence of adenotonsillar hypertrophy-associated chronic inflammation, NOS2 is stimulated by pro-inflammatory mediators and bacterial lipopolysaccharides, this enhancing NO production. A possible further source of NO in adenoidal hypertrophy might be the bacterial NOS, an enzyme that is required for normal Gram-positive bacterial growth, as well as for successful infection of a target and for defense against oxidant-based immune

response.⁹⁹ Indeed, it was also reported that nNO is significantly lower in subjects with obstructive adenoids than in those without, an apparent paradox that might be explained by the dual observation that either children with higher nNO levels also have chronic inflammation due to hypertrophy of the nasal turbinates, or that adenoids occluding the choanal lumen reduce nNO diffusion.⁵⁴ Variables such as gender, age, body mass index, and passive smoking exposure did not significantly affect nNO levels.⁵⁴ Indeed, in that study authors adopted as normal reference values for nNO those obtained from studies published before 2010, which all considered nNO levels ranging from 200 to 450 ppb as normal.⁵⁴ Other groups measured nNO in healthy children using the same technique (i.e., exhalation against resistance), and found higher nNO levels than previously obtained.^{46,47}

At present, pending further studies, it seems unlikely that nNO measurement will ever have a role in the management of children with adenoidal hypertrophy.

nNO in Cystic Fibrosis

CF, an autosomal recessive disease caused by mutations of a gene located on the long arm of chromosome 7 and known as Cystic Fibrosis Transmembrane Conductance Regulator, is by far the most common inherited pulmonary life-threatening disorder in the Caucasian population, and is characterized by several abnormalities of the epithelial surfaces.¹⁰⁰ Since NOS activity in lung tissue is increased in inflammatory airway diseases, it would be expected that CF patients have increased NO production in the respiratory tract. Conversely, it was repeatedly demonstrated that, compared to healthy subjects, CF children have significantly lower concentrations of NO in the

TABLE 4—Nasal NO From Pediatric Studies in Airway Diseases Different From Primary Ciliary Dyskinesia

Condition	Technique	Subjects n (age)	Transnasal airflow	Values (ppb) ¹	Equipment ²	Refs.
Allergic rhinitis	Breath hold	21 (5–17 yrs)	Not reported	267 ± 18	Rotork 447 [®]	26
	Not reported	27 (16–75 yrs)	Not reported	810 ± 344	R 2000 [®] , Aerocrine AB	81
	Nasal exhalation	40 (6–12 yrs)	Not reported	914 (634–1212)	CLD 77 AL Med [®] , EcoPhysics	83
	ER ³	35 (22.7 ± 8.7 yrs)	700 ml/min	388 ± 119	280 Sievers Instruments [®]	84
	Breath hold	40 (9.6–12 yrs)	Not reported	835 (713–957)	NIOX [®] , Aerocrine AB	85
	Breath hold	40 (13–20 yrs)	250 ml/min	1105 (551–2051)	LR 2000 [®] , Logan Research Ltd	29
	ER ³	40 (13 yrs)	3000 ml/min	130	NIOX [®] , Aerocrine AB	87
Rhinosinusitis	Not reported	17 (16–75)	Not reported	588	Not reported	86
	Tidal breath	16 (4–13 yrs)	700 ml/min	70 ± 8.7	CLD 700 AL Med [®] , EcoPhysics	25
	Tidal breath	12 (3–68 yrs)	660 ml/min	96 ± 72.8	CLD 700 AL Med [®] , EcoPhysics	24
	Breath hold	14 (11–75 yrs)	300 ml/min	762 (620–1013)	NIOX [®] , Aerocrine AB	94
Adenoidal hypertrophy	ER ³	35 (4–17 yrs)	330 ml/min	721 (158.5–1534.7)	CLD88 [®] , EcoPhysics	54
Cystic fibrosis	Nasal exhalation	11 (9–24 yrs)	3000 ml/min	32 (23.3–43.4)	NIOX [®] , Aerocrine AB	48
	Humming	11 (9–24 yrs)	3000 ml/min	46 (33–63.2)	NIOX [®] , Aerocrine AB	48
	Breath hold	11 (11–37 yrs)	66 ml/min	438.2 ± 225.3	280 Sievers Instruments [®]	28
	Breath hold	32 (11 ± 3.4 yrs)	330 ml/min	421 ± 254.7	CLD88 [®] , EcoPhysics	47
	ER ³	32 (11 ± 3.4 yrs)	330 ml/min	424 ± 269.2	CLD88 [®] , EcoPhysics	47
	Tidal breath	32 (11 ± 3.4 yrs)	330 ml/min	326 ± 177.5	CLD88 [®] , EcoPhysics	47
	Nasal exhalation	32 (11 ± 3.4 yrs)	330 ml/min	259 ± 165.3	CLD88 [®] , EcoPhysics	47
	Humming	32 (11 ± 3.4 yrs)	330 ml/min	402 ± 637.5	CLD88 [®] , EcoPhysics	47
	Breath hold	45 (4–50.7 yrs)	300 ml/min	416 ± 28	NIOX [®] , Aerocrine AB	46
	ER ³	10 (not reported)	300 ml/min	412 ± 76	NIOX [®] , Aerocrine AB	46
	Tidal breath	17 (not reported)	300 ml/min	243 ± 39	NIOX [®] , Aerocrine AB	46
	Breath hold	12 (11.7 ± 3.1 yrs)	300 ml/min	501 (450–608)	NIOX [®] , Aerocrine AB	51
	Breath hold	21 (3.9–23.2 yrs)	300 ml/min	501 ± 49	NIOX [®] , Aerocrine AB	50
	Tidal breath	21 (3.9–23.2 yrs)	300 ml/min	273 ± 32	NIOX [®] , Aerocrine AB	50
	Breath hold	33 (7–17 yrs)	250 ml/min	460 (114–865)	LR 2000 [®] , Logan Research Ltd	21
	Tidal breath	23 (5–32 yrs)	700 ml/min	23 ± 17	CLD 700 AL Med [®] , EcoPhysics	23
	ER ³	8 (4–14 yrs)	700 ml/min	72 ± 18	CLD 700 AL Med [®] , EcoPhysics	22
	ER ³	59 (7–55 yrs)	3000 ml/min	46	CLD 77 AL Med [®] , EcoPhysics	55
	Humming	95 (5–18 yrs)	300 ml/min	110 (5–792)	NIOX [®] , Aerocrine AB	101

¹Expressed as mean values ± Standard Deviation (SD), or as mean or median values with range or CIs in parenthesis.

²All studies used chemiluminescence stationary devices.

³ER, exhalation against resistance.

airways.^{21–23,28,46–48,50,51,55,57,101} Several hypotheses might explain this finding. First, the destruction of the airway epithelial cells leading to a loss of NO production during the course of the disease might reduce nNO levels. Second, the impaired clearance of mucus, a hallmark of CF, may result in reduced NO diffusion into the nasal lumen. Finally, despite the ongoing airways inflammation, the expression of NOS1 and NOS2 in the airway epithelium appears frankly decreased in CF, and this may contribute to the reduced NO concentrations in the upper and lower airways.^{102–104} Moreover, a recent study demonstrated that CF children undergoing functional endoscopic sinus surgery had increased nNO levels, although these were lower than controls, confirming that upper airways obstruction is crucial for explaining nNO impairment in CF.¹⁰¹

Adults and children with CF homozygous for ΔF508 or other severe mutations have lower nNO levels compared to patients with mild mutations.⁵⁵ Indeed in CF, higher nNO levels seem to be protective against *Pseudomonas aeruginosa* infection, which, conversely, further reduces NO production.⁵⁵ Moreover, in a recent study of adults and children with CF, nNO concentrations were associated with airway colonization by bacterial pathogens.¹⁰⁵ However, no

significant relationship was found between NO levels and the presence of any specific pathogen.¹⁰⁵ Management of subjects with CF relies on very reliable tools, including sweat test and genetic analysis for the diagnosis, and pulmonary function testing for patients' longitudinal monitoring. Therefore, notwithstanding the promising data on NO research in CF, and the potential role of nNO in the pathogenesis of CF upper airway disease, at present nNO measurement does not seem to have a relevant impact on the management of affected patients.

Overall, current nNO literature includes a robust list of studies of children and adolescents with non-PCD respiratory disorders. These studies are markedly heterogeneous for the equipments and techniques adopted, and for the transnasal flows used, that range from 66 to 3000 ml/min (Table 4). The lack of methodological uniformity supports the urgent need for a standardization of nNO measurement, at least in the pediatric age.

nNO in Primary Ciliary Dyskinesia

Sensory cilia are complex organelles distributed throughout the human body, whose function is to detect extracellular changes and translate them into intracellular signals.¹⁰⁶ Motile cilia, which are found along the entire respiratory tract, are also responsible for the transport of

TABLE 5—Nasal NO Values According to the Measurement Technique in Children and Adolescents With Primary Ciliary Dyskinesia

Technique	Subjects, n (age range)	Transnasal airflow	Values (ppb) ¹	Equipment ²	Refs.
Breath hold	14 (7–27 yrs)	280 ml/min	12 (7.2–19.1)	NIOX [®] , Aerocrine AB	40
	7 (6–32 yrs)	66 ml/min	72.1 ± 46.4;	280 Sievers Instruments [®]	28
	20 (11.4 ± 3.5 yrs)	330 ml/min	58 ± 41.1	CLD88 [®] Ecophysics	47
	10 (17 yrs)	300 ml/min	30 (6.7–66.7)	NIOX [®] , Aerocrine AB	41
	21 (10.8 ± 3.2 yrs)	250 ml/min	55 (3.3–959)	LR 2000 [®] , Logan Research Ltd	30
	17 (12 ± 2 yrs)	1200 ml/min	14 (6.8–27.8)	Exhalyzer D [®] , Eco Medics	35
	45 (4–65.8 yrs)	300 ml/min	142 ± 42	NIOX [®] , Aerocrine AB	46
	15 (10.3 ± 0.7 yrs)	250 ml/min	60 ± 12.2	LR 2000 [®] , Logan Research Ltd	34
	14 (12.8 ± 3.9 yrs)	300 ml/min	27 (16–76)	NIOX [®] , Aerocrine AB	51
	16 (8.4–60.9 yrs)	300 ml/min	79 ± 19	NIOX [®] , Aerocrine AB	50
	12	108 ml/min	130 ± 46.95	Not reported	109
	20 (11.4 ± 3.5 yrs)	330 ml/min	59 ± 40.9	CLD88 [®] Ecophysics	47
	Exhalation against resistance	35 (4–65.8 yrs)	300 ml/min	113 ± 42	NIOX [®] , Aerocrine AB
149 (5.1–73.0 yrs)		300–500 ml/min	20.7 ± 24.1	280i NOA Sievers [®] CLD88 [®] Ecophysics NIOX [®] , Aerocrine AB	52
Humming	14 (7–27 yrs)	3000 ml/min	5 (3.1–8.4)	NIOX [®] , Aerocrine AB	48
	14 (7–27 yrs)	3000 ml/min	3 (2.2–4.3)	NIOX [®] , Aerocrine AB	40
	20 (11.4 ± 3.5 yrs)	330 ml/min	34 ± 28.4	CLD88 [®] Ecophysics	47
Tidal breath	20 (11.4 ± 3.5 yrs)	330 ml/min	40 ± 28.7	CLD88 [®] Ecophysics	47
	54 (4–65.8 yrs)	300 ml/min	86 ± 28	NIOX [®] , Aerocrine AB	46
	16 (8.4–60.9 yrs)	300 ml/min	59 ± 14	NIOX [®] , Aerocrine AB	50
Nasal exhalation	14 (7–27 yrs)	3000 ml/min	5 (3.4–7.7)	NIOX [®] , Aerocrine AB	48
	14 (7–27 yrs)	3000 ml/min	2 (1.3–3.1)	NIOX [®] , Aerocrine AB	40
	20 (11.4 ± 3.5 yrs)	330 ml/min	30 ± 19.8	CLD88 [®] Ecophysics	47

¹Expressed as mean values ± Standard Deviation, or as mean or median values with range or CIs in parenthesis.

²All studies used chemiluminescence stationary devices.

extracellular fluid. PCD is a rare congenital disorder caused by structural and/or functional changes of the motile cilia.¹⁰⁷ There are no representative international data on the prevalence of PCD worldwide, and available information comes from case series in single countries. Therefore, reported prevalence shows large variations, with estimates between 1/2,200 and 1/40,000 in the general population.³⁷ The diagnosis of PCD is based on the demonstration of abnormal motility and ultrastructural defects of the respiratory cilia.^{19,108}

Nasal NO concentrations appear markedly reduced in PCD (Table 5; Fig. 5),^{22,28,30,34,35,40,41,46-48,50,51,109} and NOS2 expression has been found decreased.¹¹⁰ High nNO levels combined with a low risk history should rule out the diagnosis of PCD. However, nNO has been found useful for the diagnosis of patients with atypical PCD phenotype and normal ciliary ultrastructure.^{109,111} From published data it appears that nNO is by far the most effective screening tool of PCD,¹⁰⁷ with a specificity of 88%, a sensitivity of 100%, and a positive predictive value of 89% for a correct diagnosis when using an nNO cut-off level of 105 ppb.³⁵ Also, it has been recently reported that nNO less than 100 ppb would strongly suggest PCD.⁹² However, normal and raised nNO levels were found in cases with PCD,^{46,112} indicating that patients with high clinical suspicion of PCD should not be excluded from further diagnostic evaluation merely on the basis of nNO concentration. A recent pediatric study showed that non-velum closure, which requires minimal cooperation, is suitable to discriminate PCD, although the technique

yields lower nNO values than exhalation against resistance.⁴⁷

Several mechanisms for explaining the low nNO levels observed in PCD have been proposed (Fig. 6). A likely explanation is the NO trapping and subsequent breakdown in the PCD airways due to mucus accumulation and to colonizing respiratory pathogens.¹¹³⁻¹¹⁵ A reduced synthesis of nNO may also occur because of the decreased expression, or abnormal activity of NOS isoenzymes.^{28,30,116,117} Moreover, the high levels of the arginase competing with NOS isoenzymes for L-arginine previously described in patients with CF,¹¹⁸ could similarly induce a substantial reduction of NO levels even in PCD. Finally, it was also suggested either that NO is sequestered in the upper respiratory tract within blocked paranasal sinuses or that, alternatively, nNO biosynthesis or NO storage capacity is limited in PCD because of the agenesis of the paranasal sinuses.^{40,112,115}

Experience on nNO measurement in young children is still relatively scarce, and studies aimed at using nNO measurement as screening tool in infants and preschool children should be encouraged.¹¹⁹ Recurrent upper respiratory tract infections require special attention because this clinical picture may mimic PCD.^{19,45} Indeed, in non-PCD children with upper respiratory tract infections, the decreased passage of NO from the sinuses to the nasal cavity due to mucus production and consequent nasal occlusion may result in low nNO,¹¹⁶ and this can be reversed by antibiotic treatment.²⁵ These findings indicate that, before considering reduced nNO values as suggestive of PCD, it is mandatory to repeat the measurement after an appropriate treatment, or at least during a period of clinical well-being, in order to exclude false positives related to an underlying upper airways infection.

