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Characterization of the ciliary beating efficiency in primary diffuse chronic rhinosinusitis

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1 *Running title:*
2 *Ciliary efficiency in chronic rhinosinusitis*

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4 TYPE OF ARTICLE: LETTER TO THE EDITOR

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

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10 **To the Editor:**

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

34 In the 84 included subjects whose main epidemiological characteristics are reported in
35 table 1, the mean ciliary beating efficiency (CBE) index was significantly lower
36 ($p < 0.001$) in the primary diffuse CRS group (0.27 +/- 0.15 mPa) than in the control
37 patient group (0.66 +/- 0.30 mPa). The CBF did not significantly differ between the two
38 groups while the other tested parameters were significantly lower in the primary CRS
39 group compared to the control patient group. The ROC curves of all studied parameters
40 are presented in Figure 2. We found the CBE index to be the best discriminating
41 parameter with a sensitivity of 94%, a specificity of 76% for a cut-off-value of 0.34
42 mPa, and an AUC of 0.9 while the frequency showed a sensitivity of 73% and
43 specificity of 40% (AUC of 0.51). Relative ciliary density, cilia length, and metachronal
44 wave were less discriminating than the CBE index, but more discriminating than the
45 CBF with an AUC ranging from 0.7 to 0.8. Concerning the applicability of this new
46 tool, the ciliary beating parameters could be measured in 68 out of 84 (81%) nasal
47 brushing samples. When these parameters were present, it was possible to measure the
48 CBE index in 49 out of 68 (72.1%) samples.

49 While CBF is the historical and classical parameter used to evaluate ciliary beating⁽⁶⁾,

50 it did not show up in our study as an important parameter to differentiate primary diffuse
51 CRS from control patients. CBF is reflecting one very circumscribed aspect of ciliary
52 function and CBE a much broader one and that CBF is a “subdomaine” of CBE.
53 The CBE appears as a new promising index to evaluate the potential effects of any
54 ciliary dysfunction on the mucociliary clearance process in all forms of CRS. However,
55 further studies in larger cohorts are required to specify the contribution of the CBE
56 Index, when considering i) the different types of primary diffuse CRS, ii) the
57 understanding of primary diffuse non-type2 CRS physiopathology (chronic purulent
58 diffuse idiopathic rhinosinusitis excluding PCD, cystic fibrosis, immunodeficiency) iii)
59 the severity or the evolution under treatment of these diseases.

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61 Word count: 627

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Table 1

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							0.67
<i>Women</i>	24	55.8%		21	51.2%		
<i>Men</i>	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

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103 LEGENDS FOR ILLUSTRATION

104 Figure 1: Flow Chart

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

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110 LEGENDS FOR TABLE

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112 Table 1 : Epidemiological characteristics of the study population (n=84). CRS :
113 Chronic rhinosinusitis; SD : standard deviation ; AERD : Aspirin-exacerbated
114 respiratory disease. *Oral corticosteroids = the patient received oral corticosteroids
115 less than a week before nasal brushing

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117 LIST OF ABBREVIATION

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119 AERD: Aspirin-exacerbated respiratory disease

120 CBE: Ciliary beating efficiency

121 CBF: Ciliary beating frequency

122 CPs: Control patients

123 CRS: Chronic rhinosinusitis

124 HSDV: High-speed digital video microscopy

125

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

127 *Rhinosinusitis, Ciliary motility disorders/diagnosis*

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178 Final approval of the version to be published

