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# Transmission Electron Microscopy in the Diagnosis of Primary Ciliary Dyskinesia

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## ABSTRACT

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease with extensive genetic heterogeneity. Dyskinetic or completely absent motility of cilia predisposes to recurrent pulmonary and upper respiratory tract infections resulting in bronchiectasis. Also infections of the middle ear are common due to lack of ciliary movement in the Eustachian tube. Men have reduced fertility due to spermatozoa with absent motility or abnormalities in the ductuli efferentes. Female subfertility and tendency to ectopic pregnancy has also been suggested. Headache, a common complaint in PCD patients, has been associated with absence of cilia in the brain ventricles, leading to decreased circulation of the cerebrospinal fluid. Finally, half of the patients with PCD has situs inversus, probably due to the absence of ciliary motility in Hensen's node in the embryo, which is responsible for the unidirectional flow of fluid on the back of the embryo, which determines sidedness. PCD, which is an inborn disease, should be distinguished from secondary ciliary dyskinesia (SCD) which is an acquired disease. Transmission electron microscopy is the most commonly used method for diagnosis of PCD, even though alternative methods, such as determination of ciliary motility and measurement of exhaled nitric oxide (NO) may be considered. The best method to distinguish PCD from SCD is the determination

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of the number of inner and outer dynein arms, which can be carried out reliably on a limited number of ciliary cross-sections. There is also a significant difference in the ciliary orientation (determined by the direction of a line drawn through the central microtubule pair) between PCD and SCD, but there is some overlap in the values, making this parameter less suitable to distinguish PCD from SCD.

## INTRODUCTION

Primary ciliary dyskinesia (PCD) is a term used to describe a group of ultrastructural, genetically heterogenous autosomal recessive disorders affecting ciliary movement [1].

The first description of a cilia-related syndrome appeared in the beginning of the 20th century. Oeri [2] described a patient with sinusitis, bronchiectasis, and situs inversus, and Siewert [3] described a patient with bronchiectasis and situs inversus. Then, Kartagener [4] in more detail described the same triad of abnormalities (sinusitis, bronchiectasis, and situs inversus) in four patients and first recognized a clinical syndrome, which was subsequently named after him. The pathogenesis of Kartagener's syndrome began to come to light in the 1970s. Afzelius *et al*. [5] described the association of sinusitis, bronchiectasis, and situs inversus with an absence of dynein arms in the tails of the immotile spermatozoa in infertile men. Eliasson *et al*. [6] introduced the term "immotile cilia syndrome" to describe all congenital ciliary defects resulting in impaired mucociliary clearance and male infertility. Soon it was determined that lack of motility was not absolute. In some patients, ciliary movement was observed; nevertheless, it was not adequate for normal mucociliary clearance. Therefore, the syndrome name was changed to "dyskinetic cilia syndrome" [7]. Sleigh [8] proposed to use the term "primary ciliary dyskinesia (PCD)" to denote congenital ultrastructural cilia alterations and the term "secondary ciliary dyskinesia (SCD)" to describe acquired ultrastructural cilia defects.

Cilia are eukaryotic cell organelles that play important roles including cell motility, transport of mucus, other fluids, and even other cells, and function in communication with the extracellular environment [9]. There are two types of cilia: motile cilia, which propel a single cell through liquid or move fluid across the surface of a layer of cells, and immotile cilia, which usually serve as sensors [1]. Cilia are found in all animals; however, nematodes and arthropods have only immotile cilia on some sensory nerve cells. Cilia are rare in plants occurring more notably in cycads. Protozoa have only motile cilia and use them for either locomotion or to simply move liquid over their surface. In humans, motile cilia are found lining the upper and lower respiratory tract, sinuses, middle ear, the ependyma of the brain, the ductuli efferentes of males, and the female oviduct, and they are found in the uterus. Furthermore, cilia are morphologically similar to flagella of spermatozoa [10]. Motile cilia are also present in Hensen's node. They perform there an embryological function in the determination of laterality [11-13]. Motile cilia are usually present on a cell surface in large numbers.

