Nasal nitric oxide and pulmonary radioaerosol mucociliary clearance as supplementary tools in diagnosis of primary ciliary dyskinesia

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This review has been accepted as a thesis together with 2 previously published papers by University of Copenhagen December 18, 2009 and defended on January 8, 2010.

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Dan Med Bull 2010;57: (8)B4174

This thesis is based on the following two published papers, referred to by their roman numerals in the text:

I. Marthin JK, Mortensen J, Pressler T, Nielsen KG. Pulmonary Radioaerosol Mucociliary Clearance in Diagnosis of Primary Ciliary Dyskinesia. Chest 2007; 132:966–976

II. Marthin JK & Nielsen KG. Age Dependent Choice of Nasal Nitric Oxide Techniques as First Line Test in Diagnosis of Primary Ciliary Dyskinesia. Eur respir J. 2010 Jun 4. PMID: 20525709.

ABBREVIATIONS AND DEFINITIONS at end of textbody.

PART I: INTRODUCTION

Primary ciliary dyskinesia (PCD), first described by Afzelius in 1976 as a syndrome of immotile cilia[1] is a rare disorder characterized by abnormalities in ciliary ultrastructure and/or function and thus more complex than just immotility[2]. Inheritance is predominantly autosomal recessive with approximately one third of PCD patients carrying mutations of the dynein genes DNAH5 and DNAI1, resulting in outer dynein arm (ODA) defects of the ciliary microtubule[3]. Impaired mucociliary transport leads to recurrent and chronic upper and lower respiratory tract infections[2]. Approximately 40-50% of patients with PCD have situs inversus, reflecting dysfunction of embryological nodal cilia[4];[5]. Male infertility is common and reflects defects in sperm tail axonemes[6].

The prevalence of PCD is debated. Estimates range widely from 1:10,000 to 1:22,000[7] and 1:15,000 to 30,000[2] and the age at diagnosis is shown to depend on whether the child has *situs inversus* (mean age at diagnosis 3.6 years) or *situs solitus* (mean age at diagnosis 4.7 years) in a large survey of the PCD ERS task force[8], thus underlining the difficulty in pinpointing PCD patients if no specific or otherwise obvious signs are present.

Diagnosis of PCD is problematic on several levels and even more complicated in very young children: Firstly, symptoms of upper and lower airway infections are very common in young children, and very rarely caused by PCD. This may cause a natural delay in referral from the general practitioner to a secondary pediatric centre and further on to the tertiary centre. Secondly, when referred, final diagnosis is sometimes frustratingly difficult and yet not always exact as described below, which may result in need of repeated definitive diagnostic tests before final diagnosis is settled.

Difficulty in diagnosis of PCD is unfortunate as it may cause delayed or even missed diagnoses. Early diagnosis of PCD is important as initiated treatment leads to stabilization of an otherwise declining lung function[2;5;9-12]. Moreover, chronic secretory otitis media should be addressed differently in a child with PCD where indeed, conservative management is recommended[13;14].

If age at diagnosis were to be minimized, every step on the diagnostic pathway would have to be improved.

Early in this pathway, ideally, a screening test, non-invasive, cheap, safe, easy and quick to perform at any age and available at either the general practitioner or at secondary pediatric centres should exist. This test should separate high-index-of-suspicion patients with indication for further investigation of PCD at a tertiary PCD Centre from the remaining patients with mixed (non-PCD) causes of airway symptoms.

Nasal nitric oxide (nNO) measurement has been proposed as such a primary test to exclude PCD due to its high discrimination between PCD and healthy documented by several studies in patients with PCD exhibiting almost exclusively low nNO values compared to healthy [15-18]. However, certain issues still needs to be addressed before nNO can play a future central role in PCD work up:

Real life experience: prospective studies on consecutive PCD referrals are still sparse and referrals with a mix of respiratory diseases and symptoms mimicking PCD may behave differently than selected healthy controls in a comparative study. Thus, previous findings do not necessarily warrant extrapolation onto a real life setting.

Age span: recommendations for nNO measurements have been provided in 2005 by ATS/ERS[19], stressing the need for soft palate closure during sampling to avoid dilution from lower airways. However, this demands the use of a sampling technique requiring full cooperation of the child, and hence precludes the ability to measure nNO in young children and infants.

Overlap in nNO between PCD and other airway diseases with symptoms mimicking those of PCD: low levels of nNO have been reported in diffuse panbronchiolitis[20;21], sinusitis[22] and cystic fibrosis (CF) [23;24]. Among CF patients, nNO is reduced in children[]23] as well as in adults[24] and conflicting results have been reported to whether or not nNO can separate CF from PCD[17;18;25].

The final step in the diagnostic pathway of PCD work up is a combination of the definitive diagnostics confided to analyses of ciliary function (ciliary beat pattern [CBP] –and beat frequency [CBF]) and qualitative and quantitative description of ciliary ultrastructure. However, these tests may be complicated by inconclusive or false positive results of ciliary function during acute respiratory tract infections[26;27] and by the possibility of normal electron microscopic ultrastructural findings in patients with PCD[27-31], the latter with risk of excluding patients from treatment that actually have PCD. Hence supplemental diagnostic tools, also for second line PCD investigation, are still warranted with a view to obtain fewer false positives and hence both reduce inappropriate use of resources (patient's and socioeconomic) and false negative cases (under-treatment). Measurement of tracheobronchial mucociliary clearance is a potential second line test to indirectly assess the reduced or absent ciliary function, reflected by the patterns of mucociliary clearance, in all ciliated parts of the airways, although so far very diminutively investigated in patients with PCD[32-34].

AIMS OF THE PHD THESIS

The aims of this thesis were to evaluate the discriminative capacity and "real-life" clinical application of two candidates for supplemental diagnostic testing for PCD:

- Nasal nitric oxide (nNO) measurement placed as a first line test to point out probable PCD patients for further investigation, regardless of age.
- Pulmonary radioaerosol mucociliary clearance (PRMC) as a second line test for PCD investigation in children from 5 years of age.

And finally, to propose an algorithm for the pathway of diagnosing PCD based on these two studies and recommendations from the literature.

BACKGROUND: PCD AND CURRENT DIAGNOSTIC METHODS

Since the first descriptions in 1976 of PCD as a syndrome of immotile cilia due to ciliary ultrastructural defect[1;35] different modalities of diagnostic testing have been applied in the PCD work up based on knowledge of ciliary structure and ciliary movements[2;36], nasal mucociliary transport[37;38] and in the recent years nNO measurements[15;16;18;39], gene analysis[6] and immunoflourescence imaging of specific dynein arms[40].

CLINICAL MANIFESTATIONS OF PCD

The prevalence of PCD may be underreported9 and correct diagnosis is frequently delayed[41]; partly because PCD presents with symptoms such as rhinitis, secretory otitis media, cough, recurrent bronchitis which are common in healthy children[2].

