

Characterization of the ciliary beating efficiency in primary diffuse chronic rhinosinusitis

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Ciliary dysfunction may result in chronic airway inflammation and infection causing injury and structural changes to the airway epithelium, leading to a variety of diseases, like bronchiectasis and primary diffuse chronic rhinosinusitis (CRS). Currently, ciliary beating analysis has mainly been studied through the measure of the ciliary beating frequency (CBF) by high-speed digital video microscopy (HSDV). However, a normal CBF has been described in different forms of primary and acquired ciliary dyskinesia^(1,2). CBF seems therefore insufficient to provide relevant information on the quality of the cilia beating, especially as a normal frequency, associated with a poorly ciliary coordination, does not guarantee an efficient mucociliary clearance. Recently, Bottier et al. developed a new tool based upon the measurement of the velocities of micro-beads advected in the flow generated by the ciliated cells^(3,4), allowing us to assess the shear stress related to the ciliary beating on nasal human brushing samples. The objectives of this study are to characterize the ciliary beating efficiency in CRS thanks to this new tool, and to evaluate its ability to discriminate CRS from control patients. In a multicentric prospective study, we compared nasal brushing samples from an adult patient group with primary diffuse CRS (mix of primary diffuse CRS - type2 and non-type2 - with a majority of type2), according to the classification in the EPOS 2020⁽⁵⁾, and control patient group. Primary ciliary dyskinesia (PCD) patients were excluded (Figure 1). Informed consent was obtained from all patients (agreements of Ethics Committees: CPP IDF X 2016-01-01 and CPP IDF VII 2019-A00973-54). The shear stress was compared to other ciliary beating HSDV parameters

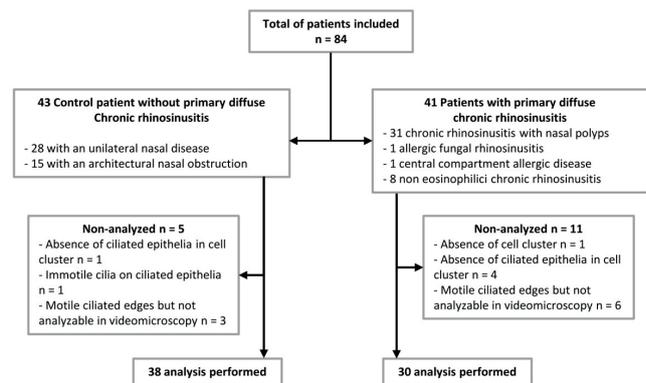


Figure 1. Flow chart.

(CBF, relative ciliary density, cilia length and metachronal wave) (More details are reported in online supplementary material). In the 84 included subjects whose main epidemiological characteristics are reported in Table 1, the mean ciliary beating efficiency (CBE) index was significantly lower ($p < 0.001$) in the primary diffuse CRS group (0.27 ± 0.15 mPa) than in the control patient group (0.66 ± 0.30 mPa). The CBF did not significantly differ between the two groups while the other tested parameters were significantly lower in the primary CRS group compared to the control patient group. The ROC curves of all studied parameters are presented in Figure 2. We found the CBE index to be the best discriminating parameter with a sensitivity of 94%, a specificity of 76% for a cut-off-value of 0.34 mPa, and

Table 1. Baseline patient characteristics and clinicopathologic data.

Characteristics	Control patients (n = 43)			Patients with CRS (n = 41)			p
	n	Percentage (%)	Mean ± SD	n	Percentage (%)	Mean ± SD	
Age	43		54.9 ± 18	41		50.6 ± 13.8	0.21
Gender							
Women	24	55.8%		21	51.9%		
Men	19	44.2%		20	48.8%		
Tobacco	4	9.3 %		6	14.6%		0.45
Respiratory allergy	5	11.6%		7	17.1%		0.48
Asthma	2	4.7%		18	43.9%		0.00002
Aspirin intolerance	2	4.7%		10	24.4%		0.01
AERD	0	0%		9	22%		0.001
Bronchiectasis	1	2.3%		5	12.2%		0.08
Oral corticosteroids*	0	0%		3	7.3%		0.07

CRS: Chronic rhinosinusitis; SD: standard deviation; AERD: Aspirin-exacerbated respiratory disease. *Oral corticosteroids = the patient received oral corticosteroids less than a week before nasal brushing.

