Characterization of the ciliary beating efficiency in primary diffuse chronic rhinosinusitis

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Running title:
Ciliary efficiency in chronic rhinosinusitis

TYPE OF ARTICLE: LETTER TO THE EDITOR

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To the Editor:

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 (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 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 disfunction 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.

Word count: 627

References

1. Chen B, Shaari J, Claire SE, Palmer JN, Chiu AG, Kennedy DW, et al. Altered sinonasal ciliary dynamics in chronic rhinosinusitis. Am J Rhinol. 2006;20(3):325-9.
2. Stannard WA, Chilvers MA, Rutman AR, Williams CD, O'Callaghan C. Diagnostic testing of patients suspected of primary ciliary dyskinesia. Am J Respir Crit Care Med. 2010;181(4):307-14.
3. Bottier M, Blanchon S, Pelle G, Bequignon E, Isabey D, Coste A, et al. A new index for characterizing micro-bead motion in a flow induced by ciliary beating: Part I, experimental analysis. PLoS Comput Biol. 2017;13(7):e1005605.
4. Bottier M, Pena Fernandez M, Pelle G, Isabey D, Louis B, Grotberg JB, et al. A new index for characterizing micro-bead motion in a flow induced by ciliary beating: Part II, modeling. PLoS Comput Biol. 2017;13(7):e1005552.
5. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology. 2020;58(Suppl S29):1-464.
6. Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. Am J Respir Crit Care Med. 2013;188(8):913-22.

Table 1

	Control patients (n = 43)			Patients with CRS (n = 41)			
Characteristics	n	Percentage (%)	Mean +/- *SD*	n	Percentage (%)	Mean +/- *SD*	p
Age	43		54.9 +/- 18	41		50.6 +/- 13.8	0.21
Gender							0.67
Women	24	55.8%		21	51.2%		
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

LEGENDS FOR ILLUSTRATION

Figure 1: Flow Chart

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

LEGENDS FOR TABLE

Table 1 : Epidemiological characteristics of the study population (n=84). 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

LIST OF ABBREVIATION

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

KEYWORDS: *Diagnostic Techniques, Respiratory system, Chronic Rhinosinusitis,*

Rhinosinusitis, Ciliary motility disorders/diagnosis

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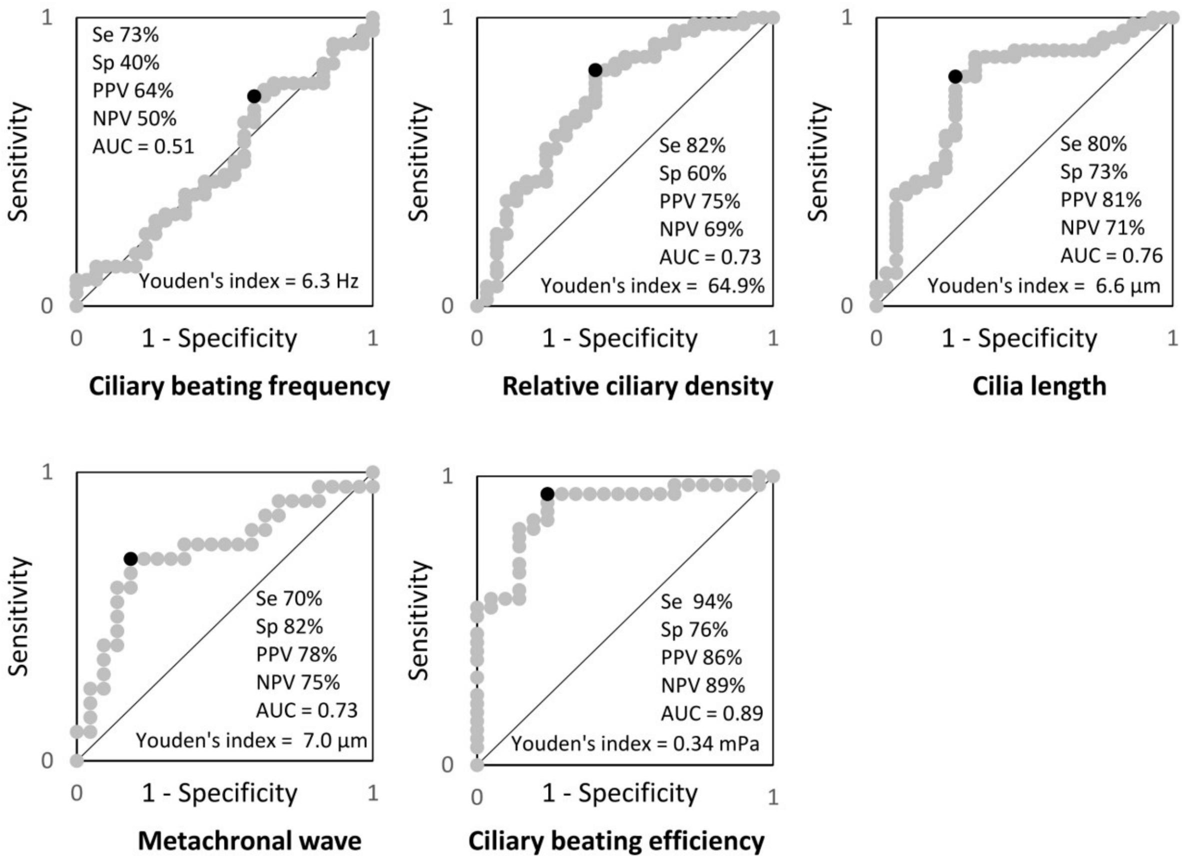
Emilie Bequignon: Conception, Design, Acquisition, Analysis; Drafting the work Final approval of the version to be published