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180 CONFLICT OF INTEREST

181 no conflict of interest

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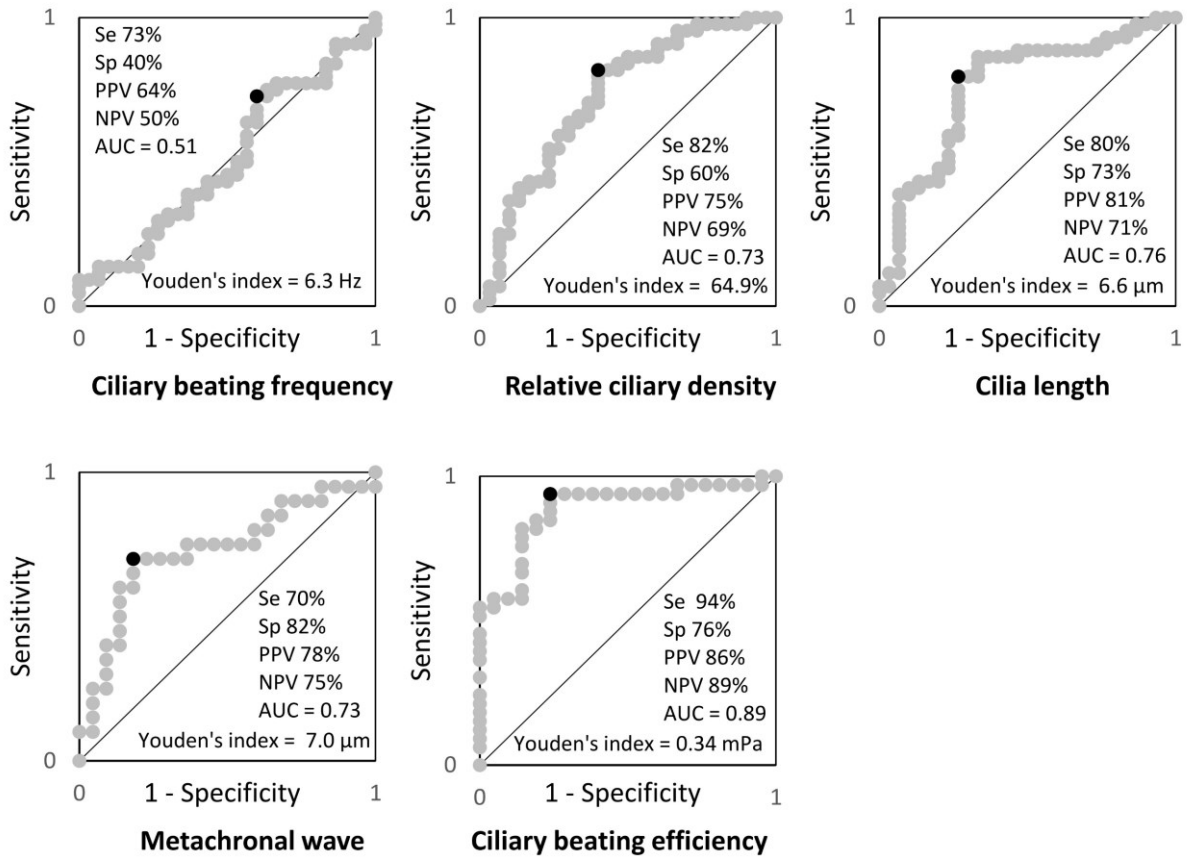