Despite the attractive role of nNO as useful non-invasive, painless and easy performed screening test, the utility of early diagnosis for improving the outcome of PCD is still controversial and deserves further comments. Based on the report that spirometry can become stable after diagnosis and referral to a PCD center for treatment,¹²⁰ until a few years ago it seemed reasonable that early diagnosis had impact on the potential of preserving lung function throughout life. Thus, diagnostic measures for PCD should be focused on the identification of young children. Yet, it has been recently reported that spirometry may be compromised even in the preschool age,¹²¹ and therefore early diagnosis of PCD might not protect against progressive lung function impairment. Pending future larger studies on the progression of PCD lung disease, all efforts at improving the diagnostic work-up of PCD should be continuously made. Other remaining issues are the possible overlap of nNO levels in nasal and pulmonary disorders sharing symptoms with PCD, and the interpretation of low nNO levels in preschool children. Indeed, also non-PCD infants have very low levels of

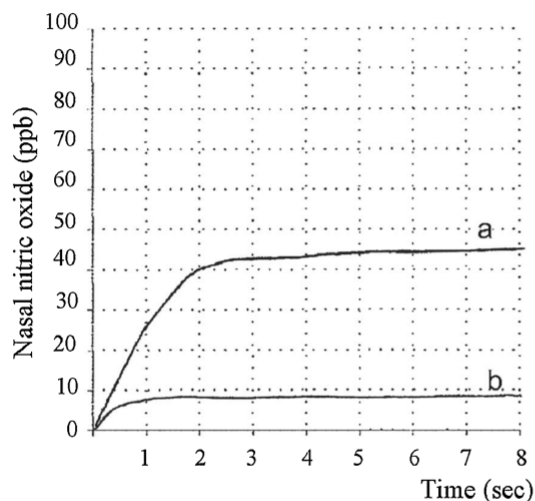


Fig. 5. Representative tracks of exhaled nasal nitric oxide in a healthy subject (a) and in a patient with primary ciliary dyskinesia (b).

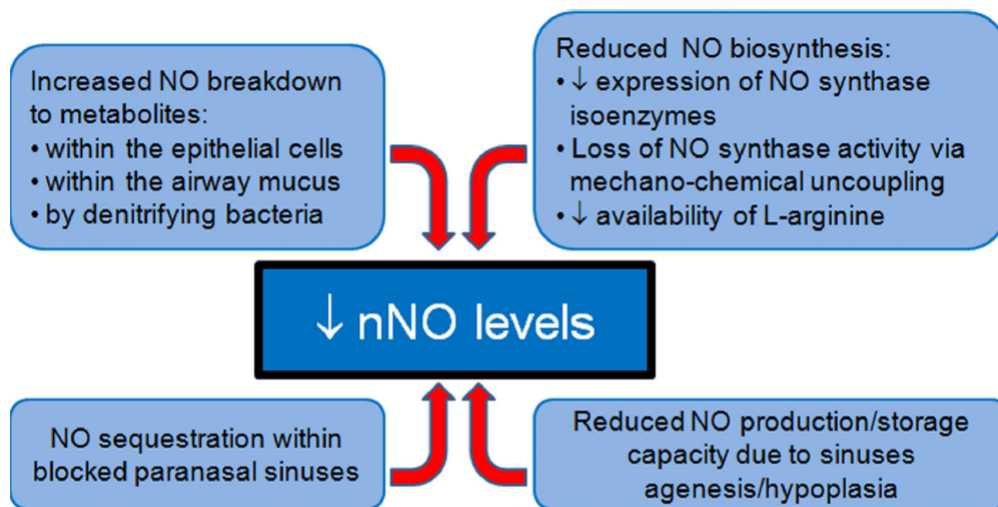


Fig. 6. Potential mechanisms for explaining low nasal nitric oxide (nNO) levels in patients with primary ciliary dyskinesia.

nNO, probably because of undeveloped paranasal sinuses, and this somehow limits its practical use to exclude PCD in this age group.

Finally, given that the prevalence of PCD is likely under-estimated worldwide, a larger diffusion of nNO portable equipments as first-line tool in the diagnostic work-up for PCD would hopefully result in a larger number of new PCD diagnosis, particularly among children and adolescents.^{43,48,62} Moreover, the markedly lower cost and the very simple use of portable devices compared to conventional stationary analyzers would make them affordable even to primary and secondary pediatric centers.

CONCLUSIONS

Nitric oxide is a sensitive indicator of the presence of airway inflammation and ciliary dysfunction. Nasal NO has been proposed as an additional tool in diagnosis, treatment, and management of several airways diseases. Measurement of nNO is easy to perform and reproducible, and the availability of either sampling techniques overcoming poor cooperation by young children or hand-held devices, that are small and inexpensive, will hopefully increase its routine application in the pediatric age. On the other hand, the various measurement techniques and the different airflows used by the available devices could substantially affect the results. The lack of a general consensus on methods and flow rates is a great limit to the comparison of the studies, and makes it difficult to clarify the role of nNO as diagnostic tool or

surrogate marker of therapeutic efficacy. Similarly to what has been previously established for FeNO, a greater effort is needed to standardize the collection methods and the flow-rate at different ages, hopefully through an international consensus on nNO in children. The current recommended method is aspiration at constant flow rate from one naris with gas entrained via the other naris.³⁷ This method samples nNO preventing the entrance of lower respiratory tract air into the nasal cavity.³⁷ In cooperative patients, velum closure is required to prevent leak of nNO via the posterior velopharyngeal aperture. Although several methods can be used, slow oral exhalation against at least 10 cm H₂O resistance is indicated as the preferred method because it reliably closes the velum and excludes oral airflow.³⁷

At present, according to the ATS/ERS recommendations, the sole clinical application of nNO measurement is PCD as it separates between non-PCD and potential PCD patients who deserve further investigation.³⁷ However, it should be considered that low nNO levels might be due to an underlying upper airway infection not associated to PCD. Regardless of patients' age, measurement of nNO can be used for screening PCD at either the general practitioner office or at secondary pediatric centers. Reduced levels of nNO, but not as much as in PCD, may be due to significant sinus obstruction, for instance due to nasal polyps. High nNO indicates upper airway inflammation in the presence of osteomeatal complex patency.

Measurement of nNO has limited value for diagnosing and/or monitoring upper airway disorders other than PCD. In particular, the routinary use of nNO as a marker

of inflammation in AR is not currently recommended, even though some studies use it as efficacy therapeutic measure. Moreover, despite nNO levels are reduced in children with rhinosinusitis, at present it is not used as a diagnostic or prognostic marker because more accurate, sensitive, and specific tools for the diagnosis (e.g., nasal endoscopy, sinus computed tomography, or magnetic resonance imaging) are available, and its use as an effect indicator of treatment awaits further confirmation. In children with adenoidal hypertrophy, further studies are needed to verify the hypothesis that nNO may be used as non-invasive marker of obstruction. Finally, since more reliable tools are currently available for CF diagnosis and monitoring, nNO measurement is not crucial for patients' management in the clinical setting, and its value at present is likely to remain within the research arena.

In conclusion, a growing body of evidence points towards the utility of nNO as a tool that guides physicians in the diagnostic work-up of pediatric airway disorders. Whilst the recent increased interest in nNO by commercial manufacturers is encouraging, uniformity of nNO measurement is mandatory. Since nNO determination is flow dependent, a general consensus from the major investigators in this area is highly desirable so that future studies will be performed with the same flow rate. A shared nNO methodology will enable to overcome the challenges that lie ahead in incorporating nNO measurement into the mainstream clinical setting of pediatric airway diseases.

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2.3 Everyday impact of respiratory disease: measuring quality of life

Monitoring chronic respiratory diseases has been traditionally focused on the periodical assessment of clinical, functional and imaging outcomes (2). However, likewise other chronic conditions, these parameters are inadequate to evaluate the impact the disease has on children's everyday physical, social, emotional and school functioning (53). Furthermore, prescribed treatments, with the related burden on patients' daily activities, is often given insufficient consideration, thus entailing an incomplete perception of disease control, comprehensively intended.

Therefore, in the last decades, the role of quality of life (QoL), particularly health-related QoL (HRQoL), has increasingly gained relevance in the management of pediatric chronic patients. This has determined an effort in standardizing evaluation tools to quantify the disease impact on patients, and therefore disease-specific questionnaires for pediatric patients or their parents have been developed (53-55).

Several tools are currently available for the assessment of QoL in chronic respiratory conditions such as asthma (53) and CF (54), and represent useful outcome measures for both clinical and research purposes. On the contrary, questionnaires specifically developed for the assessment of patient-reported outcomes (PRO) in non-CF CLD have been proposed only recently. Quittner and coworkers, moving from the experience in CF pediatric care, developed the QoL Questionnaire-Bronchiectasis (QoL-B) as the first specific tool for non-CF bronchiectasis that showed a good reproducibility and reliability in a validation study (55). Nevertheless, efforts in assessing QoL in other non-CF chronic respiratory disorders have been less remarkable, and, particularly in PCD management, a well-designed PRO measure has long been lacking.

The development and validation of a disease-specific PRO measure generally moves from the evaluation of the applicability of QoL evaluation tools originally thought for other conditions. Similar PRO measures including elements of specificity regarding the disease considered are then generally developed (55). Further steps include the assessment of reproducibility of the tools under

consideration and the evaluation of their efficacy in determining long-term QoL changes. We performed such evaluations in 20 PCD subjects followed-up at our center, in whom QoL was assessed at baseline and after 12 months by means of three commonly used questionnaires for chronic respiratory diseases, namely, the St. George's Respiratory Questionnaire, the Medical Outcomes Study Short Form 36, and the Leicester Cough Questionnaire (56). Our main finding was that, both at baseline and after 12 months, PROs were not related to functional parameters. Therefore, we concluded that the adopted PRO measures are suboptimal to longitudinally track QoL in PCD, and new tools specifically developed for PCD children are urgently needed.

Interestingly, this relevant gap in PCD management has been filled by an extremely recent study proposing the first HRQoL measure for PCD children considering general aspects common to other chronic lung disorders and also a wide spectrum of issues unique to this population (57). A major merit of this valuable QoL-PCD questionnaire is the underlying methodology, based on the recruitment of a wide, ethnically and socially heterogeneous sample of PCD subjects. Moreover, the PRO measures were evaluated at different ages, confirming the expected differences in patients' perspectives. In particular, younger children have a less evident perception of the impact their condition has on social functioning, energy levels and vitality. Given the strengths of this new instrument, it is desirable that it will be soon adopted as an end-point for monitoring health outcomes in both clinical and research settings, in the perspective of an increasingly patient-centered disease management.

Our findings on the applicability of commonly used PRO measures in PCD children have been published in *Chest*.

Long-term Assessment of Quality of Life in Primary Ciliary Dyskinesia

Time for New Tools?

To the Editor:

We deeply commend Quittner et al¹ in a recent issue of *CHEST* (August 2014) on their effort in developing the first disease-specific patient-reported outcome (PRO) measure validating the Quality of Life Questionnaire-Bronchiectasis (QOL-B). This is an area that we actively pursued in primary ciliary dyskinesia (PCD), a genetic cause of chronic suppurative lung disease with great impact on health because of abnormal mucociliary clearance leading to recurrent airway infections and bronchiectasis.²⁻⁴ Sensitive measures for tracking PCD lung disease progression have serious limitations in clinical practice since changes in spirometry may not be apparent, and repeated high-resolution CT scans increase the risk of ionizing radiation exposure.⁵

In a prospective, 1-year study of 20 subjects with PCD (median age, 16.9 years; range, 12-33.4 years) we verified whether three of the most widespread PROs used for assessing quality of life (QoL) in respiratory disorders (St. George's Respiratory Questionnaire [SGRQ], Leicester

Cough Questionnaire [LCQ], and Medical Outcomes Study Short Form 36 [SF36]) correlated with spirometry or 6-min walk test (6MWT). Patients completed SGRQ, LCQ, and SF36 and performed spirometry and 6MWT at scheduled visits (T₀, T₁₂). Table 1 summarizes the main findings.

During the study period, three respiratory exacerbations⁵ (range, 0-7) that required systemic antibiotics occurred. Eight patients (40%) needed four or more antibiotic courses. At baseline, none of the PROs was related to age at diagnosis and age at symptoms onset.

FEV₁, FVC, FEV₁/FVC, and forced expiratory flow at 25% to 75% of FVC, as well as 6-min walk distance, were not significantly related to any of the QoL assessment tools at T₀ and T₁₂. Over the 12-month period, no significant changes were found in any of the QoL outcomes considered or in spirometry or 6MWT. Despite the small sample size, our results show that SGRQ, LCQ, and SF36 are unrelated to the commonly accepted disease outcomes in PCD. Indeed, we provide the first demonstration, to our knowledge, that these tools are also suboptimal to longitudinally track QoL in PCD.

No PCD-specific QoL questionnaire has ever been validated, and the sensitivity of currently used questionnaires in detecting long-term QoL changes has not been

TABLE 1] Patient-Reported Outcomes, 6-Min Walk Test, Spirometry, and Sputum Culture Results From Patients With PCD at the Study Time Points Over 1-Year Period

Outcome	T ₀	T ₁₂	P Value ^a
SGRQ total score	15.2 (4.2-65)	14.1 (2.3-50.8)	.7
LCQ total score	16.9 (6.8-20.9)	18.2 (10.8-21)	.2
SF36 PCS	50 (30.1-60)	51.4 (26.4-60)	.4
SF36 MCS	55.3 (22.1-62.2)	55.1 (38.4-64)	.6
6MWD, % predicted	77.5 (60-89)	72 (60-84)	.2
FEV ₁ , % predicted	86.5 (45-117)	87 (41-117)	.8
FVC, % predicted	93 (64-133)	98.5 (64-134)	.8
FEV ₁ /FVC, %	74.5 (52-85)	73.5 (51-99)	.9
FEF ₂₅₋₇₅ , % predicted	56 (16-90)	54 (13-120)	.8
Positive sputum cultures, ^b %	70	30	.03

Data are presented as median and range values. 6MWD = 6-min walk distance; FEF₂₅₋₇₅ = forced expiratory flow at 25% to 75% of FVC; LCQ = Leicester Cough Questionnaire; MCS = mental component summary; PCD = primary ciliary dyskinesia; PCS = physical component summary; SF36 = Short Form-36; SGRQ = St. George's Respiratory Questionnaire.

^aMann-Whitney *U* test or Fisher exact test.

^bIncluding *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Aspergillus niger*.

evaluated so far. Pending the validation of QOL-B for measuring symptoms, functioning, and QoL also in PCD, and for evaluating the efficacy of new therapies,¹ caution in the use of tools that are not disease specific for PCD is mandatory. According to what was reported in patients with bronchiectasis,¹ QOL-B might be considered as an efficacy end point also in PCD clinical trials. We believe the need for development of a PCD-specific instrument for longitudinal QoL assessment on larger study populations is urgent.

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2.4 Management algorithms: the example of esophageal atresia survivors

In most cases, managing children with non-CF CLD means dealing with rare or at least uncommon conditions that require specialist referral to provide standardized management approaches. Nevertheless, before expert consultation, patients are often managed by primary care health professionals who are seldom trained and confident enough to correctly assess and manage the disease and its related complications (58). In this scenario, structured pathways for clinical decision-making, including diagnostic procedures and even indications or timing for specialist referral are of primary relevance. The production of different pathways and algorithms clarified how to suspect, diagnose and manage a number of conditions under the wide umbrella of pediatric CLD (40,59). Particularly, a valuable example of management algorithm has been developed for chronic cough (60). Apart from providing physicians with an easy-to-use and helpful tool, recent high quality evidence suggests that in children with chronic cough the use of algorithms also improves clinical outcomes (61).

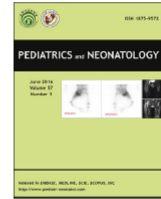
As the need for useful clinical algorithms is particularly relevant in uncommon conditions, we focused our efforts on the standardization of behaviors in the management of respiratory complications deriving from a relatively rare disease, namely esophageal atresia (EA), for which robust evidence based on large populations is lacking.

