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Clinical characteristics of the BREATHE cohort – a real-life study on patients with asthma and COPD

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ABSTRACT

Background: The BREATHE study is a cross-sectional study of real-life patients with asthma and/or COPD in Denmark and Sweden aiming to increase the knowledge across severities and combinations of obstructive airway disease.

Design: Patients with suspicion of asthma and/or COPD and healthy controls were invited to participate in the study and had a standard evaluation performed consisting of questionnaires, physical examination, FeNO and lung function, mannitol provocation test, allergy test, and collection of sputum and blood samples. A subgroup of patients and healthy controls had a bronchoscopy performed with a collection of airway samples.

Results: The study population consisted of 1403 patients with obstructive airway disease (859 with asthma, 271 with COPD, 126 with concurrent asthma and COPD, 147 with other), and 89 healthy controls (smokers and non-smokers). Of patients with asthma, 54% had moderate-to-severe disease and 46% had mild disease. In patients with COPD, 82% had groups A and B, whereas 18% had groups C and D classified disease. Patients with asthma more frequently had childhood asthma, atopic dermatitis, and allergic rhinitis, compared to patients with COPD, asthma + COPD and Other, whereas FeNO levels were higher in patients with asthma and asthma + COPD compared to COPD and Other (18 ppb and 16 ppb vs 12.5 ppb and 14 ppb, $p < 0.001$). Patients with asthma, asthma + COPD and Other had higher sputum eosinophilia (1.5%, 1.5%, 1.2% vs 0.75%, respectively, $p < 0.001$) but lower sputum neutrophilia (39.3, 43.5%, 40.8% vs 66.8%, $p < 0.001$) compared to patients with COPD.

Conclusions: The BREATHE study provides a unique database and biobank with clinical information and samples from 1403 real-life patients with asthma, COPD, and overlap representing different severities of the diseases. This research platform is highly relevant for disease phenotype- and biomarker studies aiming to describe a broad spectrum of obstructive airway diseases.

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Introduction

Randomized controlled trials (RCT) address important questions such as risk/benefit profiles of new therapies, but to improve internal validity they often report results from narrow patient groups representing less than 2% of the real-life patient population and thus hampers external validity [1,2]. Real-life studies include patients with 'real-life' co-morbidities, life-style factors, various inflammatory phenotypes, and different adherence profiles, and these patients may, therefore, elicit another response to treatment compared with the highly selected patient groups included in RCTs [3,4]. Therefore, real-life studies are pivotal to address issues concerning the entire patient population being exposed to the drugs investigated in RCTs [5,6].

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Summary: The BREATHE study provides a research platform with clinical data and biological samples from 1492 real-life patients with asthma, COPD, or both and healthy controls for development of novel biomarkers and diagnostic tools for obstructive airway disease.

Supplemental data for this article can be accessed here.

Examples of large patient–cohort studies within the field of asthma are the U-BIOPRED and the SARP studies [7,8] that included large samples of patients with severe and mild to moderate asthma. The importance of these multisite studies is considerable, but the focus has primarily been towards increasing knowledge on severe asthma. However, the frequency of severe asthma in an asthma population is less than 10% [9,10], indicating that knowledge about the majority of asthma patients must be gained from other sources. The same goes for patients with COPD and concurrent asthma and COPD, who generally have been excluded from most asthma studies.

New treatment strategies are being developed these years along with an increasing demand for individualized disease management and new biomarkers to guide treatment and follow-up and to assess comorbidities [11,12]. Therefore, in-depth knowledge of real-life respiratory disease is required to develop a scientific, evidence-based understanding of the underlying disease mechanisms driving the diseases in the entire patient population.

With the BREATHE research platform, we aimed to develop a well-characterized and comprehensive database and biobank with clinical data and samples from real-life patients with different severities of obstructive airway disease as well as a reference population of healthy controls.

Methods

Design

The study was a multicentre, descriptive cross-sectional clinical study recruiting real-life patients with asthma and/or COPD and healthy controls from five clinical centres: two specialist care units in Eastern Denmark and one specialist care unit plus two primary care units in Southern Sweden. The recruitment period was 2 years (February 2017–February 2019). The study and all related study documents were approved by the local ethics committees (H-16047428, Denmark and Dnr 2016/1069, Lund Sweden). All participants gave written informed consent prior to the study (Helsinki declaration 1964–2014 50). The study was not registered in a public domain.

Participants

The participants were either newly referred patients with suspected asthma or COPD, or patients at regular review for asthma or COPD at either specialist care units or primary care clinics; or subjects recruited as healthy controls. All healthy controls were screened by an MD to ensure no present or former respiratory disease. All participants underwent a baseline visit (visit 1a+1b), and a subset of patients and healthy controls underwent a bronchoscopy (visit 2) (Figure S1). Inclusion and exclusion criteria are described in the supplementary methods section.

