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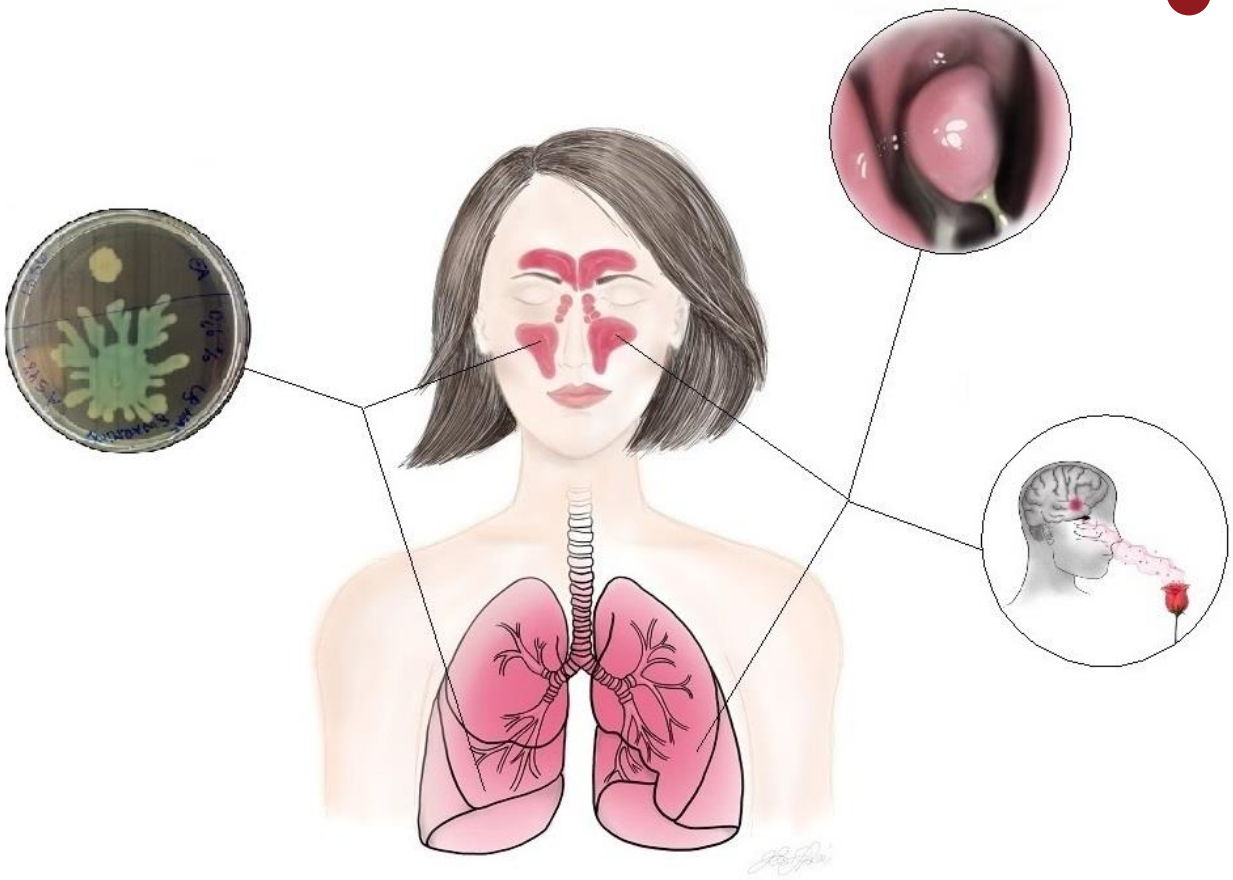

PhD thesis

Elisabeth Arndal, MD

Global airway disease in patients with primary ciliary dyskinesia and chronic obstructive pulmonary disease
- paranasal sinuses meet lungs.

Supervisors: Professor Christian von Buchwald and Professor Vibeke Backer.
This thesis has been submitted to the Graduate School of Health and Medical Sciences, University of Copenhagen 8th May 2021

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ABBREVIATIONS:

CAT	COPD assessment test
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
CRS	Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis without nasal polyps
CRSwNP	Chronic rhinosinusitis with nasal polyps
CT	Computer tomography
EPOS	European position paper on rhinosinusitis and nasal polyps
ESS	Endoscopic sinus surgery
FEV1	Forced expiratory volume in the first second
GOLD	Global initiative for chronic obstructive lung disease
HRCT	High-resolution computer tomography
HRQoL	Health-related quality of life
ID	Identification score
ICS	Inhaled corticosteroids
LM	Lund-Mackay score
MCC	Mucociliary clearance
MRC	Medical research council dyspnea scale
mMRC	Modified Medical research council dyspnea scale
MRI	Magnetic resonance imaging
ORL	Otorhinolaryngology
PA	*Pseudomonas aeruginosa*
PCD	Primary ciliary dyskinesia
PNIF	Peak nasal inspiratory flow
PROM	Patient-reported outcome measure
SIT16	Sniffin' Sticks Identification Test – 16

SNOT22 Sinonasal outcome test – 22

TDI Threshold, Discrimination, and Identification test

LIST OF PAPERS: (See appendix for full text).

Paper I:

Arndal E, Johansen HK, Haagensen JAJ, Bartel JA, Marvig RL, Alanin M, Aanæs K, Høiby N. Nielsen KG, Backer V, von Buchwald C. Primary ciliary dyskinesia patients have the same *P. aeruginosa* clone in sinuses and lungs. Eur Respir J. 2020 Jan 16;55(1). pii: 1901472. doi: 10.1183/13993003.01472-2019.

Paper II:

Arndal E, Sørensen AL, Lapperre TS, Said N; Trampedach CR, Aanæs K, Alanin M, Christensen KB, Backer V, von Buchwald C. Chronic rhinosinusitis in COPD: a prevalent but unrecognised comorbidity, impacting HRQoL. Respir Med. 2020 Aug 12;171:106092. Online ahead of print. doi:10.1016/j.rmed.2020.106092. PMID: 32846336

Paper III:

Arndal E, Sørensen AL, Christensen KB, Aanæs K, Backer V, Hummel T, von Buchwald C. COPD patients have a high prevalence of anosmia. *Submitted.*

ENGLISH SUMMARY:

In this thesis, we present our studies of global airway disease in patients suffering from chronic airways diseases, primary ciliary dyskinesia (PCD) and chronic obstructive pulmonary disease (COPD). Global airway disease describes the concept where infection, inflammation, and disease in the upper airways (nose and paranasal sinuses) impact or even induce infection, inflammation and disease in the lower airways (bronchia and lungs) and vice versa. We chose to study patients with PCD and COPD as they have decreased mucociliary clearance making them vulnerable to airway inflammation and exacerbations. They suffer from multiple comorbidities, affecting not only their quality of life but also their survival.

In Paper I we performed genotypic and phenotypic analyses of 38 *Pseudomonas aeruginosa* (PA) isolates collected from the paranasal sinuses and lungs of nine patients with PCD chronically infected with PA undergoing endoscopic sinus surgery (ESS) at our institution. We are the first to demonstrate that each patient has the same genotypic and phenotypic PA clone type in their upper and lower airways. Their sinuses can act as a bacterial reservoir, harbouring the same PA clone even years after pulmonary eradication, thus enabling reinfection of the lungs.

In Paper II, we investigated the prevalence of chronic rhinosinusitis (CRS) in 222 patients with COPD attending the COPD out-patient clinic at Bispebjerg Hospital. All patients were diagnosed according to the Global Initiative for Chronic Obstructive Lung disease 2019 (GOLD) and the European position paper on rhinosinusitis and nasal polyps 2020 (EPOS) criteria. The lower airways were evaluated by a pulmonologist and the upper airways by an otorhinolaryngologist. We found that 22.5 % (n=50) of patients with COPD suffer from CRS, of whom 82 % (n=41) were undiagnosed and untreated prior to our study. The predominant phenotype in COPD is CRS without nasal polyps (CRSsNP) (96 %), with only 4 % having CRS with nasal polyps (CRSwNP). Patients with COPD and CRS have a significantly worse health-related quality of life (HRQoL), Sinonasal outcome test (SNOT22), SNOT22-nasal symptoms subscore and COPD assessment test (CAT)) compared to patients with COPD without CRS and healthy controls. The SNOT22_nasal symptom subscore (including only nasal questions) is better than the total SNOT22 score at identifying patients with COPD who are at risk of having CRS. Multiple logistic regression analysis identified the following patients with COPD as having the highest risk of concomitant CRS: an active smoking man who uses inhaled steroids and has a high CAT and a high SNOT22_nasal symptoms subscore.

In Paper III, our screening of the olfactory function in 135 patients with COPD revealed a significantly higher prevalence of anosmia (14.1 %) than healthy controls (1.4 %) regardless of age, CRS, smoking status, and GOLD status. The high prevalence of anosmia was paralleled with a low prevalence of hyposmia and a normal level of normosmia. These results were poorly associated with patients' reply to the EPOS criteria about affected olfactory function, their grading of their smell and taste function in the SNOT22 and their olfactory (SIT16) test score.

This thesis supports global airway disease in patients with PCD and COPD, where the sinuses in patients with PCD can act as reservoirs wherefrom bacteria may reinfect the global airways. Global airway symptoms are seldom recorded in patients with COPD, requiring that physicians specifically inquire about these symptoms. Until a global airway disease, patient-reported outcome measure (PROM) has been developed the existing SNOT22_NS subscore and the CAT are short, easy to use PROMs. These PROMS will help identify patients at risk of global airways disease and should increase referral for diagnostics and treatment, preferably by a multidisciplinary unified team consisting of otorhinolaryngologists and pulmonologists.

DANISH SUMMARY (DANSK RESUME):

I denne ph.d. præsenterer vi vores studier af sygdom i de forenede luftveje hos patienter med primær cilie dyskinesi (PCD) og kronisk obstruktiv lungesygdom (KOL). Viden om sygdom i de forenede luftveje fokuserer på hvordan infektion, inflammation og sygdom i de øvre luftveje (næse-bihuler) kan påvirke og måske endda medføre inflammation, infektion og sygdom i de nedre luftveje (bronkier og lunger) og omvendt. Vi valgte at fokusere på patienter med de kronisk luftvejslidelser PCD og KOL, da de begge har nedsat mukociliær transport, hvilket øger deres sårbarhed overfor luftvejsinflammation og eksacerbationer. Den øgede infektionsrisiko sammenholdt med deres høje niveau af komorbiditet påvirker deres livskvalitet men også potentielt deres overlevelse.

I artikel I udførte vi genotypiske og fænotypiske analyser af 38 *Pseudomonas aeruginosa* (PA) isolater fra bihuler og lunger fra 9 kronisk PA inficerede patienter med PCD. Prøverne er indsamlet under endoskopisk næse-bihule kirurgi i vor afdeling. Som de første nogensinde demonstrerer vi, at hver patient har den samme genotypiske og fænotypiske PA-klon type i både deres øvre og deres nedre luftveje. Patientens bihuler formodes at fungere som et bakteriereservoir, hvori den samme klon kan overleve i årevis og muliggøre reinfektion af lungerne.

I artikel II undersøgte vi prævalensen af kronisk rhinosinuitis (CRS) hos 222 patienter med KOL under deres ambulante besøg på Lungemedicinsk afdeling, Bispebjerg Hospital. De øvre luftveje blev undersøgt af en otorhinolaryngolog og de nedre luftveje af en lungemediciner og klassificeret i henhold til de internationale retningslinjerne fra European position paper on rhinosinusitis and nasal polyps 2020 (EPOS) og Global Initiative for Chronic Obstructive Lung Disease 2019 (GOLD). Vi fandt, at 22,5 % (n=50) af patienter med KOL, led af CRS og 82 % (n=41) af disse var udiagnosticerede og ubehandlede forud for vores studie. CRS uden nasal polypose (CRSsNP) (96 %) er den dominante fænotype hos patienter med KOL, mens kun 4 % har CRS med nasal polypose (CRSwNP). Patienter med KOL og CRS har en signifikant dårligere sygdomsrelateret livskvalitet (Sinonasal outcome test (SNOT22), SNOT22_Nasal symptom subscore og COPD assessment test (CAT)) sammenlignet med patienter med KOL uden CRS og raske kontroller. SNOT22_nasal symptom subscoren er en undergruppen af spørgsmål i SNOT22, der kun er relateret til nasale symptomer. Denne undergruppe er bedre til at identificere patienter med KOL som er i risiko for at have CRS end den totale SNOT22. Multipel logistisk regressions analyser identificerede følgende

patient med KOL som havende en øget risiko for konkomitant CRS: En mandlig patient som ryger, bruger inhalations steroid og har en høj CAT og SNOT22_nasal symptom subscore.

I artikel III testede vi lugtesansen hos 135 patienter med KOL og fandt signifikant højere prævalens af anosmi (14.1 %) sammenlignet med raske kontroller (1.4 %) uanset alder, CRS, rygning og GOLD type og grad. Den høje prævalens af anosmi var ledsaget af en tilsvarende lav prævalens af hyposmi og normalt niveau af normosmi. Der var en manglende sammenhæng mellem patienternes svar på EPOS kriteriet om påvirket lugte- og smagssans, deres subjektive gradering af SNOT22 spørgsmålet omkring påvirket lugte- og smagssans og deres faktiske lugtesans test score (SIT16),

Vores studier understøtter forekomsten af sygdom i de forenede luftveje både hos patienter med PCD og KOL samt at bihulerne hos patienter med PCD kan fungere som et reservoir, hvorfra PA kan reinficere luftvejene og vice versa. Manglende afdækning af symptomer fra de forenede luftveje kræver, at læger og sygeplejersker bør spørge specifikt til symptomer fra de forenede luftveje. Indtil et forenet luftvejs spørgeskema er blevet udviklet er SNOT22_nasal symptom subscoren og CAT korte og let anvendelige spørgeskemaer, som kan hjælpe med at identificere patienter i risiko for at have sygdom i de forenede luftveje. En sådan identifikation er nødvendig for at sikre henvisning til relevant diagnostik og behandling af sygdom i de forenede luftveje. Dette kan med fordel udføres i en multidisciplinær klinik bestående af otorhinolaryngologer og lungemedicinere.

INTRODUCTION:

Despite being anatomically connected, the human airway is, in clinical practice, divided into an upper and a lower airway. Otorhinolaryngologists treat the upper respiratory tract diseases and pulmonologists treat the lower respiratory tract diseases. However, increasing evidence indicates that many patients suffer from simultaneous chronic inflammatory upper and lower airway disease with a reciprocal impact on disease severity. Therefore, the evaluation, treatment, and monitoring of patients with chronic airway disease are changing.

A problem in patients suffering from severe chronic lung disease known as cystic fibrosis (CF) increased awareness of this connection between disease in the upper and lower airways. Some of these patients with CF needed lung transplantation due to severe chronic infection with the challenging bacteria PA, which causes debilitating lung destruction. Devastatingly, post-transplantation cultures from the lungs showed regrowth of identical clonal PA in the new healthy lungs. How could that be?

The cause was global airway disease, where inflammation and disease in the upper airways (nose and paranasal sinuses) impact or even induce inflammation and disease in the lower airways (bronchia and lungs) and vice versa. Research from primary ciliary dyskinesia (PCD) and CF also revealed a bacterial reservoir in the paranasal sinuses wherefrom the bacteria may spread to the lungs (Figure 1). So, treating the paranasal sinuses may prevent lung (re)infection [1-4].

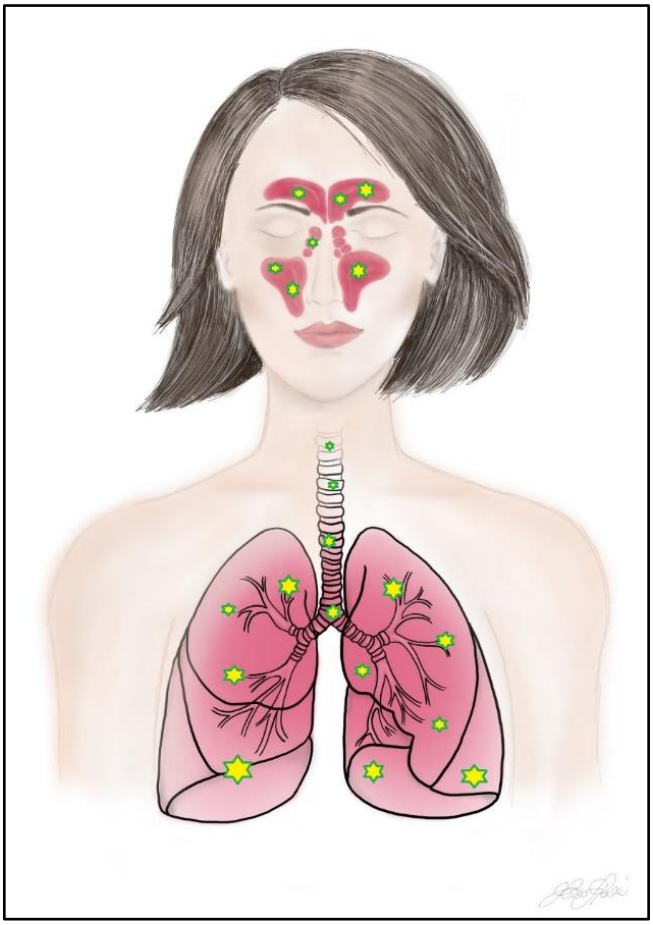


Figure 1: Schematic drawing of the global airways with possible shared pathogens between the upper and lower airways. Star symbol: pathogen. *Illustration by S. Sjöstedt 2020.* Modified by E. Arndal (insertion of pathogens symbols).

Research supports the global airway disease concept not only in CF [2, 3] but also in asthma and bronchiectasis [5, 6]. The mechanisms behind global airway disease are diverse and will be explained further in the following section on CRS. Evidence is now emerging that further supports the presence of global airway disease in PCD [7-10] and chronic obstructive pulmonary disease (COPD) [11-13].

This thesis presents our studies of global airway disease in patients with PCD and COPD (Figure 2) by examining bacteriology, CRS, and olfaction.

We chose to study patients with PCD and COPD as they are a vulnerable group of individuals prone to airway inflammation, exacerbation and they suffer from multiple comorbidities, affecting not only their quality of life but also their survival. Increased understanding of the possible interaction between upper and lower airway disease will hopefully improve treatment, prophylactic measures and, importantly quality of life in patients with global airway disease.

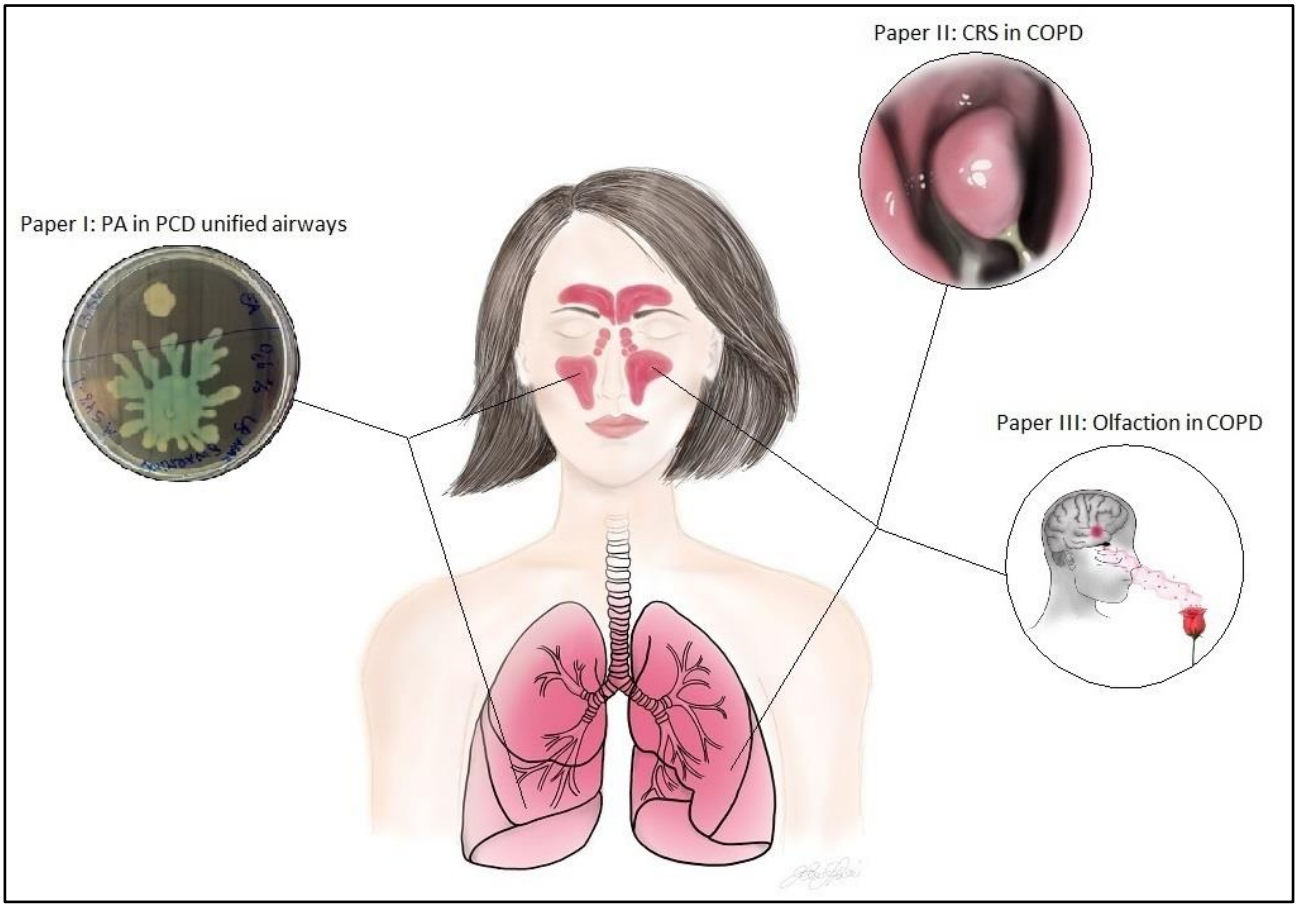


Figure 2: Schematic drawing of the global airways concept with studied topics presented in circles; from left to right: Paper I: Pseudomonas aeruginosa in PCD global airways, Paper II: CRS in COPD, Paper III: olfaction in COPD. *Illustration by S. Sjöstedt 2020.*

The focus of this thesis is global airway disease. It is the product of a multidisciplinary research collaboration between the Department of Otorhinolaryngology, Microbiology, Paediatrics, Respiratory Medicine, Radiology, Genomic Medicine, Biostatistics, Biology and Bioinformatics and the Novo Nordisk Foundation Centre for Biosustainability.

BACKGROUND:

MUCOCILIARY CLEARANCE (MCC):

MCC is a process in which cilia move in a coordinated fashion, sweeping mucous out of the airways (Figure 3) and is an important part of the global airway defence necessary to sustain a healthy airway. Despite different aetiologies, decreased mucociliary clearance (MCC) is a common denominator in both PCD and COPD and other chronic airway diseases [2, 5, 6, 14, 15]. Decreased MCC is caused by dysfunctional cilia and/or overproduction of thick mucus resulting in mucus stagnation in the airways [16-18]. This stagnation generates mucus plugs and retention of allergens or pathogens such as bacteria, viruses, and fungi, promoting local inflammation and further airway disease [19].

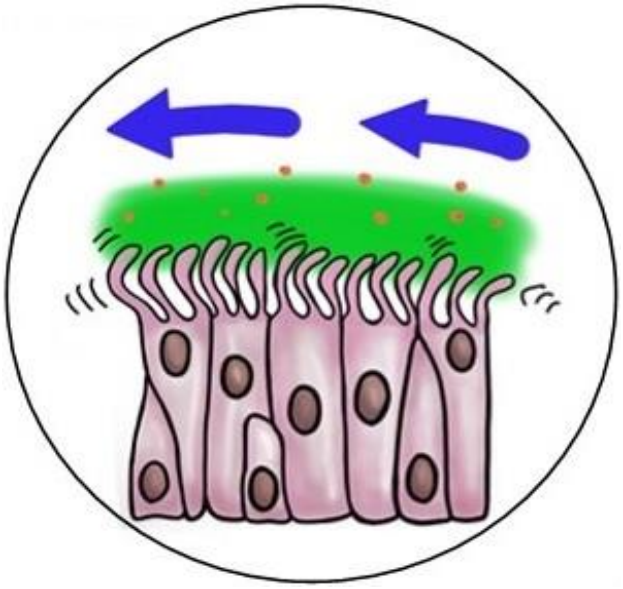


Figure 3: Schematic drawing illustrating mucociliary clearance (MCC). Airway cells with cilia that clear the mucus and any particles trapped in the mucus. *Illustration by S. Sjöstedt 2020.*

GLOBAL AIRWAY INFLAMMATION IN COPD AND PCD:

COPD and PCD patients have chronic inflammation not only in their airways but also systemically, associated with comorbidities such as diabetes, cardiovascular disease, osteoporosis in COPD and reproductive and otologic (secretory otitis media) in PCD and last but not least, the shared traits of recurrent airway infections and prevalent sinonasal symptoms [14, 15, 20]. Decreased MCC, tobacco smoke, pathogens, recurrent infections (acute nasal and pulmonary exacerbation frequency), immunodeficiency, pollutants and microbial dysbiosis all play a role in the multifactorial pathogenesis of PCD, COPD and CRS [14, 15, 21].

Both COPD and chronic rhinosinusitis (CRS) are primarily adult-onset diseases, indicating that a certain level of time and exposure is required for tissue inflammation and remodelling to occur before the disease becomes manifest [22]. Inflammatory patterns in both COPD and CRS are mixed immunological responses involving multiple cellular end chemical pathways causing mucosal inflammation and tissue remodelling. Recent 2019 Global Initiative for Chronic Obstructive Lung Disease (GOLD) [15] and European position paper on rhinosinusitis and nasal polyps 2020 (EPOS) [21] guidelines have similar phenotypic classifications for COPD and CRS with predominant eosinophilic disease versus predominantly non-eosinophilic disease; (eosinophilic/TH2 COPD and eosinophilic/Type 2 CRSwNP) versus (neutrophilic/TH1/inflammasome[1] COPD and neutrophilic/non-type2 CRSsNP) [15, 21-24]. Likewise, the inflammatory response in PCD is neutrophil dominated with increased IL-8 during pulmonary exacerbations [25].

In COPD, increased serum inflammatory markers (TNF-alfa, CRP, IL-6, CXCL8, fibrinogen and leucocytes) have been reported in 70 % of patients [26]. Multiple inflammatory cells (TH1, Th2, innate lymphocytes, macrophages, dendritic cells), chemical mediators (cytokines, chemokines.) and structural cells (fibroblasts, epithelial and endothelial cells) are involved in the immunological response and airway remodelling with mucosal metaplasia, goblet cell hyperplasia and fibrosis and

[1] IL-1β, IL-18, IL-8, TNF

loss of the small airways and alveolar walls. The degree of inflammatory and structural changes are more pronounced in patients with more advanced disease. COPD is predominantly neutrophilic but, some patients have high blood eosinophil counts associated with treatment effect (see the following COPD section) [23]. Likewise, the decreased effect of corticosteroids have been seen in some neutrophilic/non-type 2 CRS cases (EPOS) [21]. Vacher et al. found increased CD8 lymphocyte inflammation in both nasal and bronchial biopsies of COPD patients who currently smoked [27]. They also found global airway squamous cell metaplasia in inferior nasal turbinate and bronchial biopsies and increased nasal mucosal thickness in smokers with and without COPD. Of note, the participants in Vacher's study had no nasal symptoms or allergies. We would assume that the COPD patients with nasal disease such as CRS would present with more severe inflammation.