The dramatic improvement of perinatal care and of surgical techniques observed in the last decades has transformed EA, with or without tracheoesophageal fistula, from a frequently lethal malformation to a relatively treatable disease, with a consequent increase in the burden of long term complications, which more often involve the respiratory tract (62). Recurrent bronchitis, chronic cough and asthma-like symptoms represent the major clinical manifestations, together with lower respiratory tract infections, which may achieve an incidence of five episodes per year, particularly in the first years of life (63). These complications result in a respiratory impairment that, according to the available literature, is generally characterized by a restrictive rather than obstructive functional pattern and by morphological abnormalities mainly consisting of bronchiectasis and

consolidations (64). Furthermore, airway endoscopy findings, occasionally reported by some authors (65,66), have shown tracheo- and bronchomalacia to be the most frequently detected abnormalities. Despite still debated, the etiology of pulmonary manifestations following EA repair is likely multifactorial. First, the increased risk of premature birth in newborns with EA often entails the association of lung hypoplasia, tracheomalacia and other respiratory disorders typical of prematurity (67). Furthermore, available studies suggest that chronic asthma is likely elicited by a reflex mechanism, whereas repeated acid aspiration associated to gastroesophageal reflux and feeding difficulties may well explain the recurrence of lower respiratory tract infections (68,69). Finally, structural abnormalities of both the airways and the esophagus frequently persist after EA repair, contributing to alter pulmonary function (67).

Despite several articles on the long-term respiratory complications of EA survivors have been published, available literature does not provide a shared executive protocol which could guide clinicians in the management of these patients. Particularly, even though descriptive studies have summarized the most typical functional and structural features of the condition, the available diagnostic tools have never been ordered in a management algorithm. Moving from these observations, we built a basic decisional pathway whose aim is to help clinicians in assessing the degree of respiratory impairment with a reasoned use of diagnostic tools, and, consequently, to choose the most appropriate follow-up. Of course, like all algorithms, our proposal is not meant to replace clinical judgment, but its daily application is highly desirable in order to verify its efficacy in driving physicians in the systematic approach to the chronic pulmonary manifestations of EA survivors.

A review of the available literature, together with the mentioned proposal of a management algorithm for these patients, was published in *Pediatrics and Neonatology*.



REVIEW ARTICLE

Longitudinal Follow-up of Chronic Pulmonary Manifestations in Esophageal Atresia: A Clinical Algorithm and Review of the Literature

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tracheoesophageal
fistula

In the past decades improved surgical techniques and better neonatal supportive care have resulted in reduced mortality of patients with esophageal atresia (EA), with or without tracheoesophageal fistula, and in increased prevalence of long-term complications, especially respiratory manifestations. This integrative review describes the techniques currently used in the pediatric clinical practice for assessing EA-related respiratory disease. We also present a novel algorithm for the evaluation and surveillance of lung disease in EA. A total of 2813 articles were identified, of which 1451 duplicates were removed, and 1330 were excluded based on review of titles and abstracts. A total of 32 articles were assessed for eligibility. Six reviews were excluded, and 26 original studies were assessed. Lower respiratory tract infection seems frequent, especially in the first years of life. Chronic asthma, productive cough, and recurrent bronchitis are the most common respiratory complaints. Restrictive lung disease is generally reported to prevail over the obstructive or mixed patterns, and, overall, bronchial hyperresponsiveness can affect up to 78% of patients. At lung imaging, few studies detected bronchiectasis and irregular cross-sectional shape of the trachea, whereas diffuse bronchial thickening, consolidations, and pleural abnormalities were the main chest X-ray findings. Airway endoscopy is seldom included in the available studies, with tracheomalacia and tracheobronchial inflammation being described in a variable proportion of cases. A complete diagnostic approach to long-term respiratory complications after EA is mandatory. In the presence of moderate-to-severe airway disease, patients should undergo regular tertiary care follow-up with functional assessment and advanced chest imaging.

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1. Introduction

Esophageal atresia (EA) with or without tracheoesophageal fistula (TEF) occurs in one per 3000 live births.^{1–3} In recent decades, improved surgical techniques and better neonatal supportive care have resulted in reduced mortality and increased prevalence of long-term disease-related complications, the most frequent of which include respiratory manifestations.⁴

Recurrent-to-chronic respiratory symptoms may upset daily life of EA survivors,⁵ and this is the reason why the assessment of pulmonary disease is recommended in these patients. This integrative review describes the various techniques currently used in pediatric clinical practice for assessing EA-related respiratory disease. Moreover, we present a novel algorithm for the evaluation and surveillance of lung disease in EA survivors. We carried out an electronic keyword literature search for English articles published on this topic up to September 22, 2015, in the Scopus, Web of Science, PubMed, and MEDLINE databases. We excluded the studies conducted exclusively on adults, but included those with a study population comprising children (or adolescents) and adults. The terms “esophageal atresia” AND (lung OR respiratory OR pulmonary OR airway or spirometry or complications or diagnostic tools) were used as keywords in combination, and the studies found were evaluated for selecting relevant literature. In addition, a manual search was conducted to evaluate review articles’ references. Literature reviews on diagnostic procedures for EA-related pulmonary disease prompted us to develop a novel algorithm for the evaluation and surveillance of lung disease in EA survivors.

2. Etiology

The etiology of pulmonary manifestations following EA repair is multifaceted. Because newborns with EA have an increased risk of premature birth that may initiate the clinical scenario,⁶ the association with anomalies such as tracheomalacia and lung hypoplasia may significantly contribute to respiratory morbidity since birth.² Gastrointestinal symptoms (i.e., regurgitation and/or feeding difficulties with repetitive cough during swallowing, and/or dysphagia and heartburn likely due to peptic esophagitis and Barrett’s esophagus) represent the major complaints at any age, and an association of gastrointestinal and respiratory symptoms has been hypothesized to imply a correlation between esophageal and lung dysfunction.^{1,5,7} Indeed, esophageal dysmotility and gastroesophageal reflux (GER) may cause and/or worsen wheezing, bronchial asthma, and pneumonia.⁸ Although the underlying mechanisms are still being debated, literature suggests that chronic asthma is likely elicited by a reflex mechanism and that recurrent pneumonia may be explained by repetitive acid aspiration.^{9,10} Chronic airway inflammation with bouts of infection can eventually result in segmental or even lobar damage leading to the development of severe, life-threatening lung disease in a proportion of patients.^{11–14} Finally, recurrent TEF may further complicate the clinical course.¹⁵ Following EA-TEF repair, structural anomalies persist in both the trachea and the esophagus, and chest

wall deformities, exacerbated by thoracotomy, may further contribute to alter pulmonary function.²

3. Respiratory complications

Patients with EA with or without TEF experience respiratory complaints more often and more persistently than other individuals, and recurrent bronchitis, chronic cough, repeated pneumonia, and asthma-like wheezing represent the major clinical manifestations.^{11,16}

Lower respiratory tract infection is abnormally common especially in the first years of life, with more than five annual respiratory tract infections and a rate of more than three attacks of bronchitis per year of up to 78%.^{17,18} In a study from Finland, aspiration pneumonia likely related to impaired esophageal peristalsis and esophageal stricture was reported in approximately 50% of affected children, although they did not experience more current respiratory or esophageal symptoms than those without.¹²

Coughs with sputum production and recurrent bronchitis are significantly more common among patients with repaired EA than among healthy individuals,¹⁹ and although respiratory morbidity tends to improve with age, chronic cough, associated with bronchial constriction and hyperresponsiveness, can persist or even become more frequent in adulthood.²⁰ As a consequence of repeated bouts of lower airways infection, bronchiectasis may also develop.²¹

Although some respiratory complications may be accounted for by documented tracheomalacia, esophageal dysmotility, GER disease (GERD), or surgical complications, a high proportion of EA survivors have abnormal pulmonary function that is apparently unrelated to these conditions.² A restrictive pattern generally prevailing over obstructive or restrictive-obstructive airway disease has been described in up to 96% of children, adolescents, and adults previously treated for EA with or without TEF.^{1,12,22–24} Interestingly, approximately one-third of a Finnish pediatric population had restrictive or obstructive defects that were apparently unrelated to current respiratory or esophageal symptoms.¹² In the same study, bronchial hyperresponsiveness was found to be severe/moderate or mild in 26% or 52% of the cases, respectively. Airflow obstruction may be explained by several mechanisms including small airway disease or proximal obstruction due to airway malacia or epithelial damage caused by GERD and recurrent episodes of bronchitis or aspiration pneumonia worsened by poor tracheal clearance, or decreased lung growth during infancy.²⁵ Multiple potential predisposing factors to restrictive lung disease are also congenital or acquired vertebral or chest wall abnormalities (i.e., scoliosis or postoperative rib fusions), surgical trauma, aspiration, and/or recurrent chest infections.¹

Chronic asthma is considered to be common in EA survivors, with significant bronchial inflammation also occurring in patients with nonallergic asthma.^{13,26} Whatever the initial trigger is, asthma significantly contributes to respiratory morbidity in EA, and it might even worsen pre-existing GERD.¹³

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Table 1 Main findings from the 26 original articles that exclusively investigated respiratory disease in esophageal atresia survivors.

Study	Age (y)	Lung imaging	Pulmonary function tests	Bronchoscopy	Other	Main findings
Dudley & Phelan ¹⁸	1 to >9	—	—	—	Clinical outcome	Recurrent bronchitis during the first 3 y (78%)
Milligan & Levison ²²	7–18	—	Spirometry MCT	—	—	Obstructive (54%) & restrictive (21%) lung diseases
Couriel et al ³¹	8–17	—	Spirometry MCT	—	Clinical outcome	Bronchial hyperreactivity (65%) Bronchitis for > 8 y (25%) Mild restrictive lung disease
LeSouëf et al ²⁵	12–21	—	Spirometry Body plethysmography	—	—	Bronchial hyperreactivity (22%) Significant reduction of lung volumes in the pneumonia group vs. the nonpneumonia group
Chetcuti et al ³²	0–25	—	Body plethysmography	—	—	Daily cough (15%), wheezing (40%), bronchitis (34%) RV increase (77%), significant reduction in VC & FEV ₁ in patients who wheezed in the past 12 mo
Griscom & Martin ⁴⁰	2–21	CT	Spirometry	—	—	Bronchiectasis (40%) Mild restrictive (20%) & obstructive lung disease (40%)
Chetcuti et al ³³	6–37	—	Spirometry Body plethysmography	—	—	Reduced FEV ₁ (25%) & RV/TLC ratio (41%) Restrictive lung disease (18%)
Chetcuti & Phelan ¹¹	1–37	—	—	—	Clinical outcome	In the 0–5-y age group, pneumonia (50%), recurrent pneumonia (25%) Persistent cough (32% aged 0–5 y, 13% 5–10 y, 15% 10–15 y, & 9% > 15 y) Typical harsh cough (71% aged 0–5 y, 60% 5–10 y, 57% 10–15 y, & 40% > 15 y)
Beardsmore et al ³⁸	2–13 wk	—	Body plethysmography	—	—	Thoracic gas volume increase (33%), abnormalities in airway resistance pattern (78%), airway resistance increase (33%), limitation of inspiratory & expiratory airflow (11%)
Montgomery et al ³⁴	8–21	—	Spirometry Body	—	Clinical outcome	Asthma or bronchitis (39%)

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Table 1 (continued)

Study	Age (y)	Lung imaging	Pulmonary function tests	Bronchoscopy	Other	Main findings
			plethysmography Bicycle ergometer			Obstructive (44%) & restrictive (55%) lung disease
Robertson et al ³⁵	7–28	X-ray	Spirometry Body plethysmography MCT	—	Clinical outcome	Decreased maximal working capacity (53%) Respiratory symptoms (72%), bronchiectasis (4%), obstructive (12%), restrictive (36%), & mixed (4%) lung disease
Somppi et al ³⁶	3.5–30	—	Spirometry	Yes	Clinical outcome	Positive MCT (24%) Reduced FEV ₁ (67%) Tracheal inflammation (37%). Respiratory infections (29%), recurrent dyspnea (28%), & cough during the night (37%)
Agrawal et al ²⁶	7–12	—	Spirometry Body plethysmography	—	—	Restrictive lung disease (67%)
Choudhury et al ¹⁴	0 d to >30 d	—	—	—	Clinical outcome	Aspiration & pneumonia (13%) as early death causes Aspiration, tracheomalacia, & reactive airway disease as late death causes (59%)
Soto et al ³⁰	1–15	—	Spirometry	—	—	Restrictive lung disease (50%)
Little et al ²⁸	18.7	—	—	—	Clinical outcome	Respiratory infections (29%)
Sarnelli et al ¹³	0.8–14.6	X-ray HRCT Perfusion scintigraphy	—	Yes	—	Family history of atopy (40%) & allergic asthma (10%), lobar consolidations (80%), bronchiectasis (20%)
Banjar ²¹	1.25 ± 2.4	CT	Spirometry Body plethysmography	—	—	Tracheomalacia (29%), bronchiectasis (17%), obstructive (7%), restrictive (20%), & mixed lung disease (7%)
Lilja & Wester ²⁹	1–20	—	—	—	Clinical outcome	At 16–20 y, frequent cough between (36%), impaired exercise capacity (20%), respiratory infections (40%), & shortness of breath (53%)
Malmström et al ¹²	9.7–19.4	—	Spirometry Histamine challenge test	Yes	FeNO, clinical outcome	Obstructive (30%) & restrictive (35%) lung diseases Bronchial hyperreactivity (78%) Current respiratory symptoms (44%),

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Table 1 (continued)

Study	Age (y)	Lung imaging	Pulmonary function tests	Bronchoscopy	Other	Main findings
Gischler et al ¹⁷	5–6.5	—	Spirometry Treadmill test	—	FeNO, clinical outcome	wheezing (52%), pneumonia (52%) Mild (72%) & moderate bronchitis in biopsies (7%) Abnormal FeNO (23%) Reduced FEV ₁ (25%) Abnormally low maximal exercise tolerance (6.3%) Normal FeNO High proportion of patients with >5 respiratory tract infections in 5 y (74%) Reduced Rrs6 (27%) & Rrs8 (24%) FEV ₁ significantly lower in EA with TEF vs. healthy controls
Harrison et al ³⁷	7.6 ± 2.2	—	Spirometry Forced oscillation technique Body plethysmography	—	—	Obstructive lung disease (13%) FVC & TLC significantly lower in EA with TEF vs. patients with gastroesophageal reflux disease
Peetsold et al ⁸	13.2 ± 2.9	—	Spirometry Body plethysmography Cardiopulmonary exercise testing	Yes	—	Obstructive lung disease (19%) Chronic cough (19%) & dyspnea (37%) Obstructive (50%) or restrictive (11%) lung disease
Spoel et al ³⁹	24–66 wk	—	Body plethysmography	—	—	Bilateral opacities, right lower lobe infiltrate, or pleural abnormalities (10%) Obstructive (19%) & restrictive (23%) lung disease Bronchial hyperreactivity (39%) Reduced ventilation reserve (45%)
Legrand et al ²³	13.3	—	Spirometry	—	Clinical outcome	
Beucher et al ²⁴	8.5	X-ray	Spirometry MCT Bicycle ergometer	—	—	

CT = computed tomography; EA = esophageal atresia; FeNO = fractional concentration of exhaled nitric oxide; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; HRCT = high-resolution computed tomography; MCT = methacholine challenge test; Rrs6 = resistance at 6 Hz; Rrs8 = resistance at 8 Hz; RV = residual volume; TEF = tracheoesophageal fistula; TLC = total lung capacity; VC = vital capacity.

4. Management

With improved patient survival due to better neonatal care and surgery, the importance of recognition and management of pulmonary disease has increased. Table 1 summarizes the main findings from 26 original articles that exclusively investigated respiratory disease in EA survivors.

