Ciliary dyskinesia in severe asthma is not affected by chronic mucus hypersecretion

To the Editor:

Mucociliary clearance (MCC) results in an effective interaction between the mucus layer and the normal coordinated ciliary beating [1]. Ciliary dyskinesia, or abnormal ciliary beating, can be primary, or secondary to chronic infection or inflammation [2].

MCC impairment is well documented in asthma [3], a chronic inflammatory disease of the airways. Mucus alterations in asthma have been described, including a greater viscosity and modification of mucins composition [3, 4]. Respiratory cilia have been poorly studied in asthma but THOMAS *et al.* [5] demonstrated ciliary ultrastructural defects in severe asthma, and a ciliary dyskinesia in moderate and severe asthma.

Chronic cough and phlegm are reported as chronic mucus hypersecretion (CMH) [6]. In asthma, CMH is associated with increased severity [4] and recent data support a causal role for mucus plugs in the mechanism of airflow obstruction in severe asthma [7]. However, the mechanism of CMH in asthma is not well understood and it is unknown whether CMH is associated with an increased ciliary dyskinesia. In this study, we compared ciliary function in severe asthma with or without CMH.

This was a single-centre, cross-sectional study of a series of 20 adults (>18 years old) with severe asthma (according to European Respiratory Society/American Thoracic Society criteria [8]) recruited from the University Asthma Clinic of Liege between 1 January 2021 and 5 May 2021, and of 17 adult healthy controls.

We selected 10 severe asthmatics with CMH and 10 without, matched for age (mean±SD 55.8±10.3 *versus* 54.1±19.4 years, respectively; p=0.80). CMH was defined as cough and phlegm from the chest on most days for ⩾3 consecutive months, for ⩾2 consecutive years [6]. Exclusion criteria included active smoking, respiratory tract infection or oral corticosteroid use during the previous 4 weeks, and inhaled or nasal medication during the previous 24 h. Ciliated epithelial samples were obtained by nasal brushing and beating cilia were recorded using digital high-speed videomicroscopy (DHSV) at 37°C, as previously reported [9]. Ciliary function was assessed by ciliary beat frequency (CBF) and the percentage of abnormal beat pattern (CBP). The video sequences of beating cilia were reviewed at a reduced speed and the path taken by a cilium or a group of cilia during a complete beat cycle was compared with the normal beat pattern observed using DHSV. The observed movement was categorised into five distinct CBPs (normal, immotile, stiff, circular and asynchronous) [2]. For each cilium or group of cilia analysed, CBF was calculated and a distinct CBP was attributed. The percentage of abnormal CBPs was calculated ((number of abnormal CBP/total number of CBP readings for the sample) × 100)) [9]:

$$\text{Abnormal CBPs \%} = \frac{\text{Number of abnormal CBPs}}{\text{Total number of CBP readings for the sample}} \times 100$$

Ciliary dyskinesia was defined as an abnormal CBF and/or CBP.

Demographic data, quality of life and asthma control were obtained in all severe asthmatic patients by questionnaires and medical chart review. Patients underwent spirometry, exhaled nitric oxide fraction (F_{ENO}) and nasal NO (nNO) measurement (breath-hold technique) (NIOX; Aerocrine, Sweden). Sputum was induced and processed as previously described [10].
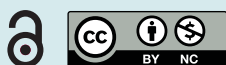
Shareable abstract (@ERSpublications)
Chronic mucus hypersecretion (CMH) is linked to increased asthma severity. Ciliary dyskinesia is present in severe asthma but CMH was not associated with a worse ciliary dysfunction, suggesting another mechanism to explain chronic cough and phlegm. https://bit.ly/3JNUgGr

Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, Inc.). Data were presented as mean±SD for continuous variables, median (range) for skewed distributions and n (%) for categorical variables. Comparisons used the t-test for parametric data, the Mann–Whitney test for nonparametric data, and the Fisher test for categorical variables. The study was approved by the ethics committee of CHU Liege (references 2020-174 and 2009-161).

Our results confirm that ciliary dyskinesia is increased in adults with severe asthma (n=20, age 55.0 ±15.2 years) compared with healthy adults (n=17, age 23.5±7.2 years), with a lower CBF (11.9±2.1 versus 14.2±2.3 Hz, p=0.002) and a higher percentage of abnormal CBPs (32.8% (15.4–55.3%) versus 17.9% (11.3–23.7%), p=0.005).

However, when comparing ciliary function between severe asthmatics with or without CMH, there was no significant difference in CBF (11.8±2.1 versus 11.9±2.2 Hz, p=0.89) or in the percentage of abnormal CBPs (30.3% (14.6–47.9%) versus 36.2% (20.7–59.1%), p=0.53).

The demographics and functional characteristics of severe asthmatic patients with or without CMH are summarised in table 1. There was no statistical difference between the groups concerning gender, body mass index, age at onset of asthma, atopic status or comorbidities. The prevalence of bronchiectasis was similar whatever the secretion status and similar to what is reported in severe asthma [11]. In our cohort of