Airway epithelial cells are susceptible to cigarette smoke and oxidative stress that induces activation of inflammatory cells, fibroblast (local fibrosis) and goblet cells (hypersecretion of mucus) and destruction of airway cilia [18,28,29]. Similarly, neutrophils also stimulate mucus secretion, all of which contributes to decreased MCC. Smoking affects the entire global airway leading to the coining of the terms smoker's lungs and smoker's nose [22, 30]. There seems to be a detrimental effect beyond smoking as macrophages of COPD patients produce more inflammatory mediators than macrophages from smokers and non-smokers. COPD patients also have impaired phagocytosis of bacteria which may explain chronic bacterial colonization [23]. Furthermore, bacterial colonization with *Hemophilus influenza, S. aureus and Streptococcus pyogenes* can maintain tissue inflammation by continuous activation of the inflammatory cascades triggering auto-inflammation and even an auto-immune response [22, 24, 25]. These are just some of the pieces of the puzzle, and the extensive work to fully understand the phenotypes of COPD, PCD, CRS, and Global airway disease are ongoing.

Airway inflammation also affects olfaction through a combination of nasal obstruction, decreasing the airflow reaching the olfactory epithelium and local inflammatory processes directly affecting the olfactory receptors [31, 32]. Research of olfaction in patients with COPD is limited, but we hypothesise that both airflow limitation and inflammation contribute to olfactory dysfunction. It is also unknown whether global airway inflammation triggers a systemic response that contributes to olfactory dysfunction beyond the local nasal inflammatory processes.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD):

COPD is one of the top five causes of death worldwide but is still believed to be severely underdiagnosed. The World Health Organisation estimates that approximately 65 million people worldwide suffer from COPD [33]. Although the age-standardised mortality of COPD is slowly declining at around 2.4 % per year, disease burden measured in disability-adjusted life years is unfortunately still increasing [34].

COPD is defined as stated in GOLD as ''*persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases*'' [15]. The chronic airflow limitation and respiratory symptoms are caused by a combination of airway obstruction and inflammation. Chronicity is caused by the destruction of the lung tissue, trapping of air in the small airways and decreased MCC leading to cough, thick airway mucous, dyspnea and recurrent respiratory infections [15, 16]. Besides the chronic lung symptoms patients with COPD may experience acute exacerbations with increased respiratory symptoms. An exacerbation may be mild, moderate, or severe and patients with severe exacerbation have a higher risk of hospitalization. Frequent exacerbations with enhanced inflammation are reported in 9—16 % of COPD patients and reduce their HRQoL and increase morbidity and mortality [35]. Acute exacerbations are commonly caused by bacteria, viruses, and air pollutants but other unknown factors are also believed to contribute.

It is important to remember the overlapping symptoms between chronic bronchitis and COPD when diagnosing a COPD patient. Patients with chronic bronchitis[2] also suffer from cough, increased sputum production, dyspnea, and exacerbations, but they have no obstructive pattern on spirometry, unlike COPD patients. However, over time patients with chronic bronchitis have an increased risk of progressing to COPD [15].

Unlike PCD, COPD is primarily caused by long-term exposure to smoke or other inhaled particles. Tobacco smoke has been reported to decrease MCC by destroying airway cilia [18, 28-29] and affecting transmembrane chloride channels (CFTR), increasing mucus viscosity [17]. Although being one of the most important causes of COPD, tobacco smoke is not the only one. Other contributing factors include air pollutants, socioeconomic status, and host factors such as impaired lung development, childhood pulmonary infections, gender, host immunologic response and genetics. The number of studies investigating genetic determinants for COPD susceptibility continues to grow. Studies have reported 40—60 % COPD heritability and genome-wide association studies have identified several genes linked with COPD [36-37]. Future studies of genetically modified animal models and human proteomics may determine which genes have a greater clinical impact.

COPD is diagnosed according to the GOLD 2019 guidelines [15] and ranges from mild (GOLD grade 1, group A) to very severe (GOLD grade 4, group D). GOLD grade stratifies according to airflow limitation (FEV_1 % predicted), while GOLD groups stratify according to symptoms and exacerbation history (Table 1)

[2] Symptoms must be present for at least 3 months in 2 consecutive years.

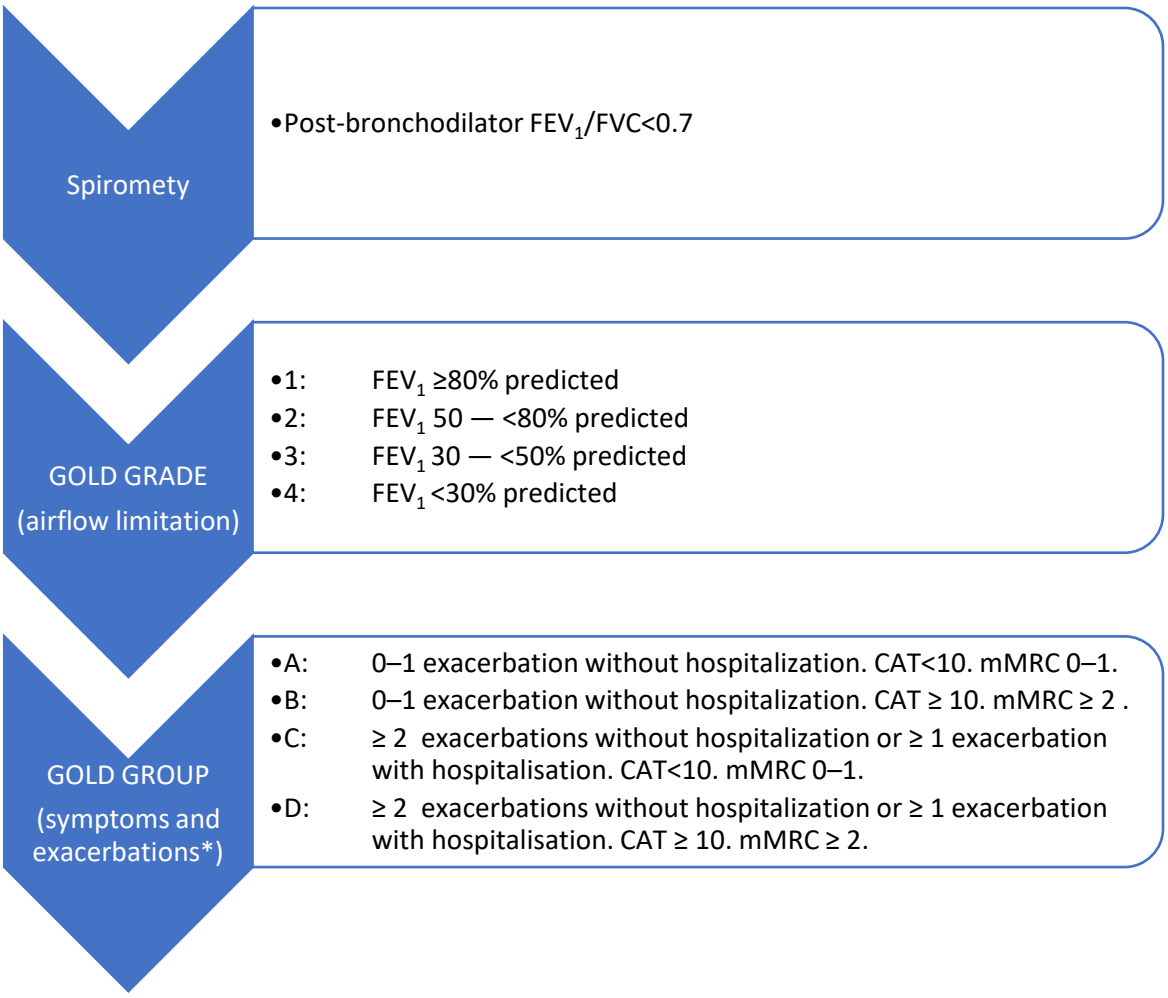


*Table 1: Overview of COPD GOLD grades and groups. FEV1: forced expiratory volume in one second. FVC: forced vital capacity. *Moderate or severe exacerbation. CAT (COPD assessment test) (Figure 7) and mMRC (modified medical research council) dyspnea scale (Table 2) are patient reported outcome measures.* *Illustration by E. Arndal 2019.*

Patient-reported outcome measures (PROM):

PROMs are important tools in monitoring the health-related quality of life (HRQoL), which is decreased in patients with COPD compared with healthy controls [38] Standard PROMs at our Respiratory Medicine out-patient clinic are the Medical Research Council dyspnea scale (MRC)[3] (Table 2) and the COPD assessment test (CAT) (Figure 7). The CAT contains eight questions on COPD HRQoL. Each scored from 0–5 with a total score of 0—40 [40]. In both PROMs higher scores equal worse HRQoL.

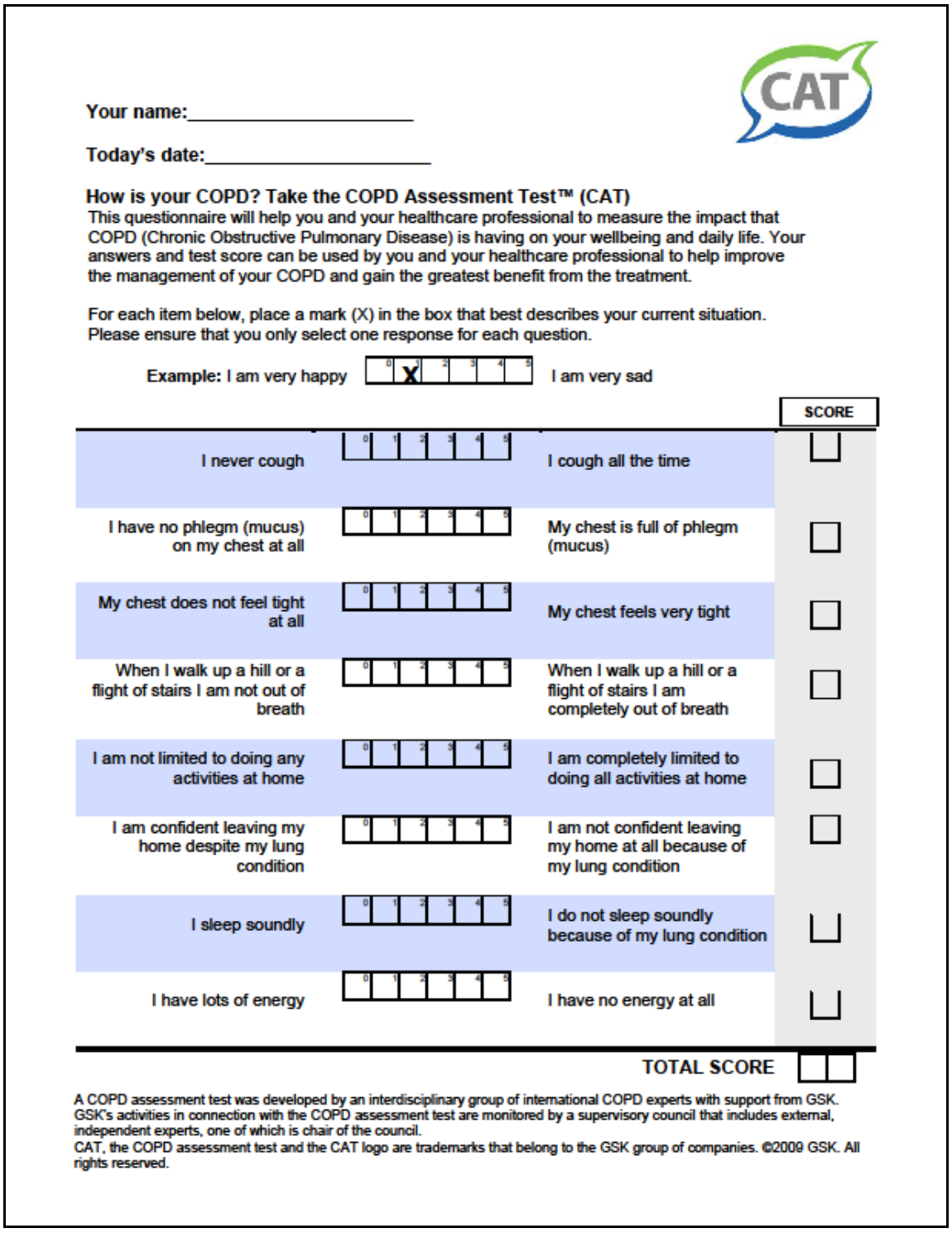
[3] The resent GOLD guideline uses the modified MRC (mMRC). The MRC contains the same five questions as the mMRC [39] with the only difference being that the MRC is divided into grade 1-5 and the mMRC into grade 0-4. In Paper II and III we have used the MRC and calculated the mMRC by subtracting 1 from the MRC score.

MRC dyspnea scale	
Grade	Dyspnea related activity
1	No shortness of breath except on strenuous exercise
2	Shortness of breath when hurrying on the level or walking up a slight hill
3	Walk slower than people of the same age on the level, stop after a mile or so or after 15 minutes of walking at own pace
4	Stops for breath after walking approximately 100 yards or after a few minutes on level ground
5	Too breathless to leave the house or breathless when (un)dressing

Table 2. MRC dyspnea scale. *It is adapted and reused with permission from the Medical Research Council.*

Severity and co-morbidities:
Patients with COPD suffer from multiple comorbidities, which contribute to increased mortality and decreased HRQoL [38, 41, 15]. They are prone to recurrent or chronic infections with Gram-negative bacteria, of which PA infection is rare but often leads to severe illness [16]. The presence of bronchiectasis increases the risk of chronic PA colonization, and one in four patients with bronchiectasis will develop chronic PA colonization over time [42]. Bronchiectasis occurs in 4—7.8 % of COPD patients and should be considered in patients with frequent exacerbations and copious sputum production. For comparison bronchiectasis is reported in 2.9 % of patients with PCD and 12.5 % in patients with CF [43-44].

Previous observational and questionnaire-based studies found daily nasal symptoms (sneezing, nasal discharge, nasal obstruction) in 75–88 % of patients with COPD [45-48]. As mentioned, a correlation between current smoking and increased sinonasal symptoms was reported in a general population study of ''non-allergic rhinitis'' and the concept of a ''smoker's nose'' was introduced [30]. Patients with COPD have decreased MCC, are prone to Gram–negative infections and have a high proportion of nasal symptoms indicating simultaneous upper airway disease. Nevertheless, knowledge of CRS in COPD is limited. The previously conducted studies do not meet the current requirements for CRS diagnostics [11-13], so further investigation is needed. To test our hypothesis that global airway disease is present in patients with COPD, we firstly studied CRS and HRQoL in COPD as described in Paper ll: “CRS in COPD: a prevalent but unrecognised comorbidity, impacting HRQoL” and secondly olfaction in COPD as presented in Paper lll: “Patients with COPD have a higher prevalence of anosmia”.



Your name:_____

Today's date:_____

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional to measure the impact that COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers and test score can be used by you and your healthcare professional to help improve the management of your COPD and gain the greatest benefit from the treatment.

For each item below, place a mark (X) in the box that best describes your current situation. Please ensure that you only select one response for each question.

Example: I am very happy [0] [X] [2] [3] [4] [5] I am very sad

			SCORE
I never cough	0 1 2 3 4 5	I cough all the time	
I have no phlegm (mucus) on my chest at all	0 1 2 3 4 5	My chest is full of phlegm (mucus)	
My chest does not feel tight at all	0 1 2 3 4 5	My chest feels very tight	
When I walk up a hill or a flight of stairs I am not out of breath	0 1 2 3 4 5	When I walk up a hill or a flight of stairs I am completely out of breath	
I am not limited to doing any activities at home	0 1 2 3 4 5	I am completely limited to doing all activities at home	
I am confident leaving my home despite my lung condition	0 1 2 3 4 5	I am not confident leaving my home at all because of my lung condition	
I sleep soundly	0 1 2 3 4 5	I do not sleep soundly because of my lung condition	
I have lots of energy	0 1 2 3 4 5	I have no energy at all	
		TOTAL SCORE	

A COPD assessment test was developed by an interdisciplinary group of international COPD experts with support from GSK. GSK's activities in connection with the COPD assessment test are monitored by a supervisory council that includes external, independent experts, one of which is chair of the council.

Figure 7: CAT: COPD Assessment Test. *Reprinted with permission from GSK.*

Treatment:

As presented in Table 1 patients with COPD are a heterogeneous group wherefore the patient's GOLD group must be considered when choosing an adequate maintenance treatment regime. Similarly, blood eosinophil count and exacerbation profile (frequency and ethology) should also be considered when choosing daily treatment with or without ICS as well as exacerbation treatment.

Thus, a patient with GOLD group D with high blood eosinophils should be treated differently than a patient with similar lung function but GOLD group A with low blood eosinophils[4]. Any comorbidities and possible contraindications must also be considered when choosing treatment [15].

General non-pharmacological treatment principles involve smoking cessation, physical exercise and pulmonary rehabilitation, adequate nutrition, mucolytics, influenza and pneumococcal vaccination (decreases the risk of airway infections).

The main goal of pharmacologic treatment is to reduce airflow limitation, alleviate symptoms and reduce the risk of disease progression. The available inhaled drugs may be used alone or in combination as dual or triple therapy. Combination therapy has a superior effect on FEV_1, symptoms and PROMs compared to monotherapy [15].

Bronchodilators:

Inhaled bronchodilators including $beta_2$-agonist and antimuscarinic antagonists, are the main treatments for COPD.

- $Beta_2$-agonists relax the smooth muscles of the airway. They can be short-acting (SABA) with a 4-6-hour duration of action or long-acting (LABA) with a 12-24-hour duration of action.

- Antimuscarinic antagonists inhibit bronchoconstriction and are either short-acting (SAMA) with a 6-9-hour duration of action or long-acting (LAMA) with a 12-24-hour duration of action.

- LAMA/LABA 12-24 hours duration of action.

Oral drugs with bronchodilatory effect:

- Methylxanthines (method of action and efficacy are debated) and Phosphodiesterase-4 inhibitors.

Anti-inflammatory drugs:

Inhaled corticosteroids (ICS) is used in combination with the abovementioned bronchodilators. The effect of ICS depends on exacerbation frequency and blood eosinophil count. Higher blood eosinophil counts predict a better effect of ICS with a cut-off of > 100 cells/µl and a maximum effect at >300 cells /µl [49]. Responsiveness to ICS has been shown to be greater in former smokers, than current smokers [50] but, results are not consistent. In some studies, combination treatment with ICS betters lung function and lowers exacerbation risk in patients with moderate and severe COPD [51-53]. ICS may increase the risk of pneumonia. Martinez-Garcia 2020 [54] found an increased risk of pneumonia in patients with low eosinophil counts and chronic bronchial infection. No

[4] Treatment according to GOLD group: group A: a bronchodilator, group B: LABA or LAMA, group C: LAMA, group D: LAMA or LAMA + LABA or ICS+LABA based on eosinophil count.

difference in pneumonia risk was observed in patients with moderate COPD and heightened cardiovascular risk than placebo [51]. Other ICS adverse effects include fungal infection, skin bruising and possibly osteoporosis.

Long-term treatment with antibiotics (low dose macrolides) may reduce the frequency of exacerbations but possibly at the risk of increasing bacterial resistance.

In patients with severe chronic hypoxemia, supplement long-term oxygen therapy may be indicated. Patients with chronic hypercapnic respiratory failure may benefit from non-invasive ventilation. Lung volume reduction surgery may be indicated in symptomatic patients with severe emphysema and air-trapping. Finally, even lung transplantation [15] may become necessary in very severe COPD patients.

Treatment effect should be monitored and adjusted accordingly. Common reasons for insufficient effect are persistent symptoms, recurrent acute exacerbations and/or progression of lung function decline.

Acute exacerbations are usually treated with a course of antibiotics if a bacterial infection is suspected, a course of oral corticosteroids short-acting bronchodilators, supplement oxygen therapy and acute non-invasive ventilation in case of acute type II respiratory failure. The use of long term (> 1 weeks) per oral corticosteroids is not recommended due to the high risk of adverse effects and lack of benefit compared to shorter courses of systemic steroid bursts [55].

CHRONIC RHINOSINUSITIS (CRS):

CRS is defined as chronic inflammation of the sinonasal mucosa lasting for more than 12 weeks [21]. It is primary[5] or secondary[6] and further subcategorised according to anatomic distribution (local[7], diffuse), endotype (Type 2, non-Type 2, inflammatory, immunological, mechanical) and phenotype (such as eosinophilic CRS (eCRS)/ CRS with nasal polyps (CRSwNP), non eCRS/ CRS without NP (CRSsNP), allergic), see Figure 2.2.1. and 2.2.2 in EPOS 2020 [21]. Patients may furthermore experience acute sinonasal infection on top of their chronic symptoms.

The literature reports divergent CRS frequencies from 2 % by doctor diagnosis to 6.7—27.9 % in a large European multicentre study based on a questionnaire on EPOS sinonasal symptoms [56]. Cultural and ethnic differences exist, which may explain some of the observed frequency variation [57, 58]. CRS symptoms may be overlooked if the patient already suffers from another severe disease such as COPD, where respiratory symptoms can be misinterpreted as originating only from the lungs. In this thesis, we will focus on CRS in adults with COPD.

We diagnosed CRS according to the EPOS2012 criteria which are identical to the EPOS 2020 criteria (Figure 8) [22, 59]. The diagnosis is based on a minimum of two symptoms, one of which

[5] CRS in asthma.
[6] CRS in CF and PCD.
[7] Tumor or fungal ball.

must be a major symptom and an otorhinolaryngologic clinical examination to establish the presence of objective findings.

Major symptoms:

- Nasal discharge
- Nasal obstruction

Minor symptoms:

- Facial pain or pressure
- Decreased sense of smell (olfaction)

Objective findings:

- Nasal endoscopic: polyps, mucosal oedema and/or secretion primarily from the middle meatus in the nasal cavity.
- If there are no endoscopic findings, a CT-sinus scan can be performed, evaluating any opacification of the paranasal sinuses and/or osteomeatal complex.

We used the Lund-Mackay sinus-CT scoring system [60] to grade the level of mucosal swelling and corresponding opacification of the paranasal sinuses (maxillary, anterior and posterior ethmoid, frontal, sphenoid) and the osteomeatal complex.

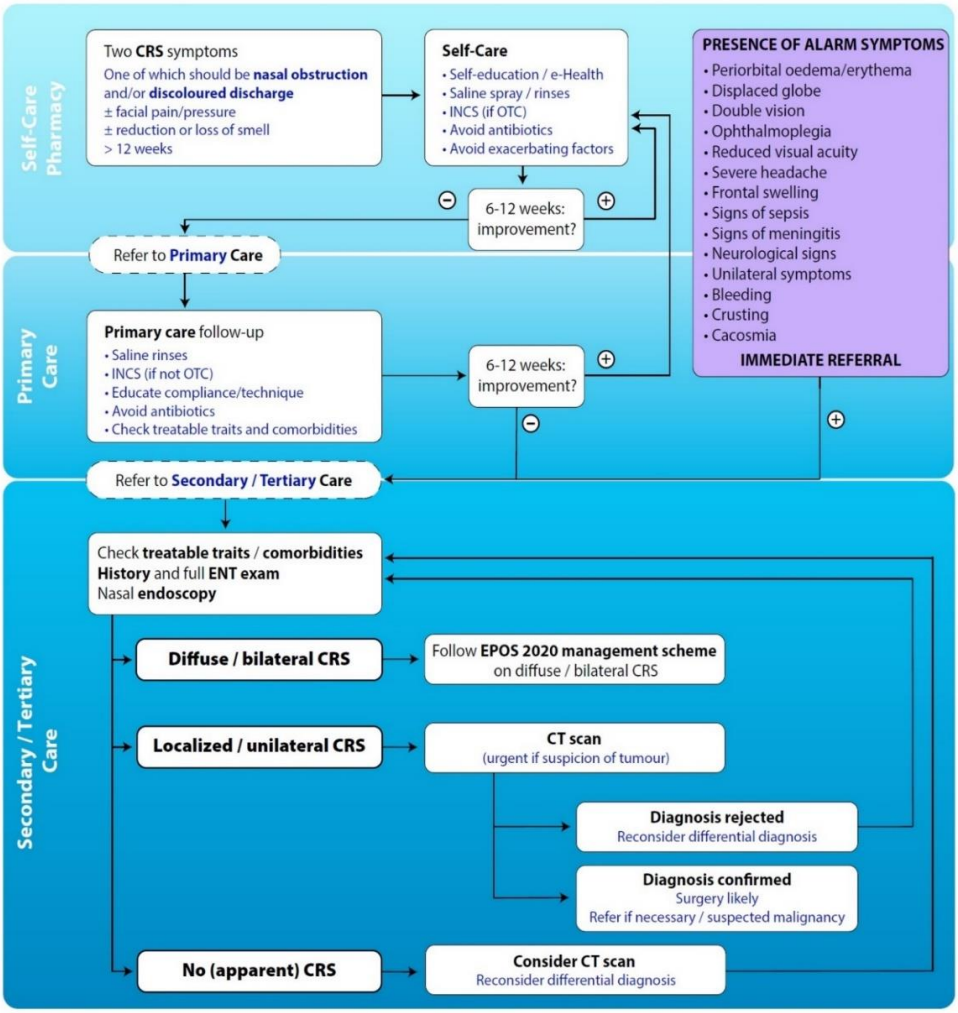


Figure 8: Flowchart for management of CRS *Reprinted from Fokkens WJ, Lund VJ, Hopkins C, et al. EPOS 2020: European position paper on rhinosinusitis and nasal polyps 2020. Rhinology. 2020;58(2):82-111. Figure 1.6.1 [21]; with permission from Rhinology.*

PROM:
One of the most widely used questionnaires to monitor sinonasal symptoms and outcomes after endoscopic sinus surgery (ESS) is the SNOT22. It contains 22 questions each of which is scored from 0–5 (0= no problem to 5=maximal problem), giving a total score of 0–110 (Paper II, Table 2). The first SNOT questionnaire [61] was inspired by the Rhinosinusitis Outcome Measure-31 [62]. Later, another two items were added to better reflect patient symptoms: "sense of taste/smell" and "blockage/congestion of the nose" [63]. The SNOT22 has been validated in a large population of patients with CRS and found to be an accurate tool for assessing disease burden [64]. The SNOT22 contains different subdomains: nasal, otologic, sleep and emotional symptoms [65] and has been validated for Danish patients [66]. Patients with CRS have decreased HRQoL affecting all subdomains of the SNOT22 [21].

SNOT22_nasal symptom questions:

- Need to blow your nose
- Sneezing
- Runny nose
- Blocked/obstructed nose
- Altered sense of taste/smell
- Cough
- Post-nasal discharge
- Thick nasal discharge
- Facial pain/pressure

There is no know cut-off value for the SNOT-22 in COPD patients. Cut-off values for SNOT-22 in CRS are described in the thesis discussion section.

Severity and co-morbidities:
CRS and upper airway inflammation have been associated with lower airway diseases such as asthma, bronchiectasis, cystic fibrosis and now also PCD [3, 5-6, 67, Paper I], but knowledge of possible global airway disease in COPD is sparse. As mentioned, CRS symptoms such as nasal obstruction and nasal discharge have been observed in up to 88 % of patients with COPD [30, 45-48]. However, most studies are only questionnaire-based with only a few studies that have clinically assessed CRS in patients with COPD [11-13] reporting a prevalence of 48–64 %.

