

## **SURVIVAL TIME OF NASAL MUCOUS MEMBRANE CILIATED CELLS: A PRELIMINARY STUDY OF CYTOLOGICAL EXAMINATION BY PHASE-CONTRAST MICROSCOPY**

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**The observation of the ciliated cell dynamics by phase-contrast microscope has allowed a profound study of some parameters of the cellular components. The aim of this preliminary study is to assess the surviving time of ciliary cells using the phase-contrast microscope. Nasal cytology was carried out on 50 healthy volunteers. The ciliary beat time was assessed by a video-camera connected with a PC. The time of ciliary beat persistence was extraordinarily long (mean 210 minutes, with range 74 -1260). The ciliary activity remained valid as rhythm, synchronism and metachronality. In conclusion, this preliminary study demonstrated that cilia has an energetic autonomy of great importance, able to allow a valid functional activity also in the case of partial sufferance of the cellular unit.**

The relevant recurrence of rhinosinusal infections, which are often complicated by a local involvement (e.g. middle ear, pharynx) (1) and/or a distal one (e.g. lower respiratory tract) (2) may suggest a dysfunction of the muco-ciliary system, mainly in the absence of an evident etiology (anatomical malformations, allergy etc.) (3). However, a complex diagnostic pathway is needed to demonstrate the muco-ciliary dysfunction (4-6).

Over time, direct investigations of ciliary activity have been the subject of research by numerous authors who have tried to establish the dynamic and physiological parameters of the ciliate movement (rate, beat phases, metachronous ciliary beat, critical temperature of ciliate stasis, etc.) and to classify the

different ciliary alterations on the basis of etiologic factors (e.g. primary ciliary dyskinesia) (1-18). Research procedures used to this end often call for the use of very expensive and complex equipment (cine-machinery connected to stroboscopes and oscilloscopes) (15-17, 19-20) which unfortunately have not proven to be of any real or practical clinical use. Consequently, researchers have turned their attention to indirect study procedures of ciliary motility, which are eminently clinical, undoubtedly easier to perform and relatively less expensive. Of these, muco-ciliary transport (TMC) represents the most used method in the rhinological field (21). Although, it provides some useful information, it does not allow to establish whether the impaired

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muco-ciliary function depends on: mucus rheological alterations (mainly in mucoviscidosis), ciliary system dysfunctions that characterize some syndromes (e.g. Kartagener), alterations secondary to viral and bacterial infections, alterations secondary to endogenous or exogenous toxicosis, or morpho-structural alterations of the nasal cavity (e.g. septal deviations and septal crests, adenoid hypertrophy, turbo-septal synechias, complete or partial choanal atresia, etc.) (21).

A particularly sophisticated method of studying ciliary system structural alterations is the use of an electron microscope (20-24), which allows to define ciliary system ultra-structural characteristics and in particular the micro-tubular system alterations (e.g. absence of dynein arms, absence of central tubules, etc.). However, this method presents some disadvantages: nasal mucous membrane biopsy is required; it is rarely used (there are few highly specialized Italian centres); it is expensive and though sensitive it is not specific because of false positives in subjects without symptoms and with normal MTC values (25).

Contrast-phase microscopy was invented in 1935 by Fritz Zernike, a Dutch physicist who was awarded the Nobel Prize in 1953. This type of microscope represents a valid option to more complex methods. Compared with an ordinary-light microscope, it is more convenient since it enables the study of cell microstructures "fresh", such as without resorting to fixation and staining which may induce morphologic alterations and chemical and physical modifications resulting in cell death.

In 1943, Zinser extended the cyto-diagnostic possibilities of contrast-phase microscopy, previously used only for experimental purposes, to clinical practice. In 1949, the technique was introduced in gynaecologic practice by Range, Voge, Haselmann and Zinser himself. In 1969, Petes Stoll and Gisela Dollenbach-Hellweg created the first microscopy atlas and so the technique began to spread. In 1994, Miniello confirmed the importance of this technique in the diagnostic strategy for gynaecological pathologies (26). This preliminary study was therefore carried out on a population of healthy subjects in order to establish parameters which may be useful for clinical and experimental practice.