In contrast to the motile cilia, immotile cilia usually occur as a single cilium per cell. All types of mammalian cells have a single immotile cilium called "primary cilium" that has been neglected for a long time. Recent studies let scientists re-evaluate its physiologi-

cal role in cell signalling and the control of cell growth and development [9]. Immotile cilia were found in the vertebrate retinal rods and cones as well as in the apical knob on olfactory neurons [14].

Ciliated cells are a part of the mucosal pseudostratified columnar epithelium lining the respiratory tract. Each ciliated cell carries approximately 200 cilia on its surface. The function of these cilia is to beat in a coordinated manner to provide normal clearance of mucus and other debris from the airways. The ciliary beat cycle consists of three sequential events: effective stroke, recovery phase, and recovery stroke. The normal ciliary beat frequency is 11-16 Hz [15, 16].

## ULTRASTRUCTURE OF CILIA

Cilia are highly complex organelles. More than 250 proteins are involved in the formation of cilia. The majority of these are components of a specific axoneme structure. There are many other proteins that are important for ciliary assembly, initiation, orientation, and control of ciliary activity [17]. The core of the motile cilium is the axoneme, which consists of nine peripheral microtubule doublets surrounding a central pair of singlet microtubules. Each of the microtubules is constructed from heterodimers of α- and β-tubulin, assembled into the 13 protofilaments of the A microtubule and the 11 protofilamets of the B microtubule (which, in addition, shares two protofilaments with the A microtubule) The microtubules of the central pair are composed of 13 protofilaments and have approximately the same orientation as the central pair of adjacent cilia, which is important in the production of a coordinated ciliary waveform [1, 9, 10, 18] (Fig.1 ). Each cilium is anchored to a

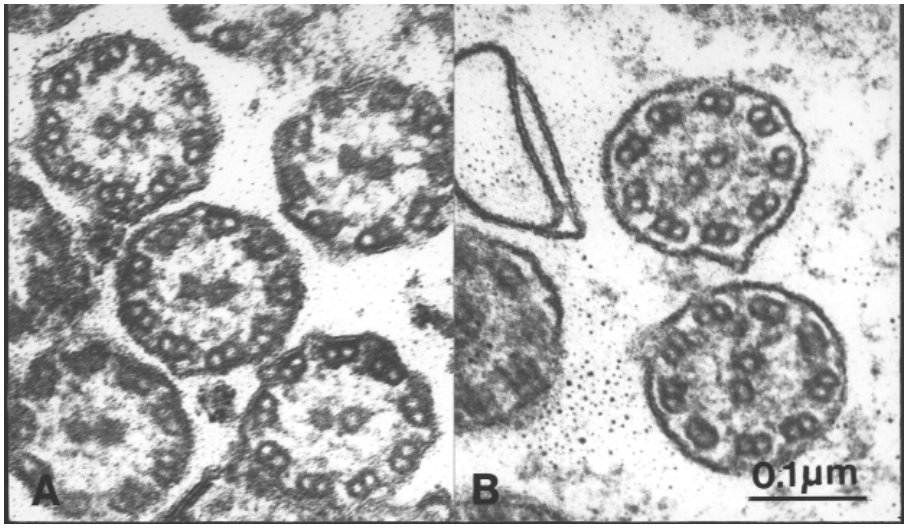


*Figure 1*. Transmission electron micrograph (a) of a patient with SCD (a 19 year-old boy) with dynein arms on the A microtubulus. The orientation of the central pair is similar in all cilia. (b) of cilia from a nasal biopsy of a patient with PCD (a four day-old baby boy with situs inversus), lacking dynein arms on the A microtubulus. Bar = 0.1 µm (same for both micrographs)

basal body in the cytoplasm near the plasma membrane. This structure is composed of nine microtubule triplets. The axoneme is held together by four sets of protein cross-links. A fibrous inner sheath surrounds the central pair of microtubules. Central singlets are connected by a bridge. Each microtubule doublet is connected to the adjacent doublets by nexin links and to the central pair by radial spokes [16].