Interview

Information on respiratory disease, allergy, family history of atopic diseases, seasonal variation in lung and nasal symptoms, and medication for respiratory diseases and/or allergy was obtained. History of tobacco consumption was recorded, and patients were classified as never smoker, former smoker (smoke-free for at least the past 6 months), or current smoker; the average number of pack-years was calculated ((average number of daily cigarettes*years)/20).

Physical examination

All participants had a health check performed with a focus on respiratory illness and co-morbidities – including measurement of blood pressure, pulse, and oxygen saturation (Visit 1). Nasal inspection for polyps and swollen mucosa was performed followed by a nasal swab of the meatus medius/medial concha.

Baseline measurements

Age, sex, weight, and height were recorded for all participants. BMI was calculated as weight in kg/(height in meters)^2.

Questionnaires

All patients answered 12 questionnaires regarding symptoms and disease-control (ACQ-5 [13], ACT [14], CAT [15], Medical Research Council dyspnoea scale (mMRC)) [16], quality-of-life (SF12 [17], miniAQLQ [18], miniRQLQ [19], CCQ [20], HADS [21]), and comorbidities (Nijmegen [22], SNOT22 [23], Epworth Sleepiness Scale (ESS) [24]) (Table 3). Patients were also asked about hospital referrals and visits to GP or specialist due to exacerbations, the onset of disease and childhood symptoms as well as socio-economic factors such as income and education level. The presence of chronic rhinosinusitis (CRS) was deduced from answers to the SNOT22 questionnaires as described [25] using a cut-off ≥3 and based on the nasal inspection patients with CRS were classified with (w) or without (s) nasal polyps (NP), i.e. CRSwNP or CRSsNP.

Pre-medication

Prior to respiratory testing, participants were asked not to use short-acting β2 agonist (SABA) for 8 h, inhaled

corticosteroid (ICS) for 12 h, long-acting β2 agonists (LABAs), long-acting muscarinic antagonists (LAMA), short-acting muscarinic antagonists (SAMA), theophylline or smoking for 24 h, leukotriene-antagonist for 1 day and antihistamines for 72 h before the visit. Patients on a stable dose of oral corticosteroids (OCS) could continue their use.

Exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) was analysed using NIOX VERO® equipment (Aerocrine AB, Solna, Sweden) and the mean of three measurements was recorded [26]. In approximately one-third of the participants, alveolar NO and bronchial flow were measured using Medisoft FENO+ (Sorinnes, Belgium).

Spirometry

Spirometry was performed according to the standards specified by the ERS and ATS [27]. Briefly, FEV1 and FVC were measured three times, with differences between the two largest FEV1 values being ≤0.150 L and the two largest FVC values being ≤0.150 L, using a Jaeger spirometer with ECCS 93 reference values (Intramedic®, Gentofte, Denmark).

Static lung volume

Participants recruited from the specialist care units had measurements of total lung capacity (TLC) and diffusion capacity for carbon monoxide (DLCO) performed using Jaeger® box (Intramedic®, Gentofte, Denmark) according to the standards specified by the ERS and ATS [28]. Predicted normal values of FEV1, FVC, FEV1/FVC ratio based on sex, height, weight, and age were calculated using reference values ECCS 93 [29].

Reversibility test

Patients with an FEV1 <70% predicted performed a short-acting β2 reversibility test. FEV1 was measured at baseline and 15 min after 0.8 mg of salbutamol (4 × 0.2 mg or 8 × 0.1 mg). The test was considered positive if FEV1 increased with at least 12% (and 200 ml) from baseline [30].

Mannitol bronchial provocation

A mannitol test was performed in participants with an FEV1 ≥70% of predicted (Aridol™; Pharmaxis, Frenchs Forest, Australia). A positive test response indicating airway hyperresponsiveness (AHR) was defined as a 15% fall or more in FEV1 at a total dose of ≤635 mg. Sensitivity to mannitol was reported as PD15, i.e. the mannitol dose that results in a 15% fall or more in FEV1, and responsiveness was reported as a response–dose ratio (RDR) defined as percent fall in FEV1/cumulative dose of mannitol [31].

Allergy testing and atopy

Specific IgEs or skin prick test (ALK-Abello®, Hørsholm) was performed with a standard panel of 10 aeroallergens. The specific IgE test was considered positive if at least one of the specific IgE levels >0.35 kU/L and the skin prick test was considered positive if at least one wheal was >3 mm observed after 15 min. Atopy was defined as a positive-specific IgE or skin prick test.

Disease severity and diagnosis

Severity of asthma was classified according to GINA guidelines [30,32] and COPD was classified according to GOLD guidelines [33]. A diagnosis of asthma, COPD, or concurrent asthma and COPD (termed 'asthma + COPD' hereafter) was based on thorough medical history, clinical evaluation, and relevant lung function and bronchoprovocation tests. Patients in whom a diagnosis of asthma or COPD could not be made were allocated to the 'Other' group.

Biological samples

Blood samples and nasal swabs (ESWAB 482C, Copan, Italy) were obtained from all participants. Leukocyte and differential cell counts were performed and blood eosinophils >0.3 × 10^9 cells/L was used as a cut-off to determine the presence of eosinophilic inflammation in the blood [34]. For the subset of participants undergoing bronchoscopy, bronchoalveolar lavage (BAL)-fluid, brushings and mucosa biopsies, faecal, urine, and saliva samples were obtained.