Unfortunately, these studies did not evaluate CRS according to the EPOS diagnostic standards and omitted nasal evaluation by an otorhinolaryngologist and the SNOT22. These limitations, especially the lack of nasal endoscopy, may have overlooked other reasons for sinonasal disease, such as deviated nasal septum and incidental findings on CT-sinus scans, overestimating the prevalence of CRS. We examined the prevalence of clinically diagnosed CRS in patients with COPD according to the highest standard recommended by EPOS (symptoms, nasal endoscopy, and sinus CT) and GOLD. Our observations are present in Paper II, '' Chronic Rhinosinusitis in COPD: a prevalent but unrecognized comorbidity, impacting HRQoL''.

Treatment:
CRS treatment is tailored according to symptom severity and the presence of any nasal polyps. It consists of a combination of topical nasal corticosteroids (spray and/or drops), nasal saline

irrigation, antibiotics, a short course of systemic[8] corticosteroids, ESS or polypectomy by polyp sling. The use of the polyp sling is a quick and effective way of re-establishing nasal airflow in patients with a high risk of post-operative complications due to anaesthesia. Other options could potentially include in-clinic ESS under local anaesthesia [(68)].

Concomitant lower airway disease such as asthma, CF and PCD are increasingly influencing CRS treatment. Biological treatment of moderate to severe recalcitrant CRSwNP alone or as part of global airway disease has been introduced and recommended [(21)], but as such standard treatment regimens for global airway disease have not yet been clearly defined.

OLFACTION:

The perception of odour (olfaction) consists of peripheral nasal receptors and cerebral registration and modulation. (Figure 9). An intact olfactory function is part of a healthy upper airway.

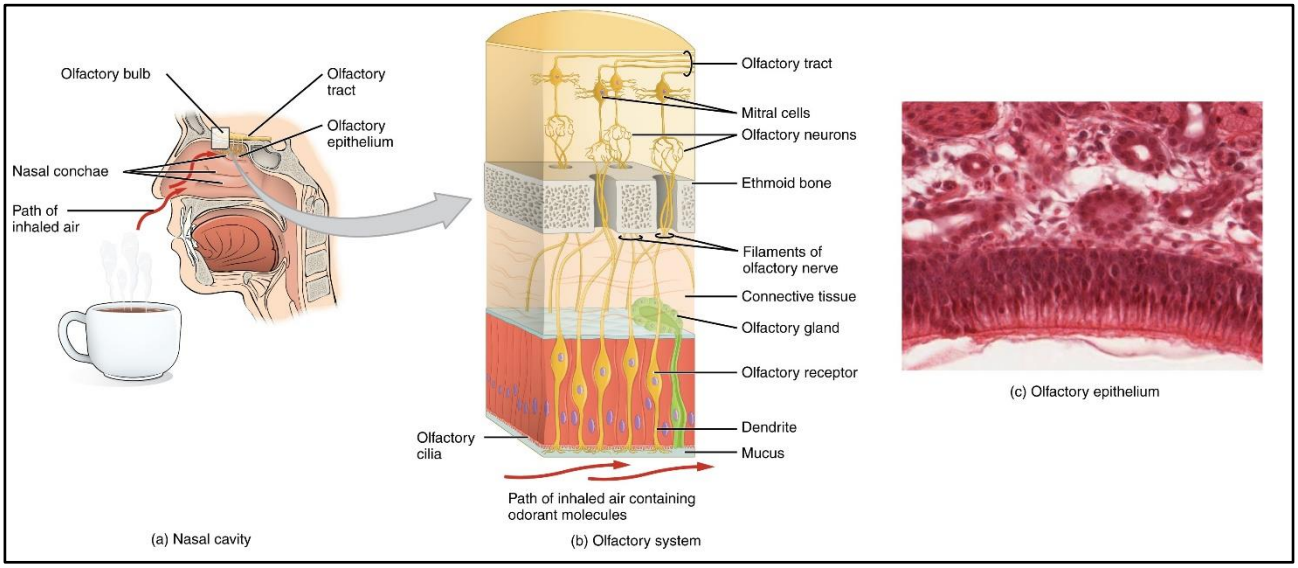


Figure 9: The olfactory system. A: odour perception in the nasal cavity and central olfactory pathways. B: Olfactory neuroepithelium and nerve. C. Microscopy of the olfactory epithelium.
Re-printed from OpenStax Anatomy and Physiology Textbook, version 8.25 by Open Stax, published 18th May 2016 © With permission from Creative Commons 4.0. No changes have been made to the original figure. (https://commons.wikimedia.org/wiki/File:1403_Olfaction.jpg).

Olfactory dysfunction is a quantitively and/or qualitatively altered sense of smell categorised as: normosmia, hyposmia, (functional) anosmia, parosmia and phantosmia [(32)]. The current prevalence in the adult population is 15 % hyposmia [(69)] and 5 % functional anosmia [(70-71)], increasing up to 62.5 % in the over 80-year-olds [(72)]. However, these numbers are expected to change in correspondence with the increasing focus on the disorder amongst clinicians.

Multiple aetiologies for olfactory dysfunction exist, such as ageing, congenital, sinonasal disease, neurological, infectious, traumatic, toxicologic and idiopathic [(31)]. In CRS, olfactory dysfunction may

[8] Per oral for 7-21 days or as a single injection.

result from chronic mucosal inflammation and swelling obstructing the nasal airflow thereby preventing odours from reaching the olfactory epithelium. Additionally, inflammatory processes may debilitate the olfactory receptors [32, 73]. A meta-analysis found that current smoking significantly increased the risk of olfactory dysfunction [74].

Diagnosis of olfactory dysfunction is based on symptoms, medical history (exploring abovementioned aetiologies), olfactory testing (psychophysical, objective[9]) and clinical examination (nasal endoscopy, CT and/or MRI scan of the nasal cavity and cerebrum).

Only one study of olfaction in COPD has previously been published [78]. They found no difference in olfactory function when comparing 40 patients with COPD to age and sex-matched healthy controls when results were adjusting for smoking.

As the subjective sense of smell correlates poorly with olfactory test results, less subjective testing is essential [79]. Multiple psychophysical olfactory tests exist [80-81] and we used the Sniffin' Sticks Identification test 16 blue (SIT16) to screen olfactory function (Figure 10).

Severity and co-morbidities:
Despite being an important symptom of CRS and included in both the EPOS criteria and the SNOT22 questionnaire, olfactory dysfunction is often overlooked. We hypothesised that this is especially true in patients with COPD, where lower respiratory symptoms are very burdensome and overshadow any olfactory symptoms.

The negative impact of olfactory dysfunction on HRQoL is substantial, anosmic and paraosmic patients being more affected than patients with hyposmia. Consequences range from danger in the case of fire, smoke, exposure to chemical fumes, consumption of spoiled foods, less olfactory gratification when eating leading to altered body weight [84] and less social dining. Additionally, olfactory dysfunction may affect the perception of own/others body odour [85], choice of partner [86-87] and mother-and-child bonding [88]. Patients suffering from olfactory dysfunction have increased mortality [89], risk of developing Alzheimer's disease [90], Parkinson's disease [91], depression [92] and reduced cognitive function [93-94].

Patients with COPD have low HRQoL, high morbidity and mortality, suffer from increasing social isolation due to declining lung function and a tendency to be underweight [48, 95]. An unrecognised olfactory dysfunction could additionally impact their nutritional state and low HRQoL. These factors prompted us to explore olfactory function in patients with COPD with and without CRS. Our results are presented in paper III, ''Patients with COPD have a higher prevalence of anosmia, a cross-sectional study of odour identification''.

Treatment: Olfactory dysfunction is treated according to aetiology but overall comprises off [32, 96]:

[9] Electroencephalography (EEG) [75], electro-olfactography (EOG) [76] and functional MRI (fMRI) [77]

- Conservative treatment /wait and see
- topical and/or systemic medication primarily with corticosteroids
- Sinonasal surgery
- olfactory rehabilitation [97-98].
- appropriate precautions (personal hygiene, smoke alarms, checking expiration dates, food preparation, influenza vaccination).

Figure 10: Sniffin' Sticks Identification test 16 blue *(SIT16) blue.* The SIT16 is culturally adapted to northern European smells and has validated Danish multiple-choice answers [32, 82, 83]. It is a suprathreshold test which means that it contains high intensity odours. The SIT16 is a subtest of the TDI test, which can be used on its own to screen olfactory function. *(https://smelltest.eu/en/product/burghart-sniffin-sticks-identification-test-16-blue/)*

PRIMARY CILIARY DYSKINESIA (PCD):

PCD is an autosomal or x-linked recessive hereditary disease affecting the ciliary structure or function and MCC in the entire respiratory and reproductive tracts [14]. This thesis will focus on PA colonization of the global airway in patients with PCD.

PCD is thought to be underdiagnosed and with large geographical differences, affecting 1:2000 — 1:40000 people. It is associated with increased morbidity and overall mortality [14, 99, 100] but with longer life expectancy in high-income countries. PCD is usually diagnosed in childhood, but late or delayed diagnosis is common. The diagnostic workup follows the international guidelines [14] consisting of medical history, nasal nitric oxide measurement, high-speed video microscopy analysis, transmission electron microscopy of a ciliary sample (Figure 4 and 5), and genetic testing.

Continuous studies of the ciliary ultrastructure and patients' genomes identifying multiple ciliary and genetic anomalies causing PCD (Figure 4) [14, 101, 102]. All these anomalies resulted in ciliary dyskinesia or ciliary immobility and decreased MCC promoting infection, inflammation and potentially tissue damage [19]. The ciliary dyskinesia in the airways makes patients with PCD prone to lower but also upper respiratory tract disease [7, 103].

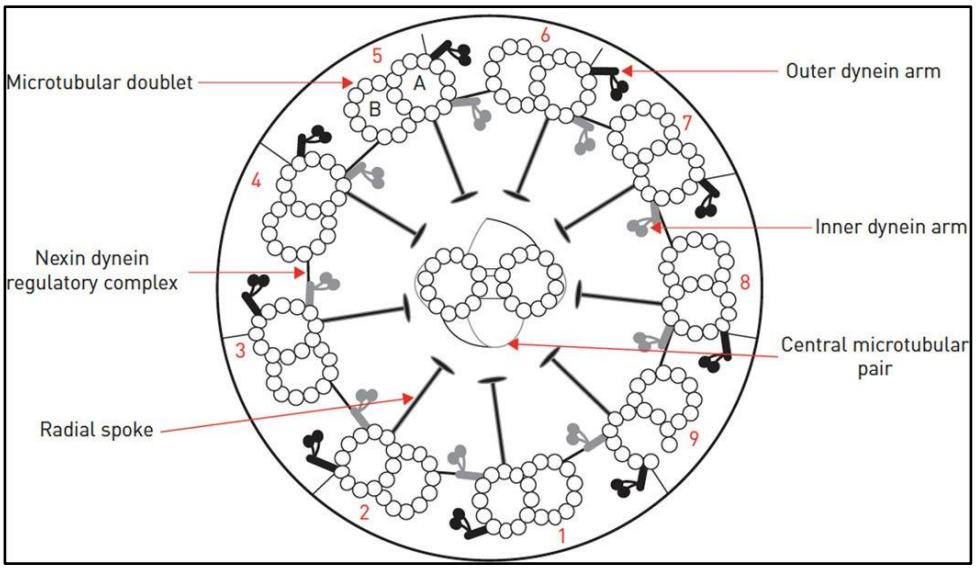


Figure 4: Schematic drawing of normal ciliary ultrastructure in transverse section.

Reprinted from Lucas JS, Barbato A, Collins SA et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. Eur Respir J 2017; 49: 1601060 doi:10.1183/13993003.01090-2016.(14)Figure 1. With permission from European Respiratory Journal.

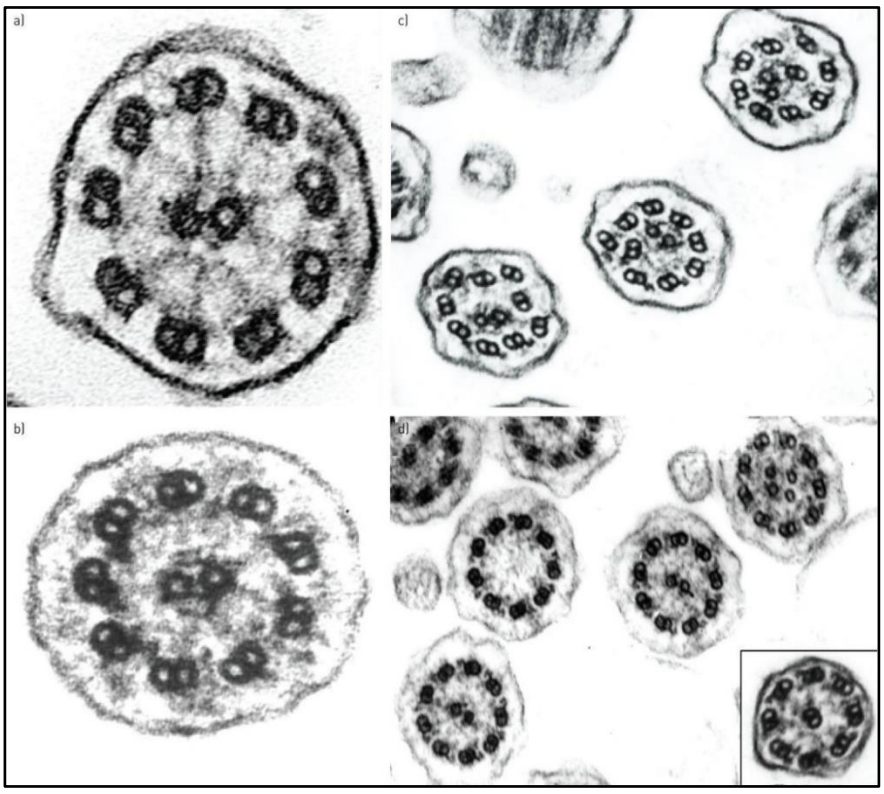


Figure 5: Electron microscopy pictures of PCD ciliary ultrastructure in transvers section. A: defect inner and outer dynein arm. B: defect outer dynein arm. C: disarrangement of inner dynein arm and microtubules. D: Defect central microtubule pair.

Reprinted from Lucas JS, Barbato A, Collins SA et al. European Respiratory Society guidelines for the diagnosis of primary ciliary dyskinesia. Eur Respir J 2017; 49: 1601060 doi:10.1183/13993003.01090-2016. (14) Figure 2. With permission from European

Severity and co-morbidities:
Morbidity of the entire respiratory tract involves an increased prevalence of upper respiratory tract disease such as CRS, otitis media with effusion and infection with *Streptococcus pneumoniae*, *Staphylococcus aureus* and especially Gram–negative bacteria such as *Hemophilus influenza*, *Moraxella catarrhalis*, and PA [104]. Respiratory tract colonisation and infection with PA increases the risk of hospitalization, the need for intensive antibiotic treatment, assisted ventilation and in the worst-case lung transplantation or death [105]. At our institution chronic PA infection is diagnosed according to the modified CF Leeds criteria where more than half of the last years sputum cultures are PA positive [106]. PA infection may initially be eradicated but over time 5–39 % [104, 107] of patients become chronically infected. PA's genetic (genotypic) and behavioural (phenotypic) traits and their role in global airway disease in patients with PCD will be described further in the following designated section. A longitudinal study of 151 patients with PCD reported an all-cause mortality rate of 4.6 % during the median 7-year follow-up period, with respiratory disease accounting for 3.3 % [108].

Studies of upper airway disease in PCD have reported sinus symptoms in 85–100 % [9, 103], purulent secretion from the sinuses in 75 % [10] and up to 50 % have CRS [101]. Alanin et al. showed that patients with PCD have PA positive culture samples from both the lungs and paranasal sinuses. This result indicates a possible sinus reservoir wherefrom bacteria could seed to the lungs and recolonise the otherwise infection-free lungs [7]. These findings inspired our research team to explore global airway disease further and resulted in Paper I: ''Primary Ciliary Dyskinesia patients have the same PA clone in sinuses and lungs''.

Treatment:
Treatment of PCD consists of chest physiotherapy, surveillance of sputum cultures and lung function, infectious control (incl. antibiotics, vaccination and prophylactic measures) and, in rare cases, lung transplantation [101].

It should be noted that PA infection status is currently based solely on lung and blood samples, leaving out sampling of the sinus. However, otorhinolaryngologists are increasingly becoming part of the multidisciplinary team treating patients with PCD based on the EPOS 2020[21] recommendations, which include Alanin et al. [67] study demonstrating that ESS eradicates the causative pathogen from the lungs in 25 % of patient and trends towards improving lung function.

PSEUDOMONAS AERUGINOSA (PA):

Intermittent airway colonisation and chronic infection with the Gram-negative-bacteria PA cause severe morbidity and mortality in vulnerable patients suffering from diseases such as CF, PCD and COPD [109, 110]. PA is capable of extensive genotypic and phenotypic adaptation in response to changes in the local environment. These changes make PA very resilient [111] and eradication treatment inherently difficult [112]. As less adapted PA bacterial strains are more easily eradicated, increased knowledge of PA's characteristics will help us treat infected patients.

PA uses quorum sensing [113], a kind of bacterial surveillance system, to sense danger and make appropriate adaptations for securing its survival [114]. Changes in the local environment include altered availability of nutrients, attack from the host immune system, the presence of antibiotics and tissue oxygen tension [115-117]. Genotypic changes involve mutations in the bacterial DNA, resulting in advantageous regulation of gene products, which in turn influences bacterial phenotype and bacterial survival [118]. Genotypic adaptation is a complex process involving multiple cascades of events affecting every step from DNA translation to the final gene product. Phenotypic characteristics such as generation time, protective biofilm formation, antibiotic resistance, protease production, and motility (swimming, swarming, twitching) are each a product of several genes. Therefore, not all genotypic mutations result in phenotypic changes as this may require mutations in multiple genes. One PA survival tactic could be to turn off most of its functions and hibernate in a protective layer of biofilm until the danger has passed (Figure 6) [119].

Less adapted PA bacterial strains are more vulnerable to attack from the host immune system and antibiotic treatment and thus more easily eradicated. Therefore, it is important to compare the level of genotypic and phenotypic adaptation of a patient's PA to a well-characterised PA reference strain, such as the commonly used PAO1, when choosing treatment.

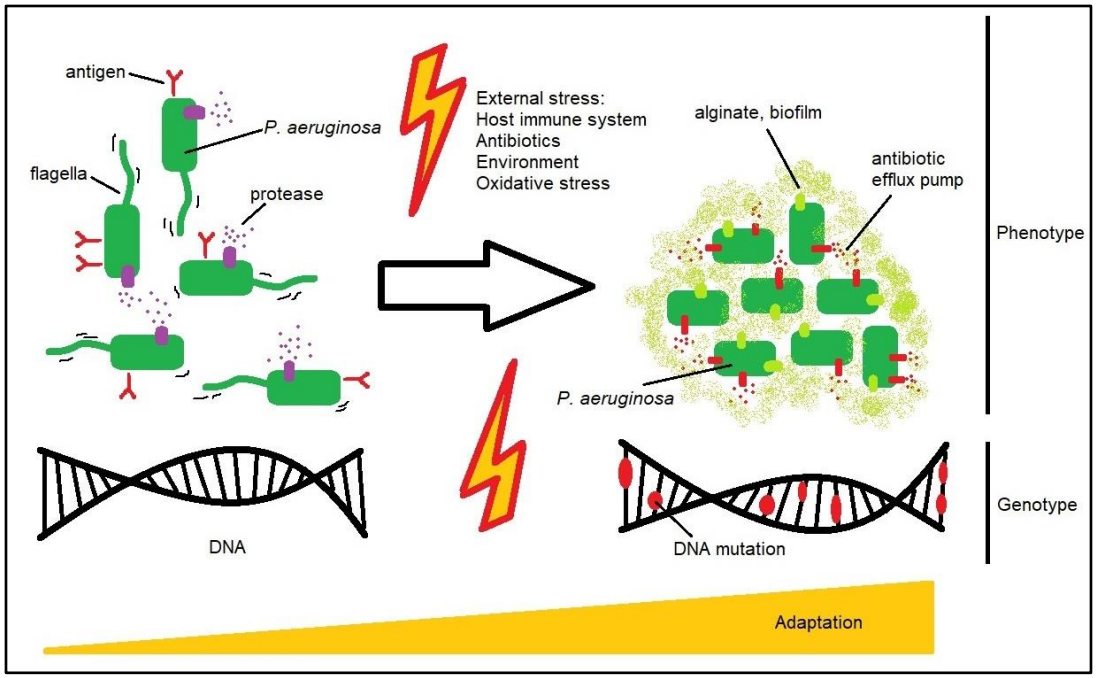


Figure 6: Schematic drawing of P. aeruginosa's phenotypic and genotypic adaptation in response to external stress. *Illustration by E. Arndal 2019.*

Previous studies from our research group have demonstrated that patients with PCD had PA positive cultures in both their lungs and sinuses [7]. However, it remains unknown whether the PA found in the paranasal sinuses is genotypically and phenotypically identical to the PA found in the lungs. If the strains are identical it is hypothesised that the bacteria from the sinuses colonise the lungs highlighting the role of the sinonasal cavity in global airway inflammation and disease. This hypothesis is investigated in Paper I: ''Primary Ciliary Dyskinesia patients have the same *P. aeruginosa* clone in sinuses and lungs''.

HYPOTHESES AND AIMS:

Overall hypothesis: that global airway disease is present in PCD and COPD.

Overall aim: to explore associations between upper and lower airway disease in these patients.

Paper I:

Hypothesis: That the paranasal sinuses (upper airways) in patients with PCD contain the same PA clone type as in their lungs (lower airways), thus acting as a bacterial reservoir.

Aim: To characterise the geno- and phenotype of PA isolates from the paranasal sinuses and lungs of PCD patients.

Paper II:

Hypothesis: That the decreased mucociliary clearance found in patients with COPD causes chronic airway inflammation and disease not only in their lungs but also in their paranasal sinuses.

Aim: To examine the prevalence and associated risk factors of CRS in COPD patients.

Paper III:

Hypothesis: That patients with COPD have decreased olfactory dysfunction.

Aim: To screen olfactory function by odour identification in patients with COPD with and without CRS.

MATERIALS AND METHODS:

Ethics: All trials were conducted according to the Helsinki declaration and approved by local ethics and data processing committees (H-4-2015-FSP; H-1-2013-032).

Here follows an abbreviated material and methods section (see Paper I-III for comprehensive details).

Paper I:

We performed genotypic and phenotypic analyses of 38 PA isolates collected from 2009 to 2017 from the paranasal sinuses and lungs of nine patients with PCD chronically infected with PA [8]. The median age at the first obtained isolate was 18 years (range 10—42 years) with a male to female ratio of 1:2. They were diagnosed according to the European Respiratory Societies guidelines [14] and either fulfilled the modified Leeds criteria for chronic infection [106] or had a PA positive sputum sample and elevated serum anti-precipitin levels [2´104]. The 21 paranasal sinus isolates were collected during ESS, which was performed by Mikkel Alanin MD, Kasper Aanæs MD, and Christian von Buchwald MD. The 17 lung samples were obtained from either bronchioalveolar lavage preceding ESS or by endolaryngeal suction or expectoration during a patient-visit to the hospitals PCD centre. In the case of patient no. 6, none of the previously PA positive lung isolates were stored, and the only available lung samples from this patient were collected after ESS. In the case of a PA positive sample (paranasal sinus or lung) patients were offered routine antibiotic treatment (see Paper I). Data on antibiotic treatment and inflammatory markers was not recorded.

Genotypic analyses
Whole-genome sequencing was performed by Rasmus L. Marvig M.Sc. PhD at the Center for Genomic Medicine, Copenhagen University Hospital, Denmark using established DNA sequencing techniques, and we compared isolate genomes to previously published PA reference genomes [8, 114, 120-124].

Phenotypic analyses
The Ph.D. student Elisabeth Arndal (EA) performed phenotypic analyses in collaboration with staff scientist Janus JA. Haagensen and Ph.D. research scientist Jennifer A. Bartell at the Novo Nordisk Foundation Center for Biosustainability. Additionally, culture and sensitivity data on all isolates were obtained. PAO1 was used as the reference strain. It is characterized by non-mucoidity, low generation time, protease secretion, full motility and susceptibility to antibiotics and biofilm formation.

Paper II – III:

All 222 patients with COPD were included from 2017–2019 during a routine visit to the Respiratory Medicine out-patient clinic at Bispebjerg Hospital, Denmark (Figure 11). Bispebjerg Respiratory

Medicine department is a tertiary centre primarily receiving patients with moderate and severe COPD, while mild COPD is seldomly referred to our centre. As the included patients represent a subgroup of all patients with COPD, our findings may not be directly applicable to the general COPD population at large but depict the prevalence of disease in this large subgroup.

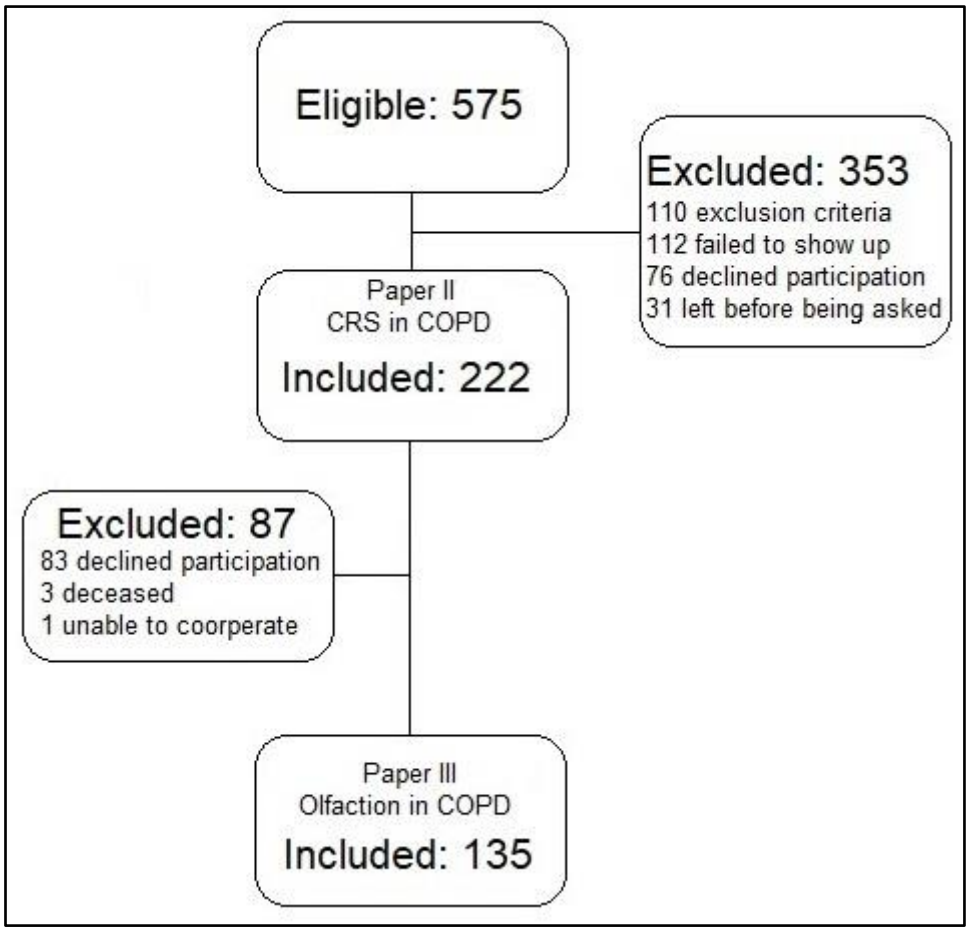


Figure 11: Flowchart of patient eligibility and inclusion for Paper II-III. COPD: chronic obstructive pulmonary disease. CRS: chronic rhinosinusitis. NCS: nasal corticosteroids.