Several studies focused only on clinical outcomes,^{27–29} whereas others also included functional assessment by spirometry,^{30–38} airway challenge tests,^{12,24} and/or lung volumes measurement.^{8,39} The restrictive pattern was generally reported to prevail over the obstructive or mixed ones, and, overall, bronchial hyperresponsiveness was found in up to 78% of patients.¹² Of note, there were a few

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studies on chest imaging findings, which were reported in only five articles.^{13,21,24,35,40} In particular, three chest computed tomography (CT) studies detected bronchiectasis^{13,40} and irregular cross-sectional shape of the trachea²¹ in a subgroup of patients, respectively. Chest CT findings may also include consolidations and/or bronchiectasis (Figure 1). By contrast, diffuse bronchial thickening, consolidations, and pleural abnormalities were the main chest X-ray findings described in a minority of patients.^{13,24,35} Airway endoscopy was seldom included in the available studies, with tracheomalacia representing a common finding^{8,12,41} and tracheobronchial inflammation being described in a variable proportion of cases.^{12,36} In addition to tracheomalacia and bronchomalacia, less common anatomic abnormalities may include ectopic or absence of bronchus and congenital bronchial stenosis.¹ Undoubtedly, evaluation of the airways structure via flexible bronchoscopy can help identify these problems in infants and children before EA/TEF repair, or also in those with persistent respiratory symptoms after EA/TEF surgery.^{12,13}

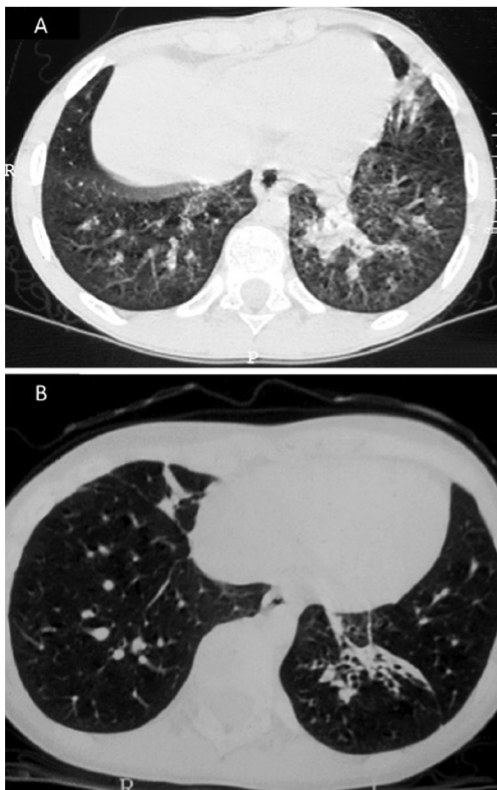


Figure 1 Chest high-resolution computed tomography from two children with esophageal atresia and tracheoesophageal fistula repaired at birth and followed at our department: (A) left lower lobe consolidations and (B) left lower lobe consolidation with bronchiectasis.

Although several articles on the main long-term respiratory complications in EA repair survivors have been published, a shared executive protocol has never been developed, nor have the available diagnostic tools been ordered in a management algorithm. Indeed, the few studies regarding the practical management of pulmonary complications in EA survivors and their heterogeneity make the development of an evidence-based operative algorithm virtually impossible. Nevertheless, due to the severe chronic complaints that some patients may experience and the impact on the healthcare costs, we propose a novel synthetic management algorithm (Figure 2), which may be helpful for clinicians dealing with lung disease secondary to EA. Basically, procedures are selected on the basis of the current clinical features. Present literature does not specify the timing of both follow-up visits and functional/chest imaging work-up. We suggest that basic procedures including transcutaneous pulse oximetry (SpO₂), chest radiographs, and lung function tests (the latter only on cooperating patients) are at least obtained in all patients at baseline. We also propose that EA survivors, with or without TEF, should be differentiated between those with mild airway disease and those with moderate-to-severe airway disease. In particular, patients with respiratory symptoms (persistent cough, recurrent-to-persistent wheezing, recurrent respiratory infections) who show slight abnormalities or normal results of SpO₂ at rest (ranging from 90% to 93%),^{42,43} and/or chest radiography, and/or spirometry (i.e., forced expiratory volume in 1 second and forced vital capacity $\geq 70\%$ predicted)⁴⁴ are defined as having mild airway disease. Conversely, patients with respiratory symptoms and more relevant abnormalities of SpO₂ at rest, and/or chest radiography, and/or spirometry are defined as having moderate-to-severe airway disease. We suggest that only the latter cases undergo regular tertiary care follow-up, including more extensive lung function assessment and advanced chest imaging (i.e., high-resolution CT and/or magnetic resonance imaging). A complete diagnostic approach to long-term respiratory complications after EA should also include tracheobronchial endoscopy with instillation of methylene blue for excluding recurrent TEF.⁵ Recurrent TEF should be corrected using laparoscopic antireflux procedures to prevent lung damage.^{45,46} These considerations lead to the conclusion that the evaluation of these patients is most efficiently accomplished in a tertiary care center where pediatric pulmonologists, gastroenterologists, radiologists, and surgeons are all available.

5. Conclusion

In patients following EA repair, recurrent-to-persistent respiratory disease represents a major feature, especially in early to middle childhood.⁴⁷ The persistence or recurrence of troublesome clinical manifestations imposes a scheduled follow-up of a large proportion of EA survivors, ideally through a multidisciplinary care approach for addressing their special needs. Pulmonary care of these patients involves managing comorbidities and preventing or minimizing damage to the lungs. Early detection and management of aspiration and other causes of recurrent-to-persistent lower airways infections in this population may

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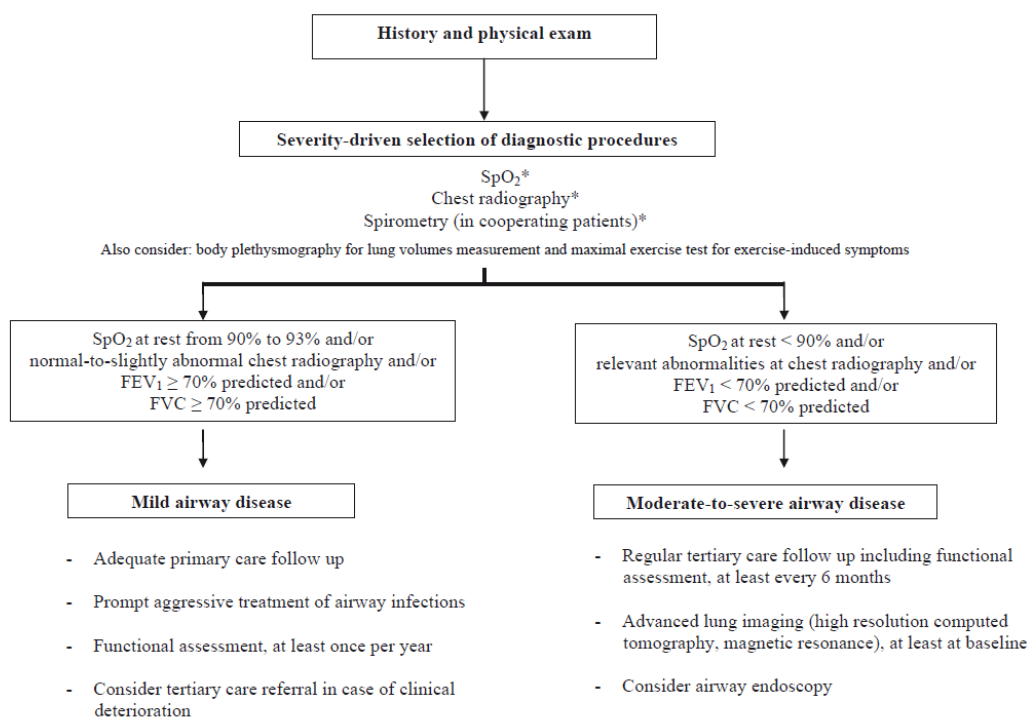


Figure 2 Algorithm for the evaluation and surveillance of chronic pulmonary manifestations in esophageal atresia survivors with or without tracheoesophageal fistula. * To be obtained in all patients. FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; SpO₂ = arterial oxygen saturation measured by pulse oximetry.

be important to prevent decrements in pulmonary function and serious long-term complications.³ A management algorithm for the evaluation and surveillance of EA-related respiratory disease based on the evidence from literature review is proposed. Like all algorithms, it is not meant to replace clinical judgment, but it should rather drive physicians to adopt a systematic approach to chronic pulmonary manifestations in EA survivors.

Ethical statement

This article does not contain any studies with human or animal subjects performed by any author(s).

Conflicts of interest

There are no financial or other relations that could lead to a conflicts of interest.

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Chapter 3

-The role of nutritional status in children with chronic lung disease-

3.1 Growth monitoring: the example of Primary Ciliary Dyskinesia

Despite apparently poorly linked, nutrition and respiratory symptoms are deeply interrelated. The raised energy expenditure deriving from the increased work of breathing typical of CLD may alter the metabolic balance of affected children significantly affecting growth. Similarly, chronic infection and poor pulmonary function also determine increased energy utilization (70). On the other hand, malnutrition decreases the strength of the respiratory muscles and impairs pulmonary defense against pathogens, increasing the risk of respiratory infections and thus leading to a dangerous “vicious circle” (70,71).

Low body mass index (BMI) has been associated with impaired respiratory function and higher risk of death in patients with chronic obstructive pulmonary disease (COPD) (70), making nutritional status a crucial aspect to be assessed in the daily management of these subjects. Similarly, nutritional status of 2-year-old children with bronchopulmonary dysplasia has been shown to affect pulmonary outcomes in childhood (72). On the other hand, impaired lung function has been reported in children with history of premature birth and intrauterine growth retardation (73).

In pediatric CF care assessment and monitoring of nutritional status represents one of the cornerstones of the clinical management. In particular, pancreatic insufficiency deeply affects somatic growth, and an association with worse respiratory function has been widely demonstrated. Indeed, nutritional deficiency secondary to malabsorption may lead to poor lung growth and increased susceptibility to infections (71), determining a more severe CF phenotype (74). Nevertheless, long-term activation of inflammation and chronic pulmonary disease with hypoxia has been shown to decrease IGF-I levels, providing another likely mechanism involved in CF children’s growth impairment (75).

Nutritional status in non-CF CLD has been less investigated and available studies are limited, also because of the difficulties in enrolling large and homogeneous populations and in progressively assessing growth for sufficient follow-up periods. Two studies have remarkably demonstrated that children with non-CF bronchiectasis show adequate somatic growth and stabilization of lung function over time (76,77). Conversely, data on the longitudinal progression of anthropometric parameters in patients with PCD are extremely sparse. Some authors have anecdotally reported progressive growth impairment during childhood (78), but the extremely limited sample size of such reports prevents from any generalization to the pediatric PCD population.

The rationale behind a possible nutritional impairment in PCD substantially derives from the chronicity of the disease and the recurrence of upper and lower respiratory tract infections. Indeed, despite several PCD features are similar to CF, malabsorption, liver disease and diabetes that play major roles in CF malnutrition (71) are not seen in this rare condition (50). In order to verify the hypothesis that PCD children and adolescents receiving centralized care do not present significant abnormalities in growth parameters, we designed a wide multicenter study involving a total of 158 PCD subjects from three European tertiary care centers, namely the Royal Brompton Hospital, London, UK, the Federico II University, Naples, Italy, and the Department of Pediatrics & Adolescent Medicine, Copenhagen, Denmark. The retrospective evaluation aimed at tracking the progression of lung function parameters and BMI over defined periods of two, four and six consecutive years. What emerged from our analysis was that early referral to a PCD center during preschool age is not associated to better spirometry or BMI. More importantly, the longitudinal assessment of our cohort showed that PCD children and adolescents receiving centralized care show steady BMI and stable spirometry during medium term follow-up.

This study, which represented a fruitful collaboration between the three mentioned European centers, was conducted during a long-term research fellowship funded by the European Respiratory Society that allowed Dr. Maglione to attend the Department of Pediatric

Respiratory Medicine at the Royal Brompton Hospital in London, UK, under the supervision of Prof. Andrew Bush. The results of this study have been published in *Pediatric Pulmonology*.

Multicenter Analysis of Body Mass Index, Lung Function, and Sputum Microbiology in Primary Ciliary Dyskinesia

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Summary. Background: No studies longitudinally, simultaneously assessed body mass index (BMI) and spirometry in primary ciliary dyskinesia (PCD). Methods: We determined BMI and spirometry in 158 PCD children and adolescents from London, UK (n = 75), Naples, Italy (n = 23) and Copenhagen, Denmark (n = 60) at first presentation and during follow-up. Annual BMI and spirometry were prospectively collected and analyzed over blocks of 2, 4, and 6 consecutive years. Sputum pathogens were recorded. Results: Age at first spirometry was 8.7 years (range, 4.2–17.4). Mean Zscores of first measured BMI, FEV₁, FVC, and FEF_{25–75} were 0.01, –1.37, –0.84, and –1.68, respectively. First spirometry was not more frequently impaired in patients referred at age ≥6 years than in those referred at preschool age (P = 0.13). There were no differences in slopes for BMI, FEV₁, FVC, or FEF_{25–75} over any time block. *H. influenzae* was the most common pathogen, isolated at least once in 65% of patients. *P. aeruginosa* was found in 58 subjects (37%) of whom 8 (5%) were chronically infected. Neither pathogens was associated with spirometry changes. Conclusions: Preschool referral to a PCD center was not associated with better spirometry or BMI. PCD children and adolescents receiving centralized care show steady BMI and spirometry during medium term follow-up. There was a high prevalence of *Pseudomonas aeruginosa* infection, but the evolution of spirometry or BMI was not affected by this microorganism in medium term. Despite our longitudinal analysis showed no differences between the three centers, the assessment of spirometry and BMI over time represents a quality improvement tool. Future studies are needed to highlight the role of spirometry and BMI in long term PCD management and identify subgroups of patients with a higher risk of early lung failure or nutritional problems. *Pediatr Pulmonol.* 2014; 49:1243–1250. © 2014 Wiley Periodicals, Inc.

Key words: body mass index; primary ciliary dyskinesia; spirometry.

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INTRODUCTION

Primary ciliary dyskinesia (PCD) is a rare autosomal recessive disorder caused by functional and ultrastructural ciliary defects, with *situs viscerum inversus* and other disorders of laterality observed in around 50% of cases.¹ Early presenting symptoms include neonatal respiratory distress, and upper airway disease. Diagnosis is often delayed, because rhinitis, cough and otitis media are common childhood issues, and a rare diagnosis like PCD is often not considered.²

Lower airways are commonly involved in PCD, and recurrent pneumonia, chronic asthma-like symptoms, and bronchiectasis are the hallmarks of the disease, especially in school-aged subjects and adolescents.¹ In contrast to early studies showing stabilization of spirometry with treatment,^{3–5} pulmonary function was subsequently reported to be reduced in some older patients.⁶ A large longitudinal study demonstrated that, although most subjects had stable or improved spirometry over time,

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Preliminary data from this study have been presented at the British Thoracic Society Winter Meeting (London, UK, December 7–9, 2011).

Conflict of interest: None.

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34% lost more than 10% of forced expiratory volume in 1 second (FEV₁) over a period of 5–10 years.⁷ More recently, in two short-term studies, spirometry deteriorated slowly in twenty apparently clinically stable children,⁸ but did not decrease in a small group with unstable disease.⁹

Although lung function and nutrition are separate issues, they are closely interrelated.¹⁰ Patients with cystic fibrosis (CF) generally show progressive lung function decline, and concomitant poor growth.¹¹ Apart from one study in non-CF suppurative lung disease which included only two PCD cases,¹² data on growth in large PCD populations are absent, and the evolution of nutritional status, in terms of BMI over time has not been described.

