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Original Research

Nasal and systemic inflammation in Chronic Obstructive Pulmonary Disease (COPD)

Nicolai Obling [a,b,*], Vibeke Backer [c,d], John R. Hurst [e], Uffe Bodtger [a,b,f]

[a] Department of Respiratory Medicine, Zealand University Hospital Næstved, Denmark
[b] Institute for Regional Health Research, University of Southern, Denmark
[c] Center for Physical Activity Research, Rigshospitalet, Copenhagen University, Denmark
[d] Department of ENT, Rigshospitalet, Copenhagen University, Copenhagen, Denmark
[e] UCL Respiratory, University College London, London United Kingdom, UK
[f] Department of Internal Medicine, Zealand University Hospital Roskilde, Denmark

A B S T R A C T

Systemic inflammation is a well-established feature of Chronic Obstructive Pulmonary Disease, COPD, but less is known about inflammation in the upper airways in the disease. In the current study, we investigated the inflammatory profile in the upper airway and in serum in a cohort of patients with COPD.

Patients were examined with inflammatory profiles measured on material from the upper airway and in serum using a 14-plex Bioplex multiplex immunoassay containing the following cytokines: IL-1-beta, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IL-18, Interferon-gamma, Tumour Necrosis Factor-alpha, Tumour Necrosis Factor beta, and GM-CSF.

We evaluated COPD disease burden using the CAT questionnaire and symptoms from the upper airways with the nasal domain of the 22 items Sino Nasal Outcome Test ($SNOT22_{nasal}$).

We included 180 patients (female 55%, age 67 (±8) years, FEV1% 52.4 (±16.6). Using a $SNOT22_{nasal}$ threshold of ≥6, we divided patients into high upper airways symptoms (high UAS), n = 74 (41%) and low upper airway symptoms (low UAS), n = 106 (59%). High UAS was significantly associated with higher levels of IL-1 beta and IL-3 in nasal samples (p = 0.016 and 0.02, respectively) and higher serum levels of IL-1 beta (p = 0.003). Upper airway scores correlated positively with nasal levels of IL-3 (rho = 0.195, p = 0.01) and serum levels of IL-1 beta (rho = 0.226, p = 0.005).

Patients with COPD and high upper airway symptoms displayed signs of eosinophilic and neutrophilic inflammation with elevated levels of IL-1 beta and IL-3 in the nose and elevated IL-1 beta in serum.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is widely recognized as a disease associated with local and systemic inflammation. The aetiology of this inflammation is multifactorial, but there is substantial evidence that exposure to harmful gases and particles play a pivotal role in initiating the inflammatory processes leading to the development of COPD. In high- and medium-income countries, tobacco smoking is considered the predominant source of these noxious elements [1]. In contrast, in low-income countries, biomass smoke exposure remains a widespread source of noxious elements and a significant risk factor for COPD [2].

The nature of airway inflammation has been subject to extensive research. While many mechanisms remain to be elucidated, it is known that inhalation of smoke in genetically susceptible individuals leads to activation of lung epithelial cells, which then secrete proinflammatory cytokines and chemokines, which are quantitatively and qualitatively different from those without a genetic predisposition. These proinflammatory mediators result in the recruitment of innate immune cells into the parenchyma and airways, where locally active enzymes degrade the lung's connective tissues resulting in small airway obstruction and emphysema [3].

In recent years the role of upper airway symptomology in obstructive lung disease has received added attention, and several studies have documented that COPD patients report symptoms from the upper airways [4,5]. The pathophysiology behind these symptoms is less well studied, and although some smaller studies have found some evidence of increased levels of inflammatory markers (IL-6+IL-8) in the upper airway of COPD patients both in stable COPD and during acute exacerbations [6,7], there has to date not be a study looking at this inflammation in detail.

In a recent study looking at 180 patients with COPD [8], we showed

* Corresponding author. Department of Respiratory Medicine, Zealand University Hospital Næstved, Denmark.
E-mail address: nao@dadlnet.dk (N. Obling).