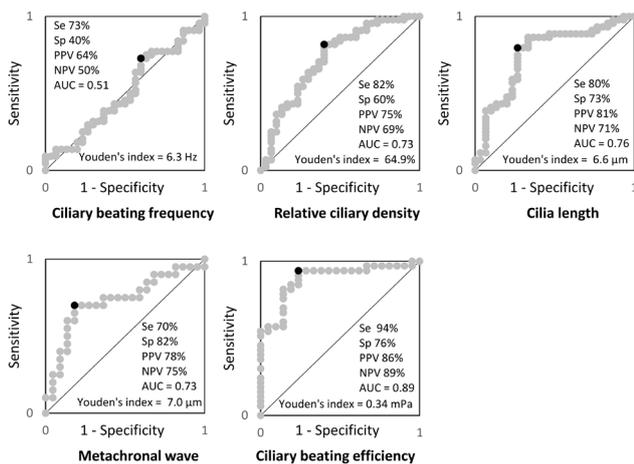


Figure 2. Performance of the ciliary beating parameters to discriminate primary diffuse chronic rhinosinusitis (CRS) from control patients (CPs).

an AUC of 0.9 while the frequency showed a sensitivity of 73% and specificity of 40% (AUC of 0.51). Relative ciliary density, cilia length, and metachronal wave were less discriminating than the CBE index, but more discriminating than the CBF with an AUC ranging from 0.7 to 0.8. Concerning the applicability of this new tool, the ciliary beating parameters could be measured in 68 out of 84 (81%) nasal brushing samples. When these parameters were present, it was possible to measure the CBE index in 49 out of 68 (72.1%) samples.

While CBF is the historical and classical parameter used to evaluate ciliary beating ⁽⁶⁾, it did not show up in our study as an important parameter to differentiate primary diffuse CRS from

control patients. CBF is reflecting one very circumscribed aspect of ciliary function and CBE a much broader one and that CBF is a “subdomaine” of CBE.

The CBE appears as a new promising index to evaluate the potential effects of any ciliary dysfunction on the mucociliary clearance process in all forms of CRS. However, further studies in larger cohorts are required to specify the contribution of the CBE Index, when considering i) the different types of primary diffuse CRS, ii) the understanding of primary diffuse non-type2 CRS physiopathology (chronic purulent diffuse idiopathic rhinosinusitis excluding PCD, cystic fibrosis, immunodeficiency) iii) the severity or the evolution under treatment of these diseases.

List of abbreviations

AERD: Aspirin-exacerbated respiratory disease, CBE: Ciliary beating efficiency, CBF: Ciliary beating frequency, CPs: Control patients, CRS: Chronic rhinosinusitis, HSDV: High-speed digital video microscopy

Authorship contribution

MR, EB : Conception, Design, Acquisition, Analysis; Drafting the work; AV, VF, , RMF, JFP: Drafting the work; EE, AC, MF, BL: Analysis; Drafting the work; All authors gave final approval of the version to be published.

Conflict of interest

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SUPPLEMENTARY MATERIAL

Materials and methods

Study population

This multicentric prospective study included adult patients with primary diffuse chronic rhinosinusitis, according to the classification in the EPOS 2020 ⁽¹⁾, and control patients (CPs) over a seven-months-period. All patients with primary diffuse CRS having a surgical indication were investigated for ex-vivo ciliary function analysis. The CPs were considered as free of primary diffuse CRS, according to clinical history, nasal endoscopy and CT-scan. They required an indication of endonasal surgery under general anesthesia for architectural nasal obstruction (e.g., septal deviation and/or inferior turbinate hypertrophy) or for unilateral sinonasal pathology (e.g., mucoceles, cerebrospinal fluid leak, skull base defect, localized inflammatory or infectious –bacterial of fungal–sinusitis). Patients with a diagnosis of PCD were excluded from the study. Informed consent was obtained from all patients and the study was approved by the Ethics Committee (CPP IDF X 2016-01-01 and CPP IDF VII 2019-A00973-54). Intrinsic and extrinsic factors that could modify the ciliary beating were collected for each patient: tobacco, asthma (i.e., according to the GINA definition of asthma ⁽²⁾), respiratory allergies, corticosteroid treatment, aspirin intolerance, aspirin exacerbated respiratory disease (AERD, according to the definition of Navarro et al. ⁽³⁾) and active sinonasal infection.

Samples of nasal epithelial cells

At the beginning of the surgical procedure under general anesthesia, a nasal brushing was performed at the middle part of the inferior turbinate with a 2 mm cytology brush (Laboratoires Gyneas, Goussainville, France) to obtain nasal epithelial cells. The nasal fossa was previously prepared with a local preparation using cotton balls with lidocaine and naphazoline. The nasal cells obtained were then suspended in a specific transport medium (DMEM-HAMF12-Penicillin-Streptomycin-Fungizone-Gentamycin) and transported to the laboratory to be examined within 3 hours. In CPs with architectural nasal obstruction, nasal brushing was performed on the side without septal deviation. In CPs with unilateral pathology, epithelial cells were sampled on the side opposite to the disease. In primary diffuse CRS patients, only in case of nasal purulent secretions, an additional sample was taken with a sterile swab, under endoscopic guidance, for bacteriological analysis.