Although some clinical symptoms are very characteristic for PCD, the clinical picture may differ between cases[42], probably reflecting heterogeneity of the disease that is still not fully understood. Many organ systems may be involved and PCD can be related to male infertility[43], rarely to hydrocephalus[44], and complex congenital heart disease, which especially presents in combination with laterality defects[45], and all three associations possibly are explained by defect ciliary motility of spermatozoa[43], ependymal cilia[44] and motile nodal embryonic cilia[44], respectively. Other rare manifestations associated with PCD such as polycystic kidney syndrome, asplenia or polysplenia and biliary atresia[44], have been reported as well as few cases of retinitis pigmentosa[46], Bardet-Biedl syndrome[44] and oral-facial-digital syndrome type 1[44;47].

However, symptoms from upper and lower airways are by far the most dominating[2]. Most characteristically, patients with PCD present with a clinical history of a chronic "wet" sounding cough in addition to chronic upper airway symptoms, especially chronic rhinitis and sinusitis and serous otitis media[42]. Neonatal respiratory distress and nasal secretion since the first day of life is very common but probably yet underreported due to recall bias[5;41]. *Situs inversus* appear in approximately 50 % of PCD patients[5], a phenomenon that can be explained by the role of motile nodal cilia during embryonic development, as they have been demonstrated to be responsible for leftward flow of extracellular fluid, and by the fact that dysfunction of monocilia at the embryonic node is associated with randomization of left-right body asymmetry[4]. Bronchiectasis are almost invariably occurring in adult patients but can also be found in a considerable fraction of children with PCD[5]. In a recent case report bronchiectasis were demonstrated in two children out of 3 children with PCD below 3 years of age, verified by high resolution CT scan and 3/3 had decreased lung function by infant pulmonary lung function testing despite the very early age at diagnosis[48]; concerning findings that stress the need for early diagnosis.

NORMAL CILIARY STRUCTURE AND FUNCTION

Cilia are hair-like structures extending from the cell membrane of the epithelial surface of various organs and can be largely grouped into either primary immotile cilia or motile cilia[49].

The motile cilia are lining the apical surface of the airway epithelium of the upper and lower respiratory tract, the ependymal cells of the choroid plexus, the retinal photoreceptor cells, the ovoid ducts and are found as the flagellum of the male spermatozoa[42]. The microtubules of the motile cilium are organised as 9 peripheral microtubule pairs ("doublets") surrounding a central

microtubule pair. This "9+2" arrangement is visible by electron microscopy (EM) in a cross-sectional cut of a motile cilium (Figure 1).

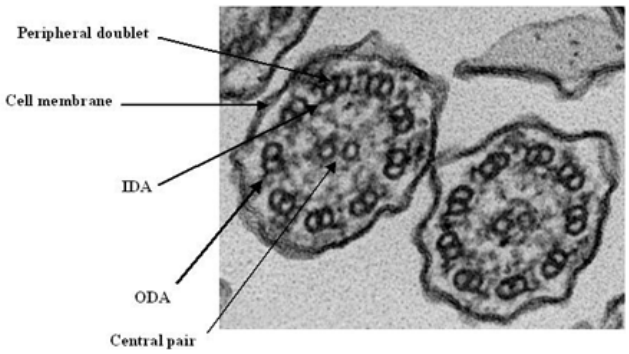

Figure 1. Cross sectional cut of two microtubules with normal 9+2 ultrastructure.
Nine peripheral microtubule doublets with inner and outer dynein arms surround a central microtubule pair. 110,000 x magnification. IDA: Inner Dynein Arm. ODA: Outer Dynein Arm.

The protein nexin links the outer microtubular doublets, and the radial spokes connect the outer microtubular doublets with a central sheath of protein surrounding the central pair of tubules. Tubulin is the main protein of microtubules and consists of a α-subunit and a β-subunit. The peripheral doublets are connected to each other by nexin links. Nine radial spokes connect each of the peripheral doublets to a protein sheath surrounding the central pair[42]. ODA and inner dynein arms (IDA) are motor protein complexes attached to the microtubules. Experimental and functional studies suggest different functions for ODA and IDA in that ODA is believed to generate sliding of the peripheral microtubules mediated by adenosine triphosphatase (ATPase) and regulate CBF, whereas IDA is believed to influence on CBP[36;50].

Cilia have been demonstrated to move in a planar motion with a forward power stroke and a backward recovery stroke[36]. Rapid (5 to 20 beats/sec[42]) coordinated wavelike ciliary movements in healthy cilia allows upward transport of mucus and inhaled particles, thus protecting the airways and considered part of the innate immune system[51;52] CBF has demonstrated to be higher in healthy infants and children compared to adults, underscoring that age-specific reference values are important[53;54]. Regulation of ciliary function is complex and not fully understood but signaling mechanisms including intracellular Ca2+, cyclic adenosine monophosphate, airway nitric oxide (NO), and L-arginine have been suggested to play key roles in the regulation of CBF[55-57].

ULTRASTRUCTURAL AND FUNCTIONAL DEFECTS OF CILIA IN PCD

Ultrastructural defects in PCD can be grouped largely into dynein arm defects, radial spoke defects, microtubular defects and other rarely occurring defects in PCD, such as ciliary disorientation and ciliary aplasia[3]. Dynein arm defects are the predominant ultrastructural defects known in PCD and seen in approximately 90% of patients diagnosed with PCD[42]. Subgroups of CBP studied in 56 children with PCD has demonstrated relation to specific ultrastructural defects; ciliary immotility being highly frequent in cases of isolated ODA (55%) and combined ODA+IDA (80%) and uncommon in cases of radial spoke defect (30%), isolated IDA defect (10%). In the mentioned study, patients with transposition defect, where a peripheral doublet replaces a lacking central pair, had CBF within normal range but abnormal CBP demonstrating a large circular beat pattern, which emphasizes the need for combined CBP –and CBF analysis in the investigation of PCD to avoid missing these patients[36]. A variant of ultrastructural pattern in PCD has furthermore been described as ciliary disorientation that implies normal axonemal structure within each cilium but with disorientation towards the other cilia and is associated with normal or near normal CBF and stiff beating cilia[28;31].

Importantly, normal ultrastructure has been identified in otherwise well characterized PCD patients[3;58].

GENETIC DEFECTS IN PCD

Knowledge on genetics of PCD is continuously evolving. However, the task is not easy. The cilium contains at least 200 proteins with multiple candidate genes located on different chromosomes[20]. To date, two major genes DNAH5 and DNAI1 have been clearly implicated in PCD, together accounting for approximately one third of patients with PCD and associated with lack of ODA, which makes testing for these two genes a new and promising tool in PCD work up[3;6;58;59]. Additionally, a small number of other minor genes have been identified in a few families: RPGR, x-linked, associated with retinitis pigmentosa and complex dynein defect60, TXNDC3, autosomal recessive, associated with situs ambiguous and partial lack of ODA[60], DNAH11, autosomal recessive, associated with normal axoneme and hypermotile ciliary movement[60;61], DNAI2, autosomal recessive, associated with lack of ODA[60;62], OFD1, identified in a single family with a novel syndrome characterized by X-linked recessive mental retardation, macrocephaly, and ciliary motility disorder[47]. Recently, three additional genes have been identified: KTU, identified in 2 families and associated with combined lack of ODA and IDA[63] and RSPH9 and RSPH4A, identified in a few patients without *situs inversus* and lack of central microtubules[64].

DIAGNOSIS

Definitive diagnosis of PCD requires combined assessment of ciliary function and electron microscopic analysis of the ciliary ultrastructure [9;42].

