

Primary ciliary dyskinesia syndrome associated with abnormal ciliary orientation in infants

E. Biggart*, K. Pritchard[#], R. Wilson[#], A. Bush*

Primary ciliary dyskinesia syndrome associated with abnormal ciliary orientation in infants. E. Biggart, K. Pritchard, R. Wilson, A. Bush. ©ERS Journals Ltd 2001.

ABSTRACT: Primary ciliary dyskinesia (PCD) syndrome associated with abnormal ciliary orientation but with normal ciliary ultrastructure has been described in adults, but there are no normal ranges for orientation in infants, despite the fact that half of all patients with PCD present in the new-born period.

Nasal brush biopsies were obtained from eight infants (three males), mean age 13.1 months, range 7–23, in order to determine ciliary orientation. They had no upper or lower airway disease and normal organ arrangement and were undergoing general anaesthesia for other reasons. Two infants with typical PCD syndrome but normal ultrastructure of individual cilia also had orientation studies.

In the eight normal subjects, a mean of 254 central pairs was examined, range 82–453. The mean ciliary orientation was 14.9 degrees, range 12.9–17.5. The two infants with PCD syndrome but normal ultrastructure of individual cilia had ciliary orientation of (Case 1) 44.5 degrees (range 10.6–64.5) in 218 central pairs; and on a second occasion, 28.9 degrees, (range 9.0–47.5) in 259 central pairs; for Case 2, 24.4 degrees, (range 13.1–38.4) in 196 central pairs.

The normal range for ciliary orientation is similar in infants to that described in other work in adults. The two cases of phenotypic primary ciliary dyskinesia in the presence of normal ciliary ultrastructure but abnormal ciliary orientation in infants supports the contention that measurement of ciliary orientation should be part of the assessment of ciliary structure and function in cases of possible primary ciliary dyskinesia, in particular when the ultrastructure of individual cilia appear to be normal.

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*Paediatric Respiratory Medicine, Imperial College of Science, Technology and Medicine at National Heart and Lung Institute, Royal Brompton Hospital, and [#]Host Defence Unit, Imperial College of Science, Technology and Medicine at National Heart and Lung Institute, London.

Correspondence: A. Bush, Dept of Paediatric Respiratory Medicine, Imperial College of Science, Technology and Medicine, National Heart and Lung Institute Thoracic Medicine, Royal Brompton Hospital, Sydney Street, London, UK, SW3 6NP. Fax: 44 207 3518763

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The syndrome of primary ciliary dyskinesia (PCD, also known as Kartagener's syndrome or immotile cilia syndrome) is a spectrum of conditions caused by a failure of mucociliary clearance related to congenital, structural or functional ciliary abnormalities. Clinically it is characterized by onset in the neonatal period of respiratory distress and rhinitis [1], and if untreated, subsequent chronic rhinosinusitis, recurrent respiratory infections and bronchiectasis, and chronic secretory otitis media [2, 3]. Half the patients have mirror image organ arrangement [2, 3]. Other features described more rarely include oesophageal atresia and severe gastro-oesophageal reflux [4]; complex congenital heart disease often with disorders of laterality [4]; biliary atresia [5]; and hydrocephalus [6, 7]. The diagnosis is important, to ensure that aggressive airway clearance and antibiotics are used to prevent the development of bronchiectasis, and that inappropriate ear, nose and throat procedures are avoided [3, 8].

The diagnosis is established firstly, by functional studies, usually a direct measurement of ciliary beat frequency on nasal epithelial cells; and secondly, from ciliary ultrastructure determined by electron microscopy [3]. Underlying structural defects include absent

inner and/or outer dynein arms [9, 10], tubular defects [11], and radial spoke defects [12]. Rarely patients with a typical PCD clinical phenotype have normal or near normal ciliary beat frequency (CBF) and normal ciliary ultrastructure, so the cause of their condition is unexplained. Recently a group of these patients were found to have a disorder of ciliary orientation [13–15], in which the individual cilia have a normal ultrastructure but their orientation with respect to each other was abnormal, leading to poor co-ordination between the beating cilia and inefficient mucociliary clearance. Although a primary abnormality of ciliary orientation was proposed, since secondary orientation defects may occur due to chronic infection [16], the existence of primary ciliary disorientation has been questioned. Furthermore, the youngest patient in the original reports was aged 10 yrs [15]; there are no normal data for younger children, making it impossible to assess the significance of ciliary orientation in the new-born period, at the very time when more than half of patients with PCD first become symptomatic [2].