In the current study, we aimed to determine the longitudinal evolution of BMI and spirometry in a large PCD population from three European centers. We hypothesized that, in PCD subjects receiving centralized care, nutrition and spirometric indices would remain stable during medium term follow-up.

MATERIALS AND METHODS

Study Design and Patients

The study was both a cross-sectional and a longitudinal analysis of clinical and functional data from a group of PCD children and adolescents attending the pediatric respiratory clinics at the Royal Brompton Hospital (RBH, London, UK, one of the three supra-regional reference centers for PCD patients in the UK)¹ at the Federico II University (Naples, Italy, the referral center for PCD in Campania, a region of Southern Italy), and at the Pediatric Pulmonary Service at Department of Pediatrics & Adolescent Medicine (Copenhagen, Denmark, the Danish PCD center). Inclusion criteria were a confirmed diagnosis of PCD,¹³ the ability to perform reliable spirometry, and the availability of annual anthropometric and spirometry data over at least three calendar years. Given the fact that the study involved merely the retrospective collation of data previously collected for clinical purposes and was a service evaluation, no ethics committee approval was required.

PCD Management

Management strategies at the three centers were very similar to each other, and, although evolving over time, were also similar to the ERS Task Force recommendations when they were published, and to which all three centers contributed.¹³ Details are reported in the online supplement.

Data Collection

A review of the electron microscopy computerized databases at RBH and the PCD Database at the Department of Pediatrics & Adolescent Medicine, Copenhagen

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and a review of the PCD clinical database of the Federico II University was carried out to identify children eligible for inclusion. For each patient the following cross-sectional data were recorded: gender; ethnic group; age at first referral to the center; ciliary ultrastructural defect; and presence of *situs viscerum inversus*. Age, height, weight, and spirometric parameters for each year were longitudinally recorded from the first measurement. Body mass index (BMI) and BMI Z-score were calculated.¹⁴ The collected data from the three centers are referred to the periods from 1990 to 2011 (UK), from 1979 to 2011 (Denmark), and from 1994 to 2011 (Italy).

Spirometry

Forced vital capacity (FVC), FEV₁, and forced expiratory flow between 25% and 75% of FVC (FEF_{25–75}) were measured according to published criteria.¹⁵ Spirometry parameters were expressed as percentage predicted and Z scores.¹⁶ FEV₁ was the primary outcome parameter, and a Z-score <−1.96 was considered abnormal.¹⁷ Further details are reported in the online supplement.

Microbiology Data

Results of sputum cultures performed during follow-up were collected. Isolation of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* was recorded and chronic pseudomonal airway infection (presence of *P. aeruginosa* for at least 6 months, with at least three positive cultures) was evaluated.¹⁸

Statistical Analysis

Study size was opportunistic and there were insufficient data to inform a power calculation. Mann–Whitney *U*-test was used to compare cross-sectional data. Longitudinal analysis of spirometric and anthropometric parameters was performed, for each subject separately, using linear regression on time since first spirometry available, yielding subject-specific estimates of slope. As the duration of follow-up widely varied among patients, in order to maximize the sample size we analyzed three consecutive measurements obtained from the subjects included in the study, and then focused on two subgroups of patients followed for 4 and 6 years, respectively, to maximize the duration of the follow-up. As few subjects had annual observations for more than 6 years, longitudinal analysis was not further extended. Pearson correlation coefficient and Spearman rank correlation coefficient (ρ) assessed correlations among continuous normally distributed and continuous non-normally distributed variables, respectively. A two-sided *P* value of <0.05 was significant. The data were analyzed with a statistical

software package (SPSS-PC, version 13.0; SPSS, Chicago, IL).

RESULTS

Demographics and Duration of Observation

The study population consisted of 158 PCD patients, whose clinical and ultrastructural data are summarized in Table 1. There were a total of 140 PCD patients from RBH, 50 patients from the Federico II University and 108 Danish subjects, from whom we could enroll 75, 23, and 60 subjects, respectively, who met the inclusion criteria. All subjects were reported non-smokers. Median duration of follow-up after first spirometry was 5 years (range, 2–16).

Cross-Sectional Analysis

Fifty-five patients (35%) had an abnormal FEV₁ when first measured. Frequency of abnormal first spirometry was similar in patients referred at age ≥6 years and those first referred at preschool age (*P* = 0.13). Specifically, first measured FEV₁ Z-score was abnormal in 18 out of 64 patients referred at preschool age (28%, median FEV₁ Z-score -1.1, range -5.18 to 0.76), versus 38 out of 94 subjects (40%, median FEV₁ Z-score -1.4, range -6.36 to 2.45) who were 6 years or older at first referral. First measured FEV₁ and FVC Z scores were not significantly related to age at referral (*r* = -0.1, *P* = 0.25, Fig. 1a, and *r* = -0.05, *P* = 0.56, Fig. 1b, respectively), but a weak, significant relationship was found with FEF_{2.5-7.5} Z-score (*r* = -0.2, *P* = 0.03).

TABLE 1—Clinical and Ultrastructural Data of the Study Population

	Study population (n = 158)
Gender (M:F)	80:78
Ethnicity, n (%)	
Caucasian	107 (68)
Pakistan	23 (14.5)
Turkish	16 (10.1)
Southern Indian	7 (4.4)
Afro-Caribbean	5 (3.2)
Situs solitus, n (%)	86 (54.4)
Age at first referral (years) ¹	6.6 (0.1–17.3)
Age at first spirometry (years) ¹	8.7 (4.2–17.4)
Cilia ultrastructure, ^{31,52} n (%)	
Outer or combined outer and inner dynein arms absence	106 (67)
Isolated inner dynein arm absence ²	15 (9.5)
Isolated axonemal disorganization	15 (9.5)
Axonemal disorganization and inner dynein arm absence	22 (14)

¹Median (range).

²All patients with an EM diagnosis of inner dynein arm absence had this finding confirmed by at least two EM tests, both performed in clinically stable conditions.

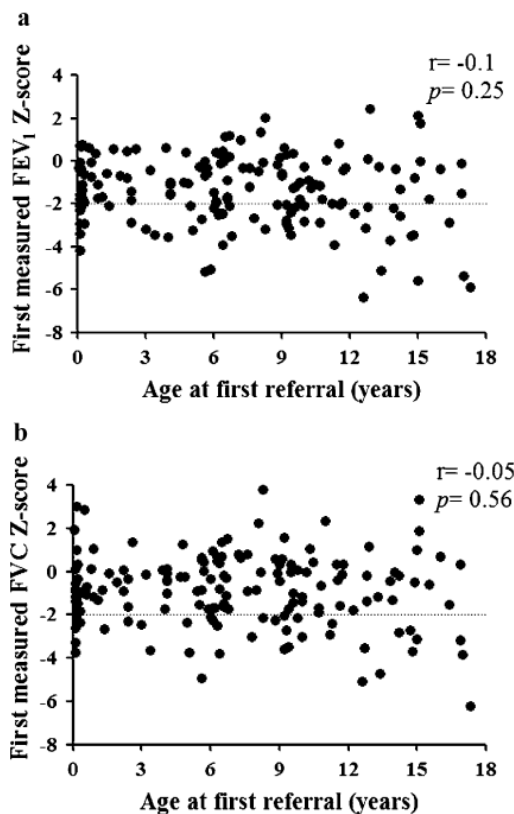


Fig. 1. Relationship between age at referral and first measured FEV₁ Z-score (a), and first measured FVC Z-score (b). The lowest value depicted for age at referral is 0.08 years, corresponding to one month of age. Dashed line, Z-score = -1.96.

Eleven subjects had an abnormally low first measured BMI (Z-score < -1.96). In 10 patients, BMI Z-score was >1.96, close to the predicted number of 8 in a normal population of equivalent size. No correlation was found between first measured BMI Z-score and age at referral (*r* = -0.03, *P* = 0.72). There was no statistically significant correlation between first measured FEV₁ and FVC Z scores with first BMI Z scores (*r* = 0.03, *P* = 0.71 and *r* = 0.05, *P* = 0.55).

Longitudinal Analysis

There were no deaths or patients lost to follow up, and no differences between the three clinics (see OLS, Fig. S2). Table 2 shows anthropometric and lung function data of the study population at first measurement and at 2, 4, and 6 years of follow-up. Evolution of FEV₁, and BMI Z scores, obtained at 3, 5, and 7 consecutive annual measurements are shown in Figures 2 and 3, respectively.

TABLE 2—Anthropometric and Spirometric Data During Follow-Up Period

	First measurement (n = 158)	Year 2 (n = 158)	Year 4 (n = 106)	Year 6 (n = 78)
Anthropometry				
Height				
cm ¹	128.9 (99.5–184)	137.3 (111–186)	148.8 (123–194)	159.8 (132–195)
Z-score ²	−0.05 (1.11)	−0.06 (1.11)	0.10 (1.11)	0.13 (1.01)
Weight				
kg ¹	27 (13.5–100)	33.7 (18.7–103)	41.5 (23–103)	50.5 (29.9–114)
Z-score ²	0.01 (1.19)	0.06 (1.23)	0.24 (1.14)	0.32 (1.08)
BMI				
kg/m ² ¹	16.5 (11.5–32.6)	17.3 (11.6–33.3)	18.5 (13.4–33.6)	19.5 (14.1–37.2)
Z-score ²	0.03 (1.27)	0.12 (1.31)	0.25 (1.22)	0.29 (1.23)
Spirometry				
FEV ₁ ²				
% pred	82.48 (20.38)	83.74 (19.50)	84.14 (16.70)	83.76 (19.0)
Z-score	−1.37 (1.63)	−1.34 (1.62)	−1.33 (1.41)	−1.39 (1.63)
FVC ²				
% pred	88.77 (20.89)	93.35 (20.44)	93.61 (15.56)	93.46 (17.17)
Z-score	−0.88 (1.68)	−0.58 (1.75)	−0.57 (1.37)	−0.60 (1.57)
FEF _{25–75} ²				
% pred	68.56 (29.64)	65.37 (30.45)	66.25(26.91)	65.22 (27.22)
Z-score	−1.58 (1.53)	−1.78 (1.59)	−1.71 (1.41)	−1.81 (1.51)

¹Median (range).²Mean (SD).

Three Consecutive Annual Measurements

Serial measurements in the 158 patients over 2 years from first measurement showed stability of FEV₁ Z-score (slope, 0.02; 95% CI, −0.07 to 0.10; $P = 0.63$; Fig. 2a), FVC Z-score (slope, 0.13; 95% CI, 0.05 to 0.23; $P = 0.31$), and FEF_{25–75} Z-score (slope, −0.03, 95% CI, −0.14 to 0.11; $P = 0.78$). No subjects had FEV₁ Z-score slopes <−1.96; slope was <−1 in six subjects. BMI Z-score did not change over the follow-up (slope, 0.05; 95% CI, 0.01–0.09; $P = 0.67$; Fig. 3a).

Five Consecutive Annual Measurements

One hundred six patients were included. Slopes of FEV₁, FVC, and FEF_{25–75} Z scores were −0.05 (95% CI, −0.10 to −0.005; $P = 0.51$; Fig. 2b), 0.02 (95% CI, −0.05 to 0.07; $P = 0.25$), and −0.04 (95% CI, −0.14 to 0.06; $P = 0.81$), respectively. In all subjects, FEV₁ Z-score slope was >−1. BMI Z-score was stable over time (slope, 0.05; 95% CI, −0.01 to 0.06; $P = 0.89$ Fig. 3b).

Seven Consecutive Annual Measurements

Seven consecutive annual evaluations were available in 78 subjects. Mean slopes were −0.05 (95% CI, −0.10 to −0.01; $P = 0.73$; Fig. 2c), −0.03 (95% CI, −0.08 to 0.02; $P = 0.47$), and −0.06 (95% CI, −0.11 to 0.02; $P = 0.90$) for FEV₁, FVC, and FEF_{25–75} Z scores, respectively. In all subjects, FEV₁ Z-score slope was >−1. BMI Z-score remained stable over 6 years (slope, 0.06; 95% CI, −0.002 to 0.05; $P = 0.81$; Fig. 3c).

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Age at referral was not related to FEV₁, FVC, FEF_{25–75}, and BMI Z scores slopes at two ($P = 0.41, 0.16, 0.11,$ and 0.89 , respectively), four ($P = 0.29, 0.39, 0.36,$ and 0.61 , respectively), and 6 years of follow-up ($P = 0.13, 0.09, 0.32,$ and 0.97 , respectively). No relationship was found between FEV₁ and BMI Z scores slopes at 2, 4, and 6 years of follow-up ($P = 0.11, 0.25,$ and 0.43 , respectively). First measured FEV₁ Z-score was not significantly related to FEV₁ Z-score slopes calculated over 2, 4, and 6 years of follow-up ($P = 0.32, 0.12,$ and 0.31 , respectively). Similarly, no significant differences in FEV₁ Z-score slopes at 2, 4, and 6 years were found between patients grouped according to the ciliary ultrastructural defect ($P = 0.77, 0.58,$ and 0.41 , respectively).

Microbiology Data

The number of sputum culture results for each patient was variable (median, 14; range 5–70). *H. influenzae* was the most commonly isolated pathogen (65% of subjects with at least one isolation), followed by *S. pneumoniae* and *S. aureus* (Table 3). *P. aeruginosa* was isolated at least once in 57 subjects (36%), and mucoid *P. aeruginosa* was detected in 11 patients (7%). Chronic *P. aeruginosa* airway infection was identified in eight cases (5%). Sputum culture results were not related to different BMI or lung function slopes (Fig. S1).