Ciliary function analysis should include both ciliary beat frequency (CBF)–and ciliary beat pattern (CBP) [36], preferentially studied by frame-by-frame analysis using high speed videocamera[65]. Ciliary function analysis is performed on living cells that are analyzed immediately after removal from the nose. Secondary ciliary defects due to viral and bacterial infection are well known and CBP resembling that of PCD are also seen in common colds[26;27]. This frequently leads to inconclusive CBF and CBP analysis with the need for subsequent repeated investigations. In difficult cases repeated CBF and CBP analysis can be performed after weeks of in vitro regeneration of the cilia[29;66]. Uncoordinated ciliary activity due to secondary defects has been reported in up to 20% of non-PCD biopsy specimens[29] and hence the analysis of CBP and CBF is not always a "single shot" event. More so, as patients referred for PCD work up almost invariably suffer from recurrent and prolonged airway infections[67]. Moreover, false positive results of CBF and CBP is a risk if disrupted epithelial strips or single cells are used for ciliary function studies[68].

Ultimately, study of the ciliary ultrastructure by electron microscopy (EM) in a large number of representative cross sections

of cilia should be used to confirm PCD diagnosis[65]. Still, normal ultrastructure can be seen in 10-20% of patients with PCD[60] with case reports dating back to the early 1980´s[69;70]. An additional smaller study in 1983 by Pedersen M reported 8 cases of normal ultrastructure and abnomal hypermotile ciliary beat pattern among 27 studied patients with PCD (29.6%)[71]. These findings have recently been supported by a study from Schwabe et al. that found a hyperkinetic beat pattern in one family of 6 patients with PCD and normal ultrastructure, all carrying DNH11 mutation[61]. Moreover, secondary reversible ultrastructural defects, the most frequent ones being membrane-deficiencies (e.g. "swollen cilia" with excessive amount of cytoplasm and compound cilia), may overlap with findings in PCD patients[72] and potentially obscure the interpretation. Hence, EM has its limitations as well. Overall, an estimate of 10 to 25% of misdiagnoses even by the combination of ciliary function analysis and EM ultrastructure analysis has been presented in a large study of more than 700 biopsy specimens that were evaluated for the presence of PCD and secondary ciliary defects[29].

The consequence of difficult diagnoses is a need for repeated investigations. This is not only cost and time consuming (for patients and staff) but also postponing the final diagnosis and initiation of necessary treatment.

Hence supplemental diagnostic tools also for second line PCD investigation are still warranted with the aim to focus on reaching fewer false positives and reduce inappropriate use of resources.

BACKGROUND: NASAL NO AND PRMC -WHAT ARE THEIR ROLES AS SUPPLEMENTARY TOOLS?

NASAL NITRIC OXIDE

In 1991 Gustafsson and colleagues[73] showed that nitric oxide (NO) is endogenously produced in the airways. In 1993 Alving et al. [74] found the fractional concentration of NO in exhaled air to be higher in asthmatic patients compared to healthy controls and shortly after, in 1994 Lundberg and colleagues[39] were the first to show NO from the sinuses (nasal NO) to be significantly lower in 4 patients with Kartagener syndrome (the triad of *situs inversus*, chronic sinusitis and bronchiectasis) a subgroup of PCD, compared to healthy controls. Since then, NO has been extensively studied for its overall importance as an innate host defense mechanism[75] as well as for its clinical role both as an inflammatory marker in asthma (exhaled NO) [76] and potential use in PCD work up due to the apparent clear cut separation of nNO levels between healthy subjects and patients with PCD[16-18;39]. In 1999 guidelines on the measurement of exhaled NO and nNO were issued by the American Thoracic Society (ATS) [77] followed by an update in 2005[19].

NO SYNTHESIS

NO is produced mainly in the epithelium of the paranasal sinuses by NO synthetase (NOS) using the amino acid L-arginine as the substrate[20]. Three isoforms of NOS (I-III) has been described: type I and type III are constitutively expressed, produce relatively low levels of NO and their activity are dependent on Ca2+ influx. The third isoform NOS II or inducible NOS (iNOS) is Ca2+ independent and its expression induced by cytokines and bacterial lipopolysaccharides[78]. NOS II is capable of generating much larger amounts of NO than NOS I and III[75].

NO has been linked to in vitro up-regulating of ciliary motility[55;56]. In an in vivo study by Loukides et al., nasally inhaled L-arginine was found to increase nNO and CBF and result in faster nasal mucociliary clearance measured by saccharin test[79]. The low NO found in patients with PCD may be related to the primary ciliary defect and several theories –none yet verified- have been proposed and summarized by Maniscalco et al[20]:

Low detected nNO level caused by impaired NO synthesis. This theory could be supported by the inducible increase in NO and improvement of ciliary beat frequency and nasal mucociliary clearance found by Loukides and colleauges[57].

Low nNO level due to close linkage between genes involved in both PCD and NOS II defects and co-inheritance of both traits. This theory however, is questioned by Maniscalco et al. as there are at least 200 proteins in the cilium and multiple candidate genes located on different chromosomes[20].

Low nNO level due to loss of ciliary function. This theory builds on the findings of Narang et al. [17] and their observations of low NO production by myocytes in patients with Duchenne's muscular dystrophy. Myocytes and cilia both have mechano-chemical ATP-ases, and since mutations in the dystrophin gene may lead to uncoupling between the contractile apparatus and NOS, as suggested by Niebroj-Dobosz[80], low detectable nNO could be the result of ciliary loss and reduced NOS activity[20].

NASAL NO SAMPLING

Measurement of nNO requires generation of air flow through the nasal cavity (transnasal air flow) that can be achieved either by aspirating or insufflating air via one nostril during soft palate closure[19]. Soft palate closure is important during nNO measurement to prevent loss of nasal NO to the lower airways or entry of lower respiratory air into the nose. NO measured from upper airways are more than 100-fold higher than NO derived from lower respiratory tract[81], the main contributing part being the paranasal sinuses[78]. Nasal NO sampling by slow oral exhalation against a resistance of at least 10 cm H_2O is recommended as the preferred method by ATS/ERS but any method that reliably demonstrates to close the velum is considered acceptable[19].

Within the same sampling technique, different maneuvers during sampling of nNO have been evaluated: humming [82-84], single breath exhalation[85], oral exhalation against a resistance (OE-R-nNO)[19;86-88], breath hold (BH-NnO) [81;89] and tidal breathing (TB-nNO)[85]. However, the use of different sampling techniques result in different nNO values[82] and hence cut off values should be interpreted in relation to the used technique. Most of the above-described techniques are not applicable in infants and young children. Daya and colleagues introduced a party "blow-out" toy for measurement of nNO in 45 children aged 3.2-17.6 years with successful measurements in all subjects[86]. In our study (Study I) we adapted the technique from Daya et al.[86] as one of three sample methods evaluated.