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that upper airway symptoms were associated with both elevated COPD Assessment Test (CAT) scores and more pronounced blood and sputum eosinophilia. In the current study, we aim to investigate the underlying inflammatory mechanisms behind these findings, and we hypothesize that upper airway symptoms in COPD can be attributed to a specific inflammatory profile involving eosinophilic cytokines.

1.1. Methods

This study was conducted as a sub-study of the cross-sectional study, "BREATHE" [9]. The study was approved by the local ethics committees in both Denmark and Sweden and by the Danish Data Protection Agency. Data collection took place between February 2017 and Marts 2019.

Patients were recruited from three respiratory outpatient clinics at Næstved Hospital and Bispebjerg Hospital in Copenhagen, in Denmark and Lund in Sweden, as well as two primary care centres in Næset and Næsby in Sweden.

We included patients according to the following inclusion criteria: age ≥40 years, a history of smoking ≥ ten pack-years of tobacco and a post-bronchodilator Forced Expiratory Volume 1 s (FEV1)/Forced Vital Capacity (FVC) index <0.70.

Exclusion criteria were self-reported or physician-diagnosed asthma and reversibility for beta 2 agonist above 400 ml and 15% from baseline FEV1 [10,11]. We defined suspected asthma as the onset of symptoms before age 40 or a medical history of respiratory symptoms in childhood or adolescence. Patients with other respiratory diseases such as sarcoidosis, pulmonary fibrosis or lung cancer were excluded.

1.2. Medical history

Patients were interviewed by one of five medical doctors. Medical history focused on information on upper and lower airway symptoms, hospital or emergency department admissions, history of exacerbations, history of asthma and other respiratory diseases, as well as other comorbidities and current use of medication.

Smoking history was recorded using pack-years of tobacco.

Exacerbations of COPD (AECOPD) were defined as self-reported worsening of respiratory symptoms requiring additional treatment with oral antibiotics and/or corticosteroids or admission to hospital equivalent to moderate and severe COPD exacerbations.

1.3. Questionnaires

The COPD Assessment Test (CAT) is a questionnaire assessing COPD symptom burden [12]. The patient rate each of the eight questions on a scale from 0 (no symptoms) to 5 (worst possible). The questionnaire has a maximum score of 40 points and has a minimal clinical important difference of 2 points (MCID) [13].

The 22-item Sino Nasal Outcome Test (SNOT22) assesses chronic rhinosinusitis symptoms and includes nasal and more general symptoms such as fatigue [14]. Each symptom is scored from 0 (no symptoms) to 5 (symptoms as bad as they can be"). The maximum score is 110, with an MCID of 9 points [15].

The SNOT22 nasal subdomain ($SNOT22_{nasal}$) consists of seven questions (no. 1–5 + 7–8), which include: "need to blow nose", "sneezing", "runny nose", "nasal obstruction", "loss of smell or taste", "post-nasal discharge" and "thick nasal discharge". A cut-off for normality (or MCID) has been not validated, however the total SNOT22 has been found to have a median score of 7 points in healthy volunteers [16].

1.4. Definition of high upper airways symptoms (UAS)

We defined high upper airway symptoms as a $SNOT22_{nasal} \geq 6$. This cut-off value was chosen since a score of 6 implies either mild symptoms in most items or moderate symptoms in several items.

1.4.1. Lung function tests

Spirometry and reversibility test for beta 2-agonist were performed according to ERS/ATS guidelines using a Jaeger Spirometer (Intramedic®, Gentofte, Denmark) with the recording of FEV1, FVC, and FEV1/FVC index [17].

1.4.2. Induced sputum

Induced sputum from the lower airways was obtained according to the European Respiratory Society (ERS) guidelines using either spontaneous production or induction by isotonic saline or hypertonic saline (3–5%) [18].