Other important structural proteins are known as microtubule-associated proteins. The most studied of them is dynein, which forms outer and inner dynein arms (Fig. 1a), structures which are absent or abnormally small in most PCD patients (Fig. 1b). Dynein is a high molecular weight protein that belongs to the group of mechanochemical ATPases [10]. Outer dynein arm complexes consist of heavy, intermediate, and light molecular weight dynein chains in addition to at least 10 other polypeptides [19]. Inner dynein arms have a more complex structure and are composed of heavy, intermediate, and light chains that are evolutionarily distinct from those in the outer dynein arms [20]. Outer and inner dynein arms have different functions in the generation of the ciliary beat. The outer dynein arms have an effect on beat frequency, while the inner dynein arms influence the beat waveform [10]. Ciliary activity has been shown to occur via ATP hydrolysis by dynein heavy chains, which causes a sliding of the A microtubule relative to the B microtubule. This results in bending of the cilia [16].

Other microtubule-associated proteins within the ciliary axoneme include proteins associated with the radial spoke complexes, the central pair apparatus, and the nexin links. Radial spoke protein 3 is responsible for the attachment of the radial spoke complex to the peripheral microtubule doublet. The proteins calmodulin and light molecular weight dynein have also been identified in the radial spoke complex and have a role in regulating $Ca2^{+}$ influx [21].

## GENETICS OF PCD

PCD is an autosomal recessive disorder with extensive genetic heterogeneity; nevertheless, a few rare cases of apparently dominant or X-linked inheritance have been reported [22-25]. All affected genes have yet to be identified. There are at least 250 proteins within a single cilium, each encoded by a separate gene, and many other genes participate in the assembly and regulation of cilia [10, 17]. Therefore, it is clear that the number of possible candidate genes is extremely large.

The first gene in which a mutation was found to be a cause of PCD was DNAI1. This gene was isolated by Pennarun *et al*. [26] from *Chlamydomonas reihardtii*, a unicellular alga with two flagella containing an axonemal structure similar to that of human respiratory cilia and spermatozoa flagella. To date, only three autosomal genes have been shown to cause PCD with defects in outer dynein arms. These genes are DNAI1, DNAH5, and DNAH11. DNAI1 encodes outer dynein arm intermediate chain. DNAH5 and DNAH11 encode outer dynein arm heavy chains [26-30]. These genes are located on human chromosomes loci 9p13, 5p15, and 7p21, respectively [1]. No mutations have yet been reported in patients with other ultra-

structural cilia abnormalities, but the gene mutations mentioned above account only for a few PCD cases. Other genes coding for axoneme dyneins, such as DNAH7, DNAH9, DNAI2, and DNAL1 were recently excluded as major causes of PCD [31-34]. Moreover, the FOXJ1 gene, encoding a transcription factor involved in ciliary development, was also excluded as a common cause of PCD [35].

Several genetic loci for PCD have been identified, and the chromosome region 19q13.3-qter is of particular interest. It is a gene rich region and contains a large cluster of zinc finger genes. This is of potential interest as the only human gene unambiguously associated with situs abnormalities so far is ZIC3, a gene encoding one of the zinc finger transcription factors. The results of linkage analysis in five families of Arabic origin provided conclusive evidence for a PCD locus on chromosome region 19q13.3-qter and confirmed this locus heterogeneity. Genetic heterogeneity is expected in PCD with regard to its wide range of ultrastructural phenotypes [10].

Recently, it was postulated that genes responsible for X-linked retinitis pigmentosa could also be involved in PCD. A non-consanguineous family was described in which a woman with retinitis pigmentosa gave birth to two boys presenting a complex phenotype associating PCD with retinitis pigmentosa. It is already known that photoreceptor and respiratory cilia have common structures. Transmission electron microscopy (TEM) showed numerous abnormal cilia in which dynein arms and central singlets were missing [36].

## CLINICAL ASPECTS OF PCD

As all tissues where ciliated cells are present might be affected, a huge variance in the severity of the clinical phenotype of PCD and the predominant symptoms at different ages is possible [10].