NASAL NO USED AS DIAGNOSTIC TOOL

Nasal NO, as non invasive, painless and fairly easy performed and with ability to discriminate between PCD and healthy[15-18] has been proposed as a screening tool to separate between PCD and non-PCD in referred patients[90] and hence aid to minimize the load of patients with need for further and more heavy investigation. There are however remaining problems to be addressed before nNO could act fully as gatekeeper for the further definitive diagnostics in all age groups: firstly, overlap in nNO levels occurs

between other nasal[91] and pulmonary disorders[20;21;92] sharing symptoms with PCD. Secondly, lack of normative data in infants and young children remains an unsolved issue. So far, studies including normal reference values in children are sparse and usually with very small groups of healthy children[17;81;86]. Non-PCD infants have been shown to exhibit very low levels of nNO, most probably caused by their undeveloped paranasal sinuses[93-95]. Real life studies are sparse and although discrimination between PCD and healthy is well documented in highly selected well-characterized populations[15-18], the practical use of nNO to exclude/detect PCD in a mixed group of referrals, all with similar symptoms, remains to be illuminated.

MUCOCILIARY CLEARANCE

Mucociliary clearance describes the process of mucus transport towards the oropharynx[96]. Intact mucociliary clearance depend on coordinated ciliary movements and represents an important host defense mechanism which is impaired in patients with PCD[97].

Ciliary function indirectly measured by means of the effectiveness of mucociliary clearance in the work up of PCD has been used through decades. Most widely implemented through times have been the saccharin test, in which a tablet of saccharin is placed in the nose, and the effect of mucociliary clearance estimated as the time until the subject reports the sweet taste of saccharin[37]. However, a high level of cooperation and need to avoid sniffing during the test makes it unfeasible for children and in the light of newer and more specific first and second line tools in PCD investigation, the saccharin test should probably now be considered an obsolete test. Derived from the same principle, the Tc-labeled albumin droplet test is another test for nasal mucociliary transport where the movement in nasopharyngeal direction of a nasally deposited 99mTc-albumin colloid droplet is detected by gamma camera and reflects the nasal ciliary function[38]. Radiation exposure is very minimal and the method is applicable even in infants. However, these methods only assess the regional nasal mucociliary clearance that may be impaired during upper airway infections[26] and are as such only useful as tests to exclude PCD if clearance is within normal range[37;38].

PULMONARY RADIOAEROSOL MUCOCILIARY CLEARANCE

Investigation of pulmonary radioaerosol mucociliary clearance (PRMC) by use of a radioactive tracer has been widely performed during the past 25 years[96;98-100] including in the assessment of effect of anti-asthmatics on pulmonary mucociliary clearance in health[101], obstructive lung disease[102] and effect on lung physiotherapy in patients with cystic fibrosis (CF)[103]. However, when it comes to investigating tracheobronchial mucociliary clearance in patients with PCD, studies have been sparse and with only few patients included. Still, tracheobronchial mucociliary clearance in PCD has showed to be extremely slow, both in comparison to healthy non-smoking subjects[33;104] and to patients with chronic (non-PCD) lung disease[32;34]: Camner et al. (20 PCD patients) found tracheobronchial clearance able to separate between PCD and non-PCD[32], Pavia et al. found a markedly reduced tracheobronchial clearance in one patient with Kartagener syndrome compared to nine healthy non-smoking control subjects[33]. Zwas et al. found lower rate of tracheobronchial clearance in 1 bronchiectatic Kartagener patient compared to 8 bronchiectatic non-PCD patients[34]. Möller et al. (7 PCD patients) found mucociliary clearance to be prolonged up to one week in patients with PCD compared to only one day for healthy non-smoking controls[104].

Different tracers can be used in the measurement of tracheobronchial clearance[96]. The principles of measuring PRMC by use of nebulized 99mTc-albumin colloid as the tracer is described briefly below, as this was the method of Study II.

When measuring PRMC, the patient inhale ultrasonically nebulized 99mTc-albumin colloid by a breathing pattern of slow inspirations followed by immediate and forceful expirations without breath hold in order to achieve as central a deposition as possible[101]. Immediately after inhalation the patient is placed against a gamma camera for detecting the lung radioactivity and measurements performed for the next 2 h as both dynamic acquisitions and static acquisitions[99]. Moreover, a static measurement after 24 h serves as an index of the deposition onto the non-ciliated airways, hence describing the alveolar deposition. Pulmonary mucociliary clearance is measured over both lungs as the whole-lung retention after 1 h (LR1) and 2 h (LR2) corrected for background and physical decay. Regional ventilation distribution is visualized by an 81mKr-gas scintigram. The initial 99mTc aerosol distribution is compared to the 81mKr ventilation distribution to indicate how far the radioaerosol penetrates into the airways and lungs, and hence reflects the length of the path that the 99mTc aerosol has to move before it is cleared from the airways. This penetration index (PI) is used to calculate each subject's predicted values of LR1 and LR2 from previously published reference equations[99]. A lung retention value of the expected +1.67 SD represents the calculated upper limit for lung retention in an individual. The difference between measured lung retention and predicted lung retention is given by the term residual SD (RSD) for LR1 and LR2, respectively. The dynamic acquisitions in the first hour serve to assess the presence of bolus transport and are given as short films (seconds) showing the upward movement of radioaerosol from the main bronchi and trachea, resulting from ciliary movement.

INTERPRETATION OF PRMC:

The conclusion of PRMC measurement is based on the following three parameters:

Whether or not LR1 and LR2 is within the predicted values;

Whether or not there is a normal bolus transport rate in the trachea; and

Whether or not focal retention of radioactive aerosol is seen in the airways after 24 h, indicating regional or general impaired mucociliary clearance.

All of these three parameters need to be in concordance for the test to be conclusive. The PRMC test results can then be interpreted as normal, abnormal, or regional abnormal (Fig. 2):

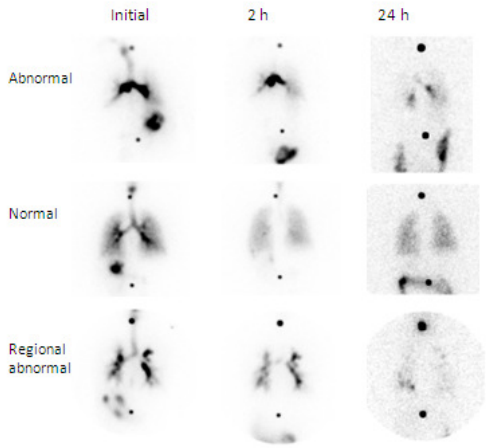

FIG 2: STATIC ACQUISITIONS IN PULMONARY RADIOAEROSOL MUCOCILIARY CLEARANCE INVESTIGATION.
Three examples showing initial aerosol deposition, the remaining aerosol deposition after 2 h and after 24 h in an abnormal, a normal, and a regionally abnormal PRMC test.
Chest 2007;132;966-976.

Cooperation: Age for this method is usually limited to children from 5 years of age as the patient should be able to cooperate to controlled inhalation of the aerosol by instruction and to lie still for the 2 full hours of acquisitions (Figure 3).

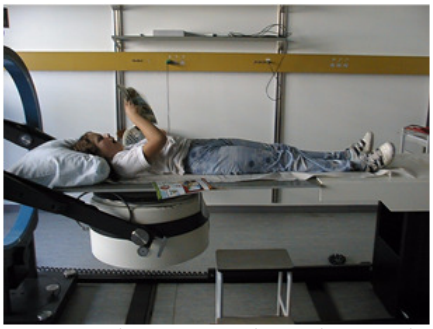

Fig 3: Zero to 2-hour acquisitions during Pulmonary Radio-aerosol Mucociliary Clearance investigation.
Following inhalation of 99m Tc albumin colloid, the child is placed above the gamma camera for 2 hours. Courtesy of Jann Mortensen.

