Phenotypes of Obstructive Lung Diseases in Elderly: A Real-Life Study in a Non-Clinical Setting

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Abstract

Background: This study aimed to characterize the clinical, functional, inflammatory and immunological features of the main obstructive lung diseases (OLD) in elderly.

Methods: We included subjects aged ≥ 65 years. In Phase I, participants answered standardized questionnaires, performed spirometry, fractional exhaled nitric oxide, an atopy assessment and blood analysis. In Phase II, patients with COPD or asthma criteria and a control group were invited to undergo a more detailed evaluation that included the determination of IL-1-β, IL-5, IL-6, IL-13, TNF-α and periostin. In Phase III was assessed the frequency of non-scheduled medical visits due to respiratory exacerbations, hospitalization and mortality.

Results: Phase I included 286 participants. The median age was 85.8 (P27-P75: 81.0 - 90.2) years and 69% were females: 27.3% had chronic obstructive pulmonary disease (COPD) without asthma (Group 1), 2.8% asthma without COPD (Group 2) and 5.2% presented asthma and COPD traits (Group 3). The remaining participants (64.7%), without asthma or COPD, were classified as Group 4. At this stage, Group 3 was more atopic and presented a lower post-bronchodilator FEV 1 and FEV 1 /FVC. In Phase II, that included 82 participants, Group 3 presented the higher median values of TNF-α (p-value: 0.072) and higher lung volumes (p=0.001). No statistically differences were found for other cytokines among groups. In Phase III, Group 3 patients needed more non-scheduled visits.

Conclusions: In our study patients with asthma and COPD traits presented more severe airflow limitation, higher values of TNF-α, higher lung volumes and more non-scheduled medical visits.

Introduction

Asthma and COPD prevalence vary widely according to survey methods and diagnostic criteria. COPD is more frequent in smokers, in those older than 40 years and in men. Different studies assessed the prevalence of COPD [1],[2],[3]. According to a systematic review, in 2010 the global COPD prevalence for the population aged 30 years or older was 11.7% (95% confidence interval: 8.4%-15.0%) [4]. In a recent meta-analysis[5], the global mean prevalence of COPD in Europe was 12.38% without significant differences among the four regions with available data. According to the Global Burden of Disease Study, COPD was one of the five leading causes of disability-adjusted life years (DALYs) in 2017 [6].

Asthma is usually considered a disease more frequent in younger people and for this reason it may be under or misdiagnosed in the elderly. Nonetheless different authors showed that asthma is also present in older people and may overlap with COPD[7]. The coexistence of asthma and COPD was until recently named as asthma-COPD overlap (ACO) [8], although the GOLD report of 2020 considered that we should no longer refer to ACO, as they constitute two different diseases that may share some common traits and clinical features.

Ageing of the population is a global problem particularly in industrialized countries. Old age is frequently associated with respiratory symptoms[9]. COPD prevalence increases with age [2],[10]. In elderly, asthma is more frequent in women[11]. Furthermore, COPD could coexist with asthma and mask the latter[12] contributing to undertreatment. In epidemiological studies conducted in United States and United Kingdom, 17–

19% of patients with obstructive airway disease reported having asthma and COPD simultaneously[13], comprising more than 50% of the patients with obstructive airway disease whose age was over 50 years[13]. Therefore, patients with concomitant diagnosis of asthma and COPD are usually older. A fully characterization of older persons with obstructive lung diseases [14] (OLD) selected from the general population is lacking in the literature, as most studies were conducted in specific samples of people with asthma or COPD followed at Health Care Departments.

The present manuscript reports results from the Obstructive Lung Disease in Elders (OLDER) study. The primary objective was to characterize in a non-clinical setting the clinical, functional, inflammatory and immunological features of older persons with the most frequent obstructive lung diseases.

Methods

Study design, setting and participants

The OLDER study took place in Lisbon, Portugal. It was an observational study, divided in three phases.

In Phase I, which took place from April to December 2016, residents of 15 Lisbon's elderly care centers (ECC) from different civil parishes were invited to participate.

In Phase I, besides spirometry, fraction of exhaled nitric oxide (FENO), an atopy assessment and blood analysis, participants answered standardized questionnaires administered by a trained interviewer and collected a blood sample.

In Phase II (April 2016 to January 2017), every patient with COPD or asthma criteria was invited to go in a two-week period after Phase I to the Nova Medical School Lung Function Laboratory, in order to perform a more detailed assessment. A subsample of participants without asthma, COPD, respiratory symptoms and with normal lung function was used as control group. The evaluation included a body plethysmography, a carbon monoxide diffusing capacity (DLCO) and additional inflammatory blood biomarkers.