Dyskinetic or completely absent motility of cilia predisposes to recurrent pulmonary and upper respiratory tract infections resulting in bronchiectasis [24]. The first symptoms of PCD in the respiratory tract may present in neonates, such as unexplained tachypnea, pneumonia with no obvious risk factors, and significant rhinitis with intense mucous production. In infants and older children, PCD may present as atypical disease or disease not responsible to treatment, for example, asthma, chronic wet cough with sputum production, already predictable rhinosinusitis, and chronic secretory otitis media. It is important that in young children chronic sinusitis cannot occur by definition because the sinuses do not reach a mature state until 6 years of age. The symptoms in adults are similar to those of older children, but the secretory otitis media is less severe [37].

In PCD, structural and functional abnormalities in respiratory cilia are frequently associated with similar defects in the tails of spermatozoa and the cilia of the epithelium in the ductuli efferentes of the testis. Therefore, the diagnosis of PCD in adult male patients is usually made as a result of the clinical presentation of infertil-

ity. Men have reduced fertility due to sperm with absent motility or abnormalities in the ductuli efferentes. The epithelium in the ductuli efferentes has cilia, which normally assist in the transportation of spermatozoa out from the testes to the vas deferens [38]. There are only very few studies of the role of these cilia in health and disease. Phillips *et al*. [39] described cilia from the ductuli efferentes, which lacked dynein arms, in a patient with Kartagener syndrome who had normal motile spermatozoa. As for spermatozoa motility, men with PCD usually have a normal ejaculate volume and sperm counts, but only 0-30% of the sperm are motile, which is not sufficient for successful fertilization [16].

Female subfertility and tendency to ectopic pregnancy have also been suggested [18]. It is considered that muscular contractions are the primary force for the transport of ova and zygotes, and the oviduct cilia participate in this process at least partially [38]. However, Halbert *et al*. [40] are of the opinion that the primary means by which the ovum is moved through the oviduct is mucociliary transport. A woman with the complete Kartagener's syndrome can be fertile or sterile, but female fertility is somewhat reduced when the cilia are immotile [38]. The first and before yet the only report of an ectopic pregnancy in a woman with PCD was provided by Lin *et al*. [41]. This patient had tried unsuccessfully to become pregnant for 8 years. A mild degree of right peritubal and periovarian adhesions was noted, but this was not likely the cause of her infertility. After adhesiolysis, she was still unable to conceive spontaneously for 2 years, until a right ovarian pregnancy occurred. The only identifiable and likely cause of the ectopic pregnancy in this patient was tubal dysfunction due to PCD [41].

Nearly half of the patients with PCD has situs inversus (the complete reversal of thoracic and abdominal organs) or rarely situs ambiguous (any other abnormalities of laterality). It has recently been proposed that the monocilia in Hensen's node determinate left-right patterning of the body. There is evidence for the role of mechanical fluid flow in left-right asymmetry. The nodal cilia normally create a leftward stream over the embryonic node of a fluid that most probably contains morphogenic factors, which specify the location of thoracic and abdominal contents in accordance with normal situs solitus [11, 12]. Inactivation of the genes associated with left-right patterning of the body should make the asymmetry random. It is also generally claimed that approximately a half of patients has situs abnormalities [6, 42]. Sometimes situs inversus is associated with complex congenital heart diseases and abnormalities of the gastrointestinal tract [37].

Hydrocephalus is a rare but well described feature of PCD. The third and fourth ventricles are connected by the aqueduct of Sylvius. Computed tomography of the brain at different time-points revealed that in some patients with PCD the aqueduct is present at birth but progressively becomes occluded, implying that the ciliated ependyma of the brain ventricles is important for a normal flow of cerebrospinal fluid in certain directions [18]. It has been reported that two-thirds of the patients with PCD suffer from chronic headaches [43]. The most obvious explanation of this symptom is abnormal circulation of cerebrospinal fluid.

Other clinical features of PCD include very severe gastrooesophageal reflux, oesophageal and extrahepatic biliary atresia, intestinal malrotation, splenic alterations (asplenia, splenic hypoplasia, and polysplenia), and renal agenesis [37, 44, 45]. These symptoms are rare and unusual manifestations of PCD. For example, intestinal malrotation accompanying Kartagener's syndrome has been previously reported twice. In these two cases, patients presented bilious vomiting and mechanical ileus [45, 46]. All these rare clinical manifestations of PCD represent the result of a single dysmorphogenic process involving the middle structures of embryo and are connected to situs inversus pathogenesis [47].