Classifications of inflammatory cells were completed after a count of 400 non-squamous cells, and the fraction of eosinophils, lymphocytes, macrophages and neutrophils were noted. Samples with >80% of non-squamous cells were classified as adequate samples [19].

1.5. Nasal examinations and samples

Patients were examined with visual nasal inspection, and biologic material from the nasal lining was obtained using a FLOQSwab nasal swap (Copan Diagnostics, Italy). The nasal swap was inserted into one of the nostrils along the floor of the nasal cavity and rotated 5–6 times, and then retracted and put into a standard transport medium containing 1 ml of buffer. The medium was then centrifuged with 2000 rpm at 4° Celsius for 10 min to produce a cell pellet (not used in this study) and 2 × 400 μL of supernatant, which was then frozen at −80° Celsius.

1.5.1. Cytokine analyses

Cytokine profiles were measured in serum (n = 146) and supernatant from the nasal swap (n = 166) using a custom 14-plex Bioplex magnetic bead-based multiplex immunoassay containing the following cytokines: Interleukin (IL)-1-beta, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IL-18, Interferon-gamma (INF-gamma), Tumour Necrosis Factor-alpha (TNF-alpha), Tumour Necrosis Factor beta (TNF-beta), and GM-CSF.

Magnetic beads coated with specific antibodies were incubated with the samples for 30 min. After the incubation, the beads were washed using a magnetic plate washer and then incubated with biotinylated detection antibodies for 30 min. Further, the beads were washed and then incubated with streptavidin-PE for 10 min. The beads were then washed, resuspended in the assay buffer and run on the BioPlex-200 instrument (Bio-Rad) to measure the analytes. All samples were analyzed in duplicates and performed in one setting. Lower limits of detection for the assays were as follows (pg/ml): IL-1-beta: 0.6, IL-3: 4.8, IL-4: 0.7, IL-5: 0.6, IL-6: 2.6, IL-8: 1.0, IL-10: 0.3, IL-13: 0.7, IL-17: 3.3, IL-18: 0.2, TNF-alpha: 6.0, INF-gamma: 6.4, GM-CSF: 2.2, TNF-beta: 0.3.

If cytokine levels were below the lower limit of detection by the assay, a value was calculated using a standard curve extrapolation. If this wasn't possible, a value of zero was assigned.

1.6. Statistical analyses

Data were analyzed using SPSS version 27 (IBM, Chicago, USA). Normally distributed data are presented with mean and standard deviation (SD). Non-normally distributed data are given as the median and interquartile range (IQR).

Categorical variables are presented with a count (*n*) and a percentage (%). If cytokine levels were below the lower limit of detection by the assay, a value of zero was assigned.

Normally distributed data were analyzed using Independent Samples T-test. Group comparisons for skewed data were calculated using the Mann-Whitney *U* test. The significance level was with an alpha of 0.05. P-values are two-tailed. Correlations were calculated using Spearman Rank Correlation for skewed data.

2. Results

We included a total of 180 patients in the study, of which 74 patients (42%) were classified as having "high" upper airways symptoms (high UAS) according to the pre-defined criterion. Table 1 shows the clinical characteristics of the cohort divided between high and low UAS. Patients in the high UAS group were more likely to be male, had slightly higher lung function and reported higher mean CAT scores than patients in the low UAS group. They also had more pronounced signs of eosinophilia in blood and sputum (data previously published) [8].

(For details of the cohort as a whole, see Supplementary Table 1).

2.1. Nasal inflammation

Table 2 outlines the concentrations of cytokines measured in the supernatant of the nasal swap across the high vs low UAS groups and shows that high UAS was significantly associated with higher median levels of IL-1-beta and IL-3 and also a trend towards higher median levels of IL-5 and IL-10, but these did not reach statistical significance.