CONFLICT OF INTEREST
no conflict of interest

FUNDING
None



1
Se 73%
Sp 40%
PPV 64%
NPV 50%
AUC = 0.51
Sensitivity
0
Youden's index = 6.3 Hz
0
1 - Specificity
1
Ciliary beating frequency
1
Se 82%
Sp 60%
PPV 75%
NPV 69%
AUC = 0.73
Sensitivity
0
Youden's index = 64.9%
0
1 - Specificity
1
Relative ciliary density
1
Se 80%
Sp 73%
PPV 81%
NPV 71%
AUC = 0.76
Sensitivity
0
Youden's index = 6.6 µm
0
1 - Specificity
1
Cilia length
1
Se 70%
Sp 82%
PPV 78%
NPV 75%
AUC = 0.73
Sensitivity
0
Youden's index = 7.0 µm
0
1 - Specificity
1
Metachronal wave
1
Se 94%
Sp 76%
PPV 86%
NPV 89%
AUC = 0.89
Sensitivity
0
Youden's index = 0.34 mPa
0
1 - Specificity
1
Ciliary beating efficiency

Figure 1

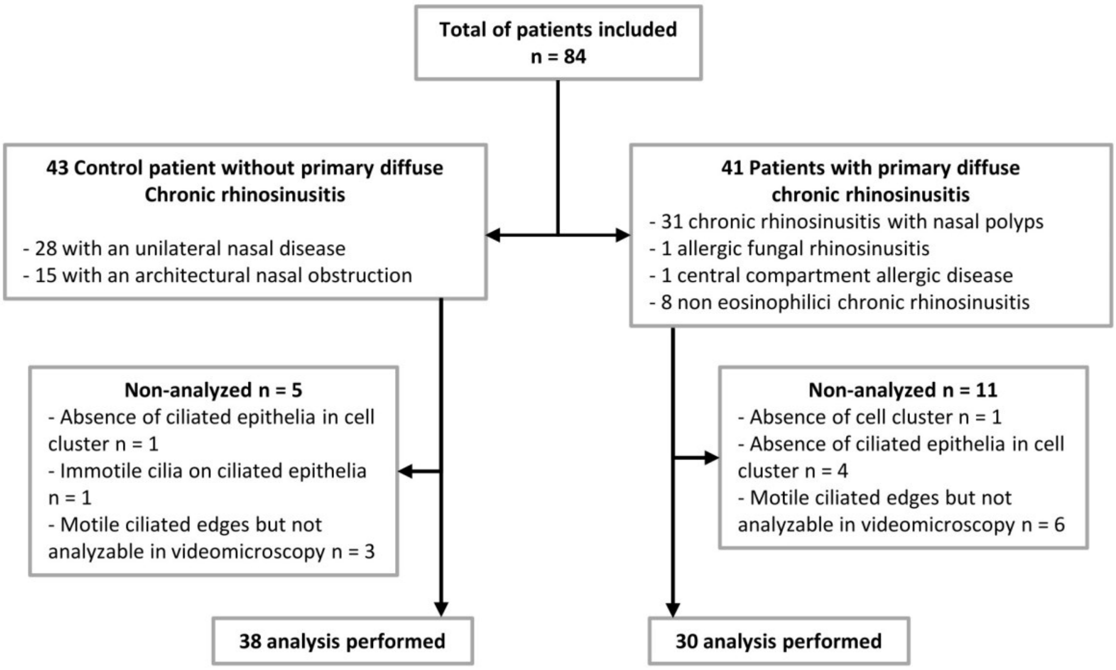

Total of patients included
n = 84
43 Control patient without primary diffuse Chronic rhinosinusitis
- 28 with an unilateral nasal disease
- 15 with an architectural nasal obstruction
41 Patients with primary diffuse chronic rhinosinusitis
- 31 chronic rhinosinusitis with nasal polyps
- 1 allergic fungal rhinosinusitis
- 1 central compartment allergic disease
- 8 non eosinophilici chronic rhinosinusitis
Non-analyzed n = 5
- Absence of ciliated epithelia in cell cluster n = 1
- Immotile cilia on ciliated epithelia n = 1
- Motile ciliated edges but not analyzable in videomicroscopy n = 3
Non-analyzed n = 11
- Absence of cell cluster n = 1
- Absence of ciliated epithelia in cell cluster n = 4
- Motile ciliated edges but not analyzable in videomicroscopy n = 6
38 analysis performed
30 analysis performed

Figure 2

Online supplementary material