Sputum induction and cell count

Sputum was obtained either spontaneously, immediately after mannitol testing or induced using isotonic (0.9%) or incremental concentrations of NaCl solutions (3%, 4%, and 5%) and processed as described [35]. Four hundred non-squamous cells were counted, and the percentages of epithelial cells, eosinophils, neutrophils, macrophages and lymphocytes were listed. A cut-off of 3% for eosinophils and 61% for neutrophils was used for sputum inflammatory phenotyping [36,37].

Data were entered in an electronic case report form (SecureCRF®, Copenhagen, Denmark).

Statistical analysis

Continuous variables were reported as mean with standard deviations for normally distributed variables, while non-normally distributed variables were reported as median with 25th and 75th percentiles. Continuous variables were tested using Kruskal–Wallis test, while categorical variables were tested using Chi-square test. Monte Carlo simulation was used if Chi-square approximation was not met (expected cell counts <5), thereby comparing the observed data to random samples.

Analyses were performed using SAS Studio (SAS Institute, Cary, NC, USA).

Results

A total of 1492 participants were recruited over a 2-year period: 859 patients with asthma, 271 patients with COPD, 126 patients with asthma + COPD, and 89 healthy controls (Figure 1 and Tables 1 and 2). Moreover, 147 patients did not have asthma or COPD ('Other' group; supplementary Table S1).

The participants were recruited from Denmark (n = 906) and Sweden (n = 591) with approximately one-third of the participants from general practitioners and two-thirds from outpatient clinics (Table 1). The age distribution was equal between sites except for participants from Copenhagen (DK), who were younger ($p < 0.001$) and BMI was higher in patients from Naestved (DK) (p = 0.003).

Gender distribution was equal across groups (Table 2). The BMI did not differ between patients with asthma and COPD, whereas FEV1 percent predicted (91% vs 56%, $p < 0.001$) was higher in patients with asthma than those with COPD.

In general, patients reported a high degree of respiratory symptoms (Table 3). The symptom burdens depicted by ACQ-5, ACT, CAT, and mMRC scores were higher in patients with COPD than in those with asthma ($p < 0.01$, all), independent of the origin of the questionnaire. Among the COPD patients, specific scores of CAT >10 and mMRC ≥2 were found in 70% and 87% of the patients, respectively, and among patients with asthma, uncontrolled disease indicated by ACQ >1.5 and ACT ≤19 was found in 44% and 49%, respectively.

Based on the quality-of-life (QoL) related questionnaire SF-12, COPD and asthma + COPD patients, in general, reported a worse health-status regarding the physical (PCS), but the mental component was comparable across groups. The same tendency was seen in the activity components from MiniAQLQ, MiniRQLQ, and CCQ, which indicated a better health status for patients with asthma and Other compared to patients with COPD and asthma + COPD ($p < 0.01$, all).

The comorbidity-related questionnaire scores from SNOT-22 and Nijmegen did not differ significantly between the four patient groups ($p = 0.45$ and $p = 0.60$, respectively), and although the score for Epworth sleepiness scale was significantly higher in

