Eur Respir J
1988, 1, 257–261

Orientation of human respiratory cilia

M.E.P. Rautiainen*

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ABSTRACT: Ciliary orientation was studied on the respiratory epithelium of the nasal cavity or the sphenoidal sinus of ten adult nonsmokers without respiratory disease. The ciliary orientation was evaluated from micrographs by measuring the angle between the plane defined by the central tubules and reference line (with a semiautomatic image analyser (IBAS I)). The standard deviation of the angles of cilia population was counted in every field. The standard deviation of the measurements described the ciliary alignment. It varied from 12.1–41.2°. The mean standard deviation was 27.3±7.4°. 58% of all measured cilia were within ±0–20° of the mean and 85% of cilia were within ±0–40°. However, a few cilia or small groups of cilia were found in most fields which differed dramatically from the main orientation. The size of these groups was always less than ten cilia. On the normal respiratory epithelium the standard deviation of ciliary orientation varies between ±10–40 (at about 97% probability). For diagnostic conclusions more than 60 cilia should be measured.
Eur Respir J. 1988, 1, 257–261.

* Department of Otorhinolaryngology, University of Kuopio, SF-70210 Kuopio, Finland.

Keywords: Ciliary orientation; respiratory cilia; ultrastructure.

Received: March 2, 1987; accepted after revision October 2, 1987.

In recent years many anomalies have been reported in ciliary ultrastructure in chronic respiratory infections [3, 6, 13, 14], in acute viral upper respiratory infections [4, 26, 34] and in bronchial asthma [7]. The immotile cilia syndrome, showing an absence of dynein arm [1], is best known and occurs in Kartagener's syndrome [2, 12, 33].

With radioisotopic methods, mucociliary activity can be measured on the respiratory epithelium [11]. Ciliary beat frequency can be measured photometrically from samples taken from respiratory epithelium [8, 25, 35]. Using a phase-contrast microscope, ciliary motility has been shown in some cases of the immotile cilia syndrome [22]. It has been suggested that this syndrome be called ciliary dyskinesia [30].

It is generally accepted that the disorientation of cilia leads to impairment of mucociliary transport. Random ciliary orientation has also sometimes been found in Kartagener's syndrome [9, 15, 16]. All reported cases of random orientation of cilia are based on small cilia populations. No paper considering ciliary orientation in normal human respiratory epithelium has been published. The obvious lack of standardization has led us to study the normal orientation of respiratory cilia with our recently created method for measuring ciliary beat direction [20]. In this paper we report our results on normal ciliary orientation.

Materials and methods

The measurements were made from samples of ten adult nonsmokers who did not suffer from any respiratory disease. Four of them were healthy volunteers and from these specimens were taken from the middle concha of the nose. Six specimens were taken, during transphenoidal hypophysectomy, from the sphenoidal sinus of patients who had a hypophyseal tumour. All the specimens were biopsies and it was impossible to distinguish whether the evaluated cilia were from the same cell or from adjacent cells of a cell sheet.

The specimens were fixed with a mixture of 4% formaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer at +4 °C for a week or less [19]. The samples were then washed in veronal acetate HCl buffer (pH 7.4), posthxed for 1 h with O_sO_4 in s-collidine buffer (pH 7.4), washed again in veronal acetate HCl buffer and stained en bloc with uranyl acetate in veronal acetate HCl buffer. The samples were embedded in Epon 812 and sectioned with a diamond knife. Ultra-thin sections were stained with uranyl acetate-lead citrate and examined in a Jeol JEM 100 S electron microscope.

From these specimens, areas with the best cross sections of cilia were chosen and these areas were photographed at the primary magnification ×15,000. The final magnification of prints was ×45,000. The size of the measured fields was 3.8 × 5.1 μm and in micrographs 17.0 × 23.5 cm. From the micrograph, the orientation of the cilia was measured from every cilia, if the two central tubules could be seen.