We included 222 patients and CRS and COPD were diagnosed as described in the background section. Patients completed the SNOT22, questions on previous CRS diagnosis/treatment and sinonasal surgery, MRC, and CAT. A pulmonologist nurse routinely recorded total MRC and CAT scores in the patient chart. Scores of the individual MRC and CAT question were not recorded. After spirometry and evaluation by a pulmonologist specialised in COPD (Nihaya Said MD, Therese Lapperre MD, Mia Moberg MD, and Julie Janner MD), patients were evaluated and examined by the Ph.D. student Elisabeth Arndal (EA). The clinical examination consisted of anterior rhinoscopy prior to decongestants, flexible nasal endoscopy after decongestant and evaluation of dental status. Patients then had a CT-sinus scan and a control lung-HRCT performed within three months. A trained radiologist evaluated both scans. CT-sinus scans were Lund-MacKay scored by a senior radiologist, who was blinded to the patient history. The presence of bronchiectasis on HRCT was

not recorded. Lund-MacKay scores of the individual sinuses range from 0=no abnormalities, 1= partial opacification and 2= total opacification. Osteomeatal scores range from 0=no obstruction; 2=obstructed. Left and right scores are added to a total score of 0—24. All patients diagnosed with CRS were prescribed standard treatment (nasal saline irrigation, nasal steroids and a one-month follow-up visit).

According to both GOLD grade and group[10], a senior pulmonologist specialised in COPD, double checked all patients' COPD classification to secure a uniform COPD classification. The new GOLD 2019 guideline recommendation on eosinophil count was introduced at the end of our study period. Therefore, we retrospectively retrieved the latest (if any) eosinophilic counts within the previous six months of the study visit (Table 2, Paper II).

Exclusion criteria: adult asthma with/without COPD, PCD, CF, lung cancer, acute pulmonary exacerbation within the last two weeks, acute common cold or odontogenic infection in the upper jaw or recent nasal surgery and inability to understand or read Danish.

Olfactory testing (Paper III):
In addition to the examination described above we screened olfactory function in 135 patients with COPD before the nasal examination. All patients answered the EPOS criteria about an affected sense of smell and completed the SNOT22 containing a question on the affected sense of smell and taste. The forced multiple-choice SIT16 identification test blue[11] was used for testing both nostrils simultaneously with the ''smelling first'' condition, where the subject smells the odour pen before reading the possible multiple-choice answer [83, 125]. As mentioned above, patients with a history of recent or current airway infection, nasal surgery or lung cancer were excluded. History of familial olfactory dysfunction and neurodegenerative disease (excluding Alzheimer's) was not recorded. Two patients had received radio therapy for laryngeal cancer, but the radiation field did not include the nasal cavity. Eleven patients reported either previous fascial trauma, toxic exposure or sinonasal surgery. No patients received oral corticosteroids up to two weeks before SIT16 testing, as recent pulmonary exacerbation, that may include oral corticosteroid treatment was an exclusion criterion. Olfactory scores were classified as normosmic: SIT16 score 12—16 correlating to a TDI ≥ 30.75, hyposmic: SIT16 score 9—11 correlating to a TDI 17—30.75and anosmic: SIT16 score 0—8 correlating to a TDI score ≤16. Results were compared with age and gender-matched normative data presented by Oleszkiewicz et al. [126], which is the largest material to date describing olfaction in healthy controls. We obtained permission to use their data in our analyses. Please see the thesis discussion section for further details regarding the controls group.

All patients with anosmia were referred for further examination, including cerebral MRI scan as per standard (data not included in this thesis).

Associate professor Karl Bang Christensen and Ph.D. student Anne Lyngholm Sørensen, Section of Biostatistics, University of Copenhagen, Denmark, performed the statistical analysis in paper II and

[10] Please note that GOLD group due to an oversight is listed as GOLD type in Paper II.
[11] Containing odors familiar to a northern European population.

III in collaboration with the Ph.D. student, Elisabeth Arndal. Included variables are listed in the corresponding papers. All variables were used in both the univariate and the multivariate logistical regression models.

We performed cross-sectional studies in Paper II and III. A cross-sectional study is an observational study used to examine the correlation between exposure (COPD) and outcome (CRS or olfaction) at one time point. It studies the scope of a given problem and enables investigation of the prevailing characteristics of a large study group at a given time point. However, it is unable to determine cause and effect. The results of a cross-sectional study can then prompt further studies to establish causality. The advantages of this study design include the evaluation of a large group of patients that can guide further studies and health care initiatives and increase focus on previously unknown correlations. However, the disadvantages include risk of recall bias, inability to determine causality, quality of the available data, and validity of any questionnaires used. To overcome some of these drawbacks, linked to the cross-sectional study design, we used validated questionnaires specifically designed to study both exposure and outcome. The questionnaires were completed during the study visit to minimise any recall bias. The physical examinations, spirometry and scans were also performed to investigate the current exposure and outcome. All collected data (including possible confounders) was based on individual patients' records securing patient-specific data [127]. As mentioned previously we did not include data on possible confounder such as bronchiectasis and other inflammatory markers. This factor may have affected our findings.

RESULTS:

Paper I**: ''Primary ciliary dyskinesia patients have the same *P. aeruginosa* clone in sinuses and lungs. ''**

Our study of nine patients with PCD chronically lung infected with PA demonstrates that each patient has the same genotypic and phenotypic clone type in both their upper and lower airways. So, albeit it being only nine patients, this demonstrates that the same clone exists in the sinuses up to years after the clone was first sampled from the lungs (Figure 1A in Paper I). The nine sinus samples and 15 lung samples depicted in Figure 1 each contained one or more PA isolates, resulting in a total of 38 PA isolates. The patient-specific isolates can be seen in Figure 1B in Paper I.

Genotypic and phenotypic analyses of the 38 PA isolates showed no consistent adaptative traits between the sinus and lung isolates overall, as well as variation within niche-specific isolates.

The genetic distances measured in single nucleotide pairs between clones can be seen in Figure 12 (below). These genetic distances are used to generate a phylogenetic tree (Figure 1B in Paper I and Figure 13A) depicting the genetic relationship between all sinus and lung isolates within and between patients and their genetic distance to the reference strain PAO1. It also shows that all sinus and lung isolates from each patient belong to the same clone type and only have very slight

genetic and phenotypic differences. Overall, isolates retained their phenotypic similarity to the reference strain PAO1 (Figure 13B).

SNP distance	P8 sample 1	P9 sample 1	P4 sample 1	P4 sample 2	P4 sample 3	P2 sample 1	P2 sample 2	P2 sample 3	P1 sample 1	P1 sample 2	P1 sample 3
P8 sample 1	0	24600	23872	23873	23872	26959	26968	26961	51385	51386	51401
P9 sample 1	24600	0	24076	24077	24076	26578	26587	26580	51493	51494	51509
P4 sample 1	23872	24076	0	3	4	26538	26547	26542	52089	52091	52103
P4 sample 2	23873	24077	3	0	5	26539	26548	26543	52090	52092	52104
P4 sample 3	23872	24076	4	5	0	26538	26547	26542	52090	52091	52104
P2 sample 1	26959	26578	26538	26539	26538	0	9	4	50138	50139	50154
P2 sample 2	26968	26587	26547	26548	26547	9	0	7	50145	50146	50161
P2 sample 3	26961	26580	26542	26543	26542	4	7	0	50138	50139	50154
P1 sample 1	51385	51493	52089	52090	52090	50138	50145	50138	0	35	130
P1 sample 2	51386	51494	52091	52092	52091	50139	50146	50139	35	0	135
P1 sample 3	51401	51509	52103	52104	52104	50154	50161	50154	130	135	0
P8 sample 2	3	24599	23871	23872	23871	26958	26967	26960	51384	51385	51400
P8 sample 3	2	24598	23870	23871	23870	26957	26966	26959	51383	51384	51399
P8 sample 4	2	24598	23870	23871	23870	26957	26966	26959	51383	51384	51399
P7 sample 1	51295	51582	52425	52426	52425	50506	50513	50506	32208	32209	32226
P5 sample 1	24289	24365	23046	23047	23046	26301	26310	26305	51611	51612	51627
P6 sample 1	28679	28356	28704	28705	28704	30082	30091	30086	51088	51089	51102
P9 sample 2	24598	4	24074	24075	24074	26576	26585	26578	51491	51492	51507
P9 sample 3	24624	26	24100	24101	24100	26602	26611	26604	51517	51514	51533
P9 sample 4	24597	3	24073	24074	24073	26575	26584	26577	51490	51491	51506
P3 sample 1	24297	24178	24341	24342	24341	26564	26573	26568	50871	50870	50887

Sample ID nr. | Close genetic relationsship | Distant genetic relationsship | Very distant genetic relationsship

Figure 12: A section of a chart showing the genetic distance measured in single nucleotide pairs (SNP) between samples from patients. The numbers within the green, yellow, and red squares list the SNP difference between the two samples. P: patient.

The median time between the first and last isolates was 3.0 years (range 0 – 5.5 years). The individual clone types of patient 1—5 and 7 were identified during a period of 3.5—5.5 years. The individual clone types of patient 6, 8 and 9 were identified during a period of 0—8 months. Only patient 1 had a hyper mutating clone type while the clone types of the remaining eight patients had no or few gene mutations. Clones from patient 2—5 and 7 did not accumulate gene mutations relative to patient 6, 8 and 9 (Figure 1b in Paper 1 and Figure 13A). We did not observe consistently high adaptation levels in isolates from patient 1—5 and 7 compared to the other patients (Figure 1b in Paper 1 and Figure 13B).

*PAO1.*These small differences are believed to be caused by the PA clones being exposed to different local environments in the sinuses and lungs [117-118, 123]. Each niche in the airway has slight differences in oxygen tension, mucus production, nutrient availability, immune and inflammatory response etc., which prompt small genotypic and phenotypic adaptations in the PA clone promoting survival within that airway niche. Besides clone DK019, we did not observe the sharing of clone types with CF patients or within our PCD patients. This result suggests effective isolation strategies and hygiene precautions at our institution. Antibiotic resistance was very low with 100 % of PA isolates being sensitive to colistin and 84 % sensitive to ciprofloxacin.

Patient no. 6 had a PA positive paranasal sinus sample before a positive lung sample. The patient was offered standard treatment but only wished to receive topical treatment (Colistin sinonasal irrigation x 2 daily). When the positive lung sample was observed, the patient then accepted standard treatment (see Paper I).

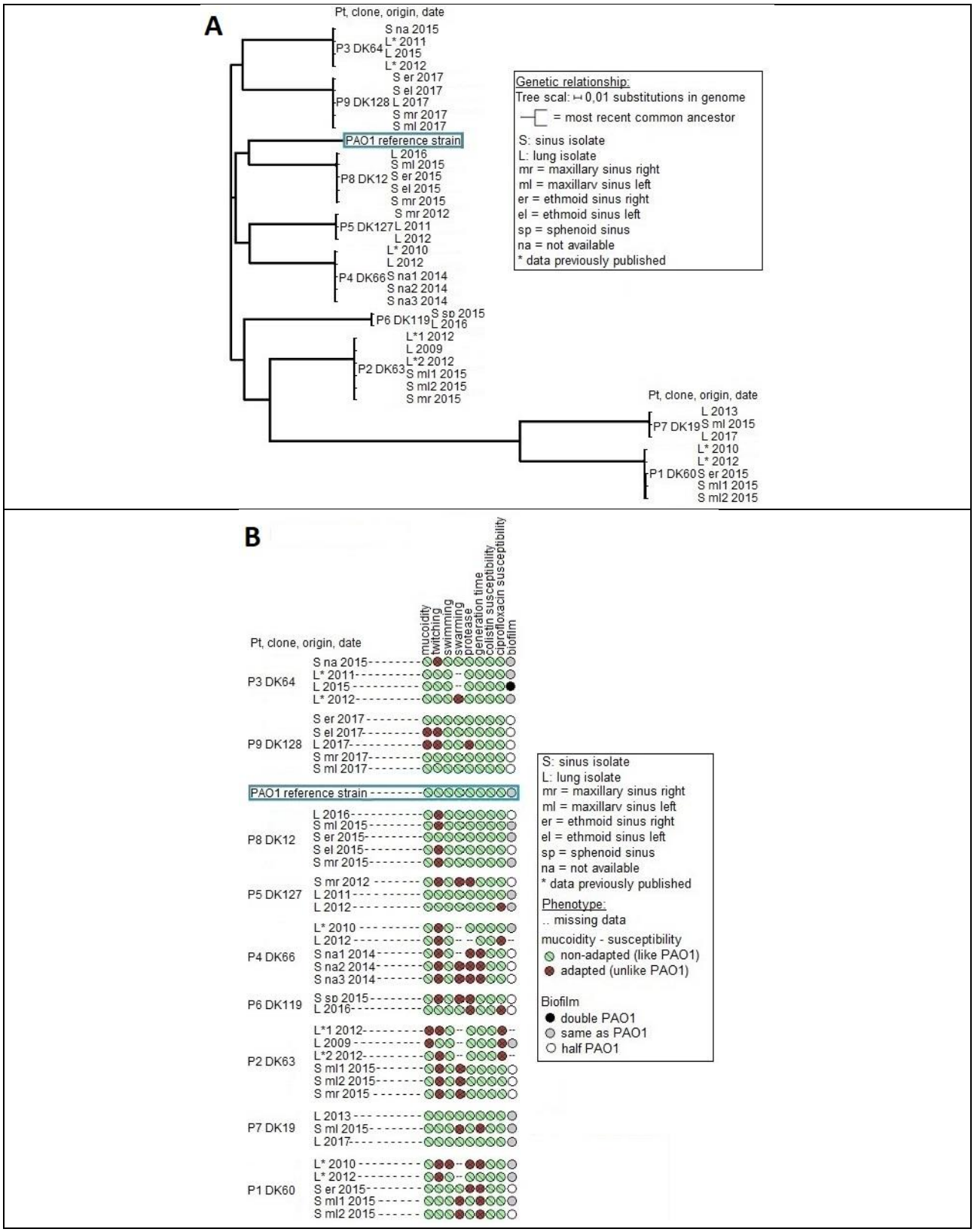


*Figure 13: **A**: The genetic relationship between paranasal sinus, lung isolates and the reference strain PAO1. The horizontal and vertical lines show the genetic distance between the isolates. Branching shows the genetic distance to the most recent common PA clone ancestor. **B**: The most common PA phenotypic traits of each paranasal sinus, lung isolate and the reference strain*

Understanding PA colonization is vital in preventing, treating, and eradicating PA from the global airways in these patients. Our findings further support the global airway disease concept in patient with PCD and emphasize that the sinuses also contain PA clones. This result supports the existence of a sinonasal bacterial reservoir. Therefore, this potential sinonasal bacterial reservoir should be evaluated in patient suffering from recurrent pulmonary infections.

Paper II**:** ''**Chronic Rhinosinusitis in COPD: a prevalent but unrecognized comorbidity impacting Health-Related Quality of Life**''.

Paper III**:** ''**COPD patients have a high prevalence of anosmia**''.

We found that 22.5 % (n=50) of patients with COPD at our centre suffered from CRS according to the EPOS2020 [21] and GOLD2019 guidelines [15]. Of these, 82 % (n=41) were undiagnosed and therefore also untreated prior to our study. The predominant CRS phenotype in COPD was CRSsNP (96 %, n=48) with only 4 % (n= 2) having CRSwNP. Overall, 55 % of all patients with COPD had a historical eosinophil count above 150 (cells X 10^9/L), respectively 65.2 % in the COPD with CRS group and 55.6 % in the COPD without CRS group (see also Table 1 in Paper II). Their present eosinophil count was not available. There was no difference in the number of frequent exacerbators between the CRS and non-CRS group (Table 2, Paper II). Additional data on previous exacerbation and recent infections were not collected. COPD patients with CRS had significantly worse HRQoL measured by PROMS: SNOT22, SNOT22-nasal symptoms and CAT, compared with COPD patients without CRS and healthy controls (Table 1, Paper II). The SNOT22 and CAT have some overlapping questions (sound sleep, energy level and cough), which address the global airway and not solely the upper or the lower airway. The SNOT22_nasal symptom subscore focusing solely on nasal symptoms was better than the complete SNOT22 score at identifying those COPD patients who had an increased risk of CRS (see table 3 Paper II). Univariate analysis exploring all variables showed that the SNOT22, the SNOT22-nasal symptoms and CAT score were significant risk factors of CRS. Multiple logistic regression analysis adjusting for gender, age, smoking and ICS use identified an active smoking male patient with COPD using inhaled steroids and having a high CAT and a high SNOT22_nasal symptoms subscores as having the highest risk of concomitant CRS (Figure 13) (Figure 2, Paper II).

Ten (2.2 %) of the 222 patients had a FEV_1 % predicted > 80 % and a post-bronchodilator FEV_1/FVC < 0.70 (Table 2 of Paper II). Two of these patients were diagnosed with CRSsNP, the remaining eight patients had no CRS. The prevalence of CRS changes from 22.5 % to 21.6 % if these two patients are excluded. The COPD diagnosis was based on chronic obstructive spirometry pattern and symptoms and the lack of change in FEV_1 on standard reversibility test. As mentioned in the method section the COPD diagnosis was reconfirmed by an additional senior COPD specialist. As there were no clinical signs of asthma the two standard asthma provocation tests (mannitol and methacholine) otherwise used at our institution were not performed [128].

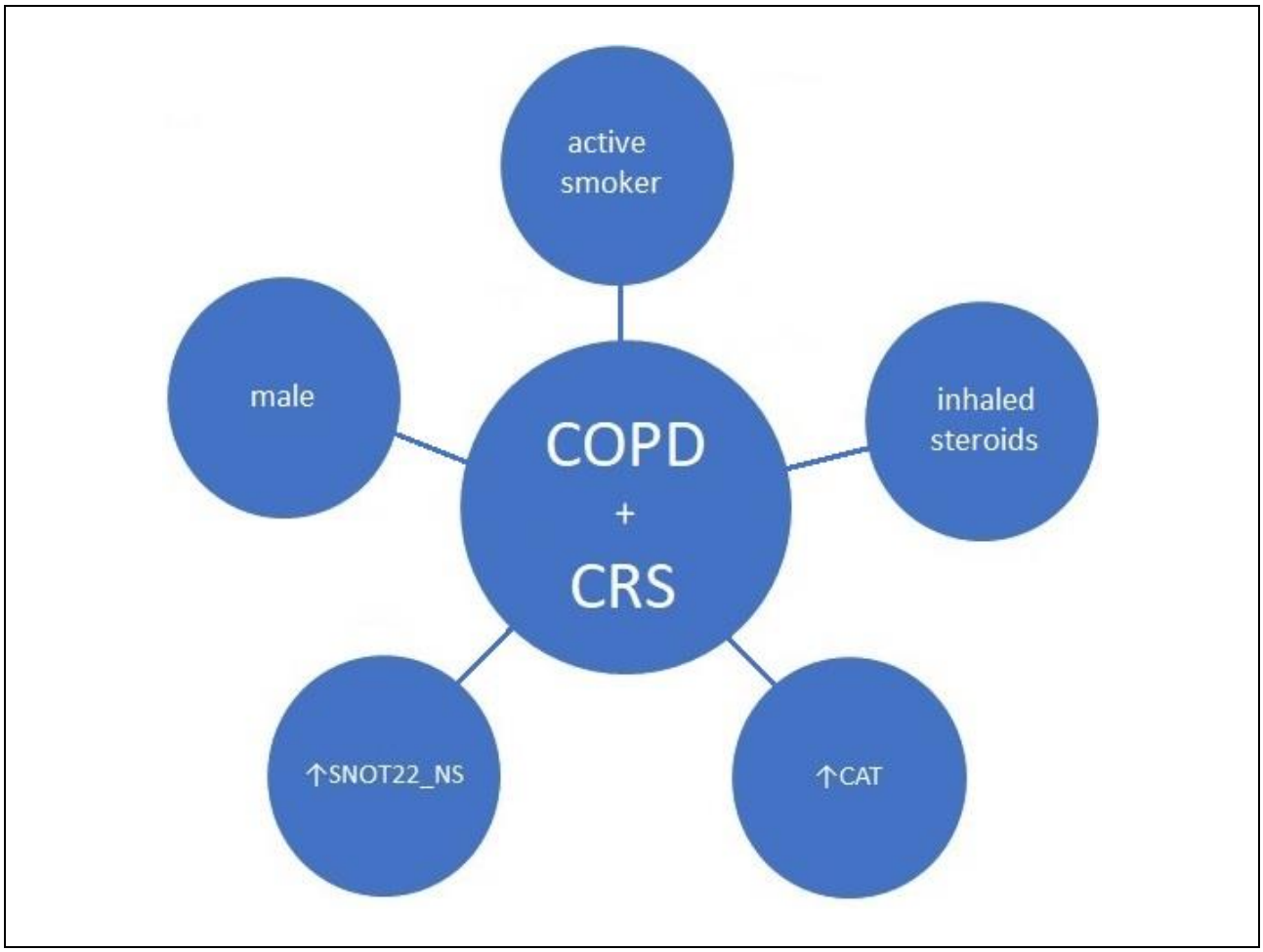


Figure 13: Risk factors for CRS in patients with COPD identified by multivariate analysis.

In addition to nasal obstruction, nasal discharge and facial pressure, olfactory dysfunction is one of the EPOS diagnostic criteria for CRS, but little is known about the olfactory function in patients with COPD. Investigation of the olfactory function in this large group of patients with COPD with and without CRS revealed a significantly higher prevalence of anosmia (14.1 %) compared to healthy controls (1.4 %) (Figure 14) regardless of age, CRS, smoking status and GOLD grade and type (Figure 2 and 3[12] in Paper III). The high prevalence of anosmia was paralleled with a low prevalence of hyposmia and a normal level of normosmia. This biphasic pattern levelled out mean SIT16 scores so that no difference was observed between patients with COPD and healthy controls.

Of the eleven patients reporting relevant history that could affect olfaction, four patients had undergone previous sino-nasal surgery. Of these, one patient with CRSsNP and a previous septorhinoplasty had a SIT16 score of 11/16 (hyposmia). The remaining three patients have normal SIT scores. Five patients reported previous facial trauma. Of these, two without CRS had a SIT16 score of 6/16 and 8/16, respectively (anosmia). Two patients had previously been exposed

[12] Supplement figure test: 3A: “type” should be grade. 3B: “grade” should be group.

to toxins (welding smoke or organic solvents). Of these, one patient with CRSwNP had a SIT16 score of 6/16 (anosmia). In total, four of eleven patients had decreased olfaction. The prevalence of anosmia changes from 14.1 % (n=19) to 12.6 % (n=17) if we exclude the two patients with anosmia without CRS, where there is another plausible reason for olfactory dysfunction. This result is still significantly higher than the 1.4 % found in the healthy control group. We choose to keep all patients in the analysis.

Patients responded to two questions about the affected sense of smell, firstly as part of the SNOT22 and secondly as part of the EPOS CRS diagnostic criteria. We observed that patients' reply to the EPOS criteria about affected olfactory function and their grading of the SNOT22 question on affected smell and taste was poorly associated with their actual SIT16 test score (Figure 15 and Figure 4[13] in Paper III).

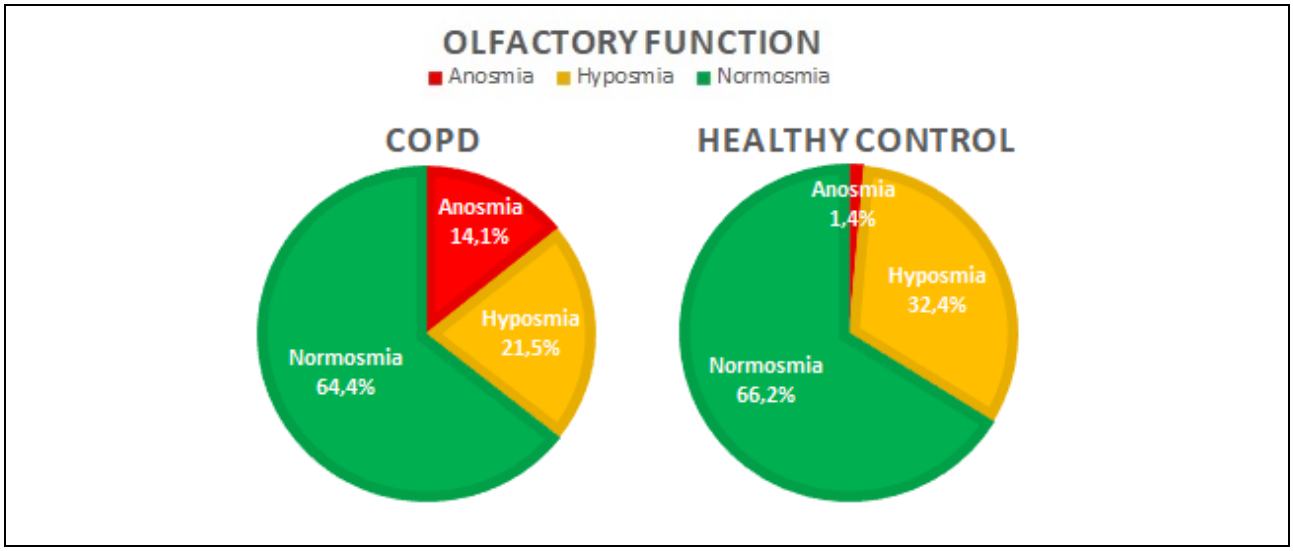


Figure 14: Olfactory function in patients with COPD and age-matched healthy controls.

[13] Figure 4 supplement test: the EPOS minor criteria are part of the diagnostic criteria for CRS, see also the background section. The minor criteria are facial pain/pressure and affected sense of smell. The figure depicts patients' answer to the EPOS question concerning affected sense of smell. Pt nr: number of patients.

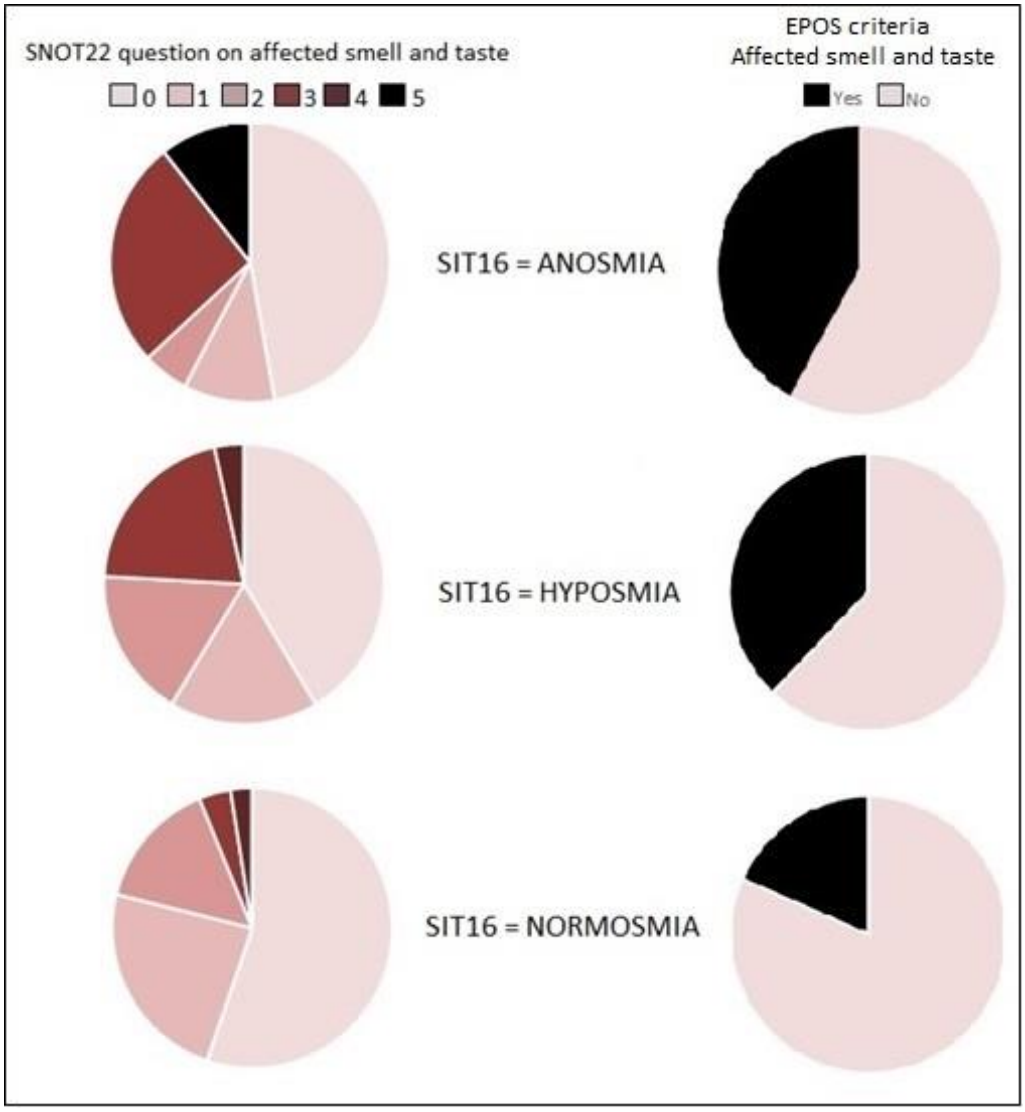


Figure 15: The mismatch between patients with COPD Sniffin Sticks Identification test (SIT16) and their answers to the EPOS olfactory criteria and the SNOT22 question on affected smell and taste. SNOT22 scale: 0= no problem to 5=maximal problem.