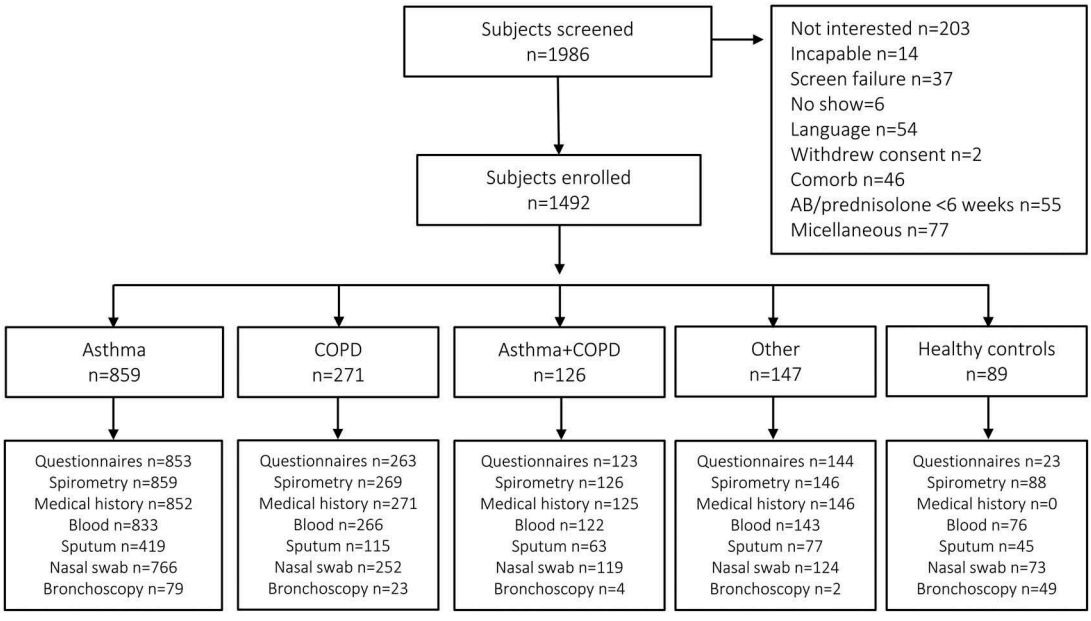

Figure 1. Consort flow diagram. Sputum was collected at specialist clinical sites only, not in primary care units; therefore, sputum samples do not exist for all participants. Bronchoscopy was performed in a subgroup of participants. Healthy controls: smokers and non-smokers. Concurrent asthma and COPD: asthma + COPD.

Table 1. Distribution of patients between countries and clinical sites in the BREATHE study.

	Copenhagen, DK	Naestved, DK	Lund, SE	General practitioners, SE	*p*-Value
Subjects (n)	675	229	108	483	
Age	44 (28–60)	62 (50–70)	63 (46–69)	57 (44–70)	<0.001
BMI	26 (5)	27 (6)	26 (5)	26 (4)	0.003
Asthma (n)	442 (65%)	70 (31%)	30 (28%)	317 (66%)	<0.001
COPD (n)	60 (9%)	98 (43%)	33 (31%)	80 (17%)	
Asthma+COPD (n)	48 (7%)	38 (17%)	4 (4%)	36 (8%)	
Other (n)	84 (12%)	20 (9%)	10 (9%)	33 (7%)	
Healthy controls (n)	40 (6%)	3 (1%)	30 (28%)	16 (3%)	

Data are presented as numbers (n), n/N (%), mean±SD, or median (interquartile range, 25th–75th).

Table 2. Baseline variables.

	Asthma	COPD	Asthma + COPD	Other	Healthy	*p*-Value	*p*-Value[#]
Subjects n	859	271	126	147	89		
Gender (females)	480 (56%)	155 (57%)	75 (60%)	89 (61%)	57 (64%)	0.49	0.66
Age (years)	45 (29–58)	68 (62–74)	64 (56–72)	51 (33–65)	42 (26–58)	<0.001	<0.001
Height (cm)	173 (10)	169 (10)	171 (10)	173 (9)	172 (10)	<0.001	<0.001
Weight (kg)	79 (17)	75 (18)	82 (19)	76 (16)	74 (16)	<0.001	0.003
BMI (kg/m2)	26.2 (5)	26.2 (6)	27.9 (6)	25.1 (4)	24.8 (4.5)	<0.001	0.94
BMI >30	158 (18%)	55 (20%)	37 (29%)	17 (12%)	10 (11%)	0.001	0.49
Smoking status							
Never smoker	517 (60%)	5 (2%)	7 (6%)	79 (54%)	53 (60%)	<0.001	<0.001
Former smoker	271 (31%)	182 (67%)	86 (68%)	52 (35%)	12 (13%)		
Current smoker	66 (8%)	84 (31%)	33 (26%)	16 (11%)	21 (24%)		
Pack-years	0 (0–5)	40 (25–50)	30 (20–42)	0 (0–12.5	0 (0–21)	<0.001	<0.001
Pack-years among former & current smokers	8 (4–20)	40 (25–50)	30 (20–44)	15 (5–30)	30 (5–43)	<0.001	<0.001
Exacerbations in previous year	0 (0–1)	0 (0–1)	0	0	0	<0.001	0.29
Atopy	452/828 (55%)	28/215 (13%)	44/112 (39%)	41/138 (30%)	9/86 (10%)	<0.001	<0.001
Total IgE (10^3 IU/L)	67 (22–219)	39 (11–162)	68 (16.9–156)	28 (9–63)	26.5 (13.7–66.5)	<0.001	0.01
Lung function							
FEV1 (L)	3.1 (0.94)	1.49 (0.66)	1.87 (0.70)	3.22 (1.08)	3.47 (0.92)	<0.001	<0.001
FEV1% predicted	91 (80–101)	55.9 (41–71)	68.5 (54–80)	100 (88–109)	102 (91–110.5)	<0.001	<0.001
FVC (L)	4.02 (3.32–4.81)	2.57 (1.99–3.20)	2.9 (2.4–3.8)	3.97 (3.28–4.87)	4.28 (3.46–5)	<0.001	<0.001
FVC % predicted	100 (88–111)	79.95 (68–92.45)	88.5 (76–702.7)	104.5 (92–114)	103 (95.5–116.5)	<0.001	<0.001
TLC (L)	6.21 (5.46–7.38)	6.63 (5.52–7.95)	6.53 (5.77–7.62)	6.08 (5.3–7.22)	5.88 (5.24–6.79)	0.003	0.004
TLC % predicted	104.5 (17)	116.3 (22.03)	116.3 (20.3)	103.4 (19.4)	99.77 (8.13)	<0.001	<0.001
DLCO (mmol/min/kPa)	8.23 (7.08–10.09)	4.29 (2.78–5.69)	5.63 (3.80–7.28)	7.8 (6.48–9.97)	7.05 (6.42–7.73)	<0.001	<0.001
DLCO % predicted	87.8 (15)	53.5 (20.6)	66.2 (17.6)	83.3 (18)	83.9 (16)	<0.001	<0.001