Levels of IL-3 in the nasal samples correlated significantly with upper airway score ($SNOT22_{nasal}$) rho = 0.195, p = 0.01, whereas IL-1 beta and IL-4 levels correlated inversely with CAT score, rho = −0.196, p = 0.01 and −0.164, p = 0.031, respectively (Table 4).

Table 1
Comparison between patients with high and low upper airway symptoms.

	High upper airway symptoms n = 74	Low upper airway symptoms n = 106	p-value
Age (years)	66 (±9)	67 (±8)	0.745
Female sex, n (%)	31 (42%)	68 (64%)	<0.01
BMI (kg/m^2)	26.0 (±6.2)	26.4 (±5.8)	0.146
Smoking status:			
Former Smoker	48 (65%)	76 (72%)	0.318
Current Smoker	26 (35%)	30 (28%)	
Tobacco Exposure (Pack Years)	50 (40–59)	43 (34–53)	0.141
Country:			
Denmark	51 (69%)	75 (71%)	0.867
Sweden	23 (31%)	31 (29%)	
SNOT22 (total score)	29 (23–37)	14 (9–22)	<0.001
$SNOT22_{nasal}$	10 (8–13)	2 (0–4)	
CAT score	17.4 (±7.5)	14.9 (±6.5)	<0.05
Inhaled medication:			
ICS use	31 (42%)	47 (44%)	0.701
Dual bronchodilator	23 (31%)	26 (25%)	0.408
Triple Therapy	27 (37%)	39 (37%)	0.843
FEV1 (L)	1.48 (±0.59)	1.31 (±0.53)	<0.05
INCS use	0 (0%)	1 (1%)	–
FEV1% predicted	53 (±16)	52 (±17)	0.629
FVC (L)	2.96 (±0.97)	2.67 (±0.88)	<0.05
FVC % predicted	82 (±17)	84 (±19)	0.403
DeltaFEV1 (ml)	110 (±132)	108 (±126)	0.905
DeltaFEV1 (%)	10 (±12)	10 (±12)	0.947
Bronchodilator Response >12% + 200 ml	13 (17%)	20 (19%)	0.824
GOLD stage (A-D)	A: 11 (15%) B: 45 (61%) C: 0 (0%) D: 18 (24%)	A: 22 (21%) B: 53 (50%) C: 4 (4%) D: 27 (26%)	0.206
Yearly exacerbations	21 (28%)	40 (38%)	0.197
≥2 moderate/severe AECOPD/year, n (%)	18 (24%)	20 (19%[e])	0.377

BMI: Body Mass Index. SNOT22: Sino Nasal Outcome Test 22, $SNOT22_{nasal}$: Nasal domain/upper airway domain of SNOT22.
CAT score: COPD Assessment Test. ICS: Inhaled Corticosteroids, FEV1: Forced Expiratory Volume 1 s. FVC: Forced Vital Capacity, RV: Residual Volume. TLC: Total Lung Capacity. DLCO: Diffusion Capacity for Carbon Monoxide. DeltaFEV1: Increase in FEV1 from baseline. GOLD: Global Initiative for Chronic Obstructive Lung Disease. AECOPD: acute exacerbations in COPD.
INCS: Intranasal corticosteroids.

Table 2
Nasal cytokine levels in patients with high and low upper airway symptoms (n = 166).

	High upper airway symptoms	Low upper airway symptoms	p value
IL-1-beta	0.29 (0.16–1.04)	0.20 (0.06–0.56)	**0.016**
IL-3	0.06 (0–0.14)	0.02 (0–0.11)	**0.020**
IL-4[a]	0 (0–0.03)	0 (0–0.04)	0.669
IL-5[a]	1.05 (0.22–1.89)	0.59 (0–1.48)	0.067
IL-6	1.17 (0.53–3.4)	1.65 (0.63–3.17)	0.511
IL-8	134.26 (75.94–277.48)	150.36 (66.53–338.51)	0.633
IL-10	0.46 (0–1.24)	0.19 (0–0.92)	0.069
IL-13[a]	0.04 (0.02-0-08)	0.04 (0–0.09)	0.557
IL-17	1.08 (0.66–2.06)	0.91 (0.54–1.78)	0.245
IL-18	279.50 (56.30–871.30)	346.84 (69.22–620.37)	0.786
IFN-gamma	0 (0–0.43)	0 (0–0.38)	0.753
TNF-beta	0.49 (0.02–1.16)	0.34 (0–0.98)	0.192
TNF-alpha	2.74 (0.78–4.91)	2.43 (1.06–4.39)	0.700
GM-CSF	0.19 (0.05–0.40)	0.23 (0.04–0.40)	0.970