PART II: STUDIES

The submitted manuscript (I) and the published article (II) are included in the appendices. Below are brief introductions to each study.

STUDY I:
Age Dependent Choice of Nasal Nitric Oxide Technique as First Line Test in Diagnosis of Primary Ciliary Dyskinesia. Eur respir J. 2010 Jun 4. PMID: 20525709.

Three nNO sampling methods were examined: sampling during breath hold (BH-nNO), oral exhalation against resistance (OE-R-nNO), and tidal breathing (TB-nNO) were performed in a real life setting for evaluation of the practical use of nNO as a first line test in PCD work up.

Online nNO measurements were performed using NIOX® (Nitric Oxide Monitoring System, Aerocrine, Sweden) equipment. Nasal NO gas was aspirated via a nasal olive probe inserted to one nostril by use of passive sampling flow rate of 5 mL/s (~ 0.3 L/min). Preferably, triple measurements were obtained for each sample method and the mean value used as result. The curves shown below each figure illustrates typical patterns of measured nNO for the specific methods.

When performing BH-nNO, the subject was instructed to inspire to total lung capacity and asked to perform a breath hold for as long as it took until a stable plateau could be read on the screen, usually 10-15 seconds. The BH-nNO curve consists of two phases: a washout-phase in which the nNO-concentration steadily increases and a plateau-phase in which the nNO-concentration has reached a maximum due to the persistent velum closure during breath hold (Figure 4). During OE-R-nNO the subject was instructed to inflate a "blow-out" toy, keep it inflated for as long as possible, and repeat inflations up to three times per sampling. Blowing against the resistance provided by the "blow out" toy caused short periods of soft palate closure due to excess pressure in the oral cavity during inflation, as previously shown by Daya et al. [86]. The intermittent episodes of soft palate closure during one sample period is illustrated on the curve by the three repeated plateaus, each representing an inflation with duration of approximately 5 seconds (Figure 5). When sampling by TB-nNO, soft palate closure was not achieved consistently, and the nNO-concentration was seen to fluctuate continuously during breathing due to dilution from the lower airways, creating online peaks that could be read directly from the screen (Figure 6).

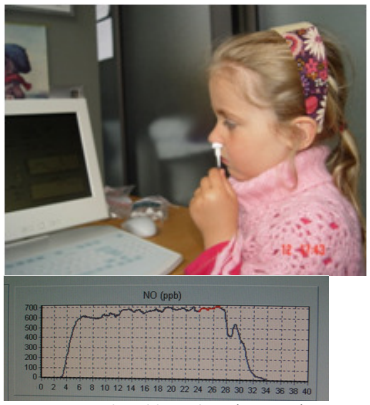

Figure 4: Breath Hold nasal NO (BH-nNO) measurement in a 6-year old girl.
During the breath hold maneuver, soft palate closure is achieved resulting in a steady nNO output, as shown by the plateau concentrations of measured nNO (curve below).

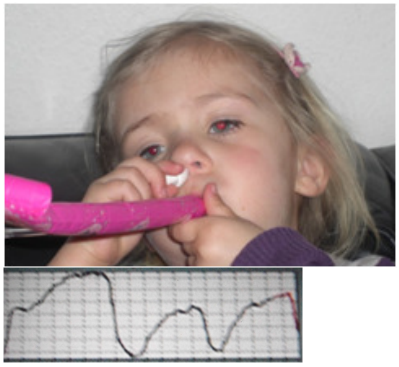

Figure 5: Nasal NO measured during Oral Exhalation against Resistance (OE-R-nNO) in a 3½-year old girl.
Repeated inflations of the "blow-out" toy create intermittent excess pressure in the oral cavity and thereby short episodes of soft palate closure and steady nNO outputs, as illustrated by the 3 repeated plateau concentrations of measured nNO (curve below).

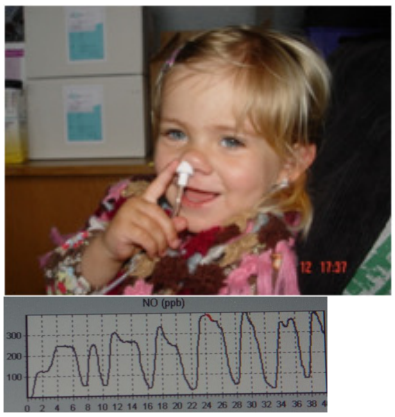

Figure 6: Tidal Breathing nasal NO (TB-nNO) measurement in a 2½-year old girl. During tidal breathing, the measured nNO concentration fluctuates (curve below) due to lack of consistent soft palate closure and resulting dilution from the lower airways.

In the evaluation of nNO measurement as a method to assist in the diagnosis of PCD regardless of age, two of the sample methods (OE-R-nNO and TB-nNO) were selected with the specific purpose to expand the age range down to young children and infants. We found all 3 sample methods to significantly discriminate between PCD and non-PCD. We suggest nNO measurement adjusted to descending age and/or level of cooperation in the following rank order: BH-nNO > OE-R-nNO > TB-nNO. We also reported a unique dataset of high, normal values of nNO in an unexpected large fraction (6.8%) of otherwise well documented PCD patients. This underlines that nNO cannot stand alone neither as a single test nor in exclusion of PCD.

STUDY II:
Pulmonary Radioaerosol Mucociliary Clearance in Diagnosis of Primary Ciliary Dyskinesia, Chest 2007;132;966-976.

In patients with PCD, mucociliary transport is impaired as a result of abnormal ciliary motion. A method based on clearance patterns after the inhalation of a radioaerosol tracer was applied as a whole-lung functional test for pulmonary radioaerosol mucociliary clearance (PRMC). The validity of PRMC for its use as an adjunctive test in the workup of patients with proven and suspected PCD was tested in a three-part study consisting of 1) a preliminary study of patients with proven PCD, 2) a consecutive study of referred patients in which PRMC was tested blinded against ciliary function and final clinical diagnosis and 3) a 1-Year implementation study of PRMC as a routine method in PCD Work up. PRMC demonstrated ability to accurately exclude PCD in selected patients referred for PCD work up and with a favorable high rate of conclusive results after the first test compared to the results of CBF –and CBP analysis. Also, in cases of discrepancy between an abnormal ciliary function test and a normal PRMC, final clinical diagnosis was in favor of the PRMC result, thus suggesting PRMC as less susceptible to secondary nasal ciliary dysfunction. Finally, PRMC demonstrated ability to detect regional clearance defects, enabling further differential diagnoses.

A cooperating child is crucial (Figure 3) and PRMC is only possible to test in patients from approximately 5 years of age.