Data are presented as numbers (n), n/N (%), mean±SD, or median (interquartile range). BMI: body mass index; FEV1: forced expiratory volume in 1 sec; FVC: forced vital capacity; TLC: total lung capacity; DLCO: diffusion capacity for CO; NA: not applicable. [#]Asthma *versus* COPD.

patients with asthma compared to COPD, asthma + COPD and Other ($p = 0.001$), all scores were within the normal range.

Comorbidities such as cardiovascular, metabolic, and orthopaedic disorders were generally more frequent in patients with COPD and asthma + COPD compared to patients with asthma and Other (all comparisons: $p < 0.01$), whereas childhood asthma (36%), atopic dermatitis (26%), and allergic rhinitis (55%) were more prevalent in patients with asthma compared to the three other patient groups ($p < 0.001$ for all) (Table 4). Furthermore, chronic rhinosinusitis without nasal polyps (CRSsNP) was more frequently observed in asthma than in the three other patient groups ($p < 0.001$).

AHR to mannitol, dose–response ratio (RDR) ($p < 0.001$, both) and PD15 ($p = 0.04$) differed across the four groups but were not significantly different between patients with asthma and COPD (Table 5). A positive mannitol test was found in 331 (48%) of the 695 tested patients with asthma and in 30/47 (64%) tested patients with asthma + COPD. Reversibility was more frequent in patients with asthma and asthma + COPD than in those with COPD and Other (45% and 47% vs 21% and 8%, $p < 0.001$).

The prevalence of blood eosinophilia (>$0.3*10^9$/L) was not different in patients with asthma, COPD, or asthma + COPD but was lower in the Other group (Table 5). However, FeNO levels were significantly higher in patients with asthma and asthma + COPD (18.0 and 16 ppb) compared to COPD and Other (12.5 ppb and 14.5 ppb). When assessing sputum eosinophils, we found a higher level in patients with asthma, asthma + COPD and Other compared to those with COPD (1.5%, 1.5%, 1.2% vs 0.75%, $p < 0.001$), whereas patients with

Table 3. Questionnaires.

	Asthma	COPD	Asthma + COPD	Other	*p*-value	*p*-value[#]
Subjects (n)	859	271	126	147		
ACQ-5	1.5 (1.2)	1.7 (1.2)	1.7 (1.2)	1.8 (1.0)	<0.001	0.007
ACQ-5 > 1.5	375 (44%)	150 (55%)	64 (51%)	51 (35%)	<0.001	0.0002
ACT	18.5 (4.8)	17.6 (4.8)	17.5 (4.9)	16.5 (3.8)	<0.001	0.004
ACT ≤ 19	420 (49%)	165 (61%)	68 (54%)	66 (45%)	0.0006	0.0001
CAT	11.1 (7.7)	15.0 (7.2)	13.4 (8)	10.2 (4.9)	<0.001	<0.001
CAT > 10	396 (46%)	190 (70%)	64 (51%)	58 (40%)	<0.001	<0.001
mMRC	1.8 (0.8)	2.6 (1.0)	2.3 (1)	1.3 (0.5)	<0.001	<0.001
mMRC ≥2	486 (57%)	235 (87%)	97 (77%)	62 (42%)	<0.001	<0.001
SF12						
PCS	46.3 (9.8)	38.4 (10.6)	41.3 (10.6)	46.6 (8.4)	<0.001	<0.001
MCS	51.0 (9.9)	51.7 (9.2)	52.5 (10.2)	55.1 (3.3)	0.09	0.5
miniAQLQ overall	5.47 (1.10)	5.44 (1.01)	5.42 (1.08)	5.85 (0.99)	<0.001	0.31
Symptoms	5.26 (1.24)	5.47 (1.10)	5.28 (1.24)	5.59 (1.16)	0.01	0.048
Activity	5.78 (1.16)	4.95 (1.40)	5.40 (1.36)	6.01 (1.14)	<0.001	<0.001
Emotional	5.40 (1.41)	5.75 (1.30)	5.43 (1.44)	5.90 (1.25)	<0.001	<0.001
Environment	5.48 (1.42)	5.75 (1.29)	5.73 (1.28)	6.03 (1.20)	<0.001	0.003
miniRQLQ overall	1.42 (1.02)	1.20 (0.85)	1.24 (0.87)	1.21 (0.99)	0.006	0.008
Activity	1.33 (1.27)	1.61 (1.42)	1.54 (1.33)	1.20 (1.23)	0.009	0.01
Eyes	0.95 (1.17)	0.75 (1.04)	0.79 (0.91)	0.81 (1.13)	0.02	0.005
Non-nose and eyes	1.57 (1.29)	1.34 (1.11)	1.49 (1.26)	1.40 (1.29)	0.10	0.04
Nose	1.68 (1.37)	1.10 (1.09)	1.12 (1.10)	1.37 (1.31)	<0.001	<0.001
Practical	1.64 (1.43)	1.17 (1.29)	1.24 (1.15)	1.35 (1.38)	<0.001	<0.001
CCQ						
Activity	3.7 (3.9)	7.0 (4.9)	5.3 (4.7)	3.8 (2.4)	<0.001	<0.001
Emotional	2.4 (2.7)	3.0 (2.9)	2.9 (2.9)	3.7 (2.3)	<0.001	0.0006
Symptoms	5.7 (4.6)	6.4 (4.2)	6.3 (4.7)	9 (5.0)	0.002	0.0023
HADS						
HADS depression	2.7 (3.0)	3.0 (2.8)	2.9 (3.4)	1.7 (1.5)	0.04	0.01
HADS anxiety	4.96 (3.9)	4.4 (3.4)	7.9 (6.7)	3.6 (2.5)	0.39	0.14
Nijmegen	15.5 (9.7)	15.6 (8.7)	15.8 (9.4)	15 (7.8)	0.60	0.42
SNOT22	20.0 (13.9)	18.9 (12.4)	18.1 (12.3)	7.5 (10.6)	0.45	0.51
ESS	6.24 (4.2)	5.3 (4.0)	5.7 (3.5)	5.6 (1.1)	0.001	<0.001