DISCUSSION

In this multicenter study, we have shown that (1) early (preschool) referral to a PCD center was not associated

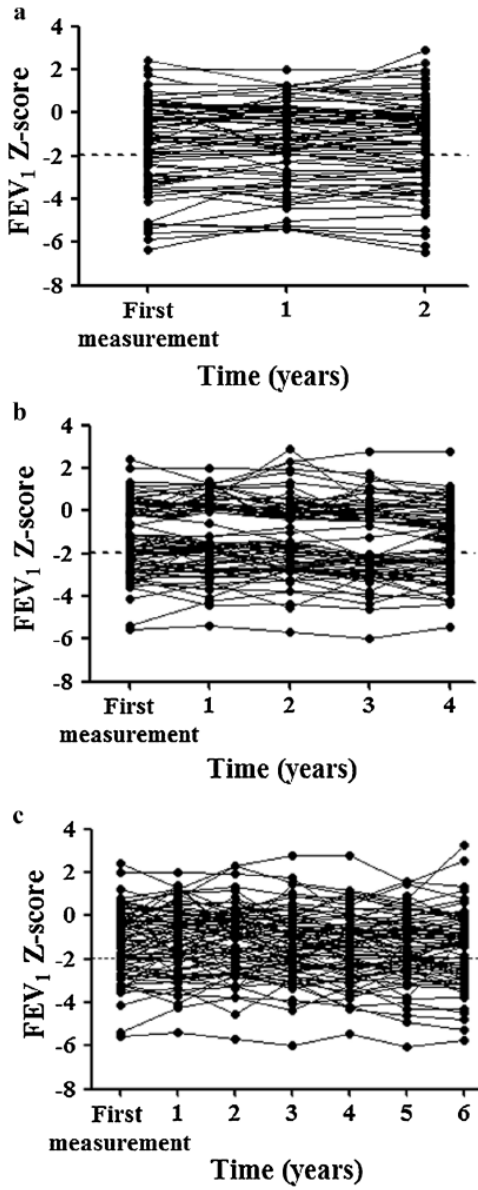


Fig. 2. Evolution of FEV₁ Z scores with time (a: 2 years, n = 158; b: 4 years n = 106; c: 6 years n = 78). Dashed line, Z-score = -1.96.

with better spirometry or BMI; (2) PCD children and adolescents receiving centralized care show steady BMI and stable spirometry during medium term follow-up; and (3) there was a high prevalence of *P. aeruginosa* infection, but unlike in CF, the evolution of lung function or BMI

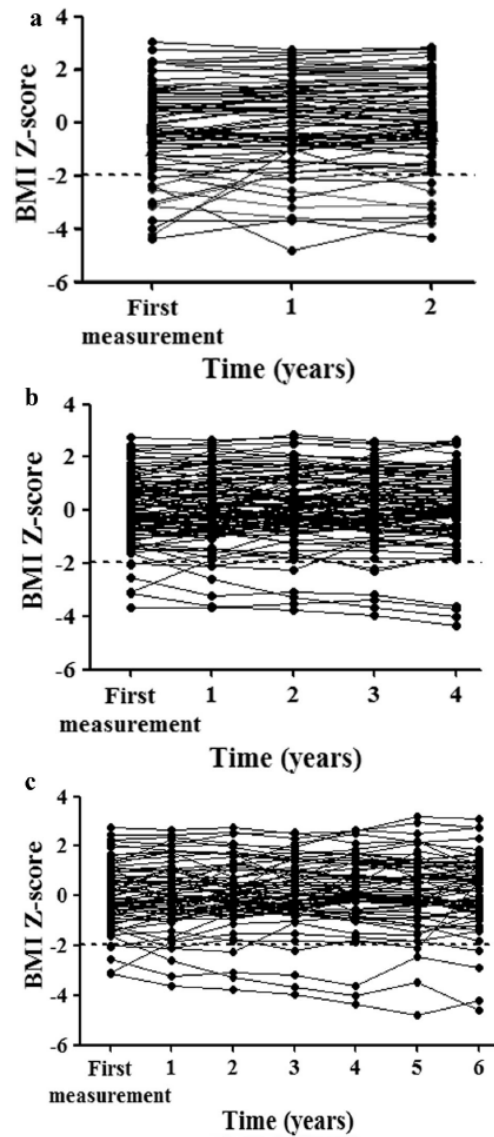


Fig. 3. Evolution of BMI Z scores with time (a: 2 years, n = 158; b: 4 years n = 106; c: 6 years n = 78). Dashed line, Z-score = -1.96.

was not affected by this micro-organism, at least in the medium term.

We have shown that PCD patients do not have significant BMI impairment. Indeed, most of our patients did not have impaired height or weight at first measurement, and so it was unlikely that we could show any improvement with PCD treatment. Nevertheless, seven of

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TABLE 3—Sputum Microbiologic Characteristics of Study Population During Follow-Up Period

	One sample ¹	Repeated samples ²
<i>H. influenzae</i> , n (%)	103 (65)	76 (48)
<i>P. aeruginosa</i> , n (%)	57 (36)	8 (5)
Mucoid <i>P. aeruginosa</i> , n (%)	11 (7)	3 (2)
<i>S. pneumoniae</i> , n (%)	82 (52)	17 (11)
<i>M. catarrhalis</i> , n (%)	58 (37)	5 (3)
<i>S. aureus</i> , n (%)	73 (46)	24 (15)

¹Results of at least one single specimen of sputum.

²Results of several (>3) sputum cultures on a subset of the same subjects.

eleven patients with a low BMI at first measurement achieved a normal BMI 2–4 years after referral, whereas in four patients BMI did not improve during the follow-up; this is close to the number that would be expected to have a BMI ≤ 1.96 Z scores in a study of this size.

The relationship between BMI and lung function impairment has been extensively investigated in other chronic pulmonary disorders. Nutritional status is a predictor of morbidity and mortality in chronic obstructive pulmonary disease (COPD),¹⁰ with low BMI significantly associated with reduced FEV₁ and increased risk of death.¹⁹ COPD increases energy expenditure because of the increased work of breathing, and malnutrition impairs pulmonary defense against pathogens, increasing the risk of infections and reducing the strength and the endurance of the respiratory muscles.¹⁹ Nutritional status at age 2 years in children who had bronchopulmonary dysplasia in infancy influences growth and pulmonary outcomes in childhood,²⁰ and prematurely born infants who have suffered intrauterine growth retardation may have impaired lung function in childhood.²¹ In CF, nutritional deficiency mainly secondary to pancreatic exocrine dysfunction can lead to poor lung growth and increased respiratory infections.¹¹ Chronic infection and poor pulmonary function also causes increased energy utilization and growth failure, thus leading to a dangerous “vicious circle.”^{10,11} Although PCD shares several features with CF, pulmonary disease is milder.²² Moreover, the malabsorption, liver disease and diabetes that play major roles in CF malnutrition,¹¹ are not seen in PCD.¹ Hence despite the burden of chest sepsis, a feature of both CF and PCD, BMI was unaffected in our study of PCD children and adolescents.

The progression of BMI and pulmonary function has been little investigated in pediatric non-CF suppurative lung disease. Two recent studies showed that children with non-CF bronchiectasis have adequate growth, and spirometry stabilizes with treatment.^{12,23} However, only two PCD patients were enrolled in one study,¹² and there

were none in the other,²³ making it difficult to generalize the findings to PCD.

Our finding that spirometry is stable over time diverges from that by Marthin et al.⁷ who found that in a third of patients with PCD, lung function deteriorates over time. This may be due to the more limited follow-up period of our study. The methods of analysis were dissimilar: Marthin et al. categorized patients according to longitudinal changes, namely whether the subject lost more or less than 10% of spirometry over time. However, we cannot exclude the possibility that, also in our study, more prolonged follow up may be associated with worsening PCD lung disease.

An important finding from our study is that approximately one third of patients who started regular follow-up at preschool age had impaired spirometry at first measurement. Although there was a trend for worse FEV₁ and FVC in those diagnosed late, this did not reach statistical significance. Nevertheless, there was evidence of worse peripheral lung function impairment (FEF_{25–75}) in late diagnosed patients; and secondly, it is known that spirometry is less sensitive than HRCT, lung volumes,^{9,24} and multiple breath washout²⁵ to PCD functional and structural lung disease. Unfortunately, these investigations were not available to us in most of the patients.

H. influenzae was the most common pathogen in our population. The prevalence of *P. aeruginosa* infection was almost twice that previously reported,⁶ highlighting the importance of this pathogen in PCD. Of note, sputum culture results did not influence the progression of growth or lung function, unlike in CF, again highlighting the importance of not extrapolating between different chronic suppurative lung diseases. Nonetheless, our data should not be interpreted as suggesting that not treating *P. aeruginosa* is clinically correct. A limitation of the study is the absence of data on any treatment given for a positive isolate, and the results of therapy. Follow up data were not sufficiently complete to be sure of the success or otherwise of any eradication therapy.

Our study has other limitations, including its retrospective nature and low sample size in the analysis beyond 6 years. Moreover, the choice to use only the best annual spirometry, considered as the most reliable estimate of the patient’s lung function in stable conditions during that year, may have biased data towards overall better spirometry, with the potential risk of underestimating lung function decline with time. On the other hand, due to recurrent chest infections, in PCD patients lung function may worsen in course of exacerbations. However, such impairment may be, at least partially, reversible after treatment.¹² Therefore, the inclusion of spirometries performed during exacerbations would have not reflected real lung function evolution. We cannot exclude that more sophisticated measures of lung function and structure may have shown changes in PCD. Furthermore, our population

was heterogeneous in ethnicity and the implications of this in terms of pulmonary outcome are still unexplored. We were not able to explore genotype–phenotype relations because only a small proportion of our patients underwent PCD mutation analysis (data not shown). Therefore, pending further studies on the genetics of PCD,¹³ we cannot exclude that different genotypes could affect the results.

Although we studied patients from three different centers using similar management strategies, and there were no differences in the results (Fig. S2), the inclusion of data from patients followed since the late 1970s, combined with the lack of formal standardization of care between PCD centers before the 2009 ERS recommendations, merits further comment. In contrast to CF, where therapeutic strategies that have progressively changed over the last decades,²⁶ the recommended PCD treatment in 2009 has not substantially evolved since the early 1980s, although likely there have been some changes. Treatment protocols always included antibiotics, bronchodilators, and regular daily airway clearance.²⁷ Indeed, a 2012 survey on management of PCD reported that airway clearance by physiotherapy and exercise, and prompt antibiotic treatment were the strategies used in the majority of the European centers including those in the current study (British Isles; Northern and Southern Europe).²⁸

In summary, this multicenter audit of data in PCD children provides encouraging results in terms of lung function and nutrition since BMI and spirometry appear stable over medium term follow-up in patients treated using standard protocols. Should future research identify significant differences in spirometry and/or BMI within groups or over time, data could be used by other centers to benchmark their results as a quality improvement exercise.²⁹ Future prospective studies should address the effects of pulmonary exacerbations and changes in chest imaging to clarify the natural history of PCD. The BESTCILIA network has been recently developed to identify all existing cross-sectional and longitudinal datasets of PCD patients from clinical databases in Europe and the US, aiming to facilitate this process.³⁰ This sort of international collaboration is needed to answer questions on clinical phenotype, genotype, disease severity, prognosis, and effect of treatments on PCD outcomes.

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SUPPORTING INFORMATION

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3.2 Micronutrients, respiratory infections and chronic lung disease

The periodical recording of anthropometric parameters represents only a partial and quite limited approach to the problem of assessing nutritional status in children and adolescents with chronic respiratory disorders. In the last years, a growing attention has been given to the evaluation of micronutrients levels in a wide group of chronic respiratory (79,80) and non-respiratory conditions (81) and even in healthy children (82), in order to assess their role in affecting a number of health outcomes.

In the field of pediatric respiratory diseases, what has gradually emerged over the last decade is that specific and sometimes multiple micronutrients deficits may significantly contribute to morbidity and mortality of respiratory disorders, as supported, for instance, by the link between acute lower respiratory infections and zinc deficiency (83).

Most studies investigating the relationship between respiratory conditions and micronutrients have been conducted in developing countries, where the higher prevalence of malnutrition and of respiratory infections makes observational reports and nutritional interventions easier to perform.

However, for most micronutrients, a robust body of evidence supporting specific effects on health outcomes is still lacking. Despite often moving from limited amount of studies, several metaanalyses and literature reviews have been published in the last years, and interesting observations for selected vitamins and oligoelements have been provided.

Zinc supplementation, for instance, has been widely investigated in order to assess its effect on the prevention of respiratory infections. Ten years ago Aggarwal and coworkers reviewed the available literature and concluded that zinc supplementation significantly decreases frequency and severity of respiratory diseases in children (84). More recently, this finding was confirmed by the analysis of 18 studies conducted in developing countries which showed a reduction in lower respiratory tract infections and in mortality due to pneumonia in children supplemented with zinc (85). On the other hand, the effect of zinc supplementation in decreasing the duration of respiratory symptoms in children with pneumonia is much more controversial, with evidence in support of the efficacy of

zinc on the clinical course (86) and a trial even suggesting a negative effect biochemical parameters (87).

Zinc supplementation has also been evaluated in association with another micronutrient whose antioxidant and immunostimulatory properties are widely recognized, namely vitamin C. Particularly, combined supplementation of the two nutrients has proven to improve symptoms and shorten duration of airway infections such as common colds and, in developing countries, pneumonia (88). Nevertheless, a recent systematic review on the effect of vitamin C on respiratory tract infections, despite confirming a protective and therapeutic effect on both children and adults with pneumonia, suggested to limit supplementation only to subjects with documented low serum levels of vitamin C (89).

Despite the raising interest towards a number of micronutrients, also including zinc and vitamin C, there is no doubt that the more relevant efforts have been dedicated to study the potential effects on respiratory and non-respiratory health outcomes of another micronutrient, namely vitamin D.

The identification of vitamin D receptors on lymphocytes, monocytes and antigen-presenting cells has made definitely clear that this micronutrient plays a role in something more complex than the calcium, phosphorus and bone metabolism, providing new insights in its multiple functions (90). In particular, the ongoing characterization of the immunomodulatory pathways in which vitamin D is involved, is providing new evidence on its links with a number of respiratory conditions. Recent studies suggest that low serum vitamin D levels are associated to a higher risk of respiratory infections (91,92) and to increased mortality secondary to pneumonia (93). Although reports on the negative effects of hypovitaminosis D on respiratory health outcomes are increasingly available, results on the efficacy of vitamin D supplementation are far more controversial. In 2013, two systematic reviews analyzing the usefulness of vitamin D supplementation in preventing respiratory infections achieved different conclusions, preventing from any definite recommendation (94,95). The only accepted indication for high-dose vitamin D is tuberculosis, as there is evidence of a significantly quicker resolution of respiratory symptoms in affected patients (96).

In this scenario, we focused our attention on the assessment of serum vitamin D levels in children with PCD, in order to assess correlations with clinical outcomes and pulmonary function. Our analysis highlighted that hypovitaminosis D is a frequent finding in PCD and is associated with poorer QoL.

The results of this study were published in the *Italian Journal of Pediatrics*.

RESEARCH

Open Access

Hypovitaminosis D: a novel finding in primary ciliary dyskinesia

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Abstract

Background: A relationship between low levels of serum vitamin D and respiratory infections has been established. No study has examined the frequency and clinical relevance of vitamin D deficiency in patients with primary ciliary dyskinesia (PCD).

Methods: Vitamin D levels were measured in 22 PCD patients (7 females, 10.5 years, range, 2–34 years). In PCD, pulmonary function tests (PFTs), sputum microbiology, self-reported physical activity (PA) level, and quality of life (QoL) by means of the Saint George's Respiratory Questionnaire (SGRQ), were also assessed.

Results: Seventy-two percent of PCD patients were vitamin-D deficient-to-insufficient and 28% were sufficient. No differences in PFTs parameters were found between vitamin D deficiency-to-insufficiency and sufficiency groups. Patients with vitamin D deficiency-to-insufficiency had significantly higher SGRQ total scores, and thus poorer QoL ($p = 0.03$). Seventy-nine percent of PCD subjects had limitations in performing vigorous activities, and 53% performed less than 3 hours of PA per week. Vitamin D deficiency-to-insufficiency and sufficiency groups did not show any differences in age at PCD diagnosis or at onset of respiratory symptoms, BMI, atopy, current asthma or bronchiectasis. However, 79% of patients with bronchiectasis had vitamin D deficiency-to-insufficiency. No differences were found in the rate of positive sputum cultures and in the number of antibiotic courses between the two groups.

Conclusions: Hypovitaminosis D is common in PCD patients, and is associated with poorer QoL. We recommend the assessment and treatment of hypovitaminosis D to be included in the routine management of PCD.

Keywords: Primary ciliary dyskinesia, Vitamin D, Quality of life

Introduction

Primary ciliary dyskinesia (PCD), a genetic disorder of cilia function and ultrastructure with *situs viscerum inversus* occurring in 50% of patients, is characterized by impaired mucociliary clearance and recurrent-to-chronic respiratory infections [1]. Early presenting symptoms include neonatal respiratory distress and upper airway disease. Lower airways are commonly involved in PCD, and recurrent pneumonia, chronic asthma-like symptoms, and bronchiectasis are the hallmarks of the disease [2]. As a consequence, pulmonary function becomes progressively impaired and respiratory failure may eventually occur [3,4].

Although vitamin D plays a major role in bone health, recent evidence suggests that low levels may contribute to several chronic diseases [5,6]. A number of studies indicate that individuals with lower vitamin D are at higher risk of respiratory infections [7-10]. Vitamin D deficiency is common also in adults with non-cystic fibrosis (CF) bronchiectasis, a condition characterized by a vicious circle of airway inflammation and infection [11].