SUMMARY OF CONCLUSIONS OF THE TWO STUDIES

The two studied methods; nNO and PRMC are two supplementary tools to exclude PCD, applicable at each end of the diagnostic pathway of PCD work up. Nasal NO measurement has its place as a first line test to define probable PCD patients and minimize load of patients referred for further and more time consuming second line definitive testing. Nasal NO measurement was feasible at all ages with the appropriate choice of sample method and demonstrated significant discrimination between PCD and non-PCD, including CF, for all three sampling methods. However, we showed an unexpected large fraction of normal high values of nNO in our PCD patients, which reassures us that nNO should not stand alone as a single test to exclude PCD if clinical history and symptoms are highly suggestive of PCD.

PRMC, on the other hand, excluded PCD with a specificity of 100% in this study, thus leaving no cases of false negatives. Important for this result may well be that coughing was monitored and not allowed for a conclusive result. Since PRMC showed a favorable rate of first time conclusive test compared to ciliary function test, PRMC has its place especially as a supplementary test to ciliary function test and EM, to rule out PCD in difficult cases with the minimum age of approximately 5 years. Moreover, by providing both dynamic and static acquisitions, PRMC measurement allows for a very detailed visual impression of the ciliary function of lower airways, including for assessment of regional clearance defects, useful for differential diagnostic considerations.

PART III: DISCUSSION

STRENGTHS AND LIMITATIONS OF THE TWO STUDIED METHODS

STRENGTHS OF NNO MEASUREMENT

Nasal NO is an easy and non-invasive test[105]. It can be quickly performed in a subject, and easily learned by medical staff personal. In Part I of the two presented studies, we demonstrated for the first time the usefulness of nNO measurement as a first wave test for PCD in a prospective study of a large group of mixed referred patients with PCD-like symptoms by demonstrating high specificity of nNO to exclude diagnosis, and also fair sensitivity to make a positive diagnosis, and by showing that nNO can be feasible in all children if sample method is adjusted to the age of the child. By inclusion of retrospective data we also confirmed earlier retrospective reports by showing significant discrimination between PCD and non-PCD[15;16;39].

LIMITATIONS OF NNO MEASUREMENT

There are some limitations that need to be considered when implementing nNO in the first line of PCD work up: Firstly, we report for the first time, a considerable proportion of normal-range nNO values in patients with clinical PCD confirmed by abnormal ciliary motility and abnormal ultrastructure, thus disqualifying nNO as a unique single test to exclude PCD. Secondly, we found a considerable overlap in the discrimination from CF of all three sample methods, in accordance with earlier studies, and emphasizing obligatory requirement for further diagnostic testing[2]. Thirdly, although nNO could be measured by TB-nNO sampling with no lower age limit, the usefulness of the test in infants and young children is still very limited as normal reference values for this age group is largely lacking.

TB-nNO demonstrated high rate of false positives especially in the younger children, probably partly due to references used that were unfit to this age group, and partly due to the less reliable values from TB-nNO sampling, as CV% was relatively high compared to BH-nNO, and agreement with BH-nNO relatively poor. By TB-nNO-sampling, soft palate closure is not achieved and the peak-values are read directly from the screen as opposed to the better-defined plateaus given when sampling by OE-R-nNO and BH-nNO. Hence, TB-nNO is not the ideal sampling method to separate PCD from non-PCD but it is still a useful alternative when neither BH-nNO nor OE-R-nNO can be performed, although a substantial part of non-PCD patients that will need further definitive diagnostic testing should be expected if relying on TB-nNO as the sole first line parameter. This stresses that a clinical history suggestive of PCD is important before performing nNO-testing and that nNO should be performed in a highly selected population of patients as it does not qualify as a screening test in less well characterized subjects and as yet, especially not in infants and young children.

Adjusting nNO sampling according to age and cooperation

We demonstrated a clear age dependent acceptability to the three methods, with very few percent of children below 6 years of age able to perform BH-nNO, whereas one fourth of children below 6 years complied with OE-R-nNO with observed minimum age of 2.5 years, and nearly all (>95%) with the TB technique. However, although OE-R-nNO and TB-nNO are technically more feasible, they demonstrated low sensitivity with a considerable fraction of non-PCD patients exhibiting nNO-values below cut off. Both these methods were also inferior to BH-nNO in terms of repeatability within occasion. Hence, OE-R-nNO and TB-nNO may be considered as second options to BH-nNO and if age and/or level of cooperation of the child is not compatible with BH-sampling. We suggest OE-R-nNO as the second choice as this method showed better reliability within occasion and closer agreement with BH-nNO and higher sensitivity and specificity than TB-nNO. Only in subjects unable to perform both BH-nNO and OE-R-nNO, TB-nNO should be the method of choice.

STRENGTHS OF PRMC

PRMC exhibited very high specificity and high sensitivity for PCD with ciliary function analysis as the reference method, and also a high rate of first time conclusive test that was superior to that of ciliary function analysis. PRMC demonstrated to be an effective method for excluding PCD in difficult cases and also a helpful tool in scrutinizing the cohort for non-PCD patients. Completely normal PRMC may be able to finally exclude PCD in referred patients with low index of suspicion of PCD but with either repeated inconclusive ciliary function tests and/or EM-tests, or conflicting results between nNO, ciliary function test and EM-test. The visual impression of ciliary function of the lower airways given by PRMC is unique due to the combination of both dynamic and static acquisitions. The high rate of detected bronchiectasis by PRMC that could also be verified by HRCT, suggested ability of PRMC to detect regional clearance defects, useful for differential diagnoses.

LIMITATIONS OF PRMC:

PRMC should be performed in selected patients, as apart from PCD, impaired mucociliary clearance does occur in other pulmonary diseases with overlapping symptoms to that of PCD: during asthmatic attacks[106], acute respiratory tract infections[97], in patients with cystic fibrosis[107;108] and in patients with bronchiectasis[109] Hence, there is a possibility of false positive results if PRMC is performed during or nearly after a lower airway infection, during an asthmatic exacerbation or if the patient is undiagnosed with CF.

Cooperation

Due to need for cooperation, PRMC cannot be performed before approximately 5 years of age.

Inconclusive tests

When a PRMC test was inconclusive for three main reasons:
Cough during the 2 hours of static acquisitions
Initial very peripheral radioaerosol deposition,
Inconsistency among LR1 and LR2 values, tracheobronchial bolus transport, and 24-h retention.

If the patient coughs during the 2 hours of static acquisitions, the measured clearance may be a result of cough clearance instead of clearance due to ciliary function and give a misleading impression of normal clearance, although this is not the case (i.e. false negative). Therefore, we monitored during acquisitions if cough and extensive throat clearing occurred; and if this was the case and clearance appeared normal, the test was interpreted as inconclusive. Initial peripheral deposition may result in an inconclusive test because peripheral deposition results in a high value of both the measured and predicted LR1 and LR2. If the range for the predicted value includes 100% retention (i.e. no clearance) it is not possible to have retention, which lies above the reference range. Thus the test is inconclusive. However, the breathing pattern of slow inspirations followed by forced expirations served to promote a preferential central deposition of the radioaerosol.

If there is inconsistency between the three parameters of interpretation: 1) the calculated LR1 and LR2 values, 2) the result of the dynamic film of bolus transport and 3) the 24 hour picture - the overall interpretation will be obscured and the result left as inconclusive.

Radiation exposure

Radiation is rather low (1 mSv) and compares favourably to the yearly background radiation (3 to 5 mSv in Denmark) or to a chest CT-scan (5-10 mSv).