All levels reported in pg/ml.
Data are presented as median and (interquartile range).
Differences between groups are analyzed with Mann Whitney *U* test.
IL: interleukin, IFN: Interferon, TNF: Tumour necrosis factor, GM-CSF: Granulocyte Macrophage Colony stimulating factor.
[a] Th2 cytokines.

Table 3
Serum cytokine levels in patients with high and low upper airway symptoms (n = 146).

	High upper airway symptoms	Low upper airway symptoms	
IL-1-beta	0.09 (0–0.17)	0 (0–0.11)	**0.003**
IL-3	0 (0–0.02)	0 (0–0.04)	0.804
IL-4[a]	0 (0–0)	0 (0–0)	0.404
IL-5[a]	0 (0–2.16)	0 (0–0.53)	0.172
IL-6	1.62 (1.10–2.49)	1.35 (0.79–2.2)	0.118
IL-8	9.34 (6.32–13.91)	8.95 (6.21–13.85)	0.748
IL-10	0 (0–0)	0 (0–0)	0.092
IL-13[a]	0 (0–0)	0 (0–0)	0.283
IL-17	0.29 (0–1.50)	0 (0–0.88)	0.182
IL-18	54.09 (35.15–79.05)	46.91 (26.07–69.86)	0.206
IFN-gamma	0 (0–0)	0 (0–0)	0.236
TNF-beta	0 (0–0)	0 (0–0)	0.937
TNF-alpha	12.60 (6.92–20.07)	10.33 (6.47–17.28)	0.303
GM-CSF	0 (0–0)	0 (0–0)	0.904

All levels are in pg/ml.
Data are presented as median and (interquartile range).
Differences between groups are analyzed with Mann Whitney *U* test.
IL: interleukin, IFN: Interferon, TNF: Tumour necrosis factor, GM-CSF: Granulocyte Macrophage Colony stimulating factor.
[a] Th2 cytokines.

2.2. Systemic inflammation

Serum levels of cytokines (Table 3) were generally lower than those in the nasal swaps, and several cytokine levels (IL-3, IL-4, IL-10, IL-13, IFN-gamma, TNF-beta and GM-CSF) were below the lower detection level in most patients. Only IL-1 beta was significantly different between groups with higher median values in the high UAS group.

Upper airway score showed a significant positive correlation with IL-1 beta, rho = 0.226, p = 0.005, but no other serum cytokine levels correlated significantly with either upper airway score or CAT score (Table 5).

2.3. Correlation between nasal and systemic inflammation

Table 6 shows that a positive correlation between nasal and serum values of the cytokines was present in 6 out of 14 analytes and included

Table 4
Correlations between upper airway score, CAT score, sputum and **nasal** cytokine levels.