The beat direction of cilia is perpendicular to the axis through the central tubules [27]. The stroke is effective in one direction only and the motion in the other direction is a recovery stroke [28, 29]. These two

parts of the motion cannot be distinguished from each other on the basis of ultrastructure of the cross-sectioned cilia. The orientation can be measured on every level of cilia because cilia do not coil around their axis [21]. The measured angles are between 0–180°, because distinction could not be made between direction of stroke and recovery stroke. In this study, the orientation of cilia was measured from every cilia by defining the angle between the reference line and the line joining the two central tubules. The reference line was chosen so that the great majority of the angles fell about the middle in the 0–180° range. The measurements were made with a semiautomatic image analyser with a digitized plate connected to a computer (IBAS I) with a cross-hair cursor. The cursor was drawn along the edge of the ruler placed to pass through the two central tubules of the cilia [20].

The standard deviation (SD) of the angles of cilia population was counted from every measured field [20]. The size of cilia population per field varied between 9 and 110 cilia. From every specimen 4–5 fields were measured.

We can also estimate the number of cilia to be measured for valid diagnostic conclusions. We may want an accuracy which allows the results to fall within ±10° from the real mean with 95% probability. In such a situation the number of cilia we need to measure can be calculated by applying the formula for 95% confidence limits [5]:

$$n = \left(\frac{1.96 \cdot SD}{10°}\right)^2$$

where n = number of cilia that should be measured, SD = standard deviation in degrees, 1.96 = factor of normal distribution corresponding to 95% confidence limits (this can be used if the sample size is larger than 30).

Results

The standard deviation in measured fields varied between 12.1 and 41.2°. In nasal samples the standard deviation varied from 12.1–38.0°. The smallest standard deviation in samples from sphenoidal sinuses was 15.0° and the biggest was 41.2° (table 1).

The mean standard deviation in a sample counted from all measured fields varied from 21.7–26.2°, in samples taken from nasal cavities. In the samples from sphenoidal sinuses the mean standard deviation varied between 26.7 and 35.5°. The mean standard deviation in all nasal samples was 23.8±6.3°. The corresponding mean standard deviation in samples from sphenoidal sinuses was 29.9±7.3°. The measured cilia populations were much bigger in the samples from the sphenoidal sinus than in the samples from the nasal cavities. In all studied fields the mean standard deviation was 27.3±7.4°.

The difference between minimum and maximum angle of measured cilia population varied between 173° (measured from the population of 75 cilia) and 43° (measured from the population of 21 cilia) from the reference line.

Ciliary orientation varied. 58.0% of all cilia studied for this report were within ±0–20° of the mean and 85.0% of cilia were within ±0–40° of the mean. In all samples, the mean of cilia which were within ±10° from the mean angle was 33.0±8.0% and 8.1±5.6% of cilia differed more than 50° from the mean angle (table 2). However, in each field, a few cilia or small groups of cilia could usually be found differing dramatically from the main orientation (fig. 1). This was obvious in large cilia populations, but had little effect on the standard deviation of the angles of orientation.

In one field of 43 cilia the standard deviation of ciliary orientation was more than 40.0°. We decided

Table 1. - Results of measurements in samples of ten healthy subjects (SD=standard deviation). Samples 1-4 are from the middle concha of the nose and samples 6-10 are from the sphenoidal sinus.

Sample	1	2	3	4	5	6	7	8	9	10
No. of fields	4	5	5	5	4	4	4	4	4	4
No. of cilia in fields	21-29	29-59	9-29	23-60	80-110	73-82	58-105	90-133	38-65	19-55
Smallest SD	12.1	19.0	17.2	18.0	22.4	28.0	32.8	23.6	18.0	15.0
Biggest SD	38.0	25.6	32.5	29.6	35.5	34.1	41.2	34.3	36.9	37.7
Mean SD	22.8	21.7	26.2	24.0	31.1	31.7	35.5	27.6	26.7	26.7
± SD	10.9	2.8	5.8	5.1	6.2	2.6	5.6	4.9	9.0	11.8

All SD's are recorded in degrees (°)

Table 2. - The ciliary disorientation (in degrees) in ten healthy adults.