In PCD, failure of mucus clearance system due to defective ciliary function results in reduced airway defense against bacteria [1]. To our knowledge, the links between vitamin D and PCD lung disease have never been investigated. We hypothesize that, likewise other chronic respiratory disorders, patients with PCD may have hypovitaminosis D. Therefore, we measured the total circulating levels of 25-hydroxy cholecalciferol [25(OH)D] in children, adolescents and adults with stable PCD lung

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disease. Moreover, we explored whether 25(OH)D concentrations were associated with pulmonary function parameters, sputum culture, patients' quality of life (QoL), and self-reported physical activity (PA) level.

Methods

Patient population

We conducted a prospective, cross-sectional study of 22 consecutive PCD subjects (median age, 10.5 years; range, 2–34 years; 7 adults; 7 females) attending the Department of Translational Medical Sciences, Federico II University, Naples, Italy, the reference center for PCD in Campania, Southern Italy. Patients lived in Naples metropolitan area (latitude, 40° 49' N; elevation, 17 m) and were evaluated from March through June 2012. Diagnosis of PCD was made at a median age of 5.9 years (range, 0.1–27) and was based on the demonstration of abnormal motility and ultrastructural defects of cilia. Eighty-two percent of patients (18/22) had *situs viscerum inversus*, nobody had heterotaxy. Table 1 summarizes the characteristics of the study population. Thirty-two percent of cases had atopy that was diagnosed on the basis of the results of skin prick tests. Current asthma occurred in 36% of patients, as assessed by standardized questionnaires [12,13]. Sixty-four percent of cases (14/22) had bronchiectasis at chest high resolution computed tomography performed in stable conditions at least in the previous 6 months for assessing disease severity at some time point during follow-up. Inclusion criteria were a confirmed diagnosis of PCD, and PCD lung disease stability was defined as previously

reported [14]. Exclusion criteria were: airway infections or asthma exacerbations during the 4 weeks prior to enrollment; current smoking; long term use of oral steroids at any dose; antibiotic treatment in the last 4 weeks before enrollment; prescription of over-the-counter calcium or vitamin-D supplements prior to, or during the study period. None of the subjects had any neoplastic, metabolic, hepatic, and cardiovascular or other concurrent medical disorders (i.e., renal or malabsorptive diseases). All participants reported neither being current smokers nor having been exposed to smoke in the previous 4 weeks.

On the study day, in the morning, patients underwent serum vitamin D levels measurement, pulmonary function tests (PFTs), deep throat or sputum culture, and completed health-related QoL and self-reported PA questionnaires. The procedures were in accordance with the Helsinki Declaration guidelines on human experimentation. The study was conducted without any support from the pharmaceutical industry, after approval by the local institutional review board. Subjects or their legal guardians gave informed written consent after extensive information about the study procedures.

Vitamin D measurement

A single determination of vitamin D levels, measured as total 25(OH)D, was performed on blood samples obtained between 08:00 a.m. and 09:00 a.m. after overnight fast, using the chemiluminescent method (Liasion, DiaSorin, Saluggia, Italy) [15]. Vitamin D levels were categorized as being sufficient when >30 ng/ml (>75 nmol/L), insufficient between 20 and 30 ng/ml (50 and 75 nmol/L), and deficient when <20 ng/ml (<50 nmol/L) [6].

Pulmonary function testing

Cooperating PCD subjects underwent PFTs (MasterScreen®Body, VIASYS Healthcare GmbH, Wuerzburg, Germany). Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), forced expiratory flow between 25% and 75% of FVC (FEF_{25–75}), functional residual capacity (FRC), and residual volume (RV) were expressed as % of predicted, while FEV₁/FVC ratio was expressed as % [16].

Quality of life and self-reported physical activity assessment

In order to assess health-related QoL, 19/22 patients with PCD (86%) aged ≥6 years completed the St. Georges Respiratory Questionnaire (SGRQ; 17), a disease-specific measure developed for asthma and chronic obstructive pulmonary disease (COPD) that has been validated also in children and adults with PCD [18,19]. SGRQ score ranges from 0 to 100 (100 indicating the maximum impairment). The youngest children were helped by their parents in answering the SGRQ questions. We also administered a

Table 1 Characteristics of patients with primary ciliary dyskinesia (n = 22)

Clinical data	
Age (yr)	10.5 [2–34] ^a
Gender (F/M)	7/15
<i>Situs viscerum inversus</i> , n (%)	18 (82)
BMI, kg/m ²	18.5 [13–37] ^a
Age to diagnosis, yrs	6 (0.1–27) ^a
Age at onset of respiratory symptoms, yrs	0.08 (0–9.5) ^a
Atopy, n (%) ^b	7 [32]
Current asthma, n (%)	8 [36]
Bronchiectasis at high resolution computed tomography, n (%)	14 (64)
Cilia ultrastructural defects, n (%)	
Outer or combined outer and inner dynein arms absence	12 (77)
Isolated inner dynein arm absence	2 [9]
Isolated axonemal disorganization	1 [5]
Axonemal disorganization and inner dynein arm absence	2 [9]

^aMedian and range values.

^bDefined on the basis of results of skin testing to the most common seasonal and perennial local allergens.

previously published questionnaire to the same 19 patients for assessing their self-reported PA [20].

Microbiological evaluation

Based on cultures results, we defined chronic bacterial colonization as persistence of specific bacteria for at least 6 months, with at least 3 positive cultures [4]. For each subject we also recorded the number of antibiotic courses performed in the past 12 months. All investigators were blinded to the other results.

Statistical analysis

Data are presented as median and range. Mann–Whitney *U* test and Fisher's test assessed comparisons among variables. A *p* value <0.05 was considered statistically significant. Data were analyzed with a statistical software package (SPSS-PC, version 13.0; SPSS; Chicago, IL).

Results

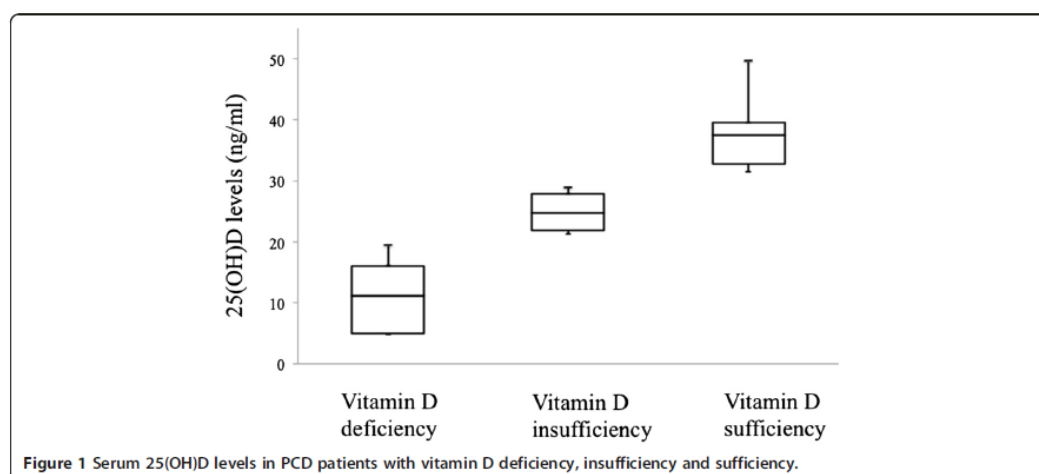
Median serum 25(OH)D levels in PCD were 25 ng/mL (4.8–49). Seventy-two percent of patients had vitamin D deficiency-to-insufficiency, with 4/22 cases (18%) exhibiting 25(OH)D levels <20 ng/ml and 12/22 patients (54%) having 20–30 ng/ml, while 6/22 cases (28%) had >30 ng/ml. Figure 1 illustrates the serum 25(OH)D levels in PCD patients with vitamin D deficiency, insufficiency and sufficiency.

Twelve cooperative PCD subjects underwent PFTs. Median FVC, FEV₁, and FEF_{25–75} were 97% (58–134), 86% (40–111), and 49% (16–100) predicted, respectively, while the FEV₁/FVC ratio was 73% (58–85). Median FRC and RV were 87% (46–173) and 74% (18–275) predicted, respectively. No significant differences in PFTs

were found between vitamin D deficiency-to-insufficiency and vitamin D sufficiency groups (*p* >0.05).

Total SGRQ score was 19 (9–65). PCD patients with vitamin D deficiency-to-insufficiency had significantly higher total scores at SGRQ (20 versus 17, *p* = 0.03) than those with vitamin D sufficiency. In the responses to the questions on PA, 10% of patients reported being moderately-to-highly limited, 26% slightly limited, and 63% not limited at all by respiratory symptoms in everyday-life activities. Fifty-two percent of cases reported moderate-to-severe limitations in performing vigorous activities, while 26% had only slight difficulties, and 21% denied any difficulty at all. Fifty-three percent of patients performed less than 3 hours of physical training every week, while only 5% spent more than 7 hours. Respiratory symptoms limited everyday-life activities at least slightly in 38% and 33% of patients with vitamin D deficiency-to-insufficiency or sufficiency, respectively (*p* = 1). Vigorous activity appeared at least slightly limited in 77% and 83% of the cases with vitamin D deficiency-to-insufficiency or sufficiency, respectively (*p* = 1). Fifty-four percent of subjects with vitamin D deficiency-to-insufficiency and 33% of patients with sufficiency reported performing > 2 hours per week of physical training (*p* = 0.6).

Vitamin D deficiency-to-insufficiency and sufficiency groups did not show any significant differences in BMI (19 versus 17 Kg/m², *p* = 0.7) and in the ages at PCD diagnosis (8.3 versus 2.3 years, *p* = 0.4) or at the onset of respiratory symptoms (13.5 versus 6.8 years, *p* = 0.7). No significant difference was found in vitamin D levels from atopic and non atopic patients (21.9 versus 27.9 ng/ml, *p* = 0.5), from asthmatic and non asthmatic subjects



(24.2 versus 26.3 ng/ml, $p = 0.4$), or from patients with or without bronchiectasis (24.5 versus 30.4 ng/ml, $p = 0.6$). Within the bronchiectasis group, 11 out of 14 cases (79%) had vitamin D deficiency-to-insufficiency and 3 patients (21%) had vitamin D sufficiency, while among the 8 cases with no bronchiectasis 5 (62%) had vitamin D deficiency-to-insufficiency and 3 (38%) had vitamin D sufficiency ($p = 0.6$).

Deep throat or sputum cultures were negative in 13/22 subjects (59%). The most frequently isolated pathogens were *Haemophilus influenzae* (27%), *Staphylococcus aureus* (14%), *Streptococcus pneumoniae* (9%), *Klebsiella spp* (9%), and *Pseudomonas aeruginosa* (4%). No significant difference in the rate of positive cultures was found between the vitamin D deficiency-to-insufficiency and the sufficiency groups (68% versus 50%, $p = 0.6$). Only 2 patients with vitamin D insufficiency met the criteria for chronic colonization by *Haemophilus influenzae*. No patients were chronically colonized by *P. aeruginosa*.

During the 12 months preceding the study, patients had undergone a median of 4 antibiotic courses (range, 0–7), but no difference was found between vitamin D deficient-to-insufficient and sufficient subjects (3.5 versus 4 courses, $p = 0.9$).

Discussion

To our knowledge, vitamin D status has never been investigated in PCD. In this pilot, cross-sectional study, only 28% of PCD patients living at a latitude of 40°52' N had sufficient 25(OH)D serum levels during spring. Our findings show that more than two thirds of PCD children have hypovitaminosis D, which is associated with worse quality of life. However, we could not find any significant relation between PCD-associated vitamin D status and pulmonary function, sputum microbiology, past exacerbations, atopy, or current asthma.

In addition to latitude and season, factors affecting vitamin D status include skin pigmentation, sun-related behavior, obesity, vitamin D dietary intake and outdoor/indoor activities, or also reduced ultraviolet B (UVB) radiations due to atmospheric pollution [5,6,21–23]. Our study was not designed to investigate the reasons for low levels of serum vitamin D. However, in our population BMI excluded obesity, but self reported PA indicated that patients were quite sedentary. A high proportion of cases (79%) had limitations in performing vigorous activities, and approximately 50% spent less than 3 hours per week doing PA, thus suggesting that PCD likely makes patients inactive [20,24].

PCD leads to chronic respiratory symptoms and loss of lung function, with great impact on health and significant restriction of life-style [25,26]. The association of vitamin D levels with quality of life in PCD patients may be explained by noncalcemic effects of vitamin D [6].

Vitamin D has an antimicrobial activity especially against airway pathogens. In bronchial epithelial cells, vitamin D increases the expression of cathelicidins which may prevent bacterial infections [27]. Cord blood levels of 25(OH)D have a strong inverse association with early life airways infections [8]. Vitamin D supplementation significantly reduced the risk of influenza A among Japanese schoolchildren [7] and of winter infections in vitamin D deficient Mongolian children [9]. Of interest is that the need for antibiotics decreases in older adults treated with oral vitamin D [10]. Finally, the resolution of inflammatory responses during tuberculosis treatment is accelerated by the addition of vitamin D [28]. Also chronic lung disorders have been associated to vitamin D status. Increased incidence of asthma and vitamin D deficiency appear related, and the decreased defense against airway pathogens triggering childhood and adulthood wheezing primarily accounts for this [29,30]. Moreover, reduced asthma control, lung function impairment and airway remodeling significantly correlate with hypovitaminosis D [31–33]. Vitamin D deficiency and chronic obstructive pulmonary disease (COPD) appear strongly associated, likely because of subjects' physical impairment and reduced PA, patients' old age which is frequently associated with low vitamin levels, continuous steroid treatment inducing vitamin D catabolism, and malnutrition [34]. Vitamin D deficiency is common in adult non-CF/non-PCD bronchiectasis and correlates with disease severity, although the mechanism is unclear [11]. Likewise non-CF bronchiectasis, the recurrent-to-chronic infectious nature of PCD pulmonary disease might be associated to vitamin D status, and this prompted us to investigate on it. Of interest is that hypovitaminosis D is almost universal in CF, due to a combination of inadequate absorption, impaired metabolism, and lack of sun exposure [35]. Chest sepsis, albeit milder in PCD than CF, is a feature of both disorders [36]. However, vitamin D malabsorption is not seen in PCD, and hence, the pathogenesis of hypovitaminosis D in PCD can be somewhat different.

Markedly reduced nasal nitric oxide (nNO) is typical of PCD [37,38]. Explanations include NO trapping and subsequent breakdown in the airways due to mucus accumulation or paranasal sinuses agenesis [39,40], or also reduced nNO synthesis due to decreased expression, or abnormal activity of nitric oxide synthase (NOS) isoenzymes [40]. Regulation of NOS potentially contributes to the antimicrobial effects of vitamin D, but conflicting data were reported, ranging from a 1,25(OH)2D3-mediated induction of NOS expression in a human macrophage-like cell line to inhibitory actions of 1,25(OH)2D3 on NOS [41,42]. These findings indicate an association between vitamin D and NO production [43], and it is tempting to suppose that the NO-vitamin D relationship plays a role also in the PCD airways host defence.