There are marked developmental changes in the upper airway in children. The sinuses do not reach a mature state until 6 yrs of age, thus in young children

by definition chronic sinusitis cannot occur. There is no reason to suppose that ciliary orientation in health or disease is necessarily the same in infants as in adults. Indeed, CBF is known to vary with age [17]. The aim of this study was: firstly, to describe the normal range of ciliary orientation in children <2 yrs of age; and secondly to describe two infants with typical PCD associated with normal ciliary ultrastructure but abnormal orientation.

Methods

Sampling of normal cilia

The study was approved by the Royal Brompton Hospital Ethics Committee. Parents of infants <2 yrs of age were approached. The infants were due to undergo general anaesthesia for cardiac catheterization. Infants with any history of significant chronic or recent acute respiratory disease were excluded. Consent was sought for nasal brush biopsy and a nasal swab for bacterial culture to be performed after induction of anaesthesia and prior to the start of the therapeutic procedure. The brushing was taken by inserting a 3 mm cytology brush into one nostril and drawing it several times across the inferior turbinate. The brush was then agitated vigorously into a fixative solution of 2.5% glutaraldehyde in 0.05 M sodium carbonate buffer (Agar Scientific Ltd, Stanstead, UK) for subsequent processing. A standard bacteriology swab was taken from the inside of one nostril and spread over a blood agar plate in the routine laboratory. The plate was cultured aerobically and read at 24 h.

Electron microscopy

Orientation was determined as previously described [15, 16]. In brief, the samples were processed for transmission electron microscopy (TEM) by washing in 0.05 M sodium cacodylate buffer, fixing for 1 h in osmium tetroxide (Agar Scientific Ltd), setting in 2% agar (oxid No.3, Basingstoke, UK) and dehydration through a series of methanol (Merke, Poole, UK) before being embedded in Araldite resin (Agar Scientific Ltd). Ultrathin sections (~90 nm) were cut using glass knives in a Reichert Ultracut E microtome. Cilia in suitable transverse sections were examined at a

magnification of $\times 20,000$ for ultrastructural defects to eliminate samples which had a dynein arm defect or possible secondary defects due to infection, *e.g.* large numbers of compound cilia or microtubular disarrangements. Provided ciliary structure was normal, orientation was assessed. Ideally for each sample, at least 10 images from different cells each containing 10 good ciliary cross sections from the same cell are required. In all but control 2 (where $n=82$ cilia were studied) at least 100 ciliary cross sections were obtained; in most cases at least $10\cdot\text{cell}^{-1}$ were able to be studied, the minimum number being seven cross sections. Each TEM image was transferred to a Macintosh computer and Image 1.33g was employed to measure orientation. A line was drawn electronically onto the screen through each central pair of microtubules, *i.e.* a minimum of 10 lines per image. The image analysis programme calculated the angle of orientation of each cilium with respect to the vertical. Thus within each image the angle of orientation of each cilium was obtained and the standard deviation of the angles calculated and finally the mean standard deviation per sample (called the ciliary orientation) was calculated using Microsoft Excel.

Results

Ciliary orientation was obtained for eight control infants (3 males), mean age 13.1 months, range 7–23. Details of the underlying diagnoses are given in table 1. Nasal swabs for bacterial culture were all negative. Primary abnormalities of ciliary ultrastructure were not recognized, nor were there any secondary abnormalities (*e.g.* compound cilia or microtubular disarrangements) seen. A mean of 254 central pairs was examined, range 82–453. The mean ciliary orientation for the eight controls was 14.9 degrees, range of orientation for the individuals 12.9–17.5.