	Upper airway symptom score	CAT score	Sputum Eosinophils (%)	Sputum Neutrophils (%)
IL-1-beta	0.138, p = 0.072	**−0.196, p = 0.010**	0.039, p = 0.744	**0.364, p = 0.001**
IL-3	**0.195, p = 0.010**	0.039, p = 0.611	0.015, p = 0.897	−0.086, p = 0.464
IL-4	0.058, p = 0.449	**−0.164, p = 0.031**	−0.081, p = 0.495	0.131, p = 0.267
IL-5	0.097, p = 0.207	0.053, p = 0.488	−0.030, p = 0.802	**−0.248, p = 0.033**
IL-6	−0.023, p = 0.761	- 0.081, p = 0.290	−0.189, p = 0.107	**0.348, p = 0.002**
IL-8	−0.054, p = 0.478	−0.028, p = 0.715	−0.134, p = 0.254	0.025, p = 0.831
IL-10	0.111, p = 0.149	−0.010, p = 0.897	0.076, p = 0.519	0.075, p = 0.526
IL-13	0.047, p = 0.537	0.088, p = 0.250	−0.184, p = 0.116	−0.072, p = 0.543
IL-17	0.054, p = 0.480	0.062, p = 0.421	**−0.233, p = 0.046**	−0.072, p = 0.544
IL-18	0.008, p = 0.920	- 0.090, p = 0.239	**−0.329, p = 0.004**	0.103, p = 0.383
IFN-gamma	−0.005, p = 0.952	0.130, p = 0.089	−0.008, p = 0.948	−0.187. p = 0.112
TNF-beta	0.043, p = 0.574	0.040, p = 0.599	−0.193, p = 0.100	−0.055, p = 0.643
TNF-alpha	0.026, p = 0.731	−0,079, p = 0.302	−0.165, p = 0.159	**0.288, p = 0.013**
GM-CSF	−0.005. p = 0.946	−0.005, p = 0.948	−0.122, p = 0.301	−0.053, p = 0.654

IL: interleukin, IFN: Interferon, TNF: Tumour necrosis factor, GM-CSF: Granulocyte Macrophage Colony stimulating factor.
Correlations presented as non-parametric Spearman's rho.
CAT: COPD Assessment Test.

Table 5
Correlations between upper airway score, CAT score, sputum and **serum** cytokine levels.

	Upper airway symptom score	CAT score	Sputum Eosinophils (%)	Sputum Neutrophils (%)
IL-1-beta	**0.226, p = 0.005**	0.117, p = 0.151	**0.498, p < 0.001**	**−0.255, p = 0.032**
IL-3	0.046, p = 0.576	0.086, p = 0.295	−0.151, p = 0.210	−0.017, p = 0.885
IL-4	0.014, p = 0.863	−0.003, p = 0.973	NA	NA
IL-5	0.123, p = 0.133	0.108, p = 0.187	**0.291, p = 0.014**	−0.203, p = 0.089
IL-6	0.142, p = 0.082	0.045, p = 0.584	−0.202, p = 0.091	0.047, p = 0.700
IL-8	0.046, p = 0.576	0.124, p = 0.128	−0.226, p = 0.059	0.185, p = 0.122
IL-10	−0.050, p = 0.544	0.077, p = 0.346	−0.091, p = 0.451	0.055, p = 0.649
IL-13	−0.039, p = 0.637	0.039, p = 0.632	−0.055, p = 0.648	−0.007, p = 0.955
IL-17	0.071, p = 0.385	0.143, p = 0.080	0.156, p = 0.195	−0.057. p = 0.637
IL-18	0.107, p = 0.192	0.065, p = 0.426	−0.078. p = 0.520	0.127, p = 0.290
IFN-gamma	−0.028, p = 0.736	0.007, p = 0.935	−0.151, p = 0.210	0.204, p = 0.088
TNF-beta	−0.029, p = 0.722	−0.066, p = 0.416	−0.035, p = 0.772	0.140, p = 0.241
TNF-alpha	0.097, p = 0.237	0.162, p = 0.047	−0.177, p = 0.139	0.069, p = 0.565
GM-CSF	−0.019, p = 0.817	0.007, p = 0.930	0.036, p = 0.768	−0.113, p = 0.347

IL: interleukin, IFN: Interferon, TNF: Tumour necrosis factor, GM-CSF: Granulocyte Macrophage Colony stimulating factor.
Correlations presented as non-parametric Spearman's rho.
CAT: COPD Assessment Test.