Data are presented as mean±SD. ACQ-5: Asthma Control Questionnaire; ACT: Asthma Control Test; CAT: COPD Assessment Test; mMRC: Medical Research Council dyspnea scale; SF-12: Health condition questionnaire; miniAQLQ: Mini Asthma Quality of Life Questionnaire; miniRQLQ: Mini Rhinoconjunctivitis Quality of Life Questionnaire; CCQ: Clinical COPD Questionnaire; HADS: Hospital Anxiety and Depression Scale; Nijmegen: Hyperventilation; SNOT22: Sino-Nasal Outcome Test; ESS: Epworth sleepiness scale. Asthma *versus* COPD.

Table 4. Comorbidities.

	Asthma	COPD	Asthma+COPD	Other	*p*-Value	*p*-Value[#]
Subject n	859	271	126	147		
Mental	37 (4%)	18 (7%)	8 (6%)	2 (1%)	0.07	0.12
Cardiovascular	86 (10%)	106 (39%)	40 (32%)	25 (17%)	<0.001	<0.001
Metabolic	55 (6%)	49 (18%)	18 (14%)	16 (11%)	<0.001	<0.001
Ortopedic	18 (2%)	18 (7%)	9 (7%)	1 (0.7%)	<0.001	0.0002
Other	95 (11%)	37 (14%)	19 (15%)	21 (14%)	0.37	0.25
Childhood asthma	311 (36%)	17 (6%)	22 (18%)	18 (12%)	<0.001	<0.001
CRSwNP[c]	5 (0.6%)	0	0	0	0.30	0.33
CRSsNP[c]	202 (24%)	26 (1%)	15 (12%)	26 (18%)	<0.001	<0.001
Atopic dermatitits	226 (26%)	39 (14%)	19 (15%)	27 (18%)	<0.001	<0.001
Allergic rhinitis	476 (55%)	94 (35%)	53 (42%)	47 (32%)	<0.001	<0.001
Severe asthma[a]	120 (14%)	3 (1%)	13 (10%)	0	<0.001	<0.001
Dysfunctional breathing[b]	174 (20%)	46 (17%)	21 (17%)	19 (13%)	0.14	0.23

CRS: chronic rhinosinusitis. [a]According to ATS/ERS guidelines. [b]Nijmegen score >23. [c]Deduced from SNOT-22. [#]Asthma *versus* COPD.

COPD had higher sputum neutrophil levels compared to the three other patient groups (66.8% vs 39.3%, 43.5% and 40.8%, $p < 0.001$) (Table 5, suppl. Figures S2 and S3).

Atopy was significantly more frequent in patients with asthma than in patients with COPD (55% vs 13%, $p < 0.001$) (supplementary Table S2).

Disease severity based on GINA classification for patients with asthma (Table 6) showed that 54% had moderate to severe disease whereas 46% had mild disease. For patients with COPD, the GOLD classification showed that 82% had groups A and B disease, whereas 18% had groups C and D disease.

Discussion

The current study represents, to our knowledge, the largest clinical real-life study on patients with obstructive airway diseases: asthma, COPD, or concurrent asthma and COPD with different degrees of severity and phenotypes. With this study, we have generated a research

Table 5. Airway hyperresponsiveness and inflammatory markers.