Phase III assessed the frequency of non-scheduled medical visits due to respiratory exacerbations (defined by deterioration of breathing symptoms that affected usual daily activities), hospitalisation and mortality in the 12 months after the inclusion in Phase I.

To be eligible to the study, participants should be ≥ 65 years, present cognitive and collaboration capabilities sufficient to perform a spirometry and should not have any contraindication for lung function tests. Sample size was calculated in order to estimate the frequency of participants with a forced expiratory volume in 1st second /forced vital capacity (FEV_1/FVC) < 0.70. According to published data for the Portuguese population[10], a prevalence of 30% was considered for this age group. For a confidence level of 95% and a 4.5% margin of error, we would need to include 293 participants. According to our experience in a previous study[15], we considered that about 70% of the ECC residents would not fulfil the inclusion criteria. In order to achieve this number, we planned to screen 1000 residents.

The procedures followed were in accordance with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). The database was registered in the Portuguese Data Protection Authority. A health

insurance was subscribed for participants.

The OLDER study was approved by the Ethics Committee of NOVA Medical School/Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Portugal. The older person and their caregivers were informed about the study and their signed consent was obtained.

Data sources for health assessment

Questionnaires

In Phase I participants answered Portuguese versions of standardized questionnaires administered by a trained interviewer. These were: 1) Bronchial Obstructive Lung Disease initiative (BOLD) questionnaire, 2) St. George's Respiratory Questionnaire (SGRQ), 3) Mini-Mental State Examination (MMSE) and 4) 15 items Geriatric Depression Scale (GDS-15). In Phase II the COPD assessment test (CAT) and the Control of Allergic Rhinitis and Asthma Test (CARAT) were also administered. Authorizations from the authors of these questionnaires were obtained when needed. SGRQ was administered only to those with COPD, asthma or ACO criteria and also to every participant who reported to have any respiratory symptom (cough, phlegm, wheezing or dyspnoea). Additionally, the Charlson comorbidity index (CCI) was calculated for each participant.

Lung function tests and fractional exhaled nitric oxide (FENO)

In Phase I a spirometry with bronchodilatation and a FENO measurement were performed according to recommendations[16],[17].

In Phase II, patients performed a body plethysmography (Master Screen Jaeger|®, Vyaire Medical Inc, Illinois, U.S.A.) and a carbon monoxide diffusing capacity (DLCO) measurement[18][19].

Atopy assessment

Atopy was assessed in Phase I by skin prick tests to common airborne allergens (Leti®, Barcelona, Spain) or, if anergy or refusal, by an inhalant panel analysis (Phadiatop®, Thermo-Fisher Scientific, Uppsala, Sweden).

Inflammatory blood biomarkers

A complete blood count (Advia120, Siemens, Munich, Germany) and a high sensitivity c-reactive protein (hs-CRP) determination (Dimension EXL200, Siemens, Munich, Germany) were carried out in Phase I.

In Phase II, blood samples drawn in Phase I were tested for different Th-1 (T helper) and Th-2 type cytokines / proteins [20][21] (IL-1-β, IL-5, IL-6, IL-13, TNF-α and periostin) using immunoassay platforms (Luminex® 200 System and Luminex® Assay-6 Plex, R&D Systems, Minneapolis, U.S.A.) according to the manufacture's protocol. Total IgE was also quantified (Phadia® 250, Thermo-Fisher Scientific, Uppsala, Sweden). Additionally, Pro–B-type natriuretic peptide (proBNP) was determined in serum as a biomarker related to cardiac failure and dysfunction (VIDAS® NT-proBNP2, Biomerieux, Marcy L'Étoile, France).

Health outcome definitions

Obstructive lung diseases (OLD) were defined as illnesses associated with airways obstruction[14]. For the present study only COPD and asthma were considered. Asthma was defined as a previous medical diagnosis

OR reported wheezing in the last 12 months plus reversibility (considered if there was a post-bronchodilator improvement in FEV_1 or FVC of at least 12% and 200 mL)[22]. A participant was classified as having COPD if had a post-bronchodilator $FEV_{1/}FVC < 0.70$, in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [8]. Global Initiative for Chronic Obstructive Lung Disease (GOLD) severity classification of airflow obstruction was also used.

Presence of allergic rhinitis was established on the basis of a previous medical diagnosis.

Smoking was assessed as more than 20 packs of cigarettes or 12 oz of tobacco in a lifetime or at least one cigarette a day for one year. A subject who smoked during the last 30 days was considered a current smoker.