## DIAGNOSIS OF PCD

A throrough medical history and carefully performed physical examination are the keystones in the successful diagnoss of PCD. Clinical features of PCD are usually present in the neonatal period. PCD should be considered in pediatric or adult patients with chronic and intractable sinopulmonary infections, as well as in infertile men. Since PCD often imitates other diseases, the investigation of individuals in whom this disorder is suspected can be divided into three stages. At first, all differential diagnoses, including cystic fibrosis and immunodeficiencies, must be excluded. Therefore, early investigations include a chest X-ray, sweat test, immunoglobulins and subclasses, cystic fibrosis genotype, and age-appropriate lung function tests. After preliminary tests have been completed, ciliary function and structure must be examined. Finally, all discovered pathologies must be evaluated [10, 15].

There are a number of ways to assess ciliary function. To screen older children and adults the saccharin test can be performed. A 1-2 mm particle of saccharin is placed on the inferior nasal turbinate 1 cm from the anterior end. The patient must sit quietly with the head bent forward, and must not speak, sneeze, cough, eat or drink for the duration of the test. The time to tasting saccharin is noted and is a measure of nasal mucociliary clearance. Normally it should be less then 60 minutes. The ability of the patient to taste saccharin must also be confirmed [15, 48].

Analysis of ciliary beat frequency and quality of ciliary movement also provides possibility to estimate ciliary function. Ciliary beat frequency can be measured with phase-contrast microscopy. The approximate normal ciliary beat frequency is 11-16 Hz. Ciliary beat patterns are usually estimated by an experienced observer; however, beat pattern analysis with digital high-speed video imaging system is also possible. If the ciliary beat frequency and beat pattern are normal, then no further investigations are required [15, 49, 50].

TEM is an important part of diagnosing PCD. It can be securely omitted only if the ciliary beat frequency is normal with a normal beat pattern [15]. Some investigators prefer to take ciliated cells for ultrastructural examination by brush biopsy whereas others strongly recommend taking a biopsy of intact ciliary epithelium attached to the basement membrane. According to Holzmann *et al*. [50], the most

trustworthy results are obtained from bronchial biopsies. However, bronchial biopsy is, in comparison with brush biopsy, more traumatic to the patient. Alternatively, biopsies can be taken from the middle turbinate or from the bronchi if bronchoscopy is being performed for some other indications [10]. During ultrastructural examination, at least five good cross-sections from each of 10 different non-adjacent cells should be studied, but more should be examined if differences between cilia are noted [15]. Short or missing dynein arms have been found to be a significant diagnostic criterion of PCD. In the majority of patients with PCD, there is absence of both outer (mean number per cilium <1.6) and inner (mean number per cilium <0.6) dynein arms. In controls, the mean number per cilium is 7.5-9.0 and 3.0-5.0 for outer and inner dynein arms, respectively. For secondary ciliary dyskinesia (SCD), 8.5 outer dynein arms per cilium and 3.0 inner dynein arms per cilium are present. The low number of inner dynein arms might be explained by their low contrast in electron microscopy [42, 51].

Recently, some new methods for the diagnosis of PCD have been proposed. Nitric oxide (NO) is produced from the respiratory tract (it is mainly synthesized in the paranasal sinuses) [52]. Patients with PCD have a very low nasal NO. Nasal NO levels are also slightly decreased in patients with cystic fibrosis. On the other hand, patients with asthma and bronchiectasis have increased exhaled NO. These observations engender the idea of a new screening test to diagnose PCD. However, it is too early to propose that solely measurement of exhaled NO can be used in the diagnosis of PCD [10]. Moreover, there is no standardized measurement method for exhaled nasal NO. It has been established that the mean NO level for the total population was 837 ppb. The mean NO level in children is lower than in adults (751 vs. 897 ppb, respectively). Recently, an attempt to determine the repeatability of exhaled NO measurements and to create a standardized nasal NO measurement technique for the diagnosis of asthma was made. It was concluded that exhaled NO measurements might provide a useful clinical tool to assess and monitor upper airways, but further investigations are needed [53].