Figure 1

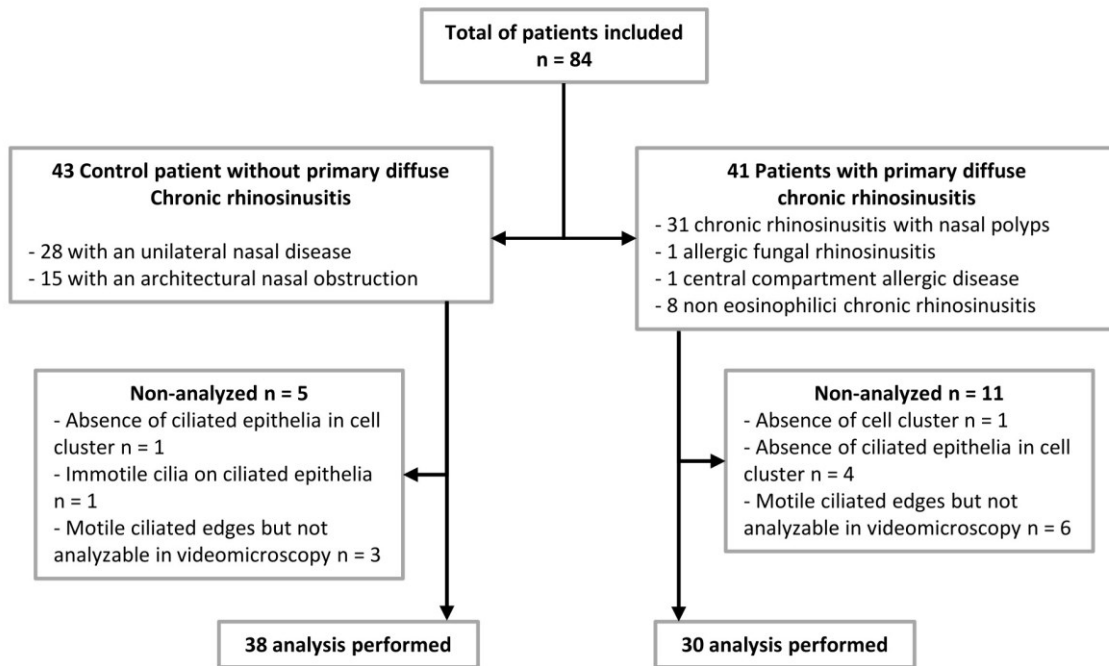


Figure 2

1 ***Online supplementary material***

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

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7 MATERIALS AND METHODS

8 *Study population*

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

26 *Samples of nasal epithelial cells*

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

39 *Digital high-speed video-microscopy*

40 In the laboratory, all ex-vivo ciliary function analyses were performed at controlled
41 room temperature (20 – 25°C). An inverted brightfield microscope was used at ×40
42 magnification. 20 µL of 4.5 µm polystyrene micro-beads (Polybead © Microspheres,
43 Polysciences, Inc., Warrington, PA, USA) at the concentration of 0.125%w/v were
44 added to 80 µL of the medium containing beating ciliated cells in suspension. 100 µL
45 of this mix medium were comprised between a microscope slide and a cover slide.
46 Ciliary beating of the samples was recorded with a digital camera (PixeLINK® A741,
47 Ottawa Canada) at a rate of 358 frames/s. Each movie was composed of 1,800 frames
48 with a definition of 256 × 192 pixels, each individual pixel being (0.32 × 0.32) µm².

49 All areas containing intact undisrupted ciliated epithelial edges larger than 50 μm ,
50 beating in the plane of the camera were recorded. As recommended,⁽⁴⁾ isolated ciliated
51 cells were excluded. For each microscopic analysis of the nasal cells, we first described
52 the presence of ciliated cell clusters and the presence of motile cilia. To determine the
53 percentage of beating ciliated edges, we identified the first 10 areas of ciliated edges
54 and scored each one as follows: an epithelial edge with a majority of cilia beating was
55 scored 10; an edge with half of cilia beating was scored 5 and an edge with a minority
56 of cilia beating was scored 0. The percentage of beating ciliated edges was defined by
57 the addition of the 10 scores of the first 10 edges (from 0 to 100). Then, 10 ciliated
58 motile edges were recorded at $\times 40$ magnification to analyze the ciliary beating
59 parameters. In a third time, cilia length was measured (10 measurements) with a $\times 100$
60 objective (pixel size $0.13 \times 0.13 \mu\text{m}^2$).