Table 6
Correlations between nasal and **serum** cytokine levels.

IL-1-beta	0.197, p = 0.016
IL-3	0.112, p = 0.182
IL-4	−0.052, p = 0.537
IL-5	**0.383, p < 0.001**
IL-6	**0.299, p < 0.001**
IL-8	**0.334, p < 0.001**
IL-10	−0.051. p = 0.541
IL-13	**0.337, p < 0.001**
IL-17	0.063, p = 0.456
IL-18	0.103, p = 0.220
IFN-gamma	−0.082, p = 0.330
TNF-beta	0.014. p = 0.867
TNF-alpha	**0.290, p < 0.001**
GM-CSF	0.093, p = 0.269

IL: interleukin, IFN: Interferon, TNF: Tumour necrosis factor, GM-CSF: Granulocyte Macrophage Colony stimulating factor.
Correlations presented as non-parametric Spearman's rho.

IL-1 beta (rho = 0.197, p = 0.016), IL-5 (rho = 0.383, p < 0.001), IL-6 (rho = 0.299, p < 0.001), IL-8 (rho = 0.334, p < 0.001), IL-13 (rho = 0.337, p < 0.001) and TNF-alpha (rho = 0.290, p < 0.001).

2.4. Correlation between nasal and pulmonary inflammation

Nasal levels of IL-17 and IL-18 correlated inversely with the percentage of eosinophils in sputum, whereas neutrophils correlated positively with both IL-1 beta, IL-6 and TNF-alpha but inversely with IL-5 (Table 4).

2.5. Correlation between systemic and pulmonary inflammation

Serum levels of IL-1 beta and IL-5 correlated positively with the percentage of eosinophils in sputum, whereas sputum neutrophils correlated inversely with serum levels of IL-1 beta (Table 5).

3. Discussion

In the current study, we sought to investigate the inflammatory profile behind upper airway symptoms in a cohort of patients with COPD. We measured the concentration of a number of cytokines in the nasal cavity and serum of patients with high and low levels of upper airway symptoms. To our knowledge, our study is the largest of its kind. We found that patients with high UAS had significantly higher levels of IL-1 beta and IL-3 in the nasal cavity and higher levels of IL-1 beta in serum.

Both IL-1 beta and IL-3 are involved in the recruitment and activity of neutrophils and would be expected in a disease where these innate immune cells are known to play a crucial role in the pathogenesis. Studies

have shown IL-1 beta concentrations in serum, BAL fluid and sputum are elevated in stable COPD compared with healthy controls and that they correlate inversely with lung function [20,21].

Our findings in the nasal cavity are confirmed, although at lower levels, in the serum of patients with high UAS and would suggest that these symptoms might be driven by inflammatory processes involving neutrophils. Evidence already exists for this connection. A Belgian study found that treating UAS with intranasal corticosteroids reduced symptoms and nasal levels of IL-8, which is a potent inducer of neutrophilic activity [22].

From our previous findings in this cohort, we expected to see a signal of cytokines involved in the eosinophilic pathways since patients with high UAS showed significantly higher levels of eosinophils in both blood and sputum. This finding is confirmed by the elevated levels of IL-3, which is also known to play a role in the activation and differentiate of eosinophils and could explain the elevated levels of eosinophils in these patients with high UAS [23]. This finding is also supported by the positive correlation (rho = 0.195) between nasal IL-3 levels and upper airway score. In addition, our results suggest a link between IL-1 beta and eosinophilic inflammation with a firm correlation between systemic IL-1 beta concentrations and the percentage of eosinophils in sputum of our cohort as a whole. This finding is contrary to the traditional perception of IL-1 beta function, which is traditionally associated with Th1 skewed immune responses [24]. However, eosinophils have been reported to secrete IL-1 beta themselves, which could explain this finding [25].