	Asthma	COPD	Asthma+COPD	Other	Healthy	*p*-value	*p*-value[#]
Subject n	859	271	126	147	89		
FeNO (ppb)	18 (11–31)	12.5 (7–20)	16 (10–25)	14.5 (9–21)	11.2 (8–16)	<0.001	<0.001
Patients FEV1 < 70%	42 (5%)	174 (64%)	48 (38%)	8 (5%)	0	<0.001	<0.001
Positive reversibility test	60/133 (45%)	46/221 (21%)	38/81 (47%)	2/24 (8%)	0	<0.001	<0.001
AHR to mannitol	331/695 (48%)	20/46 (44%)	30/47 (64%)	8/121 (7%)	4/79 (5%)	<0.001	0.59
PD15 (mg)	217.5 (80.6–413.1)	302.9 (141.2–406.4)	224.4 (47.9–320.3)	493.5 (293.4–566.4)	NA	0.04	0.15
RDR mannitol (%/mg)	0.03 (0.01–0.07)	0.02 (0.02–0.05)	0.05 (0.02–0.18)	0.01 (0.002–0.01)	0.01 (0.003–0.01)	<0.001	0.77
Blood cell count							
Eosinophils (cells*10^9/L)	0.20 (0.1–0.3)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	<0.001	0.88
Neutrophils (cells*10^9/L)	3.6 (2.8–4.5)	4.7 (3.4–5.7)	4.1 (3.2–4.8)	1.8 (1.4–2.2)	3.5 (2.7–4.6)	<0.001	<0.001
Lymphocytes (cells*10^9/L)	1.9 (1.6–2.3)	2 (1.6–2.6)	2 (1.6 – 2.5)	3.5 (2.7–4.3)	1.9 (1.6–2.4)	0.003	0.048
Eosinophils > 0.3 (cells*10^9/L)	267 (31%)	77 (28%)	45 (36%)	17 (12%)	4 (5%)	<0.001	0.34
Sputum cell count							
Eosinophils	1.5 (0.27–5.5)	0.75 (0–3.5)	1.5 (0.3–5.8)	1.2 (0.3–3.5)	0 (0–2)	<0.001	0.004
Eosinophils ≥ 3%	161/419 (38%)	34/115 (30%)	26/63 (41%)	22/77 (29%)	6/43 (14%)	0.003	0.08
Neutrophils	39.3 (15.3–64.3)	66.8 (40–81)	43.5 (22.5–68.3)	40.8 (25–61.5)	30.5 (11–63.8)	<0.001	<0.001
Sputum inflammatory phenotype							
Eosinophilic	119 (28%)	17 (15%)	19 (30%)	15 (19.5%)	2 (4.7%)	<0.001	<0.001
Neutrophilic	74 (18%)	43 (37%)	15 (24%)	13 (17%)	7 (16%)		
Mixed inflammation	42 (10%)	17 (15%)	7 (11%)	7 (9%)	4 (9.3%)		
Paucigranolocytic	184 (44%)	38 (33%)	22 (35%)	42 (54.5%)	30 (70%)		

Data are presented as numbers (n), n/N (%), mean±SD, or median (interquartile range). AHR: airway hyperresponsiveness, defined as a decrease in FEV1 ≥15%. PD15: mannitol dose that results in a 15% fall or more in FEV1. RDR: response–dose ratio defined as % fall in FEV1 per mg of mannitol. [#]Asthma *versus* COPD.

Table 6. GOLD classification of patients with COPD and GINA classification of patients with asthma.

GOLD classification of patients with COPD					
	GOLD 1	GOLD 2	GOLD 3	GOLD 4	TOTAL
Group A, n (%)	1 (0.5)	4 (1.8)	3 (1.4)	1 (0.5)	9 (4.1)
Group B, n (%)	16 (7.2)	104 (47.1)	41 (18.6)	11 (5)	172 (77.8)
Group C, n (%)	0 (0)	0 (0)	1 (0.5)	0 (0)	1 (0.5)
Group D, n (%)	1 (0.5)	18 (8.1)	16 (7.2)	4 (1.8)	39 (17.6)
Total, n (%)	18 (8.1)	126 (57.0)	61 (27.6)	16 (7.2)	221
COLD guidelines 2019					
GINA classification of patients with asthma[a]					
GINA step	GINA 1 + 2, n (%)	GINA 3, n (%)	GINA 4, n (%)	GINA 5, n (%)	Total, n
Patients	239 (46%)	80 (16%)	86 (17%)	107 (21%)	512

GINA guidelines 2019. [a]Only Danish patients, since medication doses were not available for Swedish patients.

platform with a unique database and biobank from 1492 well-characterized subjects including patients from both specialized centres and from primary care centres. Patients were recruited from both capital cities and more rural areas, and include obese and non-obese, smokers and non-smokers, as well as control subjects consisting of healthy controls, healthy smokers and patients referred with asthma or COPD symptoms but where the diagnosis cannot be confirmed, many of which are unique features of our study.