DISCUSSION:

Now that the global airway concept has been established in other patients with decreased mucociliary clearance (MCC) [2, 5-6], we found it natural to investigate further the interaction between the upper and the lower airways in patients with PCD and COPD.

We demonstrate the existence of global airway PA colonisation in patients with PCD by genetic testing of PA samples from both their upper and lower airways. Our results build on previous work by our research group [7] demonstrating the presence of PA positive cultures from the sinuses and the lungs in patients with PCD.

For the first time, we present that the same PA clone lives in the sinuses and the lungs of patients with PCD, demonstrating how identical PA bacteria colonise the entire global airway of these patients.

Despite patients with PCD receiving multiple courses of antibiotics, we still identified identical PA clones in the sinuses up to years after the initial find in the lungs. In three patients, no.6, 8 and 9, the observation period was 0—8 months. This result enabled us to conclude that their individual clone type exists in their global airways during that period. However, we cannot conclude whether they will clear or retain their clone type in future. There are different possibilities behind the continuous presence of the individual clone type in the global airways of these nine patients. It may be caused by a sinonasal bacterial reservoir, making the patient unable to clear the clone from their global airways or continuous reinfection from another source. Notably, we found no signs of cross-infection between patients and all but one clone type (DK19), were unique to PCD. DK19 belongs to the PA14 clonal complex which is an abundant and globally distributed clone type [123, 129] that has previously been found independently (i.e., without signs of cross- infection) in Danish CF patients [114]. Another alternative external source of reinfection could come from the patients' close surroundings, e.g., a local environmental source [130]. As all, but one, clone types were unique to PCD and dissimilar to known environmental sources, we find it more likely that a sinonasal bacterial reservoir is the cause of the continuous clonal presence. A reservoir wherefrom pathogens may reinfect the lungs, sustain airway inflammation and promote infection.

Why is PA not cleared from the sinuses? Possible reasons for the retainment of PA in the sinuses despite antibiotic eradication attempts are a diminished antibiotic effect in the sinuses compared to the lungs, which may be caused by less antibiotic bioavailability due to the anatomical differences between these two areas of the airways. Studies have demonstrated decreased efficacy of intravenous antibiotics in the sinuses compared with the lungs [131], with lower concentrations of antibiotics in the sinus cavity secretion than the concentrations within the sinus mucosa in patients with CRS. This result indicates that the time above the minimal inhibitory concentration and therapeutic effect decreases as the distance from the sinus mucosa increases [132]. The distances in the sinuses are much greater than in the lungs; therefore, bacteria in the sinus cavity may be less exposed to antibiotic treatment and therefore more likely to survive. The presence of human mucus has likewise been found to decrease PA susceptibility to tobramycin but not colistin [133], making eradication less likely in patients with high mucus production and perhaps, especially in the mucus-filled sinus cavities. The degree of antibiotic exposure can drive bacterial adaptation altering antibiotic susceptibility. Intensive antibiotic treatment in late CF PA lung infection stimulates extensive genotypic and phenotypic changes [119, 134] and tempero-spatial diversification within different niches of the lungs [117, 135]. Our PCD PA clones exhibited a low level of adaptation, retaining their PA01 like genotype and phenotype, perhaps due to less antibiotic stress than in CF. Other considerations in the question of PA retention include differences in antibiotic drug administration methods when treating the sinuses and the lungs (inhaled versus oral and intravenous), tissues oxygen tension and host inflammatory response. Studies of global airway disease in CF have shown regional differences in the inflammatory response dominated by high levels of IgA in the sinuses and neutrophils in the lungs [136]. A study of 20 patients with CF showed decreased oxygen tension in the sinus mucosa compared to controls, and the authors hypothesized that anoxic conditions might change the bacterial community [137]. All the above-mentioned factors may contribute to the intra-clonal regional differences seen in the global airways of patients with PCD.

Mutations in the AlgU, LasR and MigA genes involved in biofilm formation, mucoidity, quorum sensing and colistin resistance are not always directly correlated to the PA phenotype [111, 113, 138]. Other regulatory mechanisms and post transcriptional events affect the same phenotypic traits as the mutated gene, thereby cancelling its impact [139-141]. What is then the relevance of analysing PA genotype? Whole-genome sequencing examines the entire PA isolate genome including any gene mutations, to determine the exact PA clone type. Knowledge of the clone type allows for monitoring of any cross-infection between patients or other exogenous sources enabling necessary prophylactic measures to be put in place. RNA testing can help elucidate which gene mutations result in altered gene products and how it affects the phenotype. At present, genotyping and phenotyping are not performed routinely at our institution, where PA treatment is guided by culture and sensitivity testing and guidelines.

We observed limited levels of PA adaptation, which may seem surprising given that PCD is a congenital disease, and the median age of included patients was 18 years (range 10—42 years). One reason for the low level of adaptation could be that chronic PA colonization develops over time. Hence, our isolates have been exposed to the host environment for a shorter period experiencing less stress and, therefore, undergone less adaption (Figure 6). Patients initially become infected with PA and then clear the infection. However, over time, some become more prone to recurrent PA infection, less able to clear the infection and are chronically infected [2]. A study of 158 children and adolescents with PCD over a six-year time period observed PA acute colonisation in 37 % and chronic infection in 5 % [107]. Another study of 107 PCD patients (median age 17 years, range 0—74 years) found that 11—47 % were intermittently colonised, and 39 % of patients were chronically infected with PA over a period of 11 years [104]. Previously studies [8, 114] have shown that gene mutations in PA isolates from patients with PCD resemble the mutational patterns seen in early CF infection. Multiple pathoadaptive genes controlling virulence factors and bacterial fitness have been identified.

Similarly, we observed mutations in the MigA, LasR and AlgU genes but only in some matching sinus and lung isolates. In general, the genetic adaptation in our isolates was low in accordance with previous findings. Another consideration is the difference in monitoring and antibiotic treatment between patients with PCD and CF at our institution. CF patients are infected earlier with PA and have more recurrent infection than PCD increasing the antibiotic selection pressure. CF patients are also followed more closely in the out-patient clinic and receive intravenous antibiotics as first-line PA treatment compared to peroral antibiotics in patients with PCD ([3], Paper I). This difference in antibiotic treatment may cause less pressure on PA isolates in PCD patients and thereby less cause for adaptation.

The PA clones infecting our patients with PCD displayed similar characteristics as early PA infection in patients with CF [8, 114] suggesting that treatment experience from CF may be transferable to PCD. Studies of PA from patients with bronchiectasis is unlike PA from patients with CF [142]. Studies of PA infection in COPD have diverging results [109, 143] regarding PA infection comparability in CF. Further research is needed to reveal whether treatment experience from CF and PCD can be extended to include COPD PA infection.

Another point of interest is how PA colonization and lung function impact each other. Cohen-Cymberknoh et al. 2017 [105] showed that patients with PCD and low FEV_1 were prone to PA colonization. However, the observed decline in lung function did not differ significantly when compared to non-colonized patients. Contrary to this, Eden et al. 2019 [144] showed that PA positive patients with PCD had worse lung function than other patients with non-CF bronchiectasis. However, they did not discuss the possible preventive effect of early PA eradication. Further research is needed to investigate methods of preventing the inflammatory and structural damage caused by PA colonization and its effect on lung function.

Patients with PCD and global airway disease suffer from dysfunctional MCC, which alters the local sinus milieu and the local middle ear milieu augmenting the favourable conditions for continued bacterial growth and pathogen retention. These mechanisms may be relevant to other chronic inflammatory airway diseases where though airway mucus and decreased MCC are also a key factor. The study by Alanin et al. [67] reported a 25 % PA eradication rate in patients with PCD undergoing ESS combined with postoperative nasal saline irrigation and nasal steroids. The beneficial effect of ESS on the sinus environment has also been reported in patients with CF [145] and emphasises the importance of recognising and treating a bacterial focus in the sinuses.

Prospective studies of simultaneous sinus and lung PA samples are necessary to establish the route of colonisation, the interplay between colonization and infection, test if the PA colonies in the lungs and sinuses respond equally well to treatment and how the eradication of PA affects prognosis.

The presence of sinonasal symptoms, thick nasal secretion and suboptimal MCC has also been established in 42–88 % of patients with COPD [30, 45-48, 146]. These previously published questionnaire-based studies of nasal symptoms in COPD patients supported global airway disease in COPD patients. Amongst others, Caillaud et al. [146] study of 274 CPOD patients observed that 42 % had nasal symptoms (discharge, obstruction, and anosmia) and that mMRC and cumulative smoking was correlated with chronic nasal symptoms. Of the 128 patients reporting nasal symptoms, 62 % reported rhinorrhea, 43 % nasal obstruction and 16 % anosmia. Similarly, they did not find an association between exacerbation frequency, FEV_1 and nasal symptoms. Their nasal symptoms were diagnosed as chronic if they had persisted for more than six weeks per year, whereas EPOS defines chronicity as symptoms for more than 12 weeks. This difference in chronicity definitions may explain their relatively high prevalence of chronic nasal symptoms compared to our study. Their study population also included patients with COPD-asthma overlap, possibly affecting their results due to the known association between asthma and CRSwNP. It is important to remember that nasal symptoms alone are insufficient to diagnose CRS. According to EPOS guidelines nasal symptoms must be present for at least 12 weeks and must be supported by objective findings on flexible fiberscopy or CT-sinus scan. The literature observed signs of upper and lower airway associated symptoms but was less diagnostically accurate, paralleled to a decreased certainty factory compared to our study.

Interestingly this does not seem to have altered the way we as clinicians approach the care of these patients. Pulmonologists tend to the lower airways and otorhinolaryngologists to the upper airways, but they rarely focus on the global airway. Clinical research from our global airways research group has shown that 40–65 % [5, 147] of patients with CRSwNP, booked for ESS, had concomitant asthma but 50 % were unaware of this fact. Based on our findings only one in five patients with COPD suffering from CRS have been examined and treated by an otorhinolaryngologist. None of the patients with COPD have been diagnosed with CRS by a respiratory physician. This result clearly shows that increased awareness of global airway disease is needed amongst clinicians.

After examining PA colonisation of the global airways in patients with PCD, we turned our attention to the global airways in patients with COPD. Patients with COPD also have substantial chronic lower airway disease, decreased MCC and though airway mucus, which we hypothesised makes them likely candidates for having global airway inflammation and disease. We found one in four patients with COPD (22.5 %) to suffer from CRS and that over 80 % of these were undiagnosed prior to our study. This lack of diagnosis means that CRS was untreated. Considering that the WHO [33] estimates that 65 million people worldwide suffer from COPD and with a CRS prevalence of 22.5 % we potentially end up with 14.6 million people suffering from largely undiagnosed global airway disease.

Based on previous work by other researcher groups CRS frequency in COPD is reported as higher as 48.5–64 % [11-13]. Both studies diagnosed COPD according to the most current version of GOLD considering their study period (2006 and 2014 GOLD versions) [148-149]. There are some important differences between the previous and current version of GOLD except the addition of eosinophil count. GOLD 2014 is similar to GOLD 2019 as staging is based on symptoms and spirometry, while GOLD 2006 has stage I—IV, where stage I and II do not require the presence of COPD symptoms, such as cough and sputum. This situation may have led Kelemence et al. [13] to overestimate the number of study participants suffering from true COPD as 40 % of their study population had COPD stage I–II.

Furthermore, we question the accuracy of the CRS diagnosis in these studies as they do not concur with current guidelines. None of the patients included had a nasal endoscopy, which is essential in evaluating CRS and objective measures based solely on CT-sinus scans. Studies have demonstrated the unreliability of CT-sinus scans as the sole objective measure when diagnosing CRS, as non-symptomatic incidental findings are quite common on head and neck CT-scans [150-151]. Geographical and ethnical differences [58] could further contribute to the described frequency variation as the previous studies of CRS in COPD included Chinese and Turkish patients. Even within European centres questionnaire-based CRS frequency varies considerably (6.9–27.1 %) with Scandinavian countries having low frequencies of 7.8 % [56]. Our result of 22.5 % CRS in patients with COPD is much higher than the average CRS frequency in the general Scandinavian population and is in the top range in Europe, signifying a substantial disease burden. A burden which at present is largely unrecognised and uncared for. We are presently comparing CRS prevalence in patients with COPD to the general public. Therefore, tt would be interesting to know the

prevalence of patients with COPD and CRS in the abovementioned countries to secure a more accurate comparison of frequencies.

Global airway disease has been presented in many other chronic lower airway diseases. However, the clinical presentation differs, and management should be tailored to suite the phenotypic and endotypic differences. New treatment with monoclonal antibodies is indicated in patients with recalcitrant CRSwNP [21]. CRSwNP is very common in patients with asthma [5, 147], PCD (15–30 %) and CF (17–50 %) [2, 22, 152]. Contrastingly, only 4 % of our patients with COPD had CRSwNP, making them rare candidates for this treatment.

To facilitate the identification of patients with COPD who had an increased risk of CRS, we used multivariate analysis to set up a risk factor model, see Paper II. Clinicians can use these risk factors (Figure 13) to select which patients should be referred for otorhinolaryngologic evaluation and considered for treatment and monitoring in a multidisciplinary global airway team. This risk factor model was designed as a tool for pulmonologists but can equally be used by otorhinolaryngologists to screen which of their patients with CRS may suffer from undiagnosed COPD.

Somewhat surprisingly, we did not observe increased odds of having CRS in frequent exacerbators. We had expected lower airway exacerbations to correlate with increased upper airway disease [153]. A possible explanation may be that an exacerbation is an acute worsening of pulmonary symptoms. Therefore, any acute nasal symptoms may also be temporary not causing an increase in chronic symptoms. Future registration of acute nasal symptoms and/or acute rhinosinusitis during pulmonary exacerbation would be useful. We found 23 % of our study population to be frequent exacerbators, which is higher than the 9—16 % previously reported [35]. This factor may have introduced a selection bias caused by differences in the study populations where a larger part of our patients had higher GOLD grades and groups (Table 2, Paper II). But we must also consider that recent pulmonary exacerbation was an exclusion criterion, which we would expect to have lowered the number of included frequent exacerbators. This factor may affect the generalisability of our results. However, it can still be instructional in the general COPD population and directly transferable to other tertiary hospital settings.

Bronchiectasis was not recorded in our study but would be interesting to include in future studies as it increases the risk of PA infection, morbidity, and mortality. It is unknown whether bronchiectasis in COPD only affects the lungs or increases patients' risk of developing a sinonasal PA reservoir like the reservoir function seen in both CF and PCD (Paper I). Future research on the links between upper and lower airway inflammation, exacerbation, pathogen colonization and infection can supply a new piece of the global airway puzzle.

It is important to consider the possible influence of ICS on our results. The similar use of ICS found in our COPD patients with and without CRS reflects the comparable COPD severity distribution (FEV_1, GOLD and MRC) in the two groups. Inconsistent findings from previous studies of the effect of ICS on the risk of pneumonia demonstrate the complexity of ICS effects on the airways. We

observed no effect of ICS on the risk of CRS in univariate analysis. However, multivariate analysis showed that ICS combined with male gender, active smoking and high PROM scores identified the patients most likely to suffer from CRS. Studies have shown some systemic adverse effect of primarily continued high dose ICS [154-155] but the effect on the systemic immune system and its role in CRS remains unknown. Since our results are observational and therefore cannot prove causality, prospective RCT studies are necessary to determine the exact effect of ICS on the risk of developing CRS in future.

The SNOT22 is a well-established routine questionnaire in an otorhinolaryngologic setting and CAT, MRC in a pulmonologist setting but not vice versa. The SNOT22_NS [65] alone is not widely used. Besides measuring disease severity, these PROMs are used for monitoring disease control and treatment effect. The SNOT22 and SNOT22_NS were increased in more than 90 % of all patients with COPD regardless of CRS status, and the CAT was significantly higher in COPD with CRS compared with those without CRS. This result demonstrates the burden of upper airway disease in these patients. It is surprising, considering the high SNOT22 scores, that patients' sinonasal symptoms are not recorded during their clinical visits. Upper airway symptoms were also underreported in patients with PCD and CF [2, 156]. We hypothesise that dyspnea and lack of awareness of sinonasal symptoms cloud patients' and physicians' perception of symptom origin. Therefore, standardised questioning about global airway symptoms is essential and preferably using a global airway PROM. Such a global airway PROM is currently under development by parts of our research collaboration. Our data shows that the SNOT22_NS subscore is better than the SNOT22 at identifying patients with COPD at risk of CRS (see Paper II: Table 1—3 and Figure 2). This result leads us to recommend that the SNOT22_NS subscore be routinely introduced in the pulmonologist clinic alongside the CAT and MRC. These factors can quickly be combined in the risk factor model to identify those patients at risk, with minimal impact on the clinical burden and a large benefit for the patients. Equivalently, the CAT and MRC can be used in an otorhinolaryngologic setting to identify patients at risk of undiagnosed lower airway disease. These PROMs are short, easy to use and can be completed by the patient prior to their out-patient visit or in the waiting room. Therefore, we recommend that the SNOT22_nasal symptoms subscale becomes a standard questionnaire given to patients with COPD and that patients at risk should be referred for otorhinolaryngologic examination and treatment.

Patients with COPD and PCD already suffer from substantially decreased HRQoL. Undiagnosed and untreated CRS may also worsen their HRQoL and perhaps sustain airway inflammation, decrease lung function, and result in infection and increased risk of morbidity and mortality. Caillaud et al. [146] reported decreased HRQoL (measured by mMRC) in 274 COPD patients complaining of chronic nasal symptoms. The CAT, SNOT22 and SNOT22_NS subscores in our study were all significantly increased in patients with COPD and CRS compared with those without CRS indicating a substantial negative effect on HRQoL. All patients with COPD with and without CRS had a SNOT22 score above 7 which is the cut-off value for healthy controls [157], indicating a negative impact of global airway disease beyond the effect of CRS. Currently, there is no known cut-off value for the SNOT22 in patients with COPD. Therefore, there lies great potential for improving patients' quality of life by treating not only of CRS but their entire global airway disease.

Increased focus on global airway disease is important in order to diagnose, treat, and prevent progression correctly. The first step is to identify afflicted patients and then improve both treatment and quality of life. We anticipate the substantial socio-economic burden [158-159] of undiagnosed and untreated global airway disease to lessen by timely and efficient multidisciplinary patient care.

Another important symptom from the upper airways is affected olfactory function, which may be decreased in CRS. This correlation is reflected in the EPOS diagnostic criteria [22, 160]; however, olfactory function in patients with global airway disease is poorly understood. Is there an additional negative effect of global airway inflammation on olfaction that extends beyond CRS? Existing knowledge of olfactory function in COPD is sparse. Previous studies have reported subjective olfactory dysfunction in 16—24 % of COPD patients, but no olfactory testing was performed [13, 146]. Our SIT16 test results found a 10-times higher prevalence of anosmia (14.1 %) in our patients with COPD compared to healthy controls (1.4 %) (Figure 14). This increased prevalence of anosmia is in accordance with findings of anosmia in 29 % of patients with PCD [161] and 12.7 % of patients with CF with and without CRS [152].

The SIT16 score is a subtest of the full TDI test, which may raise concerns about whether the Identification test alone is enough to diagnose olfactory dysfunction. Robson et al. [162] found a significant difference between identification scores when comparing normosmic and completely anosmi patients in their nine odorant Combined olfactory Test, demonstrating the ability of the identification test to differentiate between the two groups correctly. They were no able to differentiate hyposmic from anosmic patients due to an overlap in test scores. Hummel et al. [163] demonstrated a moderate correlation between SIT16 Identification test and the N-butanol Threshold test (r = 0,54) and the Discrimination test (r=0,56). The test-retest correlation was r=0,73 for the Identification test alone, r=0,61 for the Threshold-test and r=0,54 for the Discrimination-test, showing that the SIT16 Identification test is moderately correlated to Threshold and Discrimination test scores. Correlation between Identification and TDI was not reported. Test-retest was highest for TDI (r=0,72), TD-test (r=0,66), TI-test (r=0,71) and DI-test (0,68). So, the combined TDI test had the best test-retest correlation while including the highest level of information on olfactory function Threshold, Discrimination, and Identification) while the Identification test had the best test-retest reliability if only choosing one test – therefore good for the initial screening of the olfactory function. Hummel et al. [163] showed that the Identification and Discrimination subtests are less sensitive to increasing age compared to the composite threshold test. Therefore, there is a risk that we underestimated the level of hyposmia and anosmia in our COPD patients. We would expect a full TDI score to reveal a higher percentage of hyposmia and anosmia in our patients because they were older.

The classification of olfactory function into normosmia, hyposmia and anosmia is based on the following criteria. The cut-off for normosmia is defined as the 10th percentile for healthy subjects age 16—35 years (TDI 30.75). The distinction between hyposmia and anosmia is defined as a TDI score below 16.5 based on results from 70 anosmic patients [126, 164-165]. Niklassen's et al. [166] validation of the Danish forced multiple choice SIT16 answers studied 74 healthy subjects and demonstrated 10th percentile values of TDI 29.8 /SIT16 13 for the 18-35-year-olds and TDI 29.95 /

SIT16 12 for the 16-24-year-olds as cut-off values for normosmia. We compared our results to the Oleszkiewicz [126] control group based on 9139 subjects, primarily from Germany and a subset from Brisbane, Australia. Their paper lists total TDI score and individual Threshold, Discrimination, and Identification scores for healthy controls in gender and age differentiated groups. The SIT16 is the same test used for the I subtest in the TDI, and scores are, therefore, directly comparable. We compared SIT16 scores from our participant to age and gender-matched controls. Each age and gender group were much larger (total n=9139) than the control group would have been if we had chosen to include 135 gender and age matched healthy local Danish controls. We, therefore, find that the Oleszkiewicz [126] control group is reliable and representative.

Hyposmia and anosmia are also prevalent in patients with asthma and concomitant CRS [167]. Pifferi et al. [161] found ciliary and olfactory function to be correlated, reporting higher prevalences of hyposmia and anosmia in patients with more severe ciliary defects. So, altered MCC seems to influence both CRS and olfaction prevalence. Surprisingly, the prevalence of hyposmia was lower in our COPD group compared to the control group. The reason for this is unclear, but multiple factors may have influenced the results. Firstly, yearlong smoking and global airway inflammation in our group of chronically ill patients with COPD have caused profound inflammatory damage to the olfactory receptors causing anosmia. Secondly, patients' olfactory mucosa may have a different vulnerability to tobacco smoke and airway inflammation. There may be a critical level of stress above which the olfactory mucosa is irreversibly damaged causing some patients to develop anosmia while others are more resilient and remain normosmic. We used the SIT16 score which has a 5-point interval for normosmia (12—16), 3-point (9—11) interval for hyposmia and 9-point interval (0—8) for anosmia. The narrow 3-point interval for hyposmia and the relatively small sample size may have contributed to the low prevalence of hyposmia seen in our study.

Thirdly, studies of the olfactory centres in the brain show continuous modulation of the olfactory stimuli from the nose before they are consciously recognized. Decreased stimulation of the olfactory mucosa causes decreases cerebral olfactory activity resulting in diminished cerebral awareness of olfactory stimuli [168-170]. Mouth breathing decreasing airflow through the upper airways, which then causes less stimulation of the olfactory mucosa in the nose. This bottom-up/top-down feedback mechanism could play a part in patients with COPD. Their decreasing lung function forces them to breathe through their mouth, decreasing stimulation of their olfactory epithelium.

Finally, the decreased inspiratory lung function of COPD patients may decrease their peak nasal inspiratory flow (PNIF) diminishing stimulation of the olfactory epithelium resulting in olfactory dysfunction. Studies have shown that current smoking [171], decreased peak expiratory flow [172], and FEV_1% predicted [173] all decrease peak inspiratory nasal flow (PNIF). Therefore, the increased prevalence of anosmia in our COPD patients could be caused by reduced inspiratory function and reduced PNIF. PNIF was not measured in our patients, but it would be very interesting to include such data in future studies. It could contribute to the understanding of the mechanisms involved in decreased olfaction in COPD patients. A prospective study measuring olfactory thresholds and

evaluating olfactory mucosa biopsies in newly diagnosed patients with COPD could shed light on the reason behind our dichotomous results.

Olfactory dysfunction is known to decrease HRQoL but is the negative impact larger than other COPD symptoms and comorbidities? We looked at HRQoL using CAT. Olfactory dysfunction was present in 35.6 % of our COPD patients and their mean CAT scores was 19.95 (SD 7.6; n=48) for hyposmia + anosmia and 20,13 (SD 7.7; n=19) for anosmia alone. The CAT score of COPD patients with CRS presented in Paper II was 21.8 (SD 7.6; n=50). Miravitlles's et al. [20] reported CAT scores from 3452 COPD patients from 11 Central and Eastern European countries and divided them into groups according to their CAT scores (0—10; 11—20; 21—30; 31—40). They showed that as the prevalence of COPD symptoms and comorbidities increased, so did CAT scores, indicating an affected HRQoL. When looking at Miravitlles's et al. [20], patients with similar CAT scores to ours (CAT score 11—20; n=1522), 61.8 % had chronic cough, 53.0 % chronic sputum production, 16.6 % had purulent sputum production, 22 % coronary artery disease and 63.2 % hypertension. Although we cannot directly compare these patients, it indicates that the HRQoL impact of olfactory dysfunction lies somewhere between heart disease and regular COPD symptoms such as chronic cough and sputum production. Therefore, the impact of olfactory dysfunction on HRQoL in COPD patients should not be overlooked. In future it would be interesting to focus additionally on HRQoL in COPD patients with olfactory dysfunction. The use of more extensive questionnaires would enable the evaluation of specific HRQoL areas and if they are affected more than others.