Digital high-speed video-microscopy

In the laboratory, all ex-vivo ciliary function analyses were performed at controlled room temperature (20 – 25°C). An inverted brightfield microscope was used at ×40 magnification. 20 µL of 4.5 µm polystyrene micro-beads (Polybead® Microspheres,

Polysciences, Inc., Warrington, PA, USA) at the concentration of 0.125%w/v were added to 80 µL of the medium containing beating ciliated cells in suspension. 100 µL of this mix medium were comprised between a microscope slide and a cover slide. Ciliary beating of the samples was recorded with a digital camera (PixeLINK® A741, Ottawa, Canada) at a rate of 358 frames/s. Each movie was composed of 1,800 frames with a definition of 256 × 192 pixels, each individual pixel being (0.32 × 0.32) µm². All areas containing intact undisrupted ciliated epithelial edges larger than 50 µm, beating in the plane of the camera were recorded. As recommended ⁽⁴⁾, isolated ciliated cells were excluded. For each microscopic analysis of the nasal cells, we first described the presence of ciliated cell clusters and the presence of motile cilia. To determine the percentage of beating ciliated edges, we identified the first 10 areas of ciliated edges and scored each one as follows: an epithelial edge with a majority of cilia beating was scored 10; an edge with half of cilia beating was scored 5 and an edge with a minority of cilia beating was scored 0. The percentage of beating ciliated edges was defined by the addition of the 10 scores of the first 10 edges (from 0 to 100). Then, 10 ciliated motile edges were recorded at ×40 magnification to analyze the ciliary beating parameters. In a third time, cilia length was measured (10 measurements) with a ×100 objective (pixel size 0.13 × 0.13 µm²).

Evaluation of the ciliary beating parameters and the ciliary beating efficiency index

All parameters were analyzed using in-house software (in Matlab™ platform) as previously described ^(5,6). The CBF was expressed in Hertz, the relative ciliary density in percentage, the metachronal wavelength and the length of cilia in micrometers. Microbeads were used as a marker of the flow generated by the ciliary beating, in order to evaluate the shear stress induced by cilia on the fluid (ciliary beating efficiency (CBE) index, in mPa). We evaluated the feasibility of these measurements for each parameter as the percentage of patients with successful analysis of nasal brushing samples, and the average required time to record movies and analyze the different parameters.

Statistical analysis

Chi-squared test was used to compare epidemiological and clinical characteristics between CRS patients and CPs. Mann-Whitney test was used for the comparison of ciliary beating parameters in the two groups. A p-value < 0.05 was considered as significant. These tests were performed with a statistical software package (Statistica v8; Stat Soft, France). The sensibility and specificity for each ciliary beating parameter was inferred and Receiver Operating Characteristic (ROC) curve and area under

curve (AUC) was used to determine the best discriminating parameter. An AUC=1 corresponds to a 100% sensitivity and specificity, while AUC=0.5 corresponds to a sensitivity equal to the false-positive rate. The cut-off using the Youden's index was determined for each parameter.

Flow chart

Nasal brushings from 84 patients (41 patients with primary diffuse CRS and 43 CPs) were obtained. No statistical differences were observed regarding age, sex, smoking habits, respiratory allergies, and corticosteroid treatment between primary diffuse CRS (n=3) patients and CPs. The main epidemiologic characteristics of the studied population are reported in Table 1. In primary diffuse CRS population, 31 patients had CRS with nasal polyps (CRSwNP), 8 had non eosinophilic CRS, 1 had allergic

fungal rhinosinusitis and 1 had central compartment allergic disease (Figure 1).

Fifty-six percent of primary diffuse CRS patients had underlying pulmonary disease including bronchiectasis (12.2%) and asthma (43.9%) and 22% of patients had AERD. Additionally, acute infection of upper airway was present at the time of surgery in 10 patients (24.4%) with primary diffuse CRS. In primary diffuse CRS population, bacteriological samples showed positive cultures with a single pathogen in 4 patients (i.e., *Haemophilus influenzae* n=2, *Pseudomonas aeruginosa* and n=1, *Streptococcus pneumoniae* n=1).

The analysis of the ciliary beating parameters was feasible in 30 primary diffuse CRS patients and in 38 control patients. Flow chart with details is shown in Figure 1.

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