Patients with asthma and COPD traits (previously named as ACO) were defined according to the definitions used in previous large epidemiological surveys, as the PLATINO and PUMA studies [22][23]: a post-bronchodilator $FEV_1/FVC < 0.70$ AND the presence of a previous medical diagnosis of asthma or wheezing in the last 12 months plus reversibility.

Participants were classified in groups according to these definitions[23]: Group 1 - COPD without asthma, Group 2 – Asthma without COPD, Group 3 – patients with asthma and COPD traits / clinical features. Participants without COPD or asthma were classified as Group 4.

Statistical analysis

An exploratory analysis of the variables of interest was carried out in all the sample. To compare differences between groups, Kruskal-Wallis was used for continuous variables and Chi-square or Fisher Exact tests for categorical variables.

The frequency of female gender, smoking, depression (according to GDS-15), cognitive impairment (according to MMSE), atopy, wheezing in the previous 12 months, reported asthma diagnosis, reported allergic rhinitis, frequency of $FEV_1/FVC < 0.7$ and airways reversibility were estimated in the sample.

Median, 25th (P25) and 75th (P75) percentiles were determined for age, body mass index (BMI), number of years of education, SGRQ scores (calculated for three domains: symptoms, activity and impacts as well as a total score), CARAT and CAT scores, CCI, haemoglobin level (g/dl), blood eosinophils (cells/mm^3), hs-CRP (g/dl), IL-1-β (pg/mL), IL-5 (pg/mL), IL-6 (pg/mL), IL-13 (pg/mL), TNF-α (pg/mL), periostin (ng/mL), proBNP (pg/mL), total IgE (UI/mL), post bronchodilator FEV_1 and FEV_1/FVC ratio, residual volume (RV), intra-thoracic gas volume (ITGV), total lung capacity (TLC) and DLCO. FEV_1, RV, ITGV, TLC and DLCO are presented as percentage of predicted value.

The level of significance used was 0.05, although p values greater than 0.05 and lower than 0.1 were still considered as indicating an evidence. Data analysis was performed using STATA (Stata Statistical Software: Release 12; StataCorp LP, Lakeway, TX, USA).

Results

In Phase I, 305 out of 1034 screened residents fulfilled the inclusion criteria and 286 were able to complete all the assessments planned for this phase. Non-participation was mostly related with lack of cognitive and collaboration capabilities. Descriptive analysis of these 286 participants is presented in Table 1 and 2. The median age was 85.8 (P27-P75: 81.0 - 90.2) years and 69% were females. The flow diagram is presented in Figure 1.

Eight participants (2.8%) had wheezing within the last 12 months plus reversibility and 5.6% (n=16) reported lifetime diagnosis of asthma. The combined frequency of asthma based in reported lifetime diagnosis (medical diagnosis) and wheezing within the last 12 months plus reversibility was 8%. Fifteen (65%) of these asthma patients had also COPD.

Ninety-three (32.5%) had COPD and asthma coexisted in 15 (16%) of these patients. COPD without asthma (Group1) was present in 27.3% (n=78) of the total participants. GOLD severity classification of airflow obstruction was the following: Grade 1 (mild) – 56 (71%), Grade 2 (moderate) – 16 patients (21%), Grade 3 (severe) – 4 patients (5%), Grade 4 (very severe) – two patients (3%). The frequency of asthma without COPD (Group 2) was 2.8% (n=8). Considering the whole sample, the frequency of Group 3 was 5.2% (n=15).

The remaining 185 participants (64.7%) did not present any criteria of COPD or asthma (Group 4), although 62 (21.7%) of these reported at least one respiratory symptom, namely cough and wheezing. The overlap of these groups is presented in Figure 2.

Considering only the 101 participants with COPD and / or asthma, 38% were under medication for CRD.

Comparation between groups– Phase I

Comparisons between the four groups (Tables 1 and 2) indicated the presence of statistically significant differences.

Group 1 (COPD without asthma) and Group 3 (patients with asthma and COPD traits / clinical features) reported more smoking. Wheezing in the previous 12 months was more frequent in Group 2 (asthma without COPD) and Group 3. Group 2 presented a higher BMI, worst quality of life in the activity domain, impacts domain and total score of SGRQ. Patients from Group 3 had more atopy and presented a lower FEV_1 and FEV_1/FVC post-bronchodilator.

Comparation between groups – Phase II

In Phase II we included 82 participants: 40 from Group 1, four from Group 2, six from Group 3 and 32 from Group 4. Cytokine results were available only for 78 patients.

Cytokines, lung volumes and DLCO comparisons between Groups 1, 2, 3 and 4 are presented in Table 3. Patients with both asthma and COPD (Group 3) presented the higher median values of TNF-a (differences

among groups: p-value: 0.073) and higher lung volumes (p-value: 0.001). No statistically differences were found for other cytokines among groups.