The used nebulized 99mTc colloid is approved for use for ventilation scintigraphy for all types of patients with the recommendation of pulmonary deposition of 100 MBq, leading to an absorbed dose of 12.2 mSv in the lungs and 0.5 MSv for whole body. However, when clearance is locally impaired[109], as is the case in bronchiectasis, it follows intuitively that the absorbed dose must end up being higher. And equally, in patients with PCD, where the universal clearance is impaired, absorbed dose would expectedly be higher. To our knowledge no quantitative data addressing this problem are available. Still, by PRMC, the deposited dose is approximately 10 MBq and hence only 1/10 for the quantity recommended if used for ventilation scintigraphy, thus leaving room for up to a ten-times increased absorbed dose before the recommended dose is violated.

CLINICAL IMPLICATIONS OF THE STUDIES

We propose an algorithm for the work up pathway in PCD investigation (Figure 7):

History of symptoms and clinical features that are suggestive of PCD should select patients for referral to a tertiary centre with expertise in performing and interpreting the tests, as has been suggested by other authors[65]. Once referred, a clinical evaluation at the tertiary centre should serve to clarify whether the index of suspicion is high or low and furthermore address possible differential diagnoses. Before performing nNO-measurement, CF, immunodeficiency and probably also allergic rhinitis, viral airway infections and sinusitis[20;92] should be excluded to minimize the rate of false positives. When performing nNO measurement, we suggest choice of sample method adjusted according to age and cooperation attempted in the following rank order: BH-nNO > OE-R-nNO > TB-nNO and interpreted consistent with different cut off values. Patients with nNO values below cut off should have ciliary function test as well as EM-test for assessment of ultrastructural defects performed. Due to risk of false positive results of ciliary function analysis during an airway infection[26], ciliary function testing should be timed to a window free of infection. Also, an abnormal test should be confirmed by a repeated test. In difficult cases, i.e. cases of conflicting results of nNO, ciliary function test and EM-test, or cases where the index of suspicion is low but PCD not possible to rule out, PRMC should be engaged (age >5 years) as a supplementary test as it holds very high specificity to exclude PCD. Preferentially, a window free of infection should be aimed for, as cough during PRMC usually results in an inconclusive test.

In cases of nNO values above cut off, clinical reevaluation is a mandatory step to decide whether the patient should be excluded as "non-PCD" patient or whether the index of suspicion is high enough to justify further second line investigation.

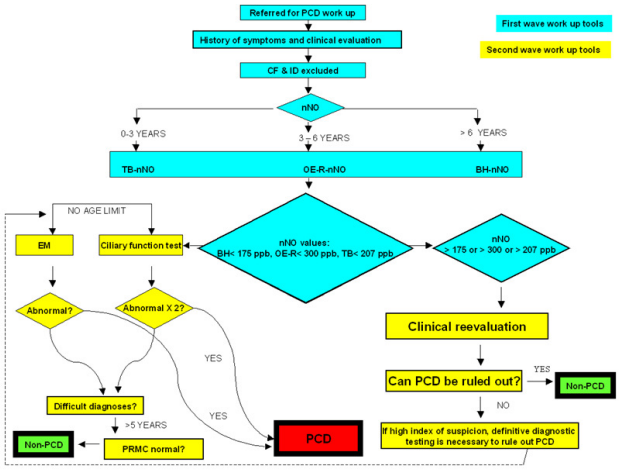

Figure 7: Suggested algorithm for the investigation of PCD

FUTURE PERSPECTIVES

The importance of early diagnosis in PCD is not yet meticulously investigated as very few studies have addressed long-term evolution of lung function in relation to age at diagnosis[10-12]. However, so far it has been demonstrated that bronchiectasis may occur even in infants with PCD[48], that FEV_1 may decline nearly up to 1 % per year if lung function is related to age in a PCD cohort (cross sectional study)[5] but also that lung function can be stabilized after established PCD diagnosis and initiated treatment[10-12]. Consequently, it seems reasonable to speculate that very early diagnosis has impact on the potential of preserving lung function throughout life, and that diagnostic measures for PCD therefore also should be focused on diagnosing young children. Aiming for as early diagnosis as possible should therefore be one of the main goals in PCD research.

We demonstrated ability to measure TB-nNO at any age and with fair repeatability and agreement with BH-nNO as reference method, thus finding nNO a potentially important tool in order to accomplish this goal. However, the usefulness of the test in infants and young children is still very limited as infant PCD nNO levels are largely uninvestigated[93;110;111]. In case reports, infant nNO in patients with PCD has been reported to be very low: 9.4 ppb in a 34 day old infant[111], 85 ppb and 115 ppb in a 4-month old and a 6-month old[93] and 7.2 ppb and 41.6 ppb in two infants reported by Piacentini[110]. In alignment with these studies we found extremely low TB-nNO values in 2 referrals with PCD diagnosed in infancy (9 ppb and 11 ppb). However, non-PCD infants do also exhibit very low levels of nNO, probably caused by their undeveloped paranasal sinuses[93-95] and studies will be needed to investigate whether nNO is able to separate between PCD and non-PCD in this age group at all.

We found a surprisingly high fraction of PCD patients with normal nNO to challenge the many previous reports suggesting that nNO is almost exclusively very low in PCD[15-18;39;112]. Phenotypic characterization and genetic data on PCD patients with normal-range nNO-values is warranted for a possibly better understanding of the disease and perhaps also enlightening for the role of NO in PCD. In our PCD patients with normal nNO, we found normal or near normal CBF in 50% of the cases. However, whether or not these patients could represent a subgroup of "mild disease" needs to be further investigated.

Scrutinizing a PCD-cohort for the exclusion of non-PCD patients in pursue for as "clean" a cohort as possible is definitely important in perspective of future research; a cohort diluted with non-PCD patients may confuse important study results. However, if the concern of including non-PCD patients in a study –with risk of gaining confusing results- leads to designing studies with very strict inclusion criteria for PCD phenotype, allowing only clear-cut cases to be included, it may also be obstructive for future knowledge. Very little is still known about the heterogeneity of PCD and "mild phenotypes" may exist that risk not being investigated. This dilemma should be acknowledged as an ongoing challenge in future studies of PCD.

When it comes to attempts of grouping PCD patients according to phenotype and genotype, conflicting results and confusion seems to arise. For instance, ODA are thought to be responsible for CBF but immotility has been reported in only 55% of isolated ODA defects–and not 100% (the rest was reported to be flickering)[36]. IDA is thought to be responsible of CBP and a normal or near normal CBF would be expected in patients with isolated IDA defect. Still, up to 10% have been reported to have immotile cilia –and not 0%[36]. Recently, evidence have been provided for the existence of PCD with normal ultrastructure[61]; knowledge that questions PCD as a disease of dysfunctional cilia exclusively caused by ultrastructural defects. Also, the low nNO found in PCD patients is yet generally unexplained. Clinical course may also

differ among patients; debut of bronchiectasis have been demonstrated in early childhood[113] as well as in adulthood[114]. Heterogeneity of the disease, poorly defined study populations due to sometimes very difficult diagnosis in patients, and small sample size may be partly the explanation of these examples of diverging findings. Hopefully, the evolving knowledge from studies on PCD-genetics will bring future answers and aid for more definite diagnostic results. Still, at present only approximately one third of PCD patients would be identified if diagnosis solely relied on testing for the two most frequent mutations[3], which underlines that so far, there is a definite need for gaining new knowledge, for evolution of new supplementary tools and for optimizing the existing ones.