Interestingly, there is a mismatch between patients' subjective rating of their olfactory function on SNOT22, their answer to the EPOS criteria of affected sense of smell and their olfactory test results (Figure 15) (Figure 4 Paper III). A similar mismatch has been reported in other patient cohorts [79, 152]. Ideally, patients' subjective ratings would match their test results and vice versa. One reason for this discrepancy may be that although the Sniffin' Sticks TDI test is good at quantifying olfactory function, it does not account for odour quality and how olfactory dysfunction impacts patients' lives and thereby how they subjectively grade their own olfactory function. Research of objective measures of qualitative odour perception is sparse but could help bridge this gap [174-175]. EOG[14] and fMRI can visualise if there is cerebral activity to peripheral odour stimulation taking testing one step further, but again the subjective angle is lost. Perhaps extensive olfactory questionnaires on odour perception and assigned subjective importance will explain the mismatch. Odour perception may vary with cerebral function, smoking, gender, culinary habits, culture, and job function. Hence, it is important to be aware of this inconsistency and not only inquiring about patients' symptoms but also use olfactory PROMs and perform olfactory testing.

In combination with the existing literature, our studies strongly support global airway disease in patients with PCD and COPD and underline the relevance of understanding how global airway disease affect disease pathogenesis and prognosis. These patients require a multidisciplinary approach to diagnostics and treatment in order to decrease airway inflammation, recurrent

[14] Electro-olfactography.

infection, olfactory dysfunction and improve prophylactics, treatment strategies and HRQoL. As morbidity and mortality are high, and HRQoL is severely affected in patients with global airway disease we as physicians should strive to improve the management of these patients, starting with an increased focus on undiagnosed global airway disease.

CONCLUSION:

In conclusion, global airway disease is present in patients with PCD and patients with COPD. The sinuses in patients with PCD act as reservoirs wherefrom bacteria may reinfect the global airways. More than 1 in 5 COPD patients suffer from CRS and more than 1 in 3 COPD patients suffer from olfactory dysfunction. Global airway symptoms are unrecorded in patients with COPD, requiring that physicians specifically inquire about these symptoms in addition to performing the guideline-recommended nasal and pulmonary assessments including nasal endoscopy, olfactory testing, and spirometry. Until a global airway PROM has been developed, the SNOT22_NS subscore and the CAT are short, easy to use PROMs, which will help identify patients at risk of global airways disease and secure referral for diagnostics and treatment, preferably by a multidisciplinary global airway team.

STRENGTHS AND LIMITATIONS:

Paper I: we included a limited number of nine patients with PCD and chronic PA infection, but the study is the first of its kind and presents results of an individual and complete collection of sinus and lung PA isolates. All sinus isolates were collected during ESS surgery, and microscopy verified lower airway representation in all lung isolates ensured accurate sampling, strengthening our results. Consecutive and simultaneous samples from both upper and lower airways, tests of possible exogenous sources of PA, inflammatory markers and registration of antibiotic treatment and effect would have strengthened our conclusion of a paranasal sinus bacterial reservoir and global airway disease in PCD.

Paper II+III: to the best of our knowledge, we conducted the largest study of multidisciplinary evaluated CRS in patients with COPD. All patients were diagnosed by otorhinolaryngologist, pulmonologists and radiologists according to the highest standard (EPOS2020[21]/ GOLD 2019[15]), including measurement of HRQoL through disease-relevant PROMs (SNOT22/SNOT22_NS/CAT/MRC). Endoscopic findings were evaluated by EA, but an additional blinded otorhinolaryngologic specialist evaluation of nasal endoscopy may have increased diagnostic accuracy. We may have over diagnosed some of the patients with less severe COPD symptoms (GOLD 1A n=3; 1B n = 6) as fixed ration FEV1/FVC is used routinely at our institution instead of the lower limit of normal [176]. It would have been interesting to see if there is a correlation between the individual scores of the MRC and CAT questions and the similar SNOT-22 questions. Although a minority of COPD have bronchiectasis (4 – 7.8 %), it would be interesting to register the correlation between bronchiectasis on HRCT and CRS in COPD patients. The presence of bronchiectasis should be included in future studies of this kind.

The lack of current blood eosinophil counts in our patients is a limitation and future studies should include a simultaneous blood eosinophil count as stated in the GOLD guidelines.

We also believe that we present the hitherto largest study addressing the olfactory function in patients with COPD with and without CRS. However, caution should be taken when considering the subgroup results presented in Figure 2 and 3 in Paper III as sample sizes in some of the groups is small (with CRS +/- current smoking n=15; with CRS and GOLD type 1+2 n=9; with CRS and GOLD grade A+C n=2). The small sample size in these subgroups increases the risk of sampling and analysis error where the observed differences are due to chance and not a real difference between the groups.

As mentioned above, an affected olfactory function is one of the cardinal symptoms in CRS, and testing should therefore be part of the CRS diagnostic workup in all global airway disease patients. Our study contributes to the sparse knowledge of olfactory function in COPD. During our study, we became aware of the need for a validated Danish PROM concerning the effects of olfactory dysfunction on patients HRQoL. We used the SIT16 test to scope of the extent of the affliction. Future studies of olfactory function in COPD patients should preferably include the composite TDI test as this is a more accurate assessment of the different aspects of olfactory function. The TDI is also more sensitive to early deterioration of olfactory function compared to the SIT16 alone [163]. We used the ''smell first'' test condition. However, in future the ''reading first'' test condition may be preferable as unqueued odour identification is more difficult, making our results less comparable to other studies which primarily use the ''reading first'' conditions [83, 125]. In clinical practice the SIT16 should be used for screening olfactory function, and if dysfunction is identified, a full TDI test is warranted.

The included patients in paper II and III primarily had more severe and more symptomatic COPD making our findings applicable to the COPD population seen in a hospital setting but our results may not be representative of disease burden in patients with the mildest form of COPD (group A, grade 1).

PERSPECTIVES:

Our results further support global airway disease in both PCD and COPD and underlines the need to improve how we care for these patients. We suggest that multidisciplinary collaboration is essential in correctly diagnosing and treating patients with global airway disease. Each speciality can contribute with their specific knowledge of how that disease entity interacts in global airways disease. The setup of a global airway disease clinic could include the following as supplements to the existing standard of care:

- Global airway disease (GAD) phenotyping
- GAD endotyping, inflammatory and immunologic markers in blood, sputum, nasal secretion.
- GAD PROMS questionnaires and apps.
- GAD microbiology
- Ciliary beat frequency analysis

- Genetic evaluation (genome, CFTR receptor function, ciliary ultrastructure, microbiome)
- Research database and biobank (blood, secretions, biopsies)
- Tailored GAD treatment
- Prophylactics
- Rehabilitation of decreased olfactory function.

Based on previous research, including papers from our group [2, 5, 67, 145], multidisciplinary global airway disease clinics have already been established at our institution for patients with severe asthma, CF, PCD and concomitant CRS.

A thorough understanding of the mechanisms and the prevalence of global airway disease in patients with decreased MCC will help physicians improve diagnostics and treatment regimes. Hopefully, increased focus on global airway disease will also decrease or even prevent morbidity and mortality and increase HRQoL in these severely chronically ill patients.

New and promising digital solution for symptom and treatment monitoring, such as the Galenus Health® app [177-178] has been developed by the European Forum for research and education in allergy and airway disease (EUFOREA) and Galenus Health. This E-health solution will also be tested at our centre as part of a EUFOREA research project. We hope that the studies presented in this thesis will promote PCD, CF, and COPD to be included in such an app and its use implemented in our daily clinical practice.

As the presented studies in Paper II and III are cross-sectional, further studies on causality are needed. Our research group presently has several studies underway within this interesting field of global airway disease. Data from our prospective global airway microbiome study, including 45 COPD patients with and without CRS with and without nasal corticosteroids, is currently being analysed. We are also investigating the effect of standard CRS treatment on SNOT22 scores in patients with COPD and CRS. A study of olfaction in hyposmic and anosmic COPD patients, including PNIF, PROMs and full TDI testing before and after olfactory rehabilitation [97] is currently being set up at our institution. Furthermore, we initiated a study of olfactory dysfunction in post-Covid-19 patients, including TDI testing and fMRI. Other potential projects could include evaluation of HRCT-lung findings and risk of CRS in patients with COPD.

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APPENDIX:

Paper I:

Primary ciliary dyskinesia patients have the same P. aeruginosa clone in sinuses and lungs

@ERSpublications
For the first time it is shown that the same Pseudomonas aeruginosa clone exists in both the upper and lower airways in patients with PCD, providing a solid support of the unified airway theory where the sinuses are a possible bacterial reservoir http://bit.ly/2kcE9tq

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To the Editor.

Similar to patients with cystic fibrosis (CF) and non-CF bronchiectasis, patients with primary ciliary dyskinesia (PCD) are prone to recurrent or chronic lung infections with Pseudomonas aeruginosa. Chronic P. aeruginosa lung infection has a prevalence of up to 39% in patients with PCD [I] and is associated with structural damage, affecting lung function. Treatment of P. aeruginosa infection is challenging because P. aeruginosa adapts to the host environment through genotypic/phenotypic changes, promoting a reduced immune response [2]. We have found previously that the paranasal sinuses in patients with CF act as bacterial reservoirs where P. aeruginosa adapts and recolonises P. aeruginosa-eradicated lungs [3, 4]. In addition, our group has reported P. aeruginosa-positive cultures from the upper and lower airways of patients with PCD [5]. However, it was unclear whether the paranasal sinuses of patients with PCD also act as bacterial reservoirs. We are investigating whether the same P. aeruginosa clone type colonises both the paranasal sinuses and the lungs, and the extent to which P. aeruginosa adapts to the host environment via genotypic/phenotypic changes.

From 2009 to 2017, we collected and analysed 38 P. aeruginosa isolates (21 paranasal sinus, 17 lung) from nine chronically lung-infected patients with PCD [6]. At least one sinus isolate and one lung isolate were collected from each patient. The mean time between first and last isolate was 3.0 years (range 0—5.5 years) (figure l). Chronic P. aeruginosa infection was diagnosed according to the modified CF Leeds criteria, or when anti-P. aeruginosa precipitin levels were elevated in combination with a P. aeruginosa-positive sample [I]. Sinus isolates were sampled from the maxillary, ethmoidal, sphenoid or frontal sinuses during endoscopic sinus surgery (ESS). Lung isolates were obtained by bronchoalveolar lavage in combination with ESS or, at a different time, by expectoration or endolaryngeal suction at our PCD center. Nine of the lung isolates have been described previously by our group [7]. Our principal eradication treatment for P. aeruginosa infection in patients with PCD is 3 weeks' inhalation of colistin and oral ciprofloxacin, and the second-line treatment is 2 weeks of intravenous aminoglycoside and ß-lactam. P. aeruginosa genotype/ phenotype was obtained from whole genome sequencing, growth rate, motility, protease secretion, biofilm formation and antibiotic susceptibility, and compared to the reference strain PAOI.

Each patient (PI—P9) had one individual P. aeruginosa clone type, which was present in both the paranasal sinuses and the lungs; thus, nine different P. aeruginosa clone types were identified (figure l). PI—P5 and P7 retained their individual clone types for years, with an observed maximum of 5.5 years. P6, P8 and P9 also retained their individual clone types over time, but their sinus isolates were collected simultaneously or within months of the lung isolates. None of the patients shared clone types and only P7's clone type had previously been identified from a patient with CF at our center. There was no evidence of cross-infection, as DK19 belongs to the environmentally abundant PA14 clonal complex.

All P. aeruginosa sinus and lung isolates from each patient's clone type had similar genotypes (figure 1). Isolates from different patients had very different genotypes. P3's clone (DK64) and P7's clone (DK19) had no gene mutations. PI's clone (DK60) was a hypermutator [8] with a total of 37 mutated genes, four of which were shared between sinus and lung isolates. The hypermutator status did not show as phenotypic changes. The remaining patients' clones had a varying, but low, number of mutated genes (mean 3.5, range 0—10). Some mutations were present in all isolates within a given clone type, while others were only present in some of the isolates. The following mutated genes were found in more than one clone type: MigA (DK66, DK119), involved in biofilm formation and colistin resistance [9]; AlgU (DK63, DK128), affecting production of alginate (biofilm matrix) and associated with a more resilient mucoid phenotype [2]; and LasR (DK60, DK66), with a central regulatory role in quorum sensing, which also improves bacterial survival [2]. We found LasR, AlgU and MigA mutations in some isolates, but saw no consistent phenotypic change in mucoidity or colistin resistance. The phenotypic characteristics were like those of PAOI, with no consistent adaptational patterns between isolates from sinuses and lungs and no clear correlation between genotype and phenotype. This may have been due to other regulatory mechanisms and post-transcriptional events that affect gene expression and gene product. Future analysis of P. aeruginosa RNA may clarify this connection.

All the isolates from each patient belonged to the same clone type, with partial yet incomplete genotypic/ phenotypic match, among isolates from the different sinuses and the lungs (figure I). This limited parallel adaptation may be caused by different local conditions in the sinuses and lungs promoting divergent adaptation of P. aeruginosa. Similarly, MARKUSSEN et al. [10] described different evolution of clonal sublineages within the same CF lungs. The genotypic/phenotypic profile in P. aeruginosa infection in early CF is less adapted than in late CF, where different sublineages develop due to temporospatial diversification [10]. We have found that the limited level of adaptation and the PAOl-like phenotype seen in our PCD P. aeruginosa isolates resembles the early P. aeruginosa infection in CF [7, Il].

Previous literature on P. aeruginosa in CF suggests that intensive antibiotic treatment promotes bacterial adaptation and antibiotic resistance [12]. We found that 100% of our isolates were sensitive to colistin, despite five isolates having a MigA mutation, and 84% were sensitive to ciprofloxacin. All sinus isolates were sensitive to ciprofloxacin while six out of 17 lung isolates had intermediate susceptibility. A possible explanation for this high level of antibiotic susceptibility could be that P. aeruginosa from patients with PCD experiences less antibiotic stress than in CF. Differences in antibiotic bioavailability and host inflammatory response between the paranasal sinuses and the lungs should also be considered. DOHT et al. [13] reported less effect of iv. antibiotics on human sinonasal

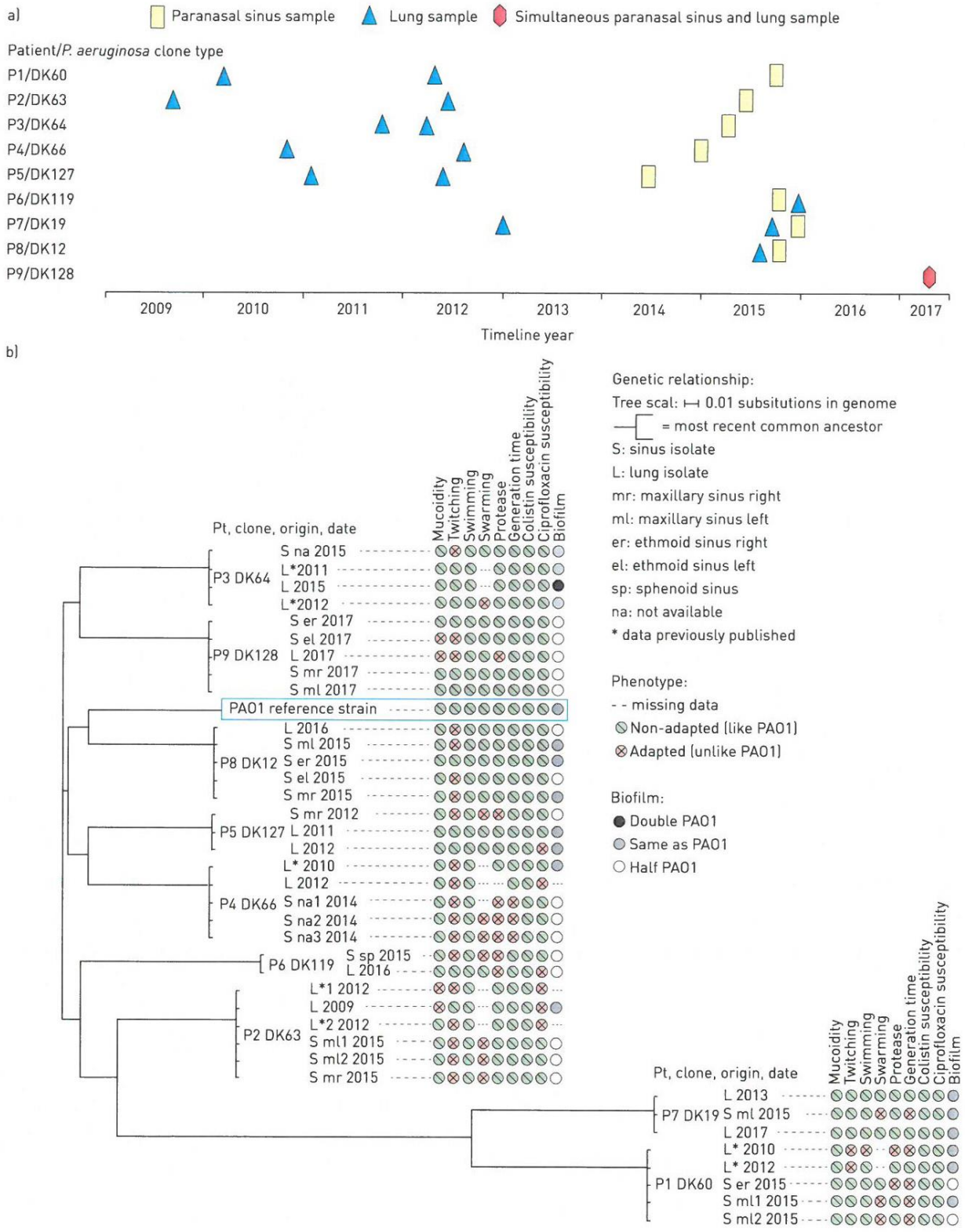

FIGURE 1 Genetic relationship and phenotype of Pseudomonas aeruginosa isolates from the paranasal sinuses and lungs in patients with primary ciliary dyskinesia. a) Date, origin and clone type of P. aeruginosa samples in each patient (PI-P9); b) phylogenetic tree showing the genetic relationship between P. aeruginosa clones and PAOI reference strain. Each branch only has isolates from one clone type. Clonal isolates are ordered according to genetic relationship. Circles after each isolate depict the phenotypic characteristics.

inflammatory markers than in the lungs. KARMA et al. [14] showed that in patients with chronic rhinosinusitis, the antibiotic concentration in sinus fluid was substantially lower than in the sinus mucosa, suggesting an insufficient dose and time inside the paranasal sinus cavities to ensure bacterial death. In CF, oxidative stress promotes bacterial genotypic/ phenotypic adaptation which increases bacterial resistance and thereby a higher likelihood of persistent infection [7]. All the aforementioned stress factors may also influence bacterial adaptation in patients with PCD.

Addressing the unified airways theory, HANSEN et al. [3] showed that patients with CF shared P. aeruginosa clones between the upper and lower airways and ALANIN et al. [5] showed the presence of P. aeruginosa in cultures from the upper and lower airvvays and a persistent P. aeruginosa clone type in the lungs of patients with PCD. We have previously shown that ESS in combination with postoperative nasal irrigation, nasal steroids and systemic antibiotics can help eradicate P. aeruginosa from the paranasal sinuses in patients with CF and PCD [4, 15]. The aforementioned literature describes P. aeruginosa infection initially in the lungs and then later in the sinuses, but is that the true chronological order? Which comes first: lung infection or sinus infection? The hypothesis of a possible sinus focus was generated because of evidence that lung-transplanted CF patients were recolonised with the same pathogen that they had had before lung transplantation. Extracted from RADEMACHER et al. [16], I l out of 34 lung-transplanted patients with non-CF bronchiectasis continued to be chronically lung-infected with the same pathogen that they had had before lung transplantation, and P. aeruginosa was the most common pathogen. Theoretically, the sinus samples in these patients may have been P. aeruginosa-positive, but this remains undetermined. However, the exact order of colonisation may be less important than recognising that P. aeruginosa colonises both the sinuses and lungs and that eradication treatment should be aimed not only at the lower airways, but at the unified airways. Furthermore, the upcoming European Position Paper on Rhinosinusitis and Nasal Polyps 2020 has an increased interest in multidisciplinary collaborations, focusing especially on the unified airways in PCD and CF.

We have shown for the first time that patients with PCD and P. aeruginosa lung infection harbour the same clone type in their paranasal sinuses and lungs, providing support for the unified airways theory in PCD. As in CF, the paranasal sinus focus in patients with PCD may be responsible for recolonising the lungs, so early eradication of the paranasal sinus colonisation could reduce lung infections. The important role of the upper airways in patients with PCD helps us to better understand the pathogenesis of P. aeruginosa infection. We therefore recommend that contributions from otorhinolaryngologists be considered in the development of PCD and CF treatments.

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Author contributions: E. Arndal, H.K. Johansen, M. Alanin, K. Aanæs, N. Høiby, K.G. Nielsen, V. Backer and C. von Buchwald developed the hypothesis and design. E. Arndal, J.A.J. Haagensen and J.A. Bartell performed the phenotypic analyses. R.L Marvig performed the genotypic analyses. M. Alanin, K. Aanæs and C. von Buchwald performed the endoscopic sinus surgery and collected sinus samples. K.G. Nielsen collected lung samples. H.K. Johansen stored sinus and lung samples. K.G. Nielsen performed updated diagnostic review of all the patients included. E. Arndal wrote the article, supervised by C. von Buchwald, and all the authors discussed the results and critically reviewed the manuscript.

Conflict of interest: E. Arndal reports grants from Candys Foundation and Copenhagen University Hospital/ Rigshospitalet Fund, during the conduct of the study. H.K. Johansen has nothing to disclose. J.A.J. Haagensen has nothing to disclose. J.A. Bartell has nothing to disclose. R.L. Marvig has nothing to disclose. M. Alanin has nothing to disclose. K. Aanaes has nothing to disclose. N. Høiby has nothing to disclose. K.G. Nielsen has nothing to disclose. V. Backer has nothing to disclose. C. von Buchwald has nothing to disclose.

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Clinical Trial Paper

Chronic rhinosinusitis in COPD: A prevalent but unrecognized comorbidity impacting health related quality of life

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ABSTRACT

Introduction: Unified airway disease where upper respiratory tract inflammation including chronic rhinosinusitis (CRS) affects lower airway disease is known from asthma, bronchiectasis, cystic fibrosis and primary ciliary dyskinesia but little is known about CRS and health related quality of life in COPD. We investigate firstly, the prevalence of CRS in COPD. Secondly the impact of CRS on HRQoL. Thirdly, risk factors for CRS in COPD. *Methods:* cross-sectional study of CRS in 222 COPD patients from 2017 to 2019 according to EPOS2012/2020 and GOLD2019 criteria. Patients completed the COPD assessment test (CAT), Medical Research Council dyspnea scale and Sinonasal outcome test 22 (SNOT22) and questions on CRS symptoms. They then had a physical examination including flexible nasal endoscopy, CT-sinus scan and HRCT-thorax. *Results:* 22.5% of COPD patients had CRS and 82% of these were undiagnosed prior to the study. HRQoL (CAT, SNOT22 and the SNOT22-nasal symptom subscore) was significantly worse in COPD patients with CRS compared with those without CRS and healthy controls. Multiple logistic regression analysis suggests that the most likely candidate for having CRS was a male COPD patient who actively smoked, took inhaled steroids, had a high CAT and SNOT22_nasal symptom subscore. *Discussion:* the largest clinical study of CRS in COPD and the only study diagnosing CRS according to EPOS and GOLD. This study supports unified airway disease in COPD. The SNOT22_nasal symptoms subscore is recommended as a standard questionnaire for COPD patients and patients at risk should be referred to an otorhinolaryngologist.

1. Introduction

Chronic obstructive pulmonary disease (COPD) has high morbidity and mortality impacting patients' health related quality of life (HRQoL). WHO estimates that 65 million people worldwide suffer from COPD (https://www.who.int/respiratory/copd/burden/en/). Experience from asthma [1], bronchiectasis [2], cystic fibrosis [3] and primary ciliary dyskinesia [4] has shown that lower airway disease is associated with upper respiratory tract inflammation including chronic rhinosinusitis
(CRS); resulting in unified airway disease. CRS is characterised by chronic sinonasal inflammation affecting 10.9% of adults (range 6.9–27.1%) in large questionnaire-based population studies [5]. Contrarily, the prevalence of clinically diagnosed CRS has been reported as low as 2% [6] demonstrating a difference between questionnaire based and clinically diagnosed CRS, suggesting potentially unrecognized and untreated CRS. CRS decreases HRQoL [7] however, very limited research exists on CRS in COPD and none according to the European position paper on rhinosinusitis and nasal polyps (EPOS2012, identical to EPOS2020) [8,9]; where clinical CRS diagnosis is based on specific symptoms and objective nasal endoscopic findings, preceding sinus Computed Tomography (CT) scan. Previous studies reported that

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Abbreviations

CAT	COPD Assessment Test
COPD	Chronic Obstructive Pulmonary Disease CRS Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis without nasal polyps
CRSwNP	Chronic rhinosinusitis with nasal polyps
GOLD	Global Initiative for Chronic Obstructive Lung Disease
MRC	Medical Research Council dyspnea questionnaire.
HRQoL	Health Related Quality of Life.
SNOT22	Sinonasal Outcome Test 22

75–88% of COPD patients have daily nasal symptoms [10–14] but, lack a systematic approach to CRS diagnostics using different criteria and either insufficient or no clinical evaluation of the sinonasal cavity [11–17]. These are important limitations. Previous studies also used the SNOT20 (sinonasal outcome test 20 items) which did not include the nasal obstruction and olfactory dysfunction items, which have been added to the SNOT22 [18]. Therefore, our research group consisting of otorhinolaryngologist, pulmonologist and radiologist, aimed to investigate firstly, the prevalence of CRS in patients with COPD based on a full diagnostic workup. Secondly, the impact of CRS on HRQoL and thirdly, risk factors for CRS in COPD patients.

Methods: A cross-sectional study of 222 patients with COPD recruited from August 2017 to March 2019 at the Respiratory outpatient clinic. Exclusion criteria: age below 18 years, asthma, CF, PCD, lung cancer, acute

common cold, acute odontogenic infection, acute pulmonary exacerbation within the last two weeks or recent nasal surgery hindering nasal examination. Flowchart of patient inclusion: see Fig. 1.

COPD was defined and graded according to GOLD2019 [19]. CRS with/without nasal polyps (CRSwNP/CRSsNP) was defined according to EPOS [8,9], as two or more symptoms with a minimum of ≥1 major symptom for more than 12 weeks AND objective findings. Major symptoms included nasal obstruction and nasal discharge. Minor symptoms were facial pain or pressure and reduced sense of smell. Flexible nasal endoscopic evaluation of objective findings: nasal polyps, discharge or mucosal oedema primarily in the middle meatus. In addition, a sinus CT-scan was performed.

HRQoL questionnaires: The SNOT22 consists of 22 questions, each scored from 0 (no problem at all) to 5 (worst possible problem) with a total score of 0–110 (Table 1). The SNOT22_nasal symptom subscore (SNOT22_NS) is one of the four symptomatic subdomains (nasal, sleep, emotional, otologic) [20]. The COPD Assessment Test (CAT) consists of 8 questions, each scored from 0 to 5, with a total score of 0–40 [21]. The Medical Research Council (MRC) dyspnea scale consist of one question with a score of 1–5 [22]. A higher score indicates worse symptoms in all the patients reported outcome measures mentioned above.