Non-scheduled visits, hospitalization and mortality – Phase III

Information about one-year non-scheduled medical visits due to respiratory exacerbations, hospitalization and mortality was available for 264 out of 286 participants from Phase I.

During the study, 27% (n=77) of the older persons needed to schedule an appointment with a doctor due to respiratory symptoms, 4.9% (n=14) were hospitalized and 4.2% (n=12) died. Four out of 12 people died as consequence of a respiratory illness. Patients with asthma and COPD traits / clinical features patients needed more non-scheduled visits to a doctor due to respiratory complaints (differences among groups: p-value = 0.046). Results are presented in Table 4.

Non-scheduled medical visits were associated with lower FEV_1 (p-value: 0.005) and a lower FEV_1/FVC post bronchodilator (p-value: 0.003). Hospitalization was associated with a lower FEV_1 post bronchodilator (p-values: 0.021). These results are presented in Table 5.

No associations were found between Th cytokine pattern and medical non-scheduled visits, hospitalization or mortality.

Discussion

In this study we aimed to characterize the clinical, lung function, inflammatory and immunological characteristics of older persons with the main OLD, namely asthma and COPD. These diseases were defined as reported by other authors and participants were allocated in different groups in order to assess differences between them. Additionally, we determined different cytokines related with Th-1 and Th-2 pathways.

Asthma prevalence, defined as the combined frequency of asthma diagnosis reported in lifetime and wheezing within the last 12 months plus reversibility was 8%, which is according to the Portuguese National Asthma Survey[24] data for this age group.

In our sample, the frequency of COPD according to GOLD definition was 32.5%. Our results are similar with data from the Portuguese BOLD study that estimated COPD prevalence in people older than 70 years of 30.8% [10], using also the fixed FEV_1/FVC ratio criteria. The fixed ratio criteria may overdiagnoses COPD in elderly, although we already showed in a previous study that in this age group, spirometry interpretation is highly influenced by the reference equations used and meaningful differences may be found[15] when using the lower limit of normality. This is in agreement with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) report 2020[8].

COPD represented the most frequent OLD, which is not surprising considering the physiologic lung function decline associated with age [25] that compromises mostly the FEV_1. This might have contributed also for the

high frequency of patients with both asthma and COPD traits, previously classified as ACO (5.2%) as compared to other authors that have used the same definition as we, namely in PLATINO [22] and PUMA [23] studies.

There were different ACO definitions available in the literature and consensual one was been not established[26][27]. This has contributed to the wide prevalence range found in population based studies and in asthma and COPD patient surveys[28]. Most definitions were based in phenotypic features and a biomarker is still lacking. In our study, higher levels of TNF-α were associated with the presence of asthma and COPD traits in the same patient. Higher levels of TNF-α have been related with the severity of airway obstruction in COPD patients [29] which may explain our findings as patients with both diseases presented more severe airways obstruction.

To the moment, no single endotype of asthma or COPD has been fully characterized in elderly. Despite the lack of strong evidence, the presence of Th-2 inflammation allows to classify asthma as Th-2 “high” or Th-2”low” endotypes, as proposed by Wenzel S. Th-2 “high” is associated with a better response to inhaled corticosteroids[30].

More recently, it was considered that type 2 inflammation may constitute also a distinct endotype in COPD, similar to the asthma Th-2 “high” endotype. Other existent COPD endotypes seem to be related with Th-1 inflammation and type 17 helper T cells [31].

Previous studies also found diverse inflammatory patterns in asthma, COPD and may overlap in certain patients, leading to a mixture of Th-2 and non-Th-2 cytokine expression. Patients with asthma and COPD may show therefore diverse inflammatory processes that overlap the characteristics of both conditions. For this reason the previously ACO entity cannot be considered a specific phenotype of asthma or COPD but a blend of both components[32], [33].

Our findings suggest that at least in this age group COPD and asthma are heterogenous in terms of immunological endotype and Th-1 and Th-2 cytokines are common independently of the considered disease, even in COPD.

Patients with both asthma and COPD traits / clinical features patients have been considered to present an increased risk of exacerbation and hospitalization [22] as they usually present more severe disease. We found lower lung function parameters and more frequent non-scheduled visits to a doctor in this group, indicating that this phenotypic presentation should raise awareness. According to our results this is particularly relevant as patients with features of both diseases presented a future risk of exacerbations. This trend for increased risk of healthcare utilizations has been previously reported[34].