61

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

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

72

73 *Statistical analysis*

74 Chi-squared test was used to compare epidemiological and clinical characteristics
75 between CRS patients and CPs. Mann-Whitney test was used for the comparison of
76 ciliary beating parameters in the two groups. A p-value < 0.05 was considered as
77 significant. These tests were performed with a statistical software package (Statistica
78 v8; Stat Soft, France). The sensibility and specificity for each ciliary beating parameter
79 was inferred and Receiver Operating Characteristic (ROC) curve and area under curve
80 (AUC) was used to determine the best discriminating parameter. An AUC=1
81 corresponds to a 100% sensitivity and specificity, while AUC=0.5 corresponds to a
82 sensitivity equal to the false-positive rate. The cut-off using the Youden's index was
83 determined for each parameter.

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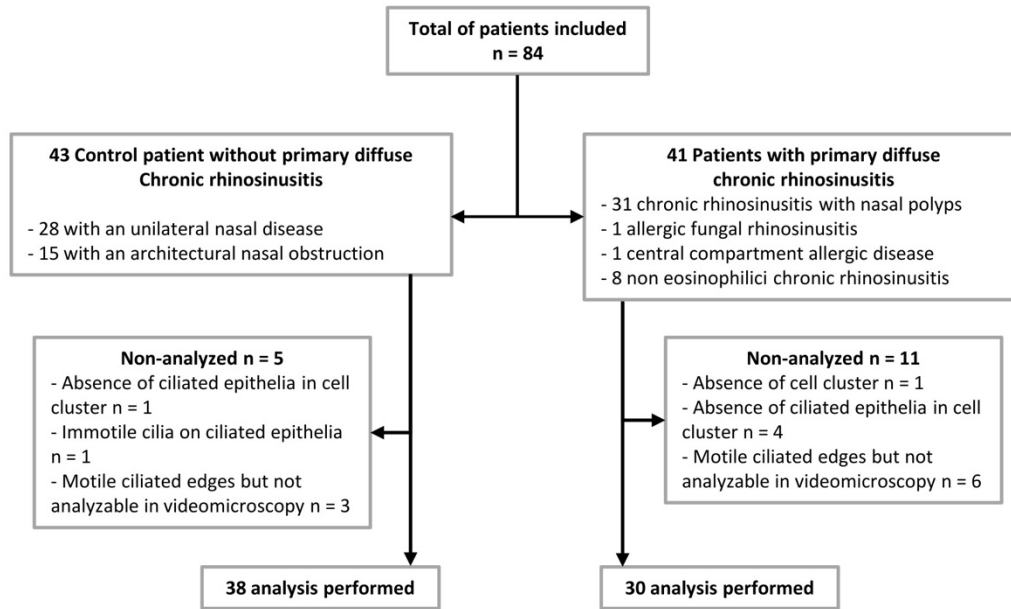
86 FLOW CHART

87 Nasal brushings from 84 patients (41 patients with primary diffuse CRS and 43 CPs)
88 were obtained. No statistical differences were observed regarding age, sex, smoking
89 habits, respiratory allergies, and corticosteroid treatment between primary diffuse CRS
90 (n=3) patients and CPs. The main epidemiologic characteristics of the studied
91 population are reported in Table 1.

92 In primary diffuse CRS population, 31 patients had CRS with nasal polyps (CRSwNP),
93 8 had non eosinophilic CRS, 1 had allergic fungal rhinosinusitis and 1 had central
94 compartment allergic disease (Figure 1).

95 Fifty-six percent of primary diffuse CRS patients had underlying pulmonary disease
96 including bronchiectasis (12.2%) and asthma (43.9%) and 22% of patients had AERD.
97 Additionally, acute infection of upper airway was present at the time of surgery in 10
98 patients (24.4%) with primary diffuse CRS. In primary diffuse CRS population,

99 bacteriological samples showed positive cultures with a single pathogen in 4 patients
 100 (i.e., *Haemophilus influenzae* n=2, *Pseudomonas aeruginosa* and n=1, *Streptococcus*
 101 *pneumoniae* n=1).
 102 The analysis of the ciliary beating parameters was feasible in 30 primary diffuse CRS
 103 patients and in 38 control patients. Flow chart with details is showed in Figure S1
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