We also found a trend towards higher values of the Th2 cytokine IL-5 in the nasal swaps, but this did not reach statistical significance ($p = 0.067$), and in serum, the trend disappeared. Our cytokine panel included other Th2 cytokines (IL-4 and IL-13), but these did not show any signal between groups, neither in serum nor in nasal swaps.

Several cytokines have been implicated in the pathophysiology of COPD, most notably IL-6, IL-8 and TNF-alpha, whose concentrations have been shown to be elevated compared to controls and to increase during acute exacerbations of COPD (AECOPD) [26]. IL-6 has also been demonstrated to rise in the nasal cavity during exacerbations [7] which suggests upper airway involvement in the inflammatory process behind COPD and AECOPDs. In our study, we did not, however, find any significant differences in serum levels of either IL-6, IL-8 or TNF-alpha between patients with high and low UAS. We did find a correlation between the systemic and nasal levels of several of the measured cytokines, which suggest that some of the systemic inflammatory processes affecting patients with COPD also affect the upper airways. It is noteworthy that these cytokines (IL-1 beta, IL-5, IL-6, IL-8, IL-13, TNF-alpha) all are mediators suspected of playing a critical role in the development of COPD [3].

When we compare the overall levels of the measured cytokines in our study with other similar studies, several differences appear. The serum levels of IL-1 beta in our study is markedly lower in both groups when we compare them to other studies [20,27]. This tendency is also true for IL-17 and IL-18, where the values in our study fall well below those reported in a study comparing asthma, COPD and asthma-COPD overlaps syndrome (ACOS) [28]. In contrast, serum values of TNF-alpha in our study appear to be at similar or higher levels than observed in previous studies, and serum values of IL-6 in our study are similar to the values reported in several studies [28,29].

4. Strength and limitations

Our study is the largest one of its kind and the first to extensively look at the inflammatory profile in COPD patients with upper airway symptoms. We used a standardized technique in the sampling of the nasal inflammatory markers, which is less prone to variation than the classical nasal wash/lavage performed in older studies. We also explored a broad panel of cytokines to investigate several potential inflammatory pathways.

The discrepancies between our reported levels of some cytokines and the existing literature can be due to several factors. Cytokine levels can be affected by freeze-thawing cycles, which can reduce levels (IL-1 beta, IL-5, IL-8, IL-17) as well as raise levels (IL-4, TNF-alpha), and although our nasal samples had only been subjected to one cycle prior to our analyses, it is impossible to rule out an effect on our measurements. The evidence does, however, suggest that the impact on levels is minimal after one cycle [30].

Individual sampling can also affect the outcome of analyses. The sampling from the nasal cavity with the nasal swap, although assumed to be less prone to variation than classical nasal lavage, could, due to patient discomfort and anxiety, still result in the sampling of variable amounts of nasal lining fluid, leading to skewed results in the final measurements. Our findings could also be affected by statistical aspects. Due to the number of analytes in our panel, there is always the risk of Type 1 errors due to multiple testing. We chose not the adjust precisely for this due to the exploratory nature of our study.

5. Conclusion

COPD patients with high upper airway symptoms showed signs of both neutrophilic and eosinophilic inflammation with elevated levels of IL-1 beta in serum and IL-1 beta and IL-3 in samples from the nasal cavity. Serum levels of IL-1 beta correlated significantly with upper airway symptoms scores, as did nasal levels of IL-3.

Declaration of interest

The authors report no conflicts of interest related to this study.

CRediT authorship contribution statement

Nicolai Obling: Formal analysis, Writing – original draft, Study design, main patient recruitment, performed data analysis and prepared main manuscript. **Vibeke Backer:** Formal analysis, Writing – original draft, Study design, data analysis and editing main manuscript. **John R. Hurst:** Formal analysis, Writing – original draft, Study design, data analysis and editing main manuscript. **Uffe Bodtger:** Formal analysis, Writing – original draft, Study design, patient recruitment, data analysis and editing main manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2022.106774.

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