Surprisingly, COPD patients reported the best SGRQ results. This might be related with the characteristics of our sample, as older patients tend to have substantial comorbidities and to be less active, which may have contributed for a wrong perception of the real burden of COPD on the quality of life.

According to the literature, comorbidities are increased in patients with asthma and COPD features compared to other obstructive airway diseases [26], mainly in smokers. In our study we could not find this association probably due to the high “baseline” degree of comorbidities among this age group.

The major strengths of our study are the inclusion of a carefully selected sample of older persons with the main OLD recruited in a non-clinical setting, the detailed characterization in terms quality of life, disease control, comorbidities, lung function, airways inflammation and cytokine assessment. Additionally, we were able to assess the future risk in these patients. To our knowledge, this is the first study providing such information in older persons.

We were not able to include a high number of patients with asthma or asthma and COPD traits and this constitute a limitation. Additionally, we do not know to which extent these results could be extrapolated. However, we found that the presence of asthma and COPD traits in the same patient is associated with worst lung function, more non-scheduled visits to a doctor and with a higher level of TNF-α.

Conclusions

Patients with asthma and COPD traits tend to have more severe airways obstruction and a higher risk to exacerbate. In older people, levels of Th-1 and Th-2 related cytokines may be similar independently of the OLD.

Abbreviations

CRD
Chronic respiratory diseases
OLD
Obstructive lung diseases

Declarations

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data sharing

The data that support the findings of this study are openly available in: https://www.dropbox.com/s/pjhpqbzh4f9pvmy/OLDER_datasetFV.xlsx?dl=0

Authors contribution:

João Gaspar-Marques: conceptualization, investigation, methodology, data validation and analysis, writing-review and editing; Iolanda Caires: investigation, resources, writing-review and editing; Teresa Palmeiro: investigation, project administration, writing-review and editing; Paula Leiria-Pinto: investigation, validation, writing-review and editing; Maria Chasqueira: investigation, resources, supervision, writing-review and editing; Paulo Paixão: investigation, resources, writing-review and editing; Maria Amália Botelho: conceptualization, methodology, writing-review and editing; António Ferreira: supervision, writing-review and editing; Isabella Annesi-Maesano: writing-review and editing; Nuno Neuparth: conceptualization, methodology, project administration, validation, writing-review and editing; Pedro Carreiro Martins: conceptualization, methodology, project administration, supervision, data validation and analysis, writing-original draft and editing.

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Tables

Table 1: Characteristics of participants in Phase I

	COPD – Group 1 (n=78)	Asthma – Group 2 (n=8)	Asthma and COPD traits – Group 3 (n=15)	Without COPD or Asthma – Group 4 (n=185)	Total (n=286)	p-value
Age - median (P25-P75) years	85.9 (80.0-90.0)	87.0 (82.3-91.0)	85.0 (77.2-88.4)	85.6 (81.0-90.4)	85.8 (81.0-90.2)	0.863
Gender (female) - %	50.0	100	66.7	75.5	69.0	0.001
BMI - median (P25-P75) kg/m^2	25.7 (23.3-28.5)	30.6 (27.2-40.2)	27.7 (23.4-28.5)	27.4 (24.6-31.2)	26.9 (24.0-30.4)	0.001
Smoking (ever) - %	38.5	10.0	33.3	15.8	22.6	0.001
Education - median (P25-P75) years	4.0 (4.0-8.0)	3.5 (1.5-4.5)	4.0 (4.0-9.0)	4.0 (4.0-6.0)	4.0 (4.0-7.0)	0.126
Cognitive impairment - %	14.1	40.0	13.3	22.8	20.6	0.147
Depression - %	17.9	20	33.3	35.3	30.0	0.139
Atopy - %	18.9	30.0	66.7	19.8	22.5	0.001
Wheezing (previous 12 months) - %	20.5	70.0	60.0	14.1	20.2	0.001
Allergic rhinitis (diagnosis) - %	12.8	40.0	6.7	13.6	13.9	0.238
CCI score - median (P25-P75)	21 (2-53)	21 (2-45)	21 (21-21)	21 (2-53)	21 (2-53)	0.153

BMI: body mass index; CCI: Charlson comorbidity index; COPD: chronic obstructive pulmonary disease; P25: 25th percentile; P75: 75th percentile

Table 2: Characteristics of participants in Phase I (continuation)