This study has some limitations. The small sample size from a single centre could affect generalizability and, perhaps, the absence of significant differences among groups. However, the condition is rare, and this, combined with the criteria of stable disease, likely restricted patients' inclusion. We did not compare stable *versus* unstable patients for determining the potential of exacerbations and/or the effect of antibiotics on vitamin D status. The cross-sectional nature of the study did not allow to evaluate vitamin D status longitudinally, particularly after adequate supplementation. We did not assess the patients' daily dietary vitamin D intake, and finally, we did not measure vitamin D-binding protein, a serum protein with immunomodulatory functions that, as well as vitamin D, could be relevant in PCD [44]. Notwithstanding these drawbacks, our study provides the novel valuable information that a high proportion of PCD subjects have vitamin D deficiency-to-insufficiency, with worse QoL than sufficient patients. Determination of vitamin D levels in the early phases of PCD might also clarify the mechanism underlying the association between the two events, including whether the former precedes the latter. In COPD, osteoporosis also due to abnormal vitamin D status increases morbidity [45]. Surprisingly, osteoporosis was never investigated in PCD. Hopefully, following our novel information, a study might be promoted for preventing or treating potential PCD-associated osteoporosis.

In conclusion, our findings show that stable PCD children and adults commonly have hypovitaminosis D, with poorer quality of life than those without. This suggests that assessment of serum vitamin D levels might be included in the management of PCD patients. However, larger studies are warranted to clarify the relationship between hypovitaminosis D and PCD lung disease.

Abbreviations

COPD: Chronic obstructive pulmonary disease; CF: Cystic fibrosis; FVC: Forced vital capacity; FEV₁: Forced expiratory volume in 1 second; FEV₂₅₋₇₅: Forced expiratory flow between 25% and 75% of FVC; FRC: Functional residual capacity; nNO: Nasal nitric oxide; NOS: Nitric oxide synthase; PCD: Primary ciliary dyskinesia; PFTs: Pulmonary function tests; QoL: Quality of life; RV: Residual volume; SGRQ: Saint George's Respiratory Questionnaire; PA: Self-reported physical activity; SPSS-PC: Statistical software package; UVB: Ultraviolet B.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Dr FS is the guarantor for the manuscript, designed the study, and contributed to writing the manuscript, FG. Dr CC contributed to interpreting the data and writing the manuscript, FG. Dr V contributed to interpreting the data, writing the manuscript, and approving the final manuscript, FG. Dr M contributed to supervising the study, analyzing the data, interpreting the data, writing the manuscript, and approving the final manuscript, ES. Dr M contributed to administering the St. Georges Respiratory and self-reported physical activity questionnaires, interpreting the data, writing the manuscript, and approving the final manuscript, FG. Dr M contributed to administering the St. Georges Respiratory and self-reported physical activity questionnaires,

interpreting the data, writing the manuscript, and approving the final manuscript, FG. Dr DM contributed to selecting patients, interpreting the data, writing the manuscript and approving the final manuscript, FG. Drs P and M performed the vitamin D assay and interpreting the data and approving the final manuscript, JY. All authors read and approved the final manuscript.

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Chapter 4

-Conclusive remarks-

Despite less frequent than CF, pediatric non-CF CLD is responsible for a relevant burden for patients and families, with a significant impact on health-related social costs. Due to the primary involvement of the respiratory system, this heterogeneous group of disorders is typically managed by pediatric pulmonologists whose approach is traditionally centered on the assessment and monitoring of lung impairment, in terms of both functional and structural damage. Nevertheless, as in most pediatric chronic conditions, the physician's task is to address the multiple aspects and implications of the disease, taking into account frequently neglected issues, such as, for instance, quality of life and nutritional status, aimed at a global approach in a patient-centered, rather than disease-centered perspective.

In this scenario, our research was first oriented towards the analysis of currently used tools for diagnosis and monitoring of pediatric non-CF CLD in order to highlight their main limits and to evaluate potential new instruments. The most relevant findings from these studies regarded: 1) the possible clinical application of chest MRI in the assessment and monitoring of lung structural impairment in children with non-CF CLD, that would entail a reduction in the ionizing radiation burden associated with periodic CT examinations; 2) the limited applicability of PRO measures created for determined respiratory conditions to other disorders, particularly PCD, and the subsequent need for disease-specific QoL assessment tools; 3) the usefulness of specific algorithms in guiding the daily management of children with chronic respiratory diseases, particularly for establishing adequate follow-up of rare conditions.

We successively focused on the evaluation of nutritional status in a subset of patients with non-CF CLD, namely PCD children and adolescents. Our data highlighted that, as hypothesized, patients receiving a centralized care show stable anthropometric parameters over a medium term follow-up. Nevertheless, when extended to the analysis of serum vitamin D levels, our evaluation showed the

deficiency of this micronutrient to be a frequent finding in PCD and to correlate to poorer QoL, suggesting the need for a systematic assessment of vitamin D status in these patients, in order to consider tailored supplementation.

Taken together, our findings strengthen the importance of a global approach to the pediatric patient with non-CF CLD. Indeed, even though lacking the multisystem involvement typical of CF, non-CF CLD management cannot ignore aspects apparently disconnected from the respiratory disease, adopting a wide and comprehensive approach aimed at improving health, globally intended.

Future research should hopefully confirm and extend our results, standardizing the proposed approach in disease-specific management algorithms, in order to verify their applicability and efficacy in improving health outcomes.

Chapter 5

-Main Materials and Methods-

MR scanning

MRI was performed with a 3.0-T MR scanner (Magnetom Trio, Siemens Erlangen, Germany), a maximum gradient strength of 40 mT/m, a slew rate of 200 mT/m/ms, and 32 radiofrequency channels. We used a dedicated 12-element integrated matrix coil system that covered the whole thorax for signal reception. It consisted of 1 anterior and 1 posterior flexible phased-array coil, each containing a set of 6 receiver elements. The applied sequence was a T2-weighted half-Fourier single-shot turbo spin-echo (HASTE) sequence, performed using an electrocardiograph-gating to reduce cardiac motion artifacts, and respiratory-gating by a navigator signal that monitored the diaphragm position. The field of view was patient-adapted. Sequence parameters were: repetition time/echo time/flip angle, infinite/92 milliseconds/150 degrees; 25 to 30 slices; slice thickness, 5 mm; distance factor, 20%; transversal orientation (matrix, 380 256); acquisition time, approximately 90 seconds. Parallel imaging was used for all measurements using the GRAPPA (Generalized Autocalibrating Partially Parallel Acquisition) algorithm with an acceleration factor of 2 and 24 reference lines. No patient required sedation. Door-to-door time was 5.5 minutes (range, 5–8). All MR studies were of diagnostic quality and were well tolerated.

HRCT scanning

The HRCT scan was performed with a 4-slice CT scanner (Aquilion, Toshiba, Japan) and a bodyweight adapted protocol (adolescents: 120 kV, 140 mAs; children over 45 kg: 120 kV, 65 mAs; children over 35 kg: 120 kV, 45 mAs; children below 35 kg: 120 kV, 30 mAs), with 1x4 mm collimation, 10 mm gap, 0.5 seconds rotation time, automatic exposure control, multiple inspiratory breath holds of 3 seconds each, with the patient in a supine position. Scanning extended from the lung apices to below the costophrenic angles. The field of view of each sequence was patient

adapted. Images were reconstructed using a high-resolution algorithm. The total time for acquisition of the images was approximately 5 minutes, including positioning of the patient. Contrast medium was not administered. For documentation of radiation exposure, the dose length product was recorded, and the effective dose and the weighted CT dose index were calculated. A lung window setting (+1500/-500 Hounsfield unit) was used for image analysis. Images were reviewed on a workstation (iMac MacOS 10.4/OsiriX v.2.7.5 32 bit).

Image Evaluation

Both CT and MR scans were scored using the morphologic scoring system which was originally developed for CF by Helbich et al. (97), later modified by Puderbach et al. (33), and recently used also for the assessment of PCD lung disease (37). Maximum achievable total score was 25, indicating the most severe lung changes. For the purpose of quantifying the severity of PCD or CF lung structure deterioration, we arbitrarily divided the total MR score into three scores subgroups, i.e. 0-9, or mild; 10-18, or moderate; and 19-25, or severe lung damage, respectively. For the analysis of the specific CT and MR parameters included in the scoring system we referred to accepted definitions described in detail elsewhere (33). Furthermore, for the categories “severity of bronchiectasis” and “severity of peribronchial wall thickening”, we recorded the most prevalent degree of severity. It was not possible to assess peribronchial wall thickening in the presence of mucous plugging, and, if mucous plugging was seen within the periphery of a lung segment, bronchiectasis was scored also in that segment. Six lobes were examined, the lingula being scored as a separate lobe. In patients with *situs viscerum inversus*, the right lung was the lung in which the middle lobar bronchus and the corresponding middle lobe were identified at scans.

Spirometry

Forced vital capacity (FVC), FEV₁, and forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅) were measured according to published criteria (98). Spirometry parameters were

expressed as percentage predicted and Z scores (99). FEV₁ was the primary outcome parameter, and a Z-score < 1.96 was considered abnormal (100).

Quality of life

In order to assess HRQoL, patients with PCD aged ≥ 6 years completed the St. Georges Respiratory Questionnaire (SGRQ; 101), a disease-specific measure developed for asthma and COPD that has been validated also in children and adults with PCD (56,102). SGRQ score ranges from 0 to 100 (100 indicating the maximum impairment). The youngest children were helped by their parents in answering the SGRQ questions.

Vitamin D measurement

A single determination of vitamin D levels, measured as total 25(OH)D, was performed on blood samples obtained between 08:00 a.m. and 09:00 a.m. after overnight fast, using the chemiluminescent method (Liasion, DiaSorin, Saluggia, Italy) (103). Vitamin D levels were categorized as being sufficient when >30 ng/ml (>75 nmol/L), insufficient between 20 and 30 ng/ml (50 and 75 nmol/L), and deficient when <20 ng/ml (<50 nmol/L) (90).

Chapter 6

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Chapter 7

-Curriculum vitae-

Main Research Fields:

- 1) Management of pediatric non-CF chronic lung disease, particularly PCD, bronchiectasis and asthma;
- 2) Assessment of lung structure abnormalities in PCD children and young adults by means of conventional and non-conventional imaging techniques, namely chest HRCT and MRI;
- 3) Respiratory manifestations in children with gastrointestinal disorders, particularly gastro-esophageal reflux disease, and esophageal atresia;
- 4) Multidisciplinary management of children with severe neurological disability and nutritional impairment;
- 5) Therapy of pediatric functional gastrointestinal disorders, particularly Irritable Bowel Syndrome.

List of publications in the years 2014-2017

- 1) **Maglione M**, Montella S, Mollica C, Carnovale V, Iacotucci P, De Gregorio F, Tosco A, Raia V, Santamaria F. *Lung structure and function similarities between primary ciliary dyskinesia and mild cystic fibrosis: a pilot study* Ital J Ped 2017; [Epub ahead of print].
- 2) Giannetti E, **Maglione M**, Sciorio E, Coppola V, Miele E, Staiano A. *Do children just grow out of Irritable Bowel Syndrome?* J Pediatr 2017; 183:122-126.e1.
- 3) **Maglione M**, Mollica C, Vallone G, Maurea S, Alberti D, Miele E, Staiano A. *Giant mesenteric haemangioma presenting with severe anaemia.* Arch Dis Child 2017;102:157-158.
- 4) Giannetti E, **Maglione M**, Alessandrella A, Strisciuglio C, De Giovanni D, Campanozzi A, Miele E, Staiano A. *A Mixture of 3 Bifidobacteria Decreases Abdominal Pain and Improves the Quality of Life in Children With Irritable Bowel Syndrome A Multicenter, Randomized, Double-Blind, Placebo-Controlled, Crossover Trial.* J Clin Gastroenterol 2017; 51:e5-e10.

- 5) Mirra V, **Maglione M**, Di Micco LL, Montella S, Santamaria F. *Longitudinal Follow-up of Chronic Pulmonary Manifestations in Esophageal Atresia: A Clinical Algorithm and Review of the Literature*. Pediatrics & Neonatology. 2016;[Epub ahead of print].
- 6) Montella S, Cantone E, **Maglione M**, Iengo M, Santamaria F. *Sodium Hyaluronate improved quality of life and nasal endoscopy features in preschool children with upper respiratory tract infections*. J Biol Regul Homeost Agents 2016;30:303-308.
- 7) Castanhinha S, Sherburn R, Walker S, Gupta A, Bossley CJ, Buckley J, Ullmann N, Grychtol R, Campbell G, **Maglione M**, Koo S, Fleming L, Gregory L, Snelgrove RJ, Bush A, Lloyd CM, Saglani S. *Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33*. J Allergy Clin Immunol 2015;136:312-322.
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- 9) Manna A, Montella S, Maniscalco M, **Maglione M**, Santamaria F. *Clinical application of nasal nitric oxide measurement in pediatric airway diseases*. Pediatr Pulmonol 2015;50:85-99.
- 10) **Maglione M**, Bush A, Nielsen KG, Hogg C, Montella S, Marthin JK, Di Giorgio A, Santamaria F. *Multicenter analysis of body mass index, lung function, and sputum microbiology in primary ciliary dyskinesia*. Pediatr Pulmonol 2014;49:1243-1250.
- 11) **Maglione M**, Montella S, Mirra V, Bruzzese D, Santamaria F. *Long-term assessment of quality of life in primary ciliary dyskinesia: time for new tools?* Chest 2014;146:e232-233.
- 12) Mirra V, **Maglione M**, Di Micco LL, Sperli F, Montella S, Santamaria F. *Micronutrienti ed infezioni respiratorie in età pediatrica*. Pneumologia Pediatrica 2014;55:24-30.

Abstracts and Communications

- 1) Giannetti E, **Maglione M**, Sciorio E, Coppola V, Miele E, Staiano A. *Children are likely to grow out of Irritable Bowel Syndrome*. XXIII National Congress of the Italian Society for Pediatric Gastroenterology and Nutrition (SIGENP), Milan, September 29-October 1, 2016.
- 2) Giannetti E, Sciorio E, Coppola V, **Maglione M**, Miele E, Staiano A. *Do children just grow out of Irritable Bowel Syndrome?* 49th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Athens, May 25-28, 2016.

- 3) **Maglione M**, Giannetti E, Montella S, Miele E, Santamaria F, Staiano A. *Magnesium Alginate plus Simethicone versus Lansoprazole in children with poorly controlled asthma: preliminary results*. 49th ESPGHAN Annual Congress, Athens, May 25-28, 2016.
- 4) **Maglione M**, Mirra V, Montella S, Sperli F, Di Micco LL, Annunziata F, Santamaria F. *Valutazione longitudinale della qualità di vita in pazienti con discinesia ciliare primitiva*. XVIII National Congress of the Society for Pediatric Respiratory Diseases (SIMRI), Florence, October 2-4, 2014.

Grants

- European Respiratory Society Long-term research fellowship at the Royal Brompton Hospital, London, UK, with the following study project: “Primary ciliary dyskinesia: raising standards through the development of a quality improvement tool”.

Invited as a speaker

- 72° National Congress of the Italian Society of Pediatrics (SIP) – Florence, November 16-19, 2016.
- XIX National Congress of the Society for Pediatric Respiratory Diseases (SIMRI) – Turin, October 22-24, 2015.
- Asmaggiorna: dal neonato all’adolescente - Naples, January 17, 2015.

Teaching Activities:

- Massive Online Open Course (MOOC): Pediatric Infectious Diseases – available at www.federica.eu since October 2016. Titles of the online video-lectures:
 - “Polmonite di comunità in età pediatrica”
 - “Gestione dell’asma bronchiale in pediatria”
 - “Gestione della tosse in pediatria”
- Professor of the Postgraduate Course in Pediatric Gastroenterology, Hepatology and Nutrition held at the Department of Pediatrics of the University of Naples “Federico II”, edition 2014;
- Professor of the Course for Specialistic Education in General Medicine – Agenzia Regionale Sanitaria (ARSAN) – Naples, December 9-11, 2015.