Case reports

Case 1. This male infant was born at term by normal vaginal delivery, birth weight 3.52 kg. The infant was described as being snuffly and having a rattley chest from birth. Mirror image organ arrangement was also noted. When first seen at the Royal Brompton Hospital at 2 weeks of age, the infant was noted

Table 1.—Control subjects and results of orientation studies

Subject number	Age months	Reason for anaesthetic	Mean (range) of ciliary orientation	Number central pairs analysed
1	10	TOF	15.7 (7.6–33.7)	316
2	8	PDA, VSD	17.5 (10.2–28.6)	82
3	11	TOF	16.6 (6.0–41.7)	128
4	11	PDA	12.3 (6.7–25.2)	453
5	23	Coarc	12.9 (7.6–18.7)	334
6	23	PDA	14.9 (7.2–24.4)	234
7	12	PA stenosis	12.9 (6.1–31.5)	188
8	7	AS	16.6 (9.1–37.7)	294

AS: aortic stenosis; Coarc: coarctation of the aorta; PA stenosis: peripheral pulmonary artery stenosis; PDA: closure of patent arterial duct; TOF: tetralogy of Fallot; VSD: ventricular septal defect.

to have rhinorrhea, a chronic cough and bilateral basal crackles. A presumptive diagnosis of PCD was made. CBF was measured on seven occasions; results were normal mean ciliary beat frequency ($n=3$; mean 12.1 Hz), and on four occasions the sample was unsatisfactory due to infection, although on three of these occasions static cilia were seen. It proved very difficult to get an adequate sample for electron microscopy, but on two occasions ciliary ultrastructure was normal (153 of 156 cilia assessed for dynein arms, 386 of 388 assessed for microtubular arrangement were normal). Ciliary orientation studies showed 44.5 degrees (range 10.6–64.5) in 218 central pairs; and on a second occasion, 28.9 degrees, range 9.0–47.5 in 259 central pairs. The subsequent infant course was typical of PCD, with continued rhinorrhea and recurrent lower respiratory tract infection. Unfortunately, grommet insertion for chronic serious otitis media was undertaken before the orientation studies had been completed, and the infant's ears discharged profusely for many months until the grommets came out. At age 6 yrs, the upper and lower respiratory tract symptoms remain unchanged.

Case 2. This male infant was born at term by normal vaginal delivery, birth weight 2.75 kg. The infant developed a severe right sided pneumonia shortly after birth (fig. 1), for which intravenous antibiotics were given and a good recovery was made. There was no mirror image arrangement. The presence of chronic rhinorrhea was also noted. A presumptive diagnosis of PCD was made. At age 2 months, a nasal brush biopsy was obtained. Light microscopy of cilia showed a mixture of static, dyskinetic and normally beating cilia. Electron microscopy showed normal ciliary ultrastructure (60 of 62 cilia assessed for dynein arms, 409 of 426 assessed for microtubular arrangement were normal). Ciliary orientation studies



Fig. 1. – Chest radiograph of Case 2, taken shortly after birth. There is extensive collapse and consolidation in the right upper zone, with mediastinal displacement to the right. The child does not have mirror image arrangement.

showed 24.4 degrees, range 13.1–38.4 in 196 central pairs. When last seen at six months of age, the infant continued to have a nasal discharge, with bilateral crackles and mild recession, but was thriving. On audiometric assessment at age 15 months, bilateral signs of fluid in the middle ear cavity with flat tympanograms were found. On distraction testing the infant only responded to sounds around 50–55 dB and has been offered hearing aids.

Discussion

This study has defined for the first time the normal range for nasal ciliary orientation in children <2 yrs of age, and described two infants with typical features of primary ciliary dyskinesia with normal ciliary ultrastructure. The mean ciliary orientation obtained in normals was 14.9 ± 0.7 degrees, range of mean orientation for the individuals 12.9–17.5, and the range of orientation considering all fields examined was 6.0–41.7. This was similar to the results obtained in normal adult subjects using the same equipment (12.8 ± 1.5 degrees) [15]. Similar results were reported by others [13].