In this population, we found that patients with asthma and asthma + COPD had higher levels of FeNO and sputum eosinophils compared to patients with COPD; moreover, patients with asthma were more atopic, more frequently had chronic rhinosinusitis and childhood asthma and were younger than patients with COPD, asthma + COPD and Other. Conversely, patients with COPD and asthma + COPD had a higher frequency of comorbidities such as cardiovascular and metabolic disorders, lower quality of life, and patients with COPD had higher levels of sputum neutrophils. Systemic inflammation measured with blood eosinophils did not differ between patients with asthma, COPD, and asthma + COPD, which contrasts with the general assumption that asthma is more eosinophilic driven whereas COPD is neutrophilic; however, this finding is in line with previous observations [38]. When further characterizing the inflammatory phenotypes in sputum, we found that patients with asthma and asthma + COPD more often had sputum eosinophilic inflammation than patients with COPD and Other, suggesting that even though the blood eosinophil counts were comparable, patients with asthma and asthma + COPD more frequently had signs of localized eosinophilic inflammation in the airways compared to patients with COPD and Other. However, sputum eosinophilia was also observed in some patients with COPD, which points to the existence of eosinophilic COPD and highlights the importance of airway sampling when assessing inflammatory phenotypes in airway disease.

The frequency of CRSwNP in patients with asthma was lower than expected probably due to the high fraction of patients with mild to moderate disease with a low degree of blood eosinophils. Former studies on CRSwNP have primarily been performed in severe asthma or in severe CRS and in both cases, the frequency of polyps was substantially higher than in unselected, real-life patients [39–41].

Airway hyperresponsiveness (AHR) measured with a mannitol challenge test did not differ between patients with asthma and COPD, but was higher in patients with asthma + COPD and lower in the Other group – however, only very few patients with COPD had the test performed due to low level of lung function (FEV1 < 70%), which may explain these findings. Moreover, COPD patients with normal lung function and with a significant smoking history could have airway inflammation with mast cells resembling the inflammation in patients with asthma [42,43]. Likewise, AHR to mannitol was also observed in four healthy asymptomatic smokers, which may be explained by the fact that inflammation caused by smoking may be sensitive to the mannitol test, as previously described [44]. These findings suggest that categorizing the diseases as asthma, COPD, or asthma + COPD based on AHR test results may be too simple, since pathology, inflammatory phenotypes, and test results tend to overlap [45].

This study is a real-life GP/specialist-based study that included patients referred or followed in these clinical settings and it is therefore designed to show the disease burden in these patients and not in a broader epidemiological setting. The patients, in general, reported a high degree of respiratory symptoms, which may reflect the fact that approximately two-thirds of the patients were recruited after being referred for specialist evaluation. The group of patients termed Other had in-between levels of inflammation, atopy, chronic rhinosinusitis and childhood asthma, few comorbidities, average age, normal weight, and normal

lung function but with many symptoms. Some had cough or dyspnoea, but most were classified with unspecific respiratory symptoms or negative bronchial provocation tests. They may represent a cluster characterized by being highly symptomatic but with a paucigranulocytic phenotype, like the cluster suggested by Haldar et al. [46], who found a group of asthma patients, who reported having many respiratory symptoms but a low degree of inflammation.

This study offered all patients with a similar medical work-up regardless of whether the referral diagnosis was asthma, COPD, or asthma + COPD. For example, all patients completed a range of questionnaires without preselecting them as patients with either asthma and/or COPD, which provides a unique opportunity to further validate these questionnaires in a broader, more real-life setting where the entire spectrum of patients with obstructive airway disease is represented. Moreover, since asthma and COPD represent a continuum of airway obstruction with heterogenous inflammatory mediators, deciding on an evaluation program based on a referral note may not be the optimal way to evaluate patients. Also, the traditional classification of these diseases based on clinical manifestations that does not distinguish between cellular and molecular mechanisms in the evaluation of obstructive airway disease may not be up to date.

Limitations to our study could be that each participating centre had its own specialist focus, which affected the patient flow and resulted in an unequal distribution of patient groups from the clinical sites. On the other hand, this approach allowed each centre to focus on recruiting patients with different severities and phenotypes of the diseases; moreover, only a few doctors at each site examined all the patients, which contributed to a uniform way of interpreting test results and diagnosing patients. We do recognize that despite thorough medication history and examinations, lung function tests and questionnaires, diagnosing obstructive airway diseases can be challenging [45,47]. In this study, we chose to use mannitol for bronchial provocation tests, which has a high specificity but low sensitivity compared to methacholine, and we are therefore at risk of under-diagnosing asthma [48]. Another limitation of our study is the bronchoscopy population, which was smaller and more selected than the general study population and may not represent the entire severity spectrum of the diseases.

Standardization of clinical and laboratory procedures across borders can be challenging, and to meet these issues, we translated all standard operating procedures (SOPs) to both Danish and Swedish, had regular meetings and central scientific coordinators in each country that ensured harmonization of procedures. However, despite these efforts, lack of laboratory facilities at the primary care centres prevented the collection of processed sputum from these sites.

In conclusion, this clinical study on real-life patients with obstructive airway disease, who are thoroughly examined independently of traditional labelling, can be the future basis of cellular and biomarker evaluation of patients with obstructive airway diseases managed in the everyday clinic.

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