Angle deviation from mean value (degrees)	Sample 1	2	3	4	5	6	7	8	9	10	Mean±SD
0–10.0°	44.6	45.1	23.6	35.6	27.6	28.1	23.3	29.2	33.3	39.3	33.0±8.0
10.1–20.0°	27.2	23.2	34.8	24.9	24.1	26.5	20.0	30.2	26.5	29.7	26.7±4.1
20.1–30.0°	10.7	16.5	16.9	20.6	18.4	17.3	14.0	15.3	17.0	15.8	16.3±2.6
30.1–40.0°	5.8	9.8	10.1	10.7	11.6	10.8	12.1	10.8	10.0	6.2	9.8±2.1
40.1–50.0°	6.8	1.8	9.0	4.6	8.2	6.7	8.4	6.4	5.9	4.1	6.2±2.2
>50.1°	4.9	3.7	5.6	3.6	10.2	10.6	22.3	8.1	7.3	4.8	8.1±5.6

All values as %

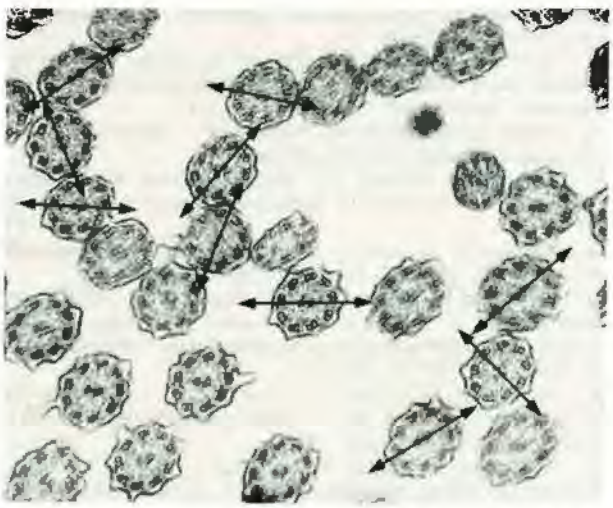

Fig. 1. Locally the ciliary orientation may also appear random in samples from healthy humans. The arrows show the direction perpendicular to the beat direction of cilia, measured in this study. This picture is from sample No. 2. Magnification ×45,000.

to use 40.0° as the upper limit for the value of normal standard deviation. Thereafter, we could calculate the number of cilia to be measured to achieve a measurement result within ±10° of the real mean value, with a probability of 95%. We found the number of cilia to be 61. We think that 60 is the minimum number of cilia which should be measured, when looking for abnormalities of ciliary orientation.

Discussion

In ciliary dyskinesia the cilia are motile but the mucociliary clearance is impaired [22–24]. Also, in the classic immotile cilia syndrome, with absence of dynein arms, motile cilia have been found [17, 18]. It has been suggested, that the lack of dynein arms in cilia is only partial in patients with ciliary dyskinesia [18]. In dynein defective cilia, the ciliary wave form is different from normal ciliary wave form [23]. Cilia with a radial spoke defect [32] and cilia with a tubulus translocation defect [31] have characteristic ciliary wave form abnormalities [23].

Random ciliary orientation has sometimes been found in Kartagener's syndrome [9, 15, 16]. These findings have been carried out in small cilia populations and we cannot consider them as reliable standards. HOLLEY and AFZELIUS [10] have measured ciliary orientation by measuring the basal foot orientation in serial sections of larger cilia populations in nasal mucosa. In Kartagener's syndrome 19–54% of cilia differed in orientation by more than ±30° from the mean. In patients with common cold 13–45% of cilia differed in orientation by more than ±30° from the mean. So there were no significant differences between these two groups. In our material 15–43% of cilia differed by more than ±30° from the mean (table 2).

The method HOLLEY and AFZELIUS [10] have applied is good but it might be too difficult for routine work. Our results suggest, however, that the measurements made from the cross-sections of cilia (scale 0–180°) reliably reflect the alignment within the scale of 0–360° also.