All patients were included during a routine visit at the COPD outpatient clinic which included spirometry and completion of the CAT and MRC questionnaire followed by an evaluation by a respiratory

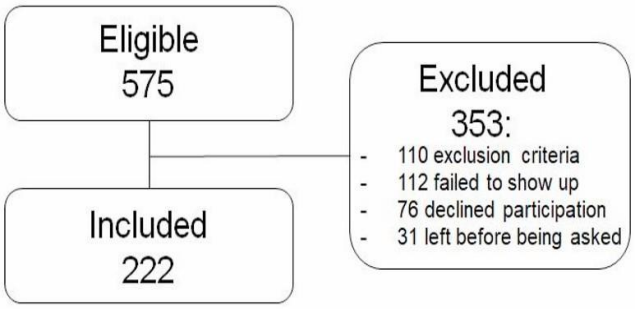

Fig. 1. Flowchart of patient in- and exclusion.

specialist. All patients completed a validated Danish version of the SNOT22 [23] and questions about previous sinonasal surgery and CRS treatment. The patients were specifically asked about major/minor symptoms by the first author, who then inspected the oral cavity, performed anterior rhinoscopy prior to decongestion followed by bilateral flexible nasal endoscopy after decongestion to evaluate the degree of mucosal oedema. Patients had a sinus CT-scan and a control High-Resolution Computed Tomography (HRCT) of the lungs within three months of the initial visit. Seventeen patients did not want a sinus CT-scan but were retained in the study as this did not alter the diagnosis (see discussion). No patients were excluded due to lung cancer. All scans were evaluated by a radiologist and all CT-sinus scans were scored using Lund-Mackay [24]. The radiologist was blinded to the patients' symptoms and endoscopic findings. The Lund-Mackay CT-sinus score assesses the degree of radiologic sinonasal disease for each paranasal sinus and osteomeatal complex with a score from 0 to 2 and a total score ranging from 0 to 24. Patients diagnosed with CRSw/sNP based on objective findings (endoscopy and/or sinus CT-scan) were offered standard medical treatment with daily nasal steroids and nasal saline irrigation. CRS patients were scheduled for a one-month follow-up visit at the Department of otorhinolaryngology.

The study was approved by the ethics committee in the Capital Region of Denmark (H-17011622) and the Danish Data Protection Agency. The study was conducted according to the Helsinki Declaration. Written

informed consent was collected from all patients prior to inclusion. The MRC dyspnea scale was used with the permission of the Medical Research Council. Permission was also obtained for the use of CAT . Statistics: Continuous variables are reported as mean (SD) or median (range); categorical variables as frequency and proportion; SNOT22 scores as median and inter-quartile range and compared across groups using the Hodges-Lehmann estimator of difference in location with corresponding 95% confidence intervals. Univariate and multivariate logistic regression evaluated CRS risk factors. The multiple logistic regression model was adjusted for gender, age, active smoking, inhaled steroids, FEV_1% predicted, SNOT22_NS and CAT score. Active smoking was defined as current smoking or smoking cessation within the last six months. Inhaled steroids were any daily inhaled steroids. Missing data: packyears (2.3%), CAT (18.0%), MRC (5.4%), eosinophils (10.4%) and Lund-Mackay (7.7%). Multiple imputation generated missing values using the R MICE package [25].

2. Results

CRS prevalence in COPD: Fifty (22.5%) out of 222 COPD patients had CRS according to EPOS (Table 2) . Only 4% (n = 9) of all COPD patients had previously been diagnosed with CRS, illustrating that 18.5% of all COPD patients and 82% of COPD + CRS patients were undiagnosed and untreated for their CRS. Nasal polyps (CRSwNP) were found in 4.1% of COPD patients with CRS. Five patients had severe nasal septal deviation and airflow obstruction and were therefore not diagnosed as CRS but referred for septoplasty. History of allergy were obtained from 146 (65.8%) of the patients and were as follows (COPD without CRS/with CRS): no allergy 63.6%/59.3%, pharmacologic 6.1%/ 13.3%, pollen 6.1%/6.2%, animal, dust mite or insects 6.1%/3.5%, food 0%/2.7%, other (such as band-aid, nickel) 6.1%/8.0% and combination of abovementioned allergies 12.1%/6.6%. Patients with allergy data did not differ significantly regarding age, gender, CRS, FEV_1 and active smoking compared to those with no available allergy information.

HRQoL in COPD ± CRS: CAT was significantly higher in COPD patients with CRS compared with those without CRS ($p < 0.0001$)

(Table 2) . There was no difference in MRC between the two groups ($p = 0.4887$). SNOT22 and SNOT22_NS scores were significantly higher ($p < 0.0001$) in the COPD + CRS compared with COPD-CRS (Table 1 and Table 2).

Risk factors for CRS: Univariate analysis identified the SNOT22_NS, CAT and SNOT22 scores as significant risk factors for having CRS

Table 1
SNOT22 scores in COPD patients with/without chronic rhinosinusitis.

SNOT22-item Median score (interquartile range)	COPD + CRS (n = 50)	COPD – CRS (n = 172)	Hodges-Lehmann estimator of difference in location (95% CI)
1 Need to blow your nose	3.0 (2.0–3.0)	1.0 (0.0–2.5)	1.0 (1.0–2.0)*
2 Sneezing	2.0 (0.0–3.0)	1.0 (0.0–2.0)	1.0 (0.0–1.0)
3 Runny nose	3.0 (1.0–3.0)	1.0 (0.0–2.0)	1.0 (1.0–2.0)*
4 Cough	3.0 (2.0–4.0)	2.0 (1.0–3.0)	1.0 (1.0–2.0)*
5 Post-nasal discharge	2.0 (1.0–3.0)	0.0 (0.0–1.0)	2.0 (1.0–2.0)*
6 Thick nasal discharge	2.0 (0.0–3.0)	0.0 (0.0–1.0)	2.0 (1.0–2.0)*
7 Ear fulness	1.0 (0.0–2.0)	0.0 (0.0–0.0)	1.0 (1.0–1.0)*
8 Dizziness	1.5 (0.0–3.0)	0.0 (0.0–2.0)	1.0 (0.0–1.0)
9 Ear pain/pressure	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
10 Facial pain/pressure	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
11 Difficulty falling asleep	1.5 (0.0–3.0)	1.0 (0.0–2.0)	0.0 (0.0–1.0)
12 Waking up at night	2.0 (2.0–4.0)	2.0 (1.0–3.0)	1.0 (0.0–1.0)
13 Lack of a good night's sleep	3.0 (1.0–4.0)	2.0 (0.0–3.0)	1.0 (0.0–2.0)
14 Waking up tired	3.0 (2.0–4.0)	2.0 (0.0–3.0)	1.0 (1.0–2.0)*
15 Fatigue during the day	3.0 (2.0–4.0)	2.0 (1.0–3.0)	1.0 (1.0–2.0)*
16 Reduced productivity	3.0 (2.0–4.0)	2.0 (1.0–3.5)	1.0 (1.0–2.0)*
17 Reduced concentration	2.0 (1.0–3.0)	1.0 (0.0–2.0)	1.0 (0.0–1.0)
18 Frustrated/restless/irritable	1.5 (0.0–3.0)	0.5 (0.0–2.0)	0.0 (0.0–1.0)
19 Sad	1.0 (0.0–3.0)	1.0 (0.0–2.0)	0.0 (0.0–1.0)
20 Embarrassed	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
21 Sense of taste/smell	1.0 (0.0–3.0)	0.0 (0.0–1.0)	1.0 (0.0–1.0)
22 Blockage of nose	3.0 (1.0–3.0)	0.5 (0.0–2.0)	2.0 (1.0–2.0)*
SNOT22 total score	44 (28.5–54.0)*	22 (13.0–36.0)	19.0 (13.0–25.0)*
SNOT22_nasal symptoms	19 (13.0–23.0)*	7.5 (4.0–12.0)	10.0 (8.0–12.0)*

In bold: SNOT22_nasal symptoms subscore (SNOT22_NS). COPD: chronic obstructive pulmonary disease. CRS: chronic rhinosinusitis. SNOT22: sinonasal outcome test 22 items. *: statistically significant differences. CI: confidence interval.

(Table 3). A 1-point increase in CAT increases the odds of having CRS with 10% (95% CI:5–15%). A 1-point increase in SNOT22_NS increases the odds of having CRS with 29% (95% CI: 20–40%) and a 1-point increase in SNOT22 increases the odds of having CRS with 7% (95% CI: 5–9%). The SNOT22_NS score was a stronger predictor of CRS than the total SNOT22 and CAT score. All other variables were non-significant.

Multiple logistic regression analysis suggested that the most likely candidate for having CRS was a male COPD patient who actively smoked, used inhaled steroids, had high CAT and SNOT22_NS scores, but only the SNOT22_NS score was statistically significant ($p < 0.001$) after mutual adjustment. The relationship between the SNOT 22_NS score and the predicted risk of CRS is illustrated in Fig. 2.

Discussion: in this comprehensive cross-sectional study we show that 22.5% of COPD patients had CRS according to current EPOS criteria and that 82% were undiagnosed prior to the study. CAT, SNOT22 and the SNOT22_NS scores were significantly higher in COPD patients with CRS compared with those without CRS. This study further supports that COPD is a unified airway disease.

To the best of our knowledge we present the largest study of clinically diagnosed CRS in COPD by a multidisciplinary collaboration of otorhinolaryngology, pulmonology and radiology specialists. It is the only study diagnosing CRS according to the EPOS2012/2020 highest standard for a full diagnostic workup: nasal symptoms in combination with nasal endoscopy and/or sinus CT-scans. Our observed CRS prevalence of 22.5% is higher than the questionnaire-based population prevalence of 10.9% (range 6.9–27.1%), where it should be noted that the Swedish, Finnish and Danish CRS prevalence was 7.8% and did not include clinical evaluation of the patients [5]. Our observed prevalence is lower than previously published CRS prevalences of 48.5% (Yang et al. China) and 53–64% (Kelemence et al. Turkey). In these studies, evaluation and nasal endoscopy by an otorhinolaryngologist was not performed, and this may have resulted in an overestimation of CRS. Differences in study population may also contribute to the observed variations in prevalence, as higher levels of air-pollution, smoking habits and ethnicity are known to affect CRS prevalence [28]. Furthermore, sinonasal changes on CT-scans have been reported in up to 40% of patients

without CRS symptoms [29], indicating that sinus CT-scans should only be used for diagnostics when patients have characteristic symptoms and a normal nasal endoscopy or prior to sinus surgery.

Only nine patients had an existing CRS diagnosis meaning that 82% of COPD patients with CRS were undiagnosed and subsequently untreated. This clearly illustrates that CRS symptoms are underreported during clinical evaluation and indicates the need for additional focus on the upper airway contribution to lower airway disease. Phenotypically 96% had CRSsNP and 4.1% had CRSwNP, which is equivalent to the background population prevalence of nasal polyps [8] but, far less compared with asthma where nasal polyps are highly prevalent i.e. more than 2/3 of patients undergoing ESS at our department [30]. Asthma is more frequently associated with CRSwNP and high blood eosinophil counts mediated via a Th2 response, while COPD predominantly is associated with CRSsNP and low blood eosinophil counts thought to be mediated via a Th1 response [9,19]. This indicates different CRS endotypes and underlying pathophysiological mechanisms in COPD and asthma referred to as type 2 and non-type 2 respectively in the new EPOS guidelines [10].

Our study shows that the SNOT22 is relevant for COPD patients as 92% off all patients in this study had a SNOT22 score above 7 which is the cut-off for healthy individuals [26]. The observed median SNOT22 scores of 44.0 in COPD + CRS is like SNOT22 scores of 42.0 recorded in CRS patients in general [18]. The SNOT22 score is also used to determine the severity of sinonasal disease and 56% of our COPD patients with CRS had SNOT22 scores above 40, signaling substantial disease. Furthermore, a SNOT22 score >30 identifies those patients most likely to benefit from endoscopic sinus surgery [9,27]; 70% of our COPD patients with CRS had scores above 30. We report baseline SNOT22 scores, but post standard medical treatment scores are needed to evaluate which patients require additional treatment and how to best treat these patients as all may not be fit for surgery due to severely affected lung function. Our findings were adjusted for smoking status and are in accordance with previous studies reporting increased sinonasal symptoms in COPD patients and smokers in general [11,12,14]. Hence, CRS has a considerable negative impact on HRQoL in COPD patients. However, our results do not support an additive negative effect of CRS when the patient is suffering from COPD.

The impact of CRS on HRQoL in COPD patients is comparable to CRS patients in general but significantly worse compared with COPD patients without CRS. Both SNOT22 and CAT scores were significantly higher in COPD patients with CRS compared with those without CRS and we observed a correlation between the two scores. This may be due to partial overlap between the questionnaires that both contain questions on cough, activity, sleep and energy levels but it could also be a sign of unified airway inflammation. Yang et al. similarly found significantly higher CAT and SNOT20 scores in their cohort of COPD patients but unlike us they also found higher modified MRC scores. We found that HRQoL in COPD with CRS is negatively affected when assessed by both the SNOT22, SNOT22_NS score and CAT, clearly indicating a potential for improving HRQoL by treating CRS. Due to the overlap between CAT and SNOT22 questions there is also a possibility to decrease CAT scores by treating the comorbid CRS. Future prospective studies are needed to explore the effect on unified airways HRQoL in COPD patients relative to treatment.

SNOT22_NS scores in combination with CAT scores identified COPD patients at risk of having CRS. However, GOLD grade, number of exacerbations, packyears and FEV_1%, age, gender, inhalation steroids and eosinophils did not increase the risk of CRS in univariate analysis. Multiple logistic regression analysis suggested that the most likely candidate for comordid CRS was a male COPD patient who actively smoked, used inhaled steroids, had a high CAT and SNOT22_NS score. Chien et al., 2015 [15] reported an increased hazard ratio of CRSsNP in COPD patients (HR = 3.24; 95% CI = 2.65–3.96; $p < 0.01$) compared with healthy controls. The SNOT22_NS score was better than the SNOT22 at identifying COPD patients at risk. We

therefore recommend that the SNOT22_NS becomes part of the standard questionnaires given to COPD patients prior to their evaluation by a respiratory specialist. At our institution a joint otorhinolaryngology and pulmonology clinic for patients with severe asthma and CRS has already been implemented. We recommend a similar set-up for COPD patients in order to further investigate this CRS comorbidity. In addition, further studies of the impact of CRS treatment on morbidity, mortality and prognosis in COPD patients are needed.

Table 2

Demographics of COPD patients±chronic rhinosinusitis (CRS)~.

Variable	COPD + CRS (n = 50)	COPD – CRS (n = 172)	All (n = 222)
Age, mean (SD)	69.3 (9.5)	70.5 (8.8)	70.2 (8.9)
Gender: male n (%)	32 (64%)	97 (56%)	129 (58%)
FEV_1% predicted, median (range)	41.5 (15–92)	39.5 (16–102)	40.0 (15–102)
Packyears*, mean (SD)	45.6 (18.5)	43.9 (18.4)	44.3 (18.3)
Active smoker, n (%)	28 (56%)	82 (47.7%)	110 (49.5%)
Exacerbations, median (range)	0 (0–9)	1 (1–8)	1 (0–9)
Frequent exacerbations ≥2, n (%)	14 (28%)	36 (21%)	50 (23%)
Inhaled steroids, n (%)	28 (56%)	103 (59.9%)	131 (59%)
Eosinophils* (cells X 10^9/L), mean (SD)	0.224 (0.155)	0.203 (0.166)	0.208 (0.164)
GOLD grade, n (%) I:			
	2 (4.0%)	8 (4.7%)	10 (4.5%)
II:	13 (26.0%)	46 (26.7%)	59 (26.5%)
III:	20 (40.0%)	77 (44.8%)	97 (43.5%)
IV:	15 (30.0%)	41 (23.8%)	56 (25.1%)
GOLD type, n (%) A			
	4 (8%)	13 (7.6%)	17 (7.7%)
B	26 (52%)	87 (50.6%)	113 (50.9%)
C	1 (2%)	6 (3.5%)	7 (3.2%)
D	19 (38%)	66 (38.4%)	85 (38.3%)
CAT*, mean (SD)	21.8 (7.6)	16.9 (6.8)	18.0 (7.3)
MRC*, mean (SD)	3.5 (0.9)	3.3 (0.9)	3.3 (0.9)
SNOT22 score, median (range)	44 (11–87)	22 (0–80)	25 (0–87)
SNOT22 median score, n (%) < 7			
∞	0 (0.0%)	17 (9.9%)	17 (7.7%)
> 20§	46 (92.0%)	95 (55.2%)	141 (63.5%)
> 30§	35 (70.0%)	53 (30.8%)	88 (39.6%)
> 40§	28 (56.0%)	30 (17.4%)	58 (26.1%)
SNOT22-nasal symptom score** median (range)	19 (3–42)	7.5 (0–24)	10.0 (0–42)
EPOS CRS criteria, n (%)			
Major symptom Congested			
	37 (74%)	17 (9.9%)	45 (24.3%)
Secretion	41 (82%)	11 (6.4%)	52 (23.4%)
Minor symptom			
Facial pressure	19 (38%)	1 (0.6%)	20 (9%)
Decreased olfaction	22 (44%)	32 (18.6%)	54 (24.3%)
Nasal endoscopic findings Oedema^, n			
(%)	44 (88%)	112 (65.2%)	156 (70.4%)
Secretion, n (%)	35 (70%)	34 (19.8%)	69 (31.1%)
Polyps, n (%)	9 (18%)	0 (0%)	9 (4.1%)
Lund-Mackay score*, mean (SD)	3.0 (3.4)	2.2 (2.9)	2.4 (3.0)

COPD: chronic obstructive pulmonary disease. CRS: chronic rhinosinusitis. SD: standard deviation. FEV_1%: forced expiratory volume in the first second, predicted. GOLD: global initiative for chronic obstructive lung disease. CAT: COPD assessment test. MRC: Medical Research Council questionnaire. SNOT22: sinonasal outcome test 22. EPOS: European position paper on rhinosinusitis. ~: symptoms of allergic and vasomotor rhinitis were excluded. *contains imputed values for missing data. ∞ Proposed cut-off in healthy controls [26]. § Proposed cut-off values for different levels of CRS disease severity [9,27]. **(see Table 2, questions in bold). ^Oedema: oedema/mucosal obstruction primarily in middle meatus. Secretion: mucopurulent discharge primarily from middle meatus.

Unified airway disease is based on the existence of one continuous epithelium from the paranasal sinuses to the tips of the lungs and clinical studies have demonstrated a link between upper and lower airway disease [1–4]. This study further supports that COPD and CRS should be regarded as a unified airway disease. Exacerbation of chronic airway disease is common and multiple factors have been reported as contributors: smoking, viral infection and microbiome composition. Firstly, tobacco smoke induces goblet cell hyperplasia and decreases ciliary beat frequency and mucociliary clearance [31,32]. Dransfield et al. [33] reported smoking induced cystic fibrosis transmembrane receptor (CFTR) dysfunction in patients with

chronic bronchitis, but also in smokers with and without COPD. Håkansson et al., 2011 [14] reported negative

Table 3

Risk factors for having chronic rhinosinusitis (CRS) in patients with COPD.

Variable	OR [95% CI]	P-value
Gender		
Male	1.40 [0.72–2.68]	0.3384
Female	1	
Age		
10-year increase	0.86 [0.61–2.64]	0.4170
Active smoker		
Yes	1.40 [0.74–2.63]	0.3011
No	1	
Inhaled steroid use		
Yes	0.85 [0.45–1.62]	0.6233
No	1	
FEV_1%		
10% decrease	1.04 [0.87–1.26]	0.6732
CAT^* one point increase	1.10 [1.05–1.15]	<0.0001
one SD increase	1.94 [1.39–2.70]	
SNOT22 nasal symptoms score* one point increase	1.29 [1.20–1.40]	<0.0001
one SD increase	6.03 [3.57–10.17]	
SNOT22 total score* one point increase	1.07 [1.05–1.09]	<0.0001
one SD increase	3.23 [2.18–4.77]	

CRS: chronic rhinosinusitis. COPD: chronic obstructive pulmonary disease. OR: odds ratio. CI: confidence interval. FEV_1: forced expiratory volume in the first second. CAT: COPD assessment test. SD: standard deviation. SNOT22: sinonasal outcome test 22. ^contains imputed values for missing data. *: statistically significant differences using univariate logistic regression.

effects of smoking on the nasal mucosa indicating the presence of not only smoker lungs but also a “smoker nose”. In the present study no significant difference in packyears or percentage of active smokers between COPD patients with and without CRS was found. Secondly, some studies have linked viral infections to increased risk of exacerbations in both CRS [34] and COPD [35,36]. Dewan et al. [37] reported that acute exacerbation was significantly more frequent in patients with a history of CRS. So, COPD in combination with CRS may lead to an increased airway susceptibility to pathogens. Thirdly, microbiome dysbiosis with reduced bacterial diversity is hypothesised to alter the mucosal environment in both CRS and COPD [38,39].

It remains unknown if microbial dysbiosis is the cause or effect of these diseases. We are currently conducting studies of both the unified airways microbiome and olfactory function in COPD.

Our study has some potential limitations. Firstly, of the 222 patients included, 17 declined a sinus CT-scan. Of those, three had a prior clinical CRS diagnosis and 14 expressed that they had no major/minor symptoms. Therefore, the lack of a CT-sinus scan did not change their diagnosis or management. Secondly, five patients were referred for septoplasty due to severe obstructive nasal septal deviation. One of these was diagnosed with CRSsNP due to bilateral symptoms of obstruction, discharge, facial pressure and decreased sense of smell which could not solely be attributed to the nasal septum deviation. Thirdly, data on gastroesophageal reflux, anxiety and depression were not obtained. This is a limitation as they may also affect CRS symptom burden [8]. Fourthly, we used the fixed FEV1/FVC ratios when diagnosing COPD and not the lower limit of normal (LLN), which may have over estimated COPD in the older patient [40].

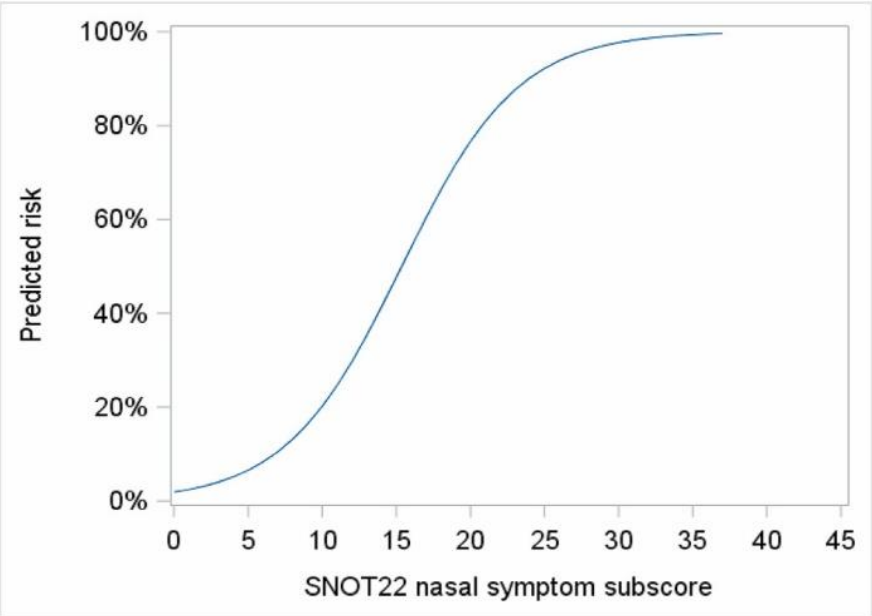

Fig. 2. Relationship between the SNOT22_nasal symptom sub scale score and the predicted risk of CRS in patient with COPD.

LLN is not routinely used at our institution. Fifthly, in this cross-sectional study, causality cannot be proven. Finally, Denmark has a free public healthcare system where general practitioners and private consultants can refer patients with more severe disease to the hospital free of charge. Our patients generally had more severe disease although all GOLD grades and types were represented and is therefore, comparable to patients in other secondary and to some extent also primary care facilities.

Interpretation: this study is the largest clinical study of CRS in COPD and the only study diagnosing CRS in COPD based on EPOS criteria, nasal endoscopy and CT-sinus scans. CRS is highly prevalent (22.5%) in patients with COPD. Nevertheless, up to 82% of those suffering from CRS are undiagnosed and untreated impacting their HRQoL. Interestingly, CRS in COPD is primarily without nasal polyps and not related to blood eosinophil counts, number of exacerbations or GOLD status. SNOT22, SNOT22_NS and CAT scores show decreased HRQoL in COPD patients with CRS. This study supports the existance of unified airway disease in COPD patients. It underlines the need for greater focus on upper respiratory tract symptoms in these patients especially as CRS treatment is well established and effective. Respiratory physicians should screen for nasal symptoms and refer patients at risk to otorhinolaryngologic evaluation, preferably as part of a multidisciplinary team consisting of respiratory and otorhinolaryngologic specialists.

Authorship contribution

EA: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing original draft and review & editing, project administration, visualization, funding acquisition. **ALS**: formal analysis, resources, data curation, writing original draft and review & editing, visualization. **TL**: methodology, investigation, resources, writing original draft and review & editing, supervision. **NS**: conceptualization, methodology, investigation, resources, writing original draft and review & editing. **CRT**: methodology, investigation, resources, writing original draft and review & editing. **KA**: conceptualization, methodology, investigation, resources, writing original draft and review & editing, supervision. **MA**: conceptualization, methodology, investigation, writing original draft and review & editing, supervision. **KBC**: formal analysis, resources, data curation, writing original draft and review & editing, visualization. **VB**: conceptualization, methodology, investigation, resources, writing original draft and review & editing, supervision, project administration, funding acquisition. **CvB**: conceptualization, methodology, investigation, resources, writing original draft and review & editing, supervision, project administration, funding acquisition.

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Declaration of competing interest

EA (none), ALS (none), TSL (none), NS (none), CT (none), KA (none), MCA (none), KBC (none), VB (none), CvB (none).

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Paper III:

COPD patients have a high prevalence of clinically diagnosed anosmia

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Abstract: olfactory dysfunction impacts nutritional state, health related quality of life (HRQoL), social interactions, morbidity and mortality but very little is known about olfaction in COPD. 135 patients with COPD 1A-4D (GOLD2019 criteria), with and without CRS (EPOS2020 criteria), age 49-89 years were tested with Sniffin' Sticks 16 odour Identification (SIT16) test after both otorhinolaryngological and pulmonological clinical examination. Both nostrils were tested simultaneously, and SIT16 scored based on forced multiple choice, after smelling the odour. Patients completed a SNOT22 questionnaire prior to testing and a flexible nasal endoscopy afterwards. Patients were stratified according to age and further subcategorised into COPD +/- Chronic Rhinosinusitis (CRS), GOLD grade, type, smoking status and compared to healthy controls. COPD patients with and without CRS had a significantly higher percentage of anosmia (14.1%) compared to healthy controls (1.4%). Similarly, higher prevalence of anosmia was observed in most subgroups (smoking status, GOLD grade and GOLD). There was no significant difference in age adjusted mean SIT16 scores in COPD patients (11.9) regardless of subgroup compared to healthy controls (11.6). Patients' answer to the EPOS criteria about affected olfaction was poorly correlated with their SIT16 score. We present the largest multidisciplinary study of odour identification and olfactory function in patients with COPD showing a higher prevalence of anosmia compared to healthy controls. Patients' subjective olfactory function correlates poorly with their olfactory test score. This emphasizes the importance of clinicians asking about and testing olfactory function to correctly diagnose and treat COPD patients with olfactory dysfunction.

Key words: odour identification, olfaction, anosmia, COPD, unified airways.