	COPD – Group 1 (n=78)	Asthma – Group 2 (n=8)	Asthma and COPD traits – Group 3 (n=15)	Without COPD or Asthma – Group 4 (n=185)	Total (n=286)	p-value
SGRQ-Total - median (P25-P75) % *	4.2 (0-8.8)	43.7 (21.1-55.3)	13.5 (4.9-62.6)	6.6 (0.3-31.2)	0.0 (0.0-6.0)	0.001
SGRQ-Symptoms - median (P25-P75) % *	15.8 (0-42.8)	40.9 (6.1-40.9)	42.4 (8.8-68.3)	34.2 (0.0-49.2)	31.9 (0.0-48.8)	0.047
SGRQ-Activity - median (P25-P75) % *	0 (0-0)	69.5 (35.5-90.8)	18.5 (0-72.8)	0.0 (0.0 – 54.4)	0.0 (0.0-43.2)	<0.001
SGRQ-Impacts- median (P25-P75) % *	0 (0-2.8)	27.0 (7.0-45.3)	3.9 (0.0-54.5)	0.0 (0.0-18.5)	0.0 (0.0-15.0)	0.001
Eosinophils - median (P25-P75) cel./mm^3	170 (90-285)	185 (120-198)	220 (170-290)	170 (120-260)	170 (120-267)	0.467
hs-CRP - median (P25-P75) mg/dL	0.30 (0.15-0.61)	0.50 (0.17-0.96)	0.33 (0.12-0.84)	0.26 (0.14-0.54)	0.29 (0.14-0.59)	0.721
FEV_1 post-BD - median (P25-P75) %	85.5 (64.0-107.2)	98.0 (79.3-121.3)	74.0 (53.0-90.0)	111.0 (92.3-137.0)	104.0 (83.0-124.5)	0.001
FEV_1/ FVC post-BD - median (P25-P75) %	0.66 (0.60-0.68)	0.76 (0.71-0.79)	0.56 (0.42-0.67)	0.76 (0.73-0.80)	0.73 (0.68-0.78)	0.001
ΔFEV_1 - median (P25-P75) %	5.0 (0-9.6)	21.2 (8.9-38.0)	12.4 (0-17.1)	4.0 (-0.8-8.3)	4.7 (-0.6-9.5)	0.001
FENO - median (P25-P75) ppb	13.0 (7.0-18.0)	19.5 (6.3-34.3)	14.0 (6.0-24.0)	11.0 (7.0-18.0)	12.0 (7.0-19.0)	0.090

BMI: body mass index; BD: bronchodilator; COPD: chronic obstructive pulmonary disease; FENO: exhaled fraction of nitric oxide; FEV_1: Forced expiratory flow in the 1st second; FVC: forced vital capacity; hs-CRP: high sensitivity c-reactive protein ;

ΔFEV_1: variation of the FEV_1 after the bronchodilator; SGRQ: St. George's Respiratory Questionnaire (SGRQ); * SGRQ was administered only to participants from Groups 1, 2 and 3 and also to every participant from Group 4 who reported any respiratory symptom (n=62)

Table 3: Phase II - Cytokines, lung volumes, DLCO, CAT and CARAT results

	Group 1 (n= 40)	Group 2 (n= 4)	Group 3 (n= 6)	Group 4 (n=32)	p-value
IL1-b - median (P25-P75) pg/mL	0 (0-0.73)	0.35 (0-0.70)	0 (0-0.37)	0 (0-0.75)	0.859
IL5 - median (P25-P75) pg/mL	0 (0-0)	0 (0-0)	0 (0-0.39)	0 (0-0.28)	0.633
IL6 - median (P25-P75) pg/mL	1.52 (1.10-2.87)	1.32 (1.07-3.06)	1.94 (1.25-3.51)	1.71(1.01-2.38)	0.956
IL13 - median (P25-P75) pg/mL	0 (0-167)	50 (0-165)	0 (0-55)	0(0-171)	0.669
TNF a - median (P25-P75) pg/mL	3.62 (2.28-4,06)	1.931 (1.48-3.30)	4.67 (3.52-4,97)	3.36 (2.61-4.72)	0.073
Periostin - median (P25-P75) ng/mLx10^3	273 (217-339)	203 (92-295)	224 (146-342)	261 (223-342)	0.352
Pro-BNP - median (P25-P75) pg/mL	399 (16-1005)	140 (76-1918)	183 (115-396)	225(113-451)	0.380
Total IgE - median (P25-P75) Ul/mL	32 (13-120)	10 (5.9-103)	47 (14-146)	31(9-91)	0.658
RV - median (P25-P75) %	109 (91-130)	104 (96-132)	138 (120-164)	90(81-106)	0.001
TLC - median (P25-P75) %	97 (89-111)	97 (85-112)	114 (103-124)	98(85-111)	0.141
TGV - median (P25-P75) %	111 (96-127)	99 (95-124)	134 (113-157)	89(82-106)	<0.001
DLCO SB - median (P25-P75) %	78 (64-89)	69 (32-97)	63 (49-109)	84(67-108)	0.226
DLCO SB/VA - median (P25-P75) %	82 (68-96)	72 (48-98)	78 (51-97)	89(77-103)	0.161
CAT Total - median (P25-P75)	6 (2-12)	13.5 (5.5-26.8)	7.5 (4.5-23)	-	0.204
CARAT-Total (lack of control) - %	25.0%	50.0%	66.7%	-	0.019