## MATERIALS AND METHODS

### *Study design*

Fifty healthy volunteers: 30 men and 20 women (aged 18 to 46 years; mean age, 28) were evaluated. The sample was thereby formed by individuals without history of nasal, otologic, or broncho-pulmonary disorders. These subjects had not undergone any local and/or systemic pharmacological treatments in the 6 months preceding the study. Skin prick-test for the most common aero/tropho allergenes was performed to exclude allergy. In addition, an active anterior rhinomanometry (using the Rhinomanometer 300 – ATMOS®) was performed to assess nasal respiratory resistance.

Nasal cytology was carried out as previously reported in detail (27). Briefly, cytological samples were obtained by scraping with a Rhino-Probe™. The samples were collected from the medial portion of the inferior middle turbinate. After fixing with absolute alcohol for 3 minutes, the samples were stained using the May-Grünwald-Giemsa and read by light microscope. Muco-ciliary Transport test was performed a week before the cytological evaluation. The test was previously described in detail (28). Briefly, a mixture of vegetal coal and saccharin is placed in the nasal fossa and then the pharynx is observed until it appears on the endoscope. This test was carried out to confirm the normality of muco-ciliary clearance.

### *Sample collection technique*

After having put the patient in the typical position for anterior rhinoscopy (sitting with the head slightly retroflexed and in contact with the headrest in order to limit probable sudden movements during sample collection), nasal mucous membrane scraping was performed by a Volkmann curette or a Rhino-Probe under careful vision with speculum. The sample was taken from the nasal fossa (contralateral to that considered for cytology) at the level of the inferior turbinate in the middle part. This part is characterized by an abundant population of ciliated cells. Local or oral decongestants or anaesthetics were avoided for possible pharmacological interferences with ciliary beat. Using the Rhino Probe, 2 or 3 movements in a postero-anterior direction along the inferior turbinate bone medial face were gently performed for extirpating only the superficial layer of the ciliated epithelium without causing any injuries to the mucous membrane. Sample collection was performed at room temperature (between 18°C and 24°C): usually this thermal range does not alter ciliary activity. Once sample collection was carried out, a chronometer was started for measuring the survival time of ciliated cells.

### *Material examination*

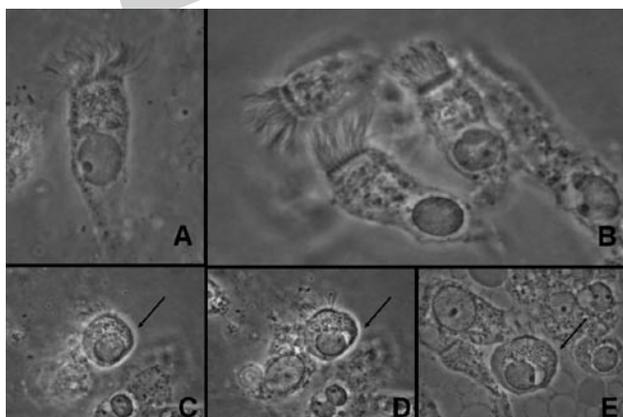
Immediately after sample collection, the content of

the cup-shaped part of the Rhino-Probe was dipped into a drop of sterile physiological saline solution that had been previously placed on a microscope slide on which a cover glass microscope slide was applied. The sample was thereafter ready to be examined by contrast-phase microscope. Once the microscope slide was put on an adequate observation turret, an examination at 400X magnification was performed using a Standard type 25 ICS contrast-phase Zeiss microscope.

Firstly, it was important to identify one cell (or syncytium of active ciliated cells) with intact cilia, cytoplasmic membrane and nucleus (such as intact morpho-functional cell unit). Ciliary beat was continuously recorded by a video-camera connected with PC until the arrest of the ciliary movements. The time elapsed between sample collection and the chronometer arrest represents the surviving time of the ciliated cell (.S.T.C.C.). Ciliated cell survival time represents a “fresh” study and for this reason microscope slides cannot be preserved.