Being a rare disease, collecting data in multi-centre trials is necessary for growing knowledge. The National Danish PCD Centre embraces a considerable number of PCD patients; children and adults, in which the patient cohort is characterized by uniform investigation, treatment and follow up with the ability of providing pilot studies potentially useful as stepping stones for further larger studies in joint venture with other centers across Europe within the newly established European Respiratory Society (ERS) Task Force. Especially with regard of effect of treatment, a field in which PCD is still very little investigated, much larger cohorts will be necessary than usually can be provided from a single centre.

CONCLUSION AND IMPLICATION OF THE STUDIES

Nasal NO and PRMC are two highly valid supplementary tools to be placed in each end of the diagnostic pathway when investigating selected patients referred for PCD work up. Nasal NO measurement, as a non-invasive, quick and easy method that can be applied regardless of age, has its obvious place as a first line test in the pathway of PCD investigation. However, nNO cannot stand alone -every patient needs tertiary centre clinical reevaluation with consideration of nNO value no matter the value of nNO. Based on these results, nNO demonstrated its role for a tertiary centre setting for first line separation in already referred –and hence a priori selected patients. PRMC, being a time consuming test applicable from 5 years of age with very high specificity to exclude PCD, has its place in second line investigation as a supplement to ciliary function test and EM-test in cases of difficult diagnoses, and as a helpful tool in narrowing the patient cohort by exclusion of non-PCD patients where definitive diagnostic testing have been inconsistent or repeatedly inconclusive and is moreover a helpful tool in assessing regional clearance defects which is useful for differential diagnoses.

PCD remains to be a diagnosis that should be made at a tertiary PCD centre, as clinical evaluation of referred patients is crucial before excluding the disease. At present, a simple reliable screening tool applicable for unselected patients in the secondary or even primary sector is not available.

SUMMARY

Primary ciliary dyskinesia (PCD) is a rare, usually autosomal recessive inherited disorder, characterized by abnormalities in ciliary structure and/or function. Frequent, intermittent or chronic airway infections precipitated by impaired airway mucociliary clearance may cause permanent lung damage and reduced lung function. Early diagnosis is considered important for the prevention of lung damage, but diagnosis is probably often delayed or even missed since diagnosis of PCD is both complex and time consuming, and yet not always exact.

The aims of this PhD thesis were to evaluate the discriminative capacity and "real-life" clinical application of two candidates for supplemental diagnostic testing for PCD:

Nasal nitric oxide (nNO) measurement placed as a first line test to point out probable PCD patients for further investigation or exclude patients, regardless of age,

Pulmonary radioaerosol mucociliary clearance (PRMC) as a second line test for PCD investigation in children from 5 years of age.

And additionally,

Proposing an algorithm for the pathway of diagnosing PCD based on these two studies and recommendations from the literature.

Nasal NO and PRMC demonstrated to be two highly valid supplementary diagnostic tools to be placed in each end of the diagnostic pathway when investigating selected patients referred for PCD work up. Nasal NO measurement demonstrated to have an obvious place as a first line test in the pathway of PCD investigation and PRMC as second line test as a supplement to ciliary function test and EM-test in cases of difficult diagnoses. Neither of these tests can stand alone in diagnosis or excluding of PCD. PCD remains to be a diagnosis that should be made at a tertiary PCD centre, as clinical evaluation of referred patients is crucial before excluding the disease.

ABBREVIATIONS AND DEFINITIONS

ABBREVIATIONS:

ATS: American Thoracic Society
AUC: Area Under Curve
BH: Breath Hold
Ca^{2+}: Calcium ion
CBF: Ciliary Beat Frequency
CBP: Ciliary Beat Pattern
CF: Cystic Fibrosis
EM: Electron Microscopy
ERS: European Respiratory Society
FEV_1: Forced Expiratory Volume in the first (1) second
H_2O: Water
HRCT: High Resolution Computerized Tomography
IDA: Inner Dynein Arm
Kr: Krypton
LR1: Lung Retention 1 hour after inhalation
LR2: Lung retention 2 hours after inhalation
MBq: Megabecquerel, unit of measure for radioactive dose

MSv: Millisievert, unit of radiation dose equivalent
NNO: nasal Nitric Oxide
NO: Nitric Oxide
NOS: Nitrogen Oxide Synthesis
ODA: Outer Dynein Arm
OE-R: Oral Exhalation against Resistance
PI: Penetration Index
PCD: Primary Ciliary Dyskinesia
ppb: parts per billion
PRMC: Pulmonary Radioaerosol Mucociliary Clearance
RSD: Residual Standard Deviation

SD: Standard Deviations
TB: Tidal Breathing
Tc: Technetium

DEFINITIONS:

Axoneme: An array of microtubules extending longitudinally through the entire cilia. In an axoneme of a normal motile cilium, the characteristic "9 + 2" configuration can be seen by electron microscopy where the microtubules are arranged by 9 peripheral doublets and a central pair (shown in Figure 1).

Compound cilia: Ultrastructural defect considered to be secondary; in a transverse electron microscopic cut the cilium is seen to have more than one –and sometimes many- axonemes within the same cell membrane.
CV%: CV –Coefficient of Variation covers the spread of a set of data as a proportion of its mean. When expressed as a percentage (CV%) it is given by the ratio of the sample standard deviation to the sample mean.

DNAI1, DNAH5, DNAH11, DNAI2 , KTU, OFD-1, RPGR, RSPH4A, RSPH9, TXNDC3: names of human genes in which mutations related to PCD have been identified.

Dynein arms: structures generating the forces of ciliary and flagellar movement

Dyneins: motor proteins responsible of the bending of the cilia.

Negative predictive value (NPV): covers the proportion of patients with a negative test result who are correctly diagnosed.

Microtubules: Protein structures that control movements of the cell.

Positive predictive value (PPV): covers the proportion of patients with a positive test result who are correctly diagnosed.

Reference method: The correct diagnosis cannot always unequivocally be reached, and for that reason, a "gold standard" reference method, can be selected to represent the correct diagnosis.

Repeatability: covers the variation of outcomes of an experiment carried out under the same conditions

ROC curve: Receiver Operating Characteristic curve covers the graph of "sensitivity versus 1-specificity" and can be used for determining the best cut-off value in a data set.

Sensitivity: covers the proportion of patients with a positive test result that are correctly identified by the test.

Situs ambiguous: (also called heterotaxia) covers the intermediate localization of thoracic and abdominal viscera where situs cannot be determined.

Situs inversus: reversal ("mirror image") of the visceral organs.

Situs solitus: normal situs of the visceral organs.

Specificity: covers the proportion of patients with a negative test result that are correctly identified by the test.

"Swollen cilia": Ultrastructural defect considered being secondary; in a transverse electron microscopic cut the cilia are seen to have excessive amount of cytoplasm.

Velum closure: soft palate closure.

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