CARAT: control of allergic rhinitis and asthma test; CAT: COPD assessment test; COPD: chronic obstructive pulmonary disease;

DLCO: carbon monoxide diffusing capacity; P25: 25^{th} percentile; P75: 75^{th} percentile;RV: residual volume; TLC: total lung capacity;

TGV: intrathoracic gas volume; SB: single breath; VA: alveolar volume; Group 1: COPD; Group 2: Asthma; Group 3: Asthma and COPD traits;

Group 4: Control group (without asthma, COPD, respiratory symptoms and with normal lung function)

Table 4: Phase III – frequency of non-scheduled visits to a doctor, hospitalizations and mortality, by OLD Groups (n=264)

	COPD - Group 1 (n= 73)	**Asthma - Group 2** (n= 8)	**Asthma and COPD traits - Group 3** (n= 15)	**Without: COPD or Asthma – Group 4** (n= 168)	p-value
Non-scheduled visit to a doctor – n (%)	26 (35.6)	1 (12.5)	8 (53.3)	42 (25)	0.046
Hospitalization – n (%)	5(6.8)	1 (12.5)	1 (6.7)	7 (4.2)	0.527
Mortality – n (%)	4 (5.5)	1 (12.5)	0 (0)	7 (4.2)	0.643

COPD: chronic obstructive pulmonary disease; OLD: Obstructive lung diseases;

Table 5: Phase III – FEV_1, FEV_1/FVC, ΔFEV_1 and FENO according the presence or absence of non-scheduled medical visits, hospitalization and mortality (n=264)

	Non-scheduled medical visits		p-value	**Hospitalizations**		p-value	**Mortality**		p-value
	Yes	No		Yes	No		Yes	No	
FEV_1 post-BD - %	93(75-118)	107 (88-135)	0.005	88(55-107)	105(84-130)	0.021	98(59-122)	105(84-129)	0.372
FEV_1/FVC post-BD - %	0.72(0.66-0.76)	0.74(0.68-0.78)	0.003	0.71(0.57-0.76)	0.73(0.67-0.78)	0.160	0.67(0.60-0.71)	0.74(0.67-0.78)	0.080
FENO - ppb	13 (8-17)	12 (7-22)	0.630	15 (8-17)	12 (7-19)	0.601	13 (9-17)	12 (7-19)	0.761

FENO: exhaled fraction of nitric oxide; FEV1: Forced expiratory flow in the 1st second; FVC: forced vital capacity; ΔFEV_1: variation of the FEV_1 after the bronchodilator; Results are presented as medians (P25-P75)