The only previous studies of ciliary orientation were in adults [13–16]. The normal range described might not be relevant to infants. It is known that the development of the paranasal sinuses takes place after infancy. It is possible that the cumulative effects over time of recurrent viral colds and other infections suffered by even normal adults might in some way affect ciliary orientation. Furthermore, one could hypothesize that the developing epithelium might function less efficiently than mature adult epithelium; for example, ciliary beat frequency varies with age [17]. For all these reasons ciliary orientation might not be the same throughout life. The study could be criticized because of the relatively small number of normals studied, the use of anaesthetized infants rather than absolutely normal controls, and the absence of viral studies in the control infants. Although the numbers are small, the confidence intervals are very narrow, and it is not believed that the use of a larger number would substantially have altered the conclusions. Furthermore, orientation was found to be similar to that found in adults, implying a uniform normal range across all ages. Nasal brushings in the control group were performed during a general anaesthetic because the procedure causes discomfort and it was felt to be more ethical to take every opportunity to minimize any distress to the parent and child. It is unlikely that a brief period of anaesthesia could influence ciliary orientation (the studies were performed immediately after induction of anaesthesia). Although a normal CBF would have added weight to the contention that these were truly normal infants, measurement was not attempted in the samples obtained, in the present study, for a number of reasons. Firstly, normal ranges for CBF have already been established in this group [17]. Secondly, anaesthetic agents may reduce CBF [18]. Thirdly, orientation studies ideally require a large sample of epithelium, and the authors were keen to maximize their chances of obtaining a good sample.

Finally, a normal CBF cannot by itself exclude a ciliary problem [3]. Attempts to minimize the chances of inadvertently studying an infant with unsuspected PCD by excluding those with any significant respiratory history and those with any disorder of laterality were made [4].

The congenital cardiac lesions in the study group have not to the best of the authors' knowledge been associated with PCD, nor did these infants have any other feature suggestive of the diagnosis. Nasal nitric oxide has also been proposed as a diagnostic test for PCD [19, 20], but both the subjects and controls were too young for measurements, with the technology then available to the authors, to be made. Ideally polymerase chain reaction for viral detection would have been performed, but this was not available. All the normal controls were evaluated for viral infection by the anaesthetist, who would have deferred the procedure had the child been unwell. Taking all this into consideration, the authors felt it was a reasonable study design.

A further possible control group would have been infants with chronic infective or allergic rhinitis. It is probable that at least some of these infants would have exhibited secondary disorientation. Such infants would however, have been unlikely to be permitted to undergo anaesthesia for elective surgery. There is no question that it would be unwise to diagnose PCD on disorientation alone [3] without carefully considering the clinical picture, which is of paramount importance in this condition. Of particular note, neonatal onset of symptoms is very typical of PCD [2, 3], but virtually unheard of in infective or allergic rhinitis.

Abnormal ciliary orientation in the study by RAYNER *et al.* [16] was correlated with delayed mucociliary clearance, presumably due to inefficient ciliary beating. Whether abnormal ciliary orientation alone could lead to PCD syndrome is still not certain. The possibility that the PCD syndrome in the two infants described in this paper was secondary to an ultrastructural defect too subtle to be detected cannot be excluded. Cilia contain >200 different proteins [21], but only a relatively few abnormal structural patterns have been described using electron microscopy. It seems likely that some subtle structural defects have yet to be described, possibly because the changes are outside the resolving power of the electron microscope, and these changes could influence orientation which is known to be abnormal in cases with an ultrastructural abnormality [13]. In favour of this possibility is that static and dyskinetic cilia were observed in both the cases reported here, but not in the adult series reported by RAYNER *et al.* [15]. However, the differences may merely have been due to secondary infection in the paediatric cases.

To conclude, the present study has described two infants with the clinical features of primary ciliary dyskinesia, but with no structural abnormality in the individual cilia. The orientation of cilia with respect to each other was abnormal, presumably resulting in inefficient mucociliary clearance and possibly leading to the clinical disease. The authors were able to make this diagnosis by describing for the first time the normal range of ciliary orientation in infancy. The description

of abnormal ciliary orientation in infants lends further weight to the view that this is a real clinical entity, and that measurement of ciliary orientation should be part of the assessment of ciliary structure and function in cases of possible primary ciliary dyskinesia, in particular when the ultrastructure of individual cilia appear to be normal.

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