TABLE 1 Demographic, functional, clinical and inflammatory characteristics of severely asthmatic adults with or without chronic mucus hypersecretion

| | Adults with severe asthma | | |
	With chronic mucus hypersecretion	Without chronic mucus hypersecretion	p-value
Patients	n=10	n=10	
Males	n=5	n=4	>0.99
Age, years	55.8±10.3	54.1±19.4	0.81
Age of onset, years	12.5 (5.5–44.3)	20.0 (4.0–47.5)	0.90
BMI, kg·m^{-2}	28.3±4.2	28.3±3.4	0.97
Atopy	60%	60%	>0.99
Biologic therapy	n=8	n=9	>0.99
Anti-IL-5	n=7	n=6	0.58
Anti-IgE	n=0	n=3	0.21
Anti-IL-4/13	n=1	n=0	0.47
FEV$_1$, % pred	77.3±24.0	69.7±20.4	0.46
FVC, % pred	79.1±19.7	80.9±16.5	0.83
FEV$_1$/FVC, %	75.6±9.6	67.7±12.0	0.12
$F_{ENO_{50}}$, ppb	29.5 (15.5–47.0)	38.5 (30.3–60.8)	0.23
nNO, nL·min^{-1}	169.9±105.2	199.4±80.8	0.49
Sputum inflammation	n=9	n=4	0.06
Eosinophils, %	5.0 (0.7–7.8)	4.3 (0.5–10.3)	0.85
Neutrophils, %	72.8 (58.5–81.6)	69.6 (50.0–86.7)	0.94
Blood eosinophils per mm^3	n=8	n=10	0.47
	120 (63–658)	50 (8–175)	0.14
ACT	16.3±6.4	20.2±3.7	0.11
ACQ	1.50 (1.00–4.03)	1.36 (1.25–2.14)	0.64
AQLQ	4.58±1.57	5.47±1.04	0.15
Chronic rhinitis	70%	60%	>0.99
Chronic rhinosinusitis	60%	50%	>0.99
Nasal polyps	30%	50%	0.65
Bronchiectasis	10%	10%	>0.99

Data are presented as mean±SD for continuous variables or median (range) for variables with a skewed distribution, unless otherwise stated. Comparisons between groups used the t-test for parametric data, the Wilcoxon–Mann–Whitney test for nonparametric data and the Fisher test for categorical variables. A p-value of <0.050 was taken as the threshold for statistical significance. BMI: body mass index; IL: interleukin; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; $F_{ENO_{50}}$: exhaled nitric oxide fraction measured at a flow rate of 50 mL·s^{-1}; nNO: nasal nitric oxide; ACT: Asthma Control Test; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire.

severe asthmatics, CMH was associated with a lower control and quality of life, but the difference was not statistically different. Type-2 biomarkers (F_{ENO}, sputum and blood eosinophils) were not significantly different between both groups. Mean nNO (185 ± 93 nL·min^{-1}) was not significantly different between the CMH and non-CMH groups.

In conclusion, our study confirms a higher ciliary dyskinesia in severe asthma, compared with healthy subjects. Moreover, CMH was not associated with a worse ciliary dysfunction in severe asthma.

Ciliary beating was evaluated using DHSV, a technique used to diagnose primary ciliary dyskinesia (PCD), a rare genetic disease characterised by abnormal ciliary function. DHSV has a high sensitivity and specificity to diagnose PCD when performed by experienced scientists following standardised protocols [12]. However, recent PCD diagnosis guidelines state that CBP and MCC measurements are required, as CBF alone is not sufficient [13]. Furthermore, CBP might help to confirm the pathogenicity of variants in PCD-causing genes but DHSV is not standardised across centres in order to be used as a confirmatory diagnostic test alone [13]. Interestingly, while nNO is decreased in PCD, nNO was within the normal range [14] in our severe asthmatic population.

Previous studies have described MCC impairment in asthma [3, 5] and our results confirm that ciliary beating is abnormal in adults with severe asthma. CBF was significantly lower in our severe asthmatic population than in our healthy controls but within previous published normal values [1]. One limitation of our study is the age difference between the healthy and severe asthma groups. This might explain the lower CBF in the severe asthma group but not the higher percentage of abnormal CBPs, as previous data described a CBF decrease with age from infancy to adulthood [15, 16], but also a decreased dyskinesia after 19 years of age [16].

Our understanding of CMH in different respiratory diseases remains limited [4]. 25–50% of asthmatic patients report chronic sputum production [4, 17] that may sometimes be attributed to post-nasal drips in patients combining asthma and chronic rhinosinusitis. However, in our cohort, the prevalence of rhinosinusal comorbidities was similar in the CMH and non-CMH groups, and higher than previously reported in literature [11].

CMH in asthma has been linked with a higher disease burden: a greater airflow obstruction, a lower quality of life, a poor asthma control and an increased exacerbation rate [4, 17, 18]. However, the physiopathology of CMH in asthma has poorly been studied. To our knowledge, this is the first study aiming to evaluate the relationship between CMH and ciliary function in severe asthma. Our results do not support that CMH in asthma is linked to increased ciliary dyskinesia, suggesting that another mechanism explains chronic cough and phlegm. There are few data regarding the association between CMH and the composition of mucus in asthma. Previous studies suggest a link between CMH and Th2 inflammation in asthma, with a higher blood and bronchoalveolar lavage eosinophilia [18], but in our small cohort, we did not find any significant difference in type-2 biomarkers. One limitation of our study is that most patients received biologic therapies at inclusion, mainly interleukin (IL)-5, which might modify the severity of the disease [19] as well as the relationship between type-2 biomarkers and CMH. Furthermore, diverse cytokines influence ciliary function. In particular, it has been shown that IL-5 leads to a CBF increase *in vitro* [20]. It would be interesting to evaluate ciliary function in severe asthmatics not treated with biologic therapies, to evaluate whether this modifies ciliary beating *in vivo*. To understand the mechanisms of abnormal ciliary beating in asthma, it would also be interesting to study ciliary beating in non-severe asthma and after cell culture of respiratory ciliated epithelium.

In summary, our results confirm an abnormal ciliary beating in severe asthma and suggest that CMH in asthma is not explained by an increased ciliary dyskinesia. The next step is to understand the pathophysiology of ciliary dyskinesia in asthma, which might be innate, or linked to chronic infection or inflammation.

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