## RESULTS

This study was carried out without causing any problems for the recruited subjects. In 43 of the subjects it was sufficient to perform just one sample collection at the level of the inferior turbinate to obtain a cell sample valid for the purpose of the research. A second sample collection was necessary only in 7 subjects and was performed at the level



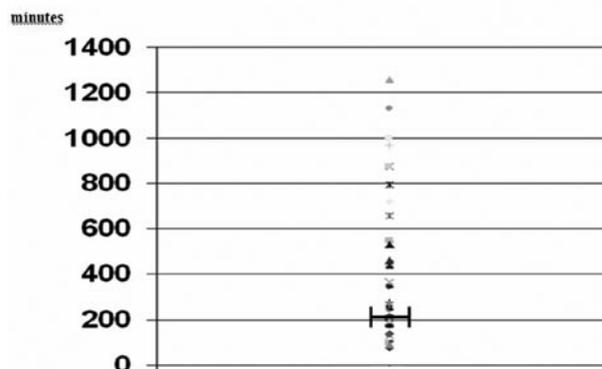
**Fig. 1.** Microscopic cytology assessed by phase-contrast microscopy (1000x). Microscopic features are representative of various cellular situations: *A* and *B* correspond to normal ciliated cells with a well conserved ciliary apparatus; *C*, *D* and *E* show ciliated cells with progressive vacuolar degeneration phenomena

of the middle turbinate. The cell population was usually represented by columnar ciliated cells and mucous-secreting goblet cells, in the typical ratio 5:1. Leukocytes or erythrocytes were rarely found (Fig. 1). Mean ciliated cell surviving time was 210 minutes, ranging between 74 and 1,260 (Fig. 2).

A reduced ciliary activity was still valid, concerning beat ratio, rhythmicity, synchronism and methachronous ciliary beat, even when some cell degeneration phenomena were already present after  $15 \pm 5$  minutes, affecting both cytoplasm and nucleus (such as a vacuolar degeneration); Fig. 1, C, D and E show these details. Moreover, a reduced ciliated cell surviving (about 20 minutes) was present in 15 subjects as scraping content included not only the most superficial mucous membrane layer but also a significant amount of mucus (this can occur when sample collection is not preceded by a delicate nasal cavity cleaning). There was no significant relationship between survival time and several parameters, including age, nasal resistance, mucus ciliary transport, and quantity of mucus.

## DISCUSSION

This study provides a surprising finding: the long persistence of the ciliary movement (210 mins on average) in respect to the nucleus-cytoplasm unit viability, which was about 30 minutes. Moreover, evident cell distress signs appeared 15 mins after sample collection, and clear vacuolar degeneration phenomena occurred, suggesting serious anoxic



**Fig. 2.** Ciliated cell surviving time is expressed in minutes after cell sampling. The mean was 210 minutes, ranging between 74 and 1260.

damage. These findings clearly show that cilia have an energy and functional autonomy that is significant and superior to that of the cell itself. However, it is important to verify, from both an experimental and clinical point of view, the effectiveness of the movement itself after cell death and consequently the functional efficacy (Muco-Ciliary Transport). Studies are ongoing to verify this fact.

A limiting factor for ciliary motion may be related to mucus rheological alterations (visco-elasticity), as previous studies and clinical practice have already shown in cystic fibrosis (29, 31). In fact, if there is a greater extent of mucus in the specimen (easily detectable by the torpidity of the cytological preparation), ciliated cell survival time is significantly reduced (15-41 minutes) (32-33). Contrast-phase microscopy is therefore satisfactory for investigation of ciliated cell activity if proper procedures are followed: correct sample collection technique, processing, standardization method, and adequate magnifications. In fact, contrast-phase microscope can detect the ciliary beat until its exhaustion demonstrating nuclear and cytoplasmic alterations occurring simultaneously. The assessment of ciliary beat might directly suggest probable disorders, such as immobile cilia syndrome, primitive or secondary dyskinesias, avoiding the necessity to immediately resort to ultra-structural investigations by electron microscope. It is extremely important to emphasize the low cost of this technique: less expensive than electron microscopy.

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