It is possible that randomly orientated cilia may also occur in other ciliary diseases. PEDERSEN *et al.* [18] have suggested that ciliary dyskinesia is caused by partial lack of dynein arms. Random ciliary orientation can also be a cause of ciliary dyskinesia. If ciliary ultrastructure is normal but cilia are randomly orientated, the wave form of each cilium may appear normal but metachronicity would be absent and mucociliary clearance impaired.

The estimation of ciliary orientation has been subjective because there have been no standardized

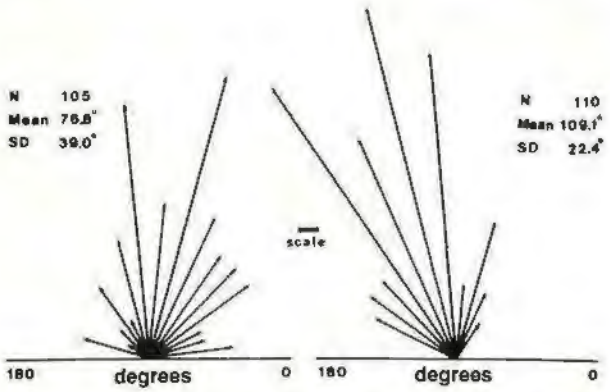

Fig. 2. Vectorial representation of fields with small and large standard deviation; the length of each vector indicates the number of cilia in the corresponding beat direction class. The scale between the diagrams indicates the length caused by one cilium to the vector beating direction. The absolute values of the mean orientation are not meaningful because they depend on the reference line chosen. On the other hand, the standard deviation characterizes each cilia population reliably.

methods for this purpose. Insufficient attention has been paid to ciliary orientation in studies concerning diseases of cilia. The method we have applied [20] is easy enough for routine work and will potentially increase interest in measurements of ciliary orientation.

It has been suggested that ciliary orientation varies normally within 5–25° [9]. In our preliminary results these limits proved to be too narrow [20]. Ciliary orientation may vary much more in healthy human respiratory epithelium (fig. 2). In particular, if we study small populations of less than ten cilia, the ciliary orientation may appear random. If there are only a few cilia which dramatically differ from the main ciliary orientation, no conclusion in respect to disease can be drawn. This is due to the fact that such aberrations also occur in normal respiratory epithelium without any respiratory disease. The results in this paper are parallel to our preliminary results [20]. On the normal respiratory epithelium the standard deviation of ciliary orientation varies between ± 10–40° (at about 97% probability).

In order to obtain reliable results for diagnostic purposes, the orientation of cilia should be measured from a population of more than 60 adjacent cilia. Evaluation of smaller populations of cilia may provide erroneous results. We feel that each field evaluated should contain at least 20–30 adjacent cilia.

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RÉSUMÉ: L'orientation ciliaire a été étudiée sur l'épithélium respiratoire de la cavité nasale ou du sinus sphénoïdal chez 10 adultes non fumeurs qui ne souffraient d'aucune maladie respiratoire. L'orientation ciliaire a été mesurée à partir de micrographies en mesurant l'angle entre le plan défini par les tubules centraux et la ligne de référence au moyen d'un analyseur semi-automique d'images (IBAS 1). La déviation standard des angles de la population ciliaire a été calculée dans chaque champ. La déviation standard des mesures décrit l'alignement ciliaire. Dans nos résultats, la déviation standard varie de 12.1 à 41.2°. La déviation standard moyenne était de 27.3 + 7.4°. 58% de tous les cils mesurés se situaient à l'intérieur d'une variation de 0 à 20° de la moyenne et 85% des cils étaient à l'intérieur d'une variation de 0 à 40°. Toutefois, on a trouvé dans la plupart des champs quelques cils ou quelques petits groupes ciliaires qui étaient dramatiquement différents de l'orientation principale. La taille de ces groupes était toujours inférieure à 10 cils. Sur l'épithélium respiratoire normal, la déviation standard de l'orientation cilliaire varie de 10 à 40° à environ 97% de probabilité. Pour pouvoir permettre des conclusions diagnostiques, il faut examiner plus de 60 cils.