Introduction:

Imagine not being able to smell fresh coffee, your partner's scent, your own body odour, if there is a fire or that the food you are about to eat has gone bad. These are just some of the consequences of olfactory dysfunction which includes both quantitative and qualitative changes in the sense of smell (olfaction). Common causes of olfactory dysfunction are age, male gender, chronic rhinosinusitis (CRS), viral infection, trauma, drug induced, iatrogenic, congenital and idiopathic (1,2). An altered sense of smell impacts health related quality of life (HRQoL), nutritional state, social interactions, safety, morbidity and 5-year mortality (1, 3-4, 5) and is correlated with depression, neurological disease such as Alzheimer's and Parkinson's disease, loss of cerebral grey matter and reduced cognitive function (1, 6). Very little is known about olfaction in patients with chronic obstructive pulmonary disease (COPD). According to the World Health Organisation COPD is estimated to affect 65 million people worldwide. COPD patients have a high prevalence of chronic rhinosinusitis (CRS) ranging from 22.5 when diagnosed according to European Position Paper on rhinosinusitis (EPOS) 2020 (7) guidelines and up to 48.5 % based on EPOS guidelines but lacking the important diagnostic tool of nasal endoscopy (8,9). CRS is a sinonasal inflammatory and obstructive disease that can also affect olfaction. The general health and HRQoL of patients with COPD (10,11) are severely affected and an undiagnosed and untreated olfactory dysfunction may cause additional negative effects. In the general population hyposmia affects approximately 15% and anosmia 5% (12,13) both of which increase with age with up to 62.5% olfactory dysfunction in those of 80-years or older (2). Qualitative olfactory disorders dealing with the patients' odour perception are beyond the scope of this article (1). Multiple culturally adapted psychophysical smell tests exist and the SIT16 is widely used in Europe (1, 14-15). Objective measurements are not standard use (16,17). Management of olfactory dysfunctions is possible and includes spontaneous recovery, CRS treatment, pharmacologic treatment and recently the very promising olfactory rehabilitation through olfactory training (1, 18). Only one study has compared olfaction in 40 COPD patients with/without nasal oxygen treatment with healthy controls and showed a significant decrease in olfaction in half of the patients with COPD and long-term nasal oxygen, but it became non-significant when they adjusted for smoking behaviour in terms of packyears (19). They did not perform nasal examination.

Hence, it is not clear whether COPD patients exhibit olfactory loss. Because of the potentially severe consequences of olfactory dysfunction, it is important to examine whether olfactory dysfunction is present and if so, to manage it correctly. We hypothesised that COPD patients have decreased olfactory function and a higher prevalence of hyposmia, and functional anosmia compared to normative data. We expected that smoking (20) and CRS (1) would further impair this dysfunction.

Material and methods:

In this cross-sectional study of 135 COPD patients, 49-89 years-old, olfactory function was tested using the psychophysical Sniffin' Sticks Identification test containing 16 odours (SIT16, blue) (14). Each odour was presented in a pen-like dispenser below the nasal columella as previously described (21) testing both nostrils simultaneously. SIT16 scores ranging from 0-16 were based on the validated Danish forced multiple choice answers (21), where each answer was chosen after smelling the odour. Olfactory function is classified as normosmia: normal sense of smell and then two degrees of decreased sense of smell which is hyposmia: quantitatively reduced and anosmia: so quantitatively reduced that olfaction has no function in daily life or no olfactory function at all. Out research team consisted of both otorhinolaryngologists and pulmonologist. Patients were included from January 2018 – March 2019 during a routine COPD visit to the out-patient clinic at the Department of Respiratory Medicine, Bispebjerg Hospital, Denmark. Patients were enrolled from a larger previously published study on the prevalence of otorhinolaryngologically diagnosed CRS, in patients with COPD (8). Of the 222 patients included in the previous study, 87 declined being smell tested resulting in 135 included patients (Figure 1). There were more females in the included group (47%) compared to the excluded group (39%). Age, FEV1%, smoking status, packyears, SNOT22 score and CRS did not differ significantly between included and excluded patients. COPD was categorized according to GOLD2019 criteria (22) and all patients completed the Sinonasal outcome test-22 item (SNOT22) questionnaire (23) and COPD assessment test (CAT) (24) prior to SIT16 testing and flexible nasal endoscopy with decongestant after testing. All patients had a CT-sinus scan which was Lund-Mackay scored (25). With permission our study results were compared to normative data of Sniffin Sticks' mean SIT16 scores and olfactory function based on Threshold Discrimination and Identification (TDI) scores from healthy subjects that were age matched and with a similar gender distribution previously reported by Oleszkiewicz et al. (26), henceforth

referred to as healthy controls. The following values for olfactory function were used: normosmia SIT16 > 11 equivalent to a TDI ≥ 30.75; hyposmia SIT16 score 9-11 equivalent to a TDI 17-30.75 and anosmia SIT16 score ≤ 8 equivalent to a TDI score ≤16. All anosmic patients were recommended further examination and treatment according to local guidelines. Written informed consent was obtained from all patients prior to inclusion. The study was approved by the local ethic committee (H-17011622) and complies with the Declaration of Helsinki for Medical Research involving Human Subjects.

Statistical analysis:

Demographic and clinical variables are reported as mean and SD or frequency and proportion, as appropriate. For odour identifications (SIT16) scores mean, SD, range, percentiles, and group mean differences with corresponding 95% confidence intervals are reported. Olfactory function was grouped into anosmia, hyposmia, and normosmia and compared across groups using Fishers exact and Chi-squared tests. Missing data: The following variables had missing observations: CAT (13.3%), eosinophils (9.6%) and Lund-Mackay CT-sinus score (5.2%). We used multiple imputation for missing values using the MICE package from R (27).

Results:

Half of the patients with COPD were current smokers and the majority of these suffered from COPD with a substantial level of respiratory symptoms (B and D) (Table 1). As seen in Table 1 the frequency of CRS was 22.2%, of whom the far majority were without nasal polyps (CRSsNP). The median number of pulmonary acute exacerbations were one (range 0-9), and the mean (SD) eosinophilic cell count was 0.16 (0-0.84) (Table 1). Further patient demographics are listed in Table 1.

There was no significant difference in age adjusted mean SIT16 scores in patients with COPD compared to healthy controls (table 2A). COPD patients had a statistically significant higher percentage of functional anosmia (14.1%) compared to healthy controls (1.4%) (table 2B). They similarly had a lower percentage of hyposmia and corresponding higher percentage of normosmia compared to age matched healthy controls.

Patients were subgrouped into with/without CRS and stratified according to smoking status, GOLD type and GOLD grade. We observed no statistically significant effect of smoking status (current versus former) on mean SIT scores (95% CL mean) in COPD patients with CRS: 11.93 (10.66-

13.19) versus 11.00 (9.02-12.97), p=0.40 and in COPD patients without CRS: 12.22 (11.44-13.09) versus 12.03 (11.11-12.95) p=0.75, respectively. There was also no statistically significant difference when comparing these subgroups to age matched healthy controls. There was a statistically significant difference in mean SIT16 scores between GOLD type 1+2: 12.76 (11.98-13.53) and GOLD type 3+4: 11.63 (10.97-12.29), p=0.046; as patients with worse lung function (GOLD type 3+4) scored lower than patients with better lung function (GOLD type 1+2). However, GOLD type 1+2 scored 1.33 points higher than age matched healthy controls p=0.0001 (0.65-2.01); while there was no difference between GOLD type 3+4 and healthy controls. There was no statistical difference in mean SIT16 scores between GOLD grade AC: 12.87 (11.13-14.61) and GOLD grade BD: 11.86 (11.31-12.41), p=0.21. GOLD grade AC scored 1.16 points higher than the age matched healthy controls, p=0.04 (0.04-2.28). There was no difference between GOLD grade BD and the age matched healthy controls.

We found a significantly altered olfactory distribution with higher prevalence of anosmia and normosmia and lower prevalence of hyposmia in all smoking status subgroups except "CRS and current smoker" compared to healthy controls (Figure 2).

Similarly, we compared the olfactory distribution of COPD patients with and without CRS stratified into GOLD type (Figure 3A) and GOLD grade (Figure 3B) to age matched healthy controls. Again, we found a statistically significantly altered olfactory distribution with higher prevalence of anosmia and normosmia and lower prevalence of hyposmia in all GOLD type and grade subgroups except "without CRS and GOLD type 1+2" and "with CRS and GOLD grade AC". Regardless of CRS status there was no difference in olfactory distribution between GOLD type 1+2 and type 3+4. Nor was there any difference between GOLD grade AC and BD.

We then paired the SIT16 score with the patients answer to the EPOS minor criteria on olfactory function (Figure 4). Overall patients' answers to the EPOS criteria were poorly correlated with their SIT16 score.

Discussion:

to our knowledge we present the largest study of odour identification in patients with COPD, based on clinical evaluation by both an Otorhinolaryngologist and a Pulmonologist. Out study substantially contributes to the sparse knowledge of olfactory function in these patients. We present

SIT16 data from 135 COPD patients with and without CRS, all of whom were examined by flexible nasal endoscopy and CT-scans of the paranasal sinuses, in accordance with GOLD and EPOS guidelines (14,18).

COPD patients overall have an altered olfactory distribution with higher percentages of both anosmia and normosmia compared to age matched healthy controls. The higher percentage of anosmia was not evident in the mean SIT16 scores due to the simultaneous high percentage of normosmia which level out the score. The corresponding low level of hyposmia may be affected by the duration of airway inflammation. We would have expected a gradual tapering off olfactory function as COPD and unified airway inflammation progresses, but this may only become apparent in a prospective study of newly diagnosed COPD patients.

The only previously published study of olfactory function in 40 COPD patients (20 with and 20 without long term oxygen treatment via nasal cannules) compared UPSIT (University of Pennsylvania Smell Identification Test) olfactory scores to healthy controls and found no difference in the sense of smell when adjusting for smoking (19). In Dewan's study patients with a history of nasal symptoms (allergy, sinusitis) were excluded and no nasal examination was performed despite COPD patients having a higher prevalence of nasal symptoms (28) and CRS (8, 9).

Patients with more severe COPD (measured by both GOLD type and grade) had significantly lower mean SIT16 scores than patients with mild COPD, but still did not differ from healthy controls. This was caused by the mean SIT16 scores of patients with better lung function and fewer lung symptoms surprisingly being 1.33 and 1.16 points higher than age matched healthy controls. However, despite the higher scores of patients with mild COPD both they and healthy controls were within the normosmic range. We had expected the COPD patients to have a decreased mean SIT16 score due to the negative inflammatory effects of smoking and CRS on their unified airways (29). These findings contrast with the meta-analysis of current versus former and never-smoker which showed that current smokers have an increased risk of olfactory dysfunction (20).

The olfactory distribution of patients with mild COPD (measured by both GOLD type and grade) with and without CRS was diverging as some but not all subgroups were significantly different than healthy controls. On the other hand, patients with severe COPD regardless of CRS status all had a significantly altered olfactory distribution with higher percentage of anosmia. COPD patients with CRS who are current smokers unexpectedly did not have a different olfactory function distribution compared to healthy controls. A previously published prospective population-based study

controversially showed that current smoking decreased allergic sensitization (30). The authors hypothesized that low level smoking could potentially have an anti-inflammatory effect. Contrary to this a recent systematic review and meta-analysis found that current smoking was associated with a significant risk of olfactory dysfunction (20). Similarly, Håkansson et al. (31) introduced a "smoker nose" with increased inflammatory rhinitis symptoms in patients with chronic bronchitis and decreased lung function who currently smoked.

So, there seems to be an effect of COPD on olfaction which is not caused solely by age, GOLD type, GOLD grade, smoking or CRS. Perhaps the olfactory epithelium in some COPD patients is more vulnerable to unified airway inflammation and therefore changes more rapidly from normosmic to anosmic bypassing hyposmia. Patients in our study have had their COPD for many years so, it is possible that disease duration may play a role in the observed distribution of olfactory function. Longer duration of the disease and more severe COPD also promotes breathing through the mouth thereby bypassing the airflow through the nasal cavity and decreasing the stimulation of the olfactory epithelium. The brain is known to pay less attention to olfactory impulses if the olfactory epithelium is not stimulated in what is called top-down modulation (6, 32) and this may also contribute to our observed higher percentage of anosmia.

Our results caused also by affected by the test conditions. Sorokowska et al. (33) reported a significant difference between the "read first" and "smell first" (reading the answers before smelling the odour and vice versa) test conditions in normosmic subjects but not in hyposmic or anosmic subjects. We used the "smell first" conditions and found a statistically significant different distribution of norm-, hypo- and functional anosmia compared to healthy controls (26). As the smelling first test condition is reported not to affect hyposmic and anosmic subjects (33) we find the observed distribution to be reliable despite our relatively smaller sample size compared to the normative data.

CRS diagnosis is based on the EPOS2020 major and minor criteria in combination with clinical findings. One of the minor criteria is affected olfaction. The patients' reply reflects their subjective perception of odour. Our results show that patients' answers to the EPOS minor criteria about affected olfaction does not correspond with their SIT16 test results (Figure 4). This is in accordance with previous studies demonstrating that patients' ability to evaluate their own olfactory function is unreliable (34). This raises the question of solely using a subjective answer which does not directly

correlate to actual olfactory function as a diagnostic criterion. Preferably an olfactory test score should supplement the patient's subjective odour perception when diagnosing CRS.

Olfactory dysfunction is associated not only with HRQoL but also cognition (35), depression (5) and the grey matter of the limbic system linking olfactory dysfunction to neuro degenerative disease such as Alzheimer's and Parkinson's (6, 18, 36). Patients with COPD have high morbidity and mortality because of their chronic lung disease but the increasing number of publications, such as this one, is making it more and more apparent that COPD morbidity is much more divers than previously thought. This means that we as physicians need to recognize COPD as not only a lower airway disease but as a unified airway disease. Multimodal treatment possibilities exist for COPD patients with olfactory dysfunction due to comorbidities such as mucosal inflammation with swelling and CRS (7). We propose that patients and physicians alike will benefit from multidisciplinary teams consisting of pulmonologists and otorhinolaryngologists.

Strengths/limitations:

We performed the odour Identification (I) test but not Threshold (T) and Discrimination (D) (TDI). Full TDI testing is time consuming and was not performed as this was a preliminary study exploring olfactory function in COPD; where we found SIT16 testing to be sufficient. The Identification and Discrimination tests both contain high level odours, so called suprathreshold odours in contrast to the Threshold test which contains odours at low concentrations. The Threshold and Discrimination tests are known to be influenced by age and CRS related changes in olfaction earlier than the Identification test (37) so, a future study of longitudinal TDI scores in patients with COPD would add new knowledge.

This is the first combined otorhinolaryngological and pulmonological study of odour identification and olfactory function in patients with COPD showing a significantly higher percentage of anosmia compered to healthy controls. This was also true for nine out of 12 subgroups (smoking status, CRS, GOLD grade and GOLD type). Patients' answer to the EPOS2020 criteria about affected olfaction was poorly correlated with their SIT16 score. Our results emphasise the importance of both asking about and testing olfactory function. This group of patients is already severely marked by the morbidity and mortality correlated with COPD and the opportunity to improving their HRQoL (38,39) should not be overlocked. Future prospective studies will show if patients with COPD and olfactory dysfunction may also benefit from olfactory training (40).

Declaration of interest: there were no conflicts of interest for any of the authors.

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Author contributions: EA had full access to all data in the study and takes responsibility for the integrity of the data. KCB, ALS and EA take responsibility for the accuracy of the data analysis. EA, KA, VB, TH and CvB contributed substantially to the study design and all authors contributed substantially to data analysis, interpretation and the writing of the manuscript.

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Table 1. Demographics of 135[#] COPD patients undergoing odour identification testing.

n (%)	135[#] (100%)
Male gender, n (%)	83 (61.5%)
Age in years, mean (SD),	69.3 (8.0)
Current smoker, n (%)	68 (50.4%)
Packyears, mean (SD)	44.4 (18.5)
FEV1% predicted, median (range)	41.0 (17-102)
CAT*, mean (SD)	18.6 (7.8)
GOLD grade (symptom severity), n (%) A + C (few symptoms) - A - C B + D (many symptoms) - B	 85 (63.0%) - 12 (8.9%) - 73 (54.1%) 50 (37.0%) - 4 (3.0%)

- D	- 46 (34.0%)
GOLD type, n (%) I + II (mild – moderate decreased lung function) - L - II III + IV (severe – very severe decreased lung function) - III - IV	 42 (31.2%) - 4 (3.0%) - 38 (28.2%) 93 (68.8%) - 60 (44.4%) - 33 (24.4%)
Inhaled steroids, n (%)	74 (54.8%)
Chronic rhinosinusitis (CRS)^, n (%) - without nasal polyps (CRSwNP) - with nasal polyps (CRSsNP)	30 (22.2%) - 25 (83.3%) - 5 (16.7%)
SNOT22, median (range)	26.0 (0-87)
Eosinophils* (cells X 10^9/L), median (range)	0.16 (0-0.84)
Lund-MacKay score*, median (range)	1 (0-14)
COPD: chronic obstructive pulmonary disease. SD: standard deviation. FEV_1%: forced expiratory volume in the first second, predicted. CAT: COPD assessment test. GOLD: global initiative for chronic obstructive lung disease. SNOT22: sinonasal outcome test 22. ¨^ CRS diagnosed according to EPOS2020: European position paper on rhinosinusitis. *contains imputed values for missing data; CAT n=18 (13.3%), eosinophils n=13 (9.6%), Lund-Mackay score n=7 (5.2%). #: demographic data included in a previously published paper (8).	

Table 2: Sniffin' Sticks odour identifications (SIT16) scores and olfactory function distribution in COPD patients compared to healthy controls.

A: SIT16 scores		
Group	COPD	Healthy controls [26]
n SIT16, mean (SD) Min-max.	135 11.9 (3.0) 3-16	2396 11.6 (2.0) 2-16
Difference (95% CI)	-0.4 (-2.4 – 1.6)	
B: Olfactory function distribution		
Anosmia, n (%) Hyposmia, n (%) Normosmia, n (%)	19(14.1) 29(21.5) 87(64.4)	16 (1.4) 372 (32.4) 760 (66.2)
Fishers exact test*	**p<0.0001**	
COPD: chronic obstructive pulmonary disease. SD: standard deviation. (26): Oleszkiewicz *et al*. 2019, table 2. **In bold**: statistically significant differences. *adjusted to match the age distribution in the COPD group.		

Figure 1: Flowchart of patient in- and exclusion.

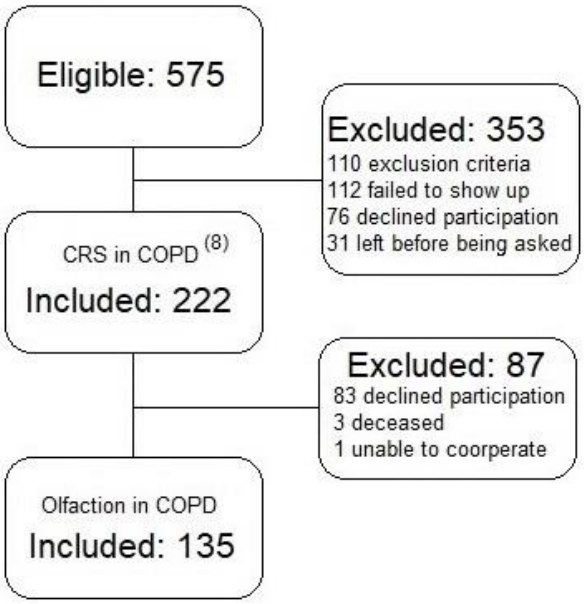
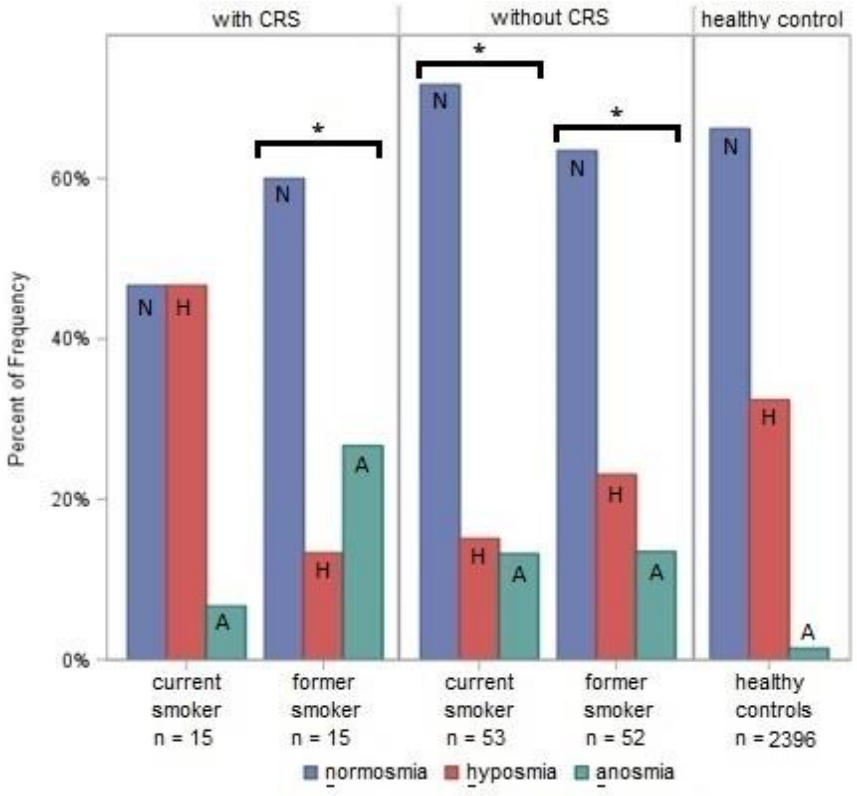
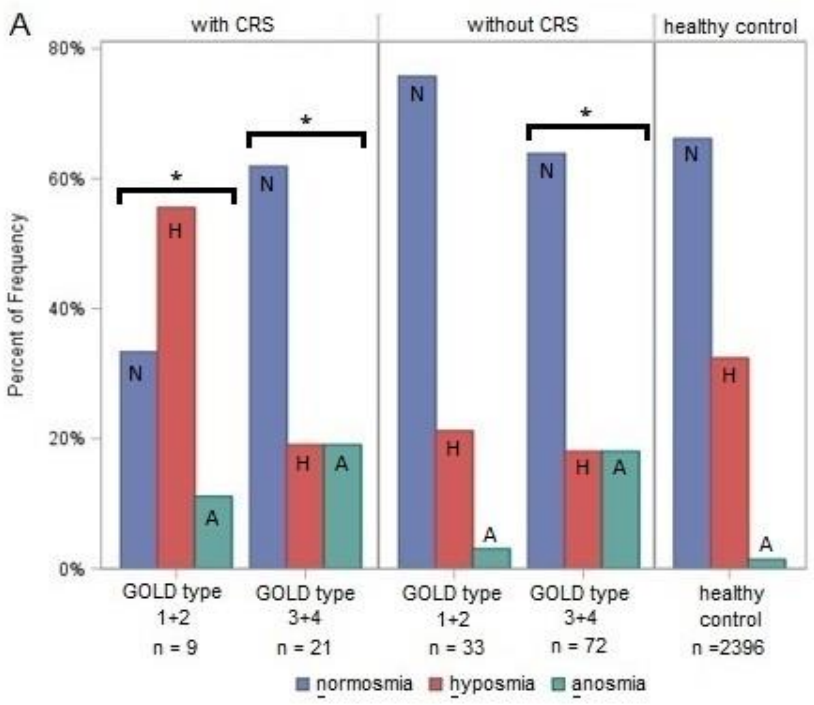


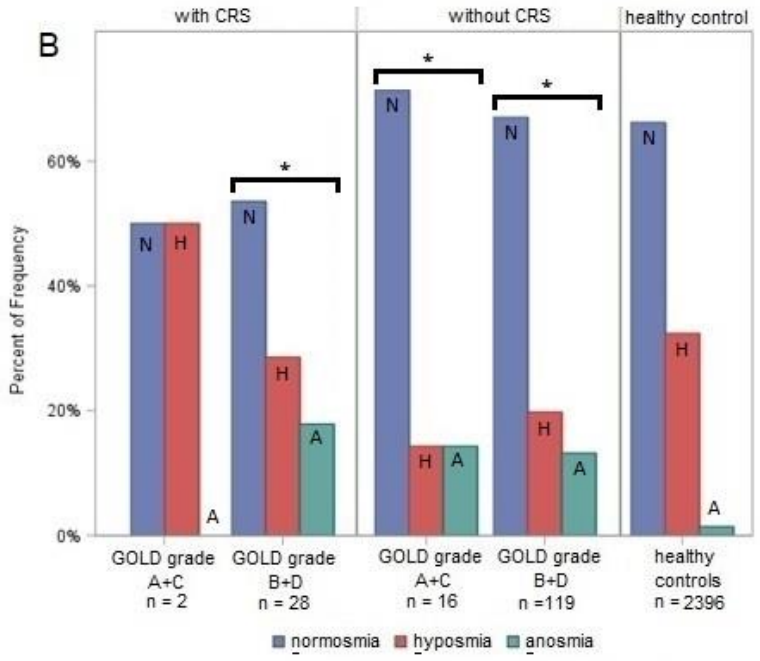
Figure 2: Olfactory function distribution in COPD patients with/without CRS and current/former smoking compared to age-matched healthy controls [26].



COPD: chronic obstructive pulmonary disease. CRS: chronic rhinosinusitis. [26]: Oleszkiewicz *et al*. 2019. *statistically significant on Chi-Square test (p<0.001).

Figure 3: Olfactory function distribution in COPD patients with/without CRS grouped according to GOLD type and grade compared to age-matched healthy controls [26].

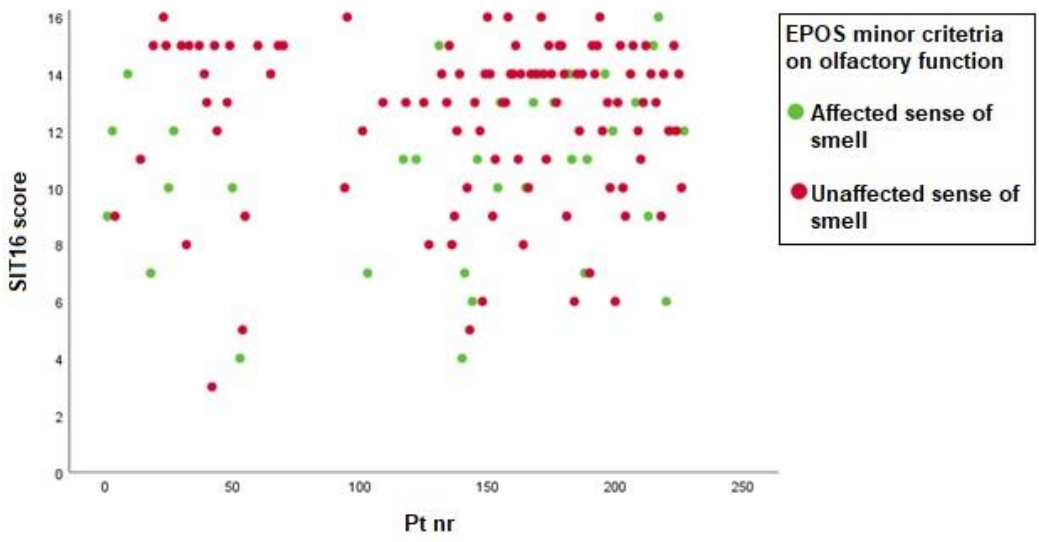




COPD: chronic obstructive pulmonary disease. CRS: chronic rhinosinusitis. GOLD: global initiative for chronic obstructive lung disease. [26]: Oleszkiewicz *et al*. 2019. *statistically significant on Chi-Square test ($p<0.001$). Panel A: stratified according to GOLD type. Panel B: stratified according to GOLD grade.

Figure 4. Odour identification (SIT16) scores in COPD patients

according to EPOS minor criteria on olfactory function



SIT16: Sniffin' Sticks identification test 16. COPD: chronic obstructive pulmonary disease. EPOS: European Position paper on rhinosinusitis (7).