Figures

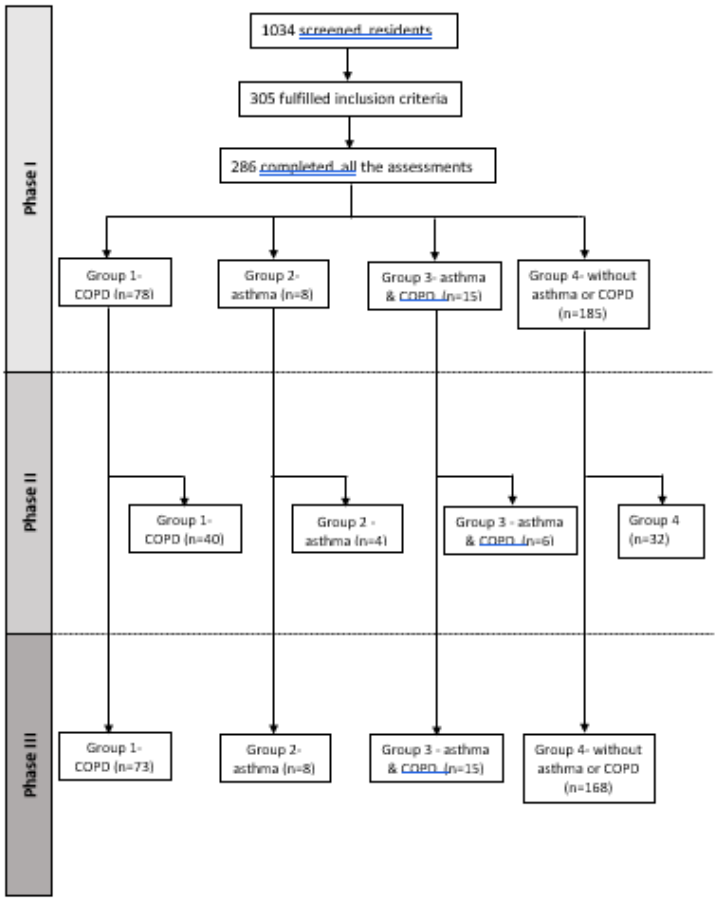

Figure 1

Flow diagram of the study

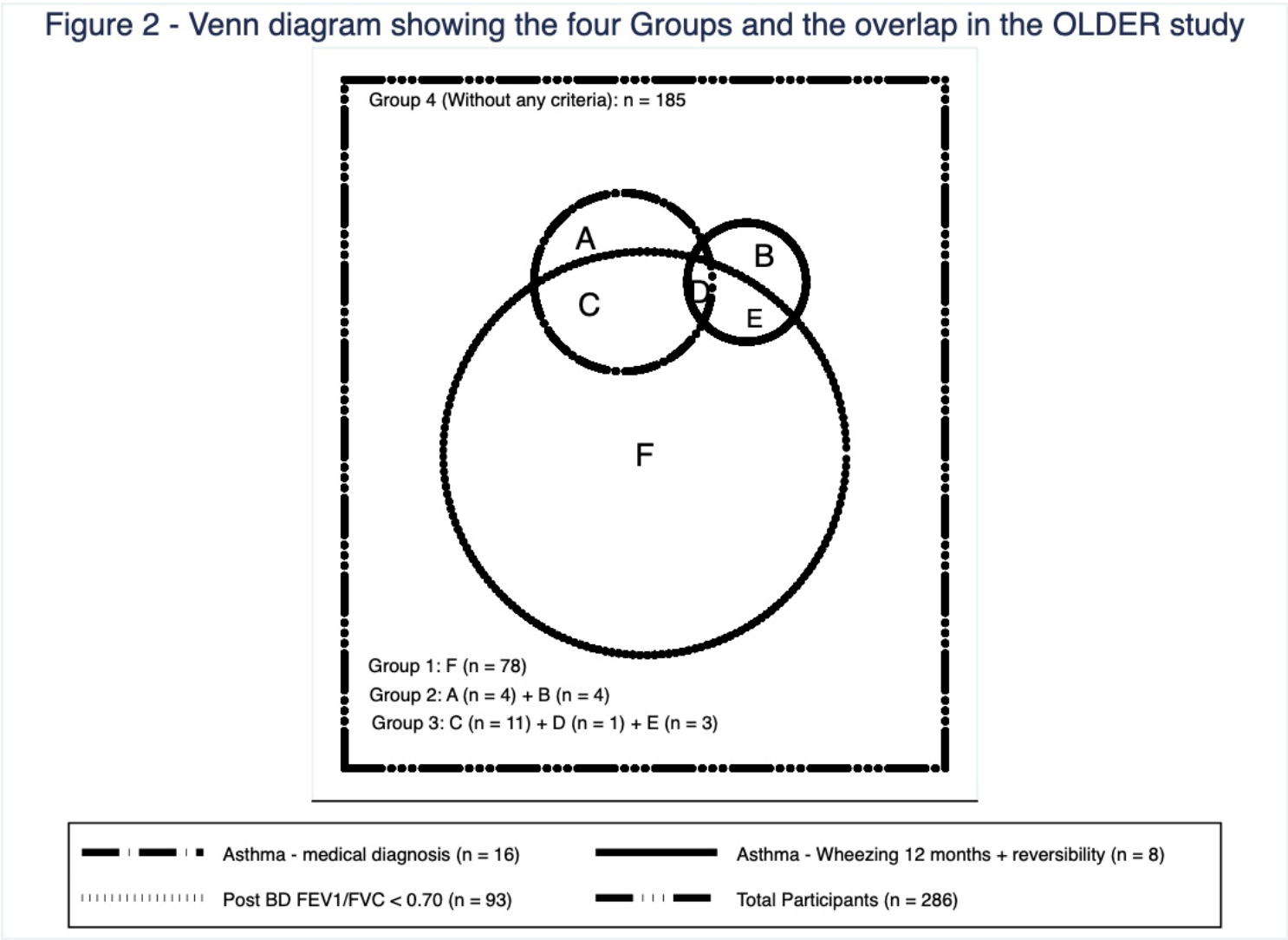

Figure 2

[See figure]