Another suggestion for the diagnostics of PCD is not less exciting and concerns the prenatal diagnosis of PCD. It has been reported that slightly enlarged brain ventricles might be used as a prenatal sonographic marker of Kartagener's syndrome, recommended when the foetus has either situs inversus or a sibling with the disease [54]. It is most likely that mild foetal ventriculomegaly might persist in foetus in accordance with hydrocephalus, a very rare manifestation of PCD.

## DIAGNOSTIC PROBLEMS OF PCD

From radiographic studies, it has been estimated that the prevalence of PCD in the general population is approximately 1:20,000. The number of actually identified cases is an order of magnitude lower [10].

The clinical PCD phenotype is broad and often mimics other chronic airways dis-

eases, such as cystic fibrosis, asthma or immunologic disorders. PCD is the differential diagnosis in the case of neonatal respiratory distress syndrome of unknown cause, neonatal pneumonia with no history of maternal illness or prolonged rupture of the membranes, significant and prolonged nasal discharge, bronchiectasis of unknown cause, severe or atypical asthma, nasal polyps, hydrocephalus, and infertility [15].

Ultrastructural alterations in cilia may be both primary and secondary. It is vital to differentiate them to choose the most appropriate treatment. Absent or reduced dynein arms, absent radial spokes, translocation of microtubule doublets, and absent central singlet pair are considered primary ciliary defects and the cause of PCD. Other abnormalities, such as compound cilia, addition or deletion of peripheral microtubules, disorganized axonemes, ciliary disorientation, discontinuity of axoneme membrane, and some other are non-specific secondary structural abnormalities [55]. Secondary defects usually occur due to respiratory infections, mechanical injury of the mucosa, or locally applied drugs. These alterations are reversible [56]. TEM usually provides significant data on the ciliary structure and distinguishes primary ciliary defects from non-specific abnormalities called SCD. It has been reported that short dynein arms are found to be a significant ultrastructural finding of PCD [57]. In some cases, it is not possible to distinguish primary and SCD without in vitro cell culturing [58].

## CILIARY ORIENTATION AS A POSSIBLE DIAGNOSTIC CRITERION OF PCD

PCD patients usually have a disorder of ciliary orientation (COR) [10]. Sometimes patients with typical PCD symptoms have a normal ciliary ultrastructure and normal or near normal ciliary beat frequency. A group of these patients were found to have a disorientation of cilia, in which the individual cilia have a normal ultrastructure but their orientation with respect to each other was abnormal [37, 59]. Ciliary disorientation has been reported in many cases of SCD and in a very few cases as the single abnormality in PCD [60]. COR is an important parameter of mucociliary clearance. Only when cilia beat in the same direction a metachronal waveform is possible. This wave sweeps the mucus layer from the airways towards the oropharynx, where it is swallowed [10].

COR has to be measured by TEM from cross-sections of ciliary shafts. Lines are drawn through the central pairs of a number of cilia arising from a single cell. These lines should normally all be more or less parallel with each other [15]. Internationally accepted normal values for COR are ≤20°. COR values of 20-35° indicate increased disorientation. COR values >35° represent a random orientation [60]. Therefore, the standard deviation (SD) of these angles should be small. Disorientation results in a larger standard deviation [15].

It is conceivable that COR values might be affected by the way in which respiratory mucosa is taken for ultrastructural examination. There are two tissue-obtaining

techniques mainly used: nasal biopsy and bronchial biopsy. Significant differences in the SD of the ciliary angles in the specimens taken from brush biopsies and bronchial biopsies were not found [61, 62]. Other factors influencing ciliary beat measurements have been reported. Curette biopsies were compared to forceps biopsies and ciliary beat frequency values did not differ for these two biopsy techniques [63].

In a retrospective study on biopsies taken from 15 patients with PCD and 15 patients with SCD at Uppsala University Hospital (Shebani et al., unpublished results) the ciliary orientation (COR) was measured by TEM from cross-sections of ciliary shafts. Micrographs of cilia were taken at magnification of $^{x}$30,000. A TEM image of each patient was transferred into the digital form and processed with the image analysis software Leica IM 4.0 (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK).