Title: Characterization of the ciliary beating efficiency in primary diffuse chronic rhinosinusitis

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 x 0.13 µm²).

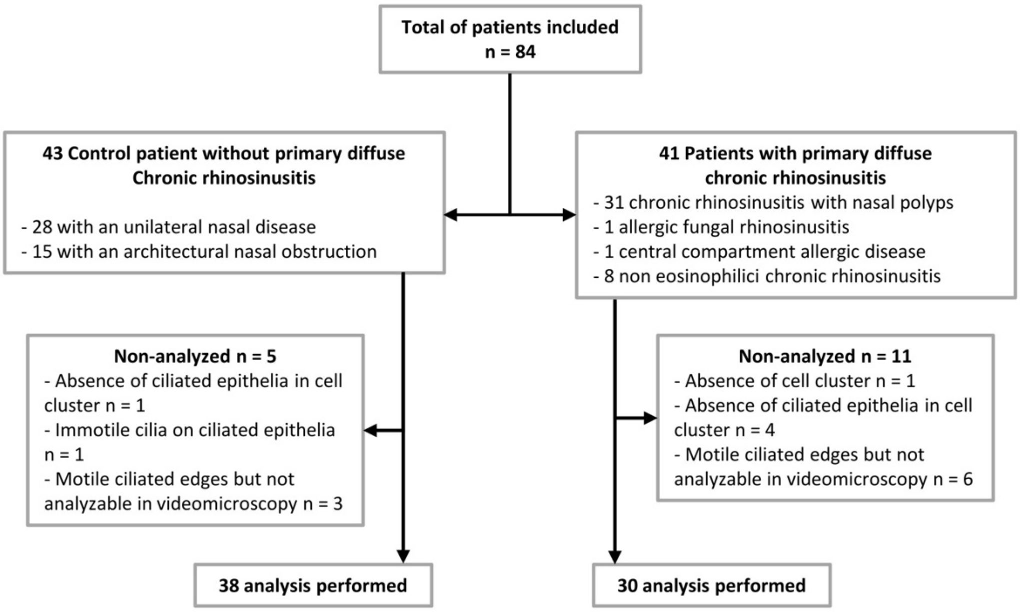
Evaluation of the ciliary beating parameters and the ciliary beating efficiency index
All parameters were analyzed using in-house software (in Matlab™ platform) as previously described.[5, 6] The CBF was express in Hertz, the relative ciliary density in percentage, the metachronal wavelength and the 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 showed in Figure S1



1. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology. 2020;58(Suppl S29):1-464.

2. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. available from: https://ginasthmaorg/wp-content/uploads/2023/05/GINA-Main-Report-2022-WMSApdf. 2022.

3. Navarro AM, Delgado J, Munoz-Cano RM, Dordal MT, Valero A, Quirce S, et al. Allergic respiratory disease (ARD), setting forth the basics: proposals of an expert consensus report. Clin Transl Allergy. 2017;7:16.

4. Thomas B, Rutman A, O'Callaghan C. Disrupted ciliated epithelium shows slower ciliary beat frequency and increased dyskinesia. Eur Respir J. 2009;34(2):401-4.

5. Bottier M, Blanchon S, Pelle G, Bequignon E, Isabey D, Coste A, et al. A new index for characterizing micro-bead motion in a flow induced by ciliary beating: Part I, experimental analysis. PLoS Comput Biol. 2017;13(7):e1005605.

6. Bottier M, Pena Fernandez M, Pelle G, Isabey D, Louis B, Grotberg JB, et al. A new index for characterizing micro-bead motion in a flow induced by ciliary beating: Part II, modeling. PLoS Comput Biol. 2017;13(7):e1005552.