Only cilia in which two central microtubules could be seen, were used for determination of COR. For each cilium, electronically two lines were drawn. The first line was drawn through the pair of singlet microtubules. The second one was drawn along the vertical axis of the micrograph. The angle of its intersection with the first line had to be acute. The angle is defined by sides that are directed upwards. Measuring COR angles in this manner, only acute angles between -90˚ (to the left of the vertical axis) and +90˚ (to the right of the vertical axis) can be obtained. In some cases, the angle of COR was equal with 90˚. In these cases, the sign (- or +) of this angle depended on other COR angles on the particular micrograph. If the majority of angles were negative, this angle was regarded as a right negative angle. In the opposite case, the angle of 90˚ was regarded as a right positive angle.

In each case, the SD of COR angles was calculated. This represented an index of COR for particular patient. For each group, a mean±SD value was obtained from all the SD values in this group, which represented an index of COR for particular group (PCD or SCD).

The mean value of COR in PCD group was 43.61±12.85˚ (range: 17.55 to 70.89˚). The mean value of COR in SCD patients was 21.79±11.34˚ (range: 10.93 to 43.56˚). This means that PCD patients had significantly more manifested disorientation of cilia compared to SCD patients ($p<0.0001$). Nevertheless, there was a noticeable overlap between PCD patients and SCD patients, which means that this parameter is not suitable as a diagnostic criterion. The PCD and SCD patients were also divided into pediatric patients and adult patients, according to their age (pediatric patients < 18 years of age). Differences in the mean values of COR between pediatric and adult patients with corresponding diagnoses as well as between males and females with the same diagnosis were not statistically significant.

The number of dynein arms is a better criterion to distinguish between PCD and SCD. Shebani et al. (unpublished results) found that the average number of inner respectively outer dynein arms per cilia for the PCD patients was 1.4 respectively 1.5, for the SCD patients 5.9 respectively 8.1, and healthy controls had 5.2 respectively 7.9 inner respectively outer dynein arms. PCD patients had significantly ($p<$

0.0001) fewer inner and outer dynein arm-like structures compared to SCD patients and healthy controls. There was no significant difference between SCD patients and healthy controls. Figure 2 shows the means of the number of outer dynein arms per cilia after consecutive measurements on 1 to 30 cilia, for a PCD patient and an SCD patient. As shown in the graph the measurement on 10-15 cilia is sufficient to make a good estimate of the number of outer dynein arms and a diagnosis.

Diagnosis of PCD is far from being a matter of mere academic interest. Delayed PCD diagnosis leads to inadequate therapy and usually results in bronchiectasis with a marked reduction in quality of the patient's life [49]. Improved diagnostic techniques therefore are of significant benefit to the patient.

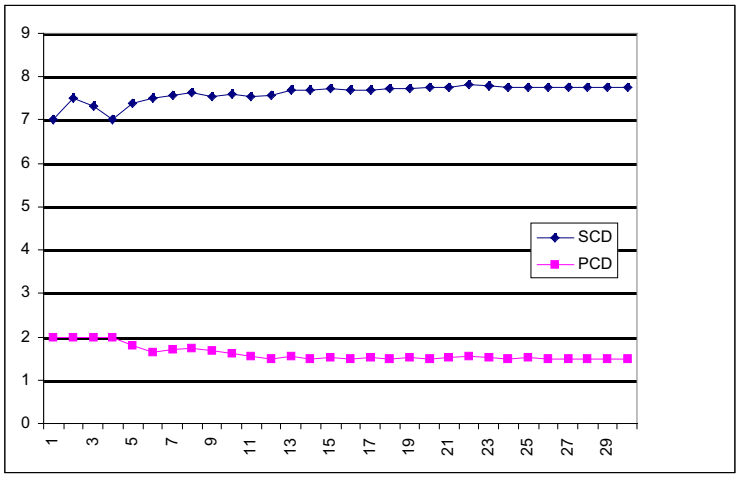


*Figure 2*. Average number of outer dynein arms in the patient with PCD shown in Fig. 1b, and the patient with SCD shown in Fig. 1a, respectively, as a function of the number of cilia (consecutive measurements) on which this parameter was determined. The graph shows that a good estimate is obtained by the measurement of about 10-15 cilia.

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