

Asthma-chronic obstructive pulmonary disease overlap: Diagnostic stability and inflammatory characteristics

To the Editor

The term asthma-chronic obstructive pulmonary disease (COPD) overlap (ACO) was initially described as a syndrome, but in the current guidelines, it is considered as the coincidence of features of both diseases.¹ One of the main issues that is still under consideration is whether ACO represents a stable entity or changes through time. Treating patients with ACO is mainly based on personal clinical experience, but taking into consideration that one of the main aspects of ACO is the asthmatic component, then it is quite reasonable to initiate inhaled corticosteroids (ICS) as a treatment option.² Most of the studies related to ACO support the presence of airway and systemic eosinophilic inflammation, while some others report a predominance of neutrophil-derived mediators.^{3,4} Taking all these points together, we identified three major gaps that need to be addressed: first, the stability of ACO in a proper follow-up under a specialized clinical guidance; second, which is the inflammatory pattern, after using established treatment modalities; and third, whether this inflammatory pattern drives an underlying mechanism that may affect some of the ACO clinical and prognostic features.

We initially recruited 164 newly diagnosed patients, all treatment naïve—seventy-four with ACO, sixty with asthma (30 with smoking history) and thirty with COPD. ACO was diagnosed using the criteria summarized in Table 1.

From 74 patients initially recruited with ACO, only 25 continued to fulfil the criteria used at baseline and remained in that group after one year of follow-up (Appendix S1 figure). The remaining 49 patients were finally considered as asthmatics, mainly due to the normalization of their lung function parameters, leading to the absence of airway obstruction. Demographic characteristics are summarized in the Appendix S1 table. Inflammatory characteristics are shown in Figure 1 and summarized in an Appendix S2 table. Blood eosinophils as absolute count, serum periostin and sputum IL-13 were significantly higher in ACO, and sputum IFN- γ was significantly lower in ACO compared to the other three groups. Sputum eosinophils did not significantly differ within groups while sputum neutrophils were higher in COPD and asthma smokers. We then performed a regression analysis for the four aforementioned significantly differing parameters where we observed the following: blood eosinophil counts were significantly affected by the presence of ACO, without any other significant determinants ($r^2 = 0.221$, $P = 0.032$); and periostin showed a positive association with IL-13 levels in sputum supernatant ($r^2 = 0.199$, $P = 0.041$), while the presence of ACO represented the strongest covariate ($r^2 = 0.43$, $P < 0.001$). Finally, sputum IFN- γ showed a significant negative association with the presence of ACO ($r^2 = 0.271$, $P = 0.002$).

In this prospective study, we clearly showed that ACO lacks diagnostic stability after one year of follow-up and using the proposed criteria. Furthermore, it seems that optimally treated patients with ACO showed a persistent T2-high inflammatory process predominantly characterized by increased blood eosinophils and serum periostin. Finally, the low values of sputum IFN- γ may reveal an impaired immune process.

In our study, the optimization of treatment and the close follow-up resulted in low diagnostic stability for ACO, mainly attributed to non-optimally treated asthma. Another critical issue is the underlying inflammatory process that characterizes the ACO entity. We are questioning ourselves whether ACO represents the additive or even the synergistic inflammatory process of the two entities or represents an independent inflammatory process where specific inflammatory features predominate mainly in the direction of a T2 high response. In a cross-sectional study including patients with asthma, COPD and ACO, T2-related cytokines such as interleukin-5 were significantly elevated in asthma while T2 low like IL-8 were significantly higher in COPD.⁵ Interestingly, using a network analysis, IL-13 was found to be the most connected node in the above network. This is partially supported by our results, where periostin—a signature T2 downstream molecule of IL-13⁶—seems to clearly differentiate ACO from its components. The most valuable finding of our study regarding blood eosinophils is the persistent significantly higher absolute count. This finding combined with the high levels of periostin indicates a persistent T2-related systemic profile. This may partially explain the higher number of exacerbations since T2 persistent inflammatory process driven by blood eosinophils is closely related to the rate of exacerbations either in asthma or in COPD.^{7,8} As for airway inflammation, the pattern of the cellular profile observed in this study may reveal the heterogeneity of this process. In our study, two major cytokines drive the airway inflammatory pattern in patients

TABLE 1 ACO criteria

Major criteria

Positive bronchodilator test (increase of FEV₁ $\geq$ 15% and $\geq$ 400 mL over baseline)

Personal history of atopy

Positive bronchodilator test (increase of FEV₁ $\geq$ 12% and $\geq$ 200 mL over baseline) on two or more occasions

Reported asthma before the age of 40 y

Note: A person > 40 y old with airway obstruction (postbronchodilation FEV₁/FVC $\leq$ 70%) and a predisposing factor for COPD [mainly smoking > 10py] with onset of symptoms at the age of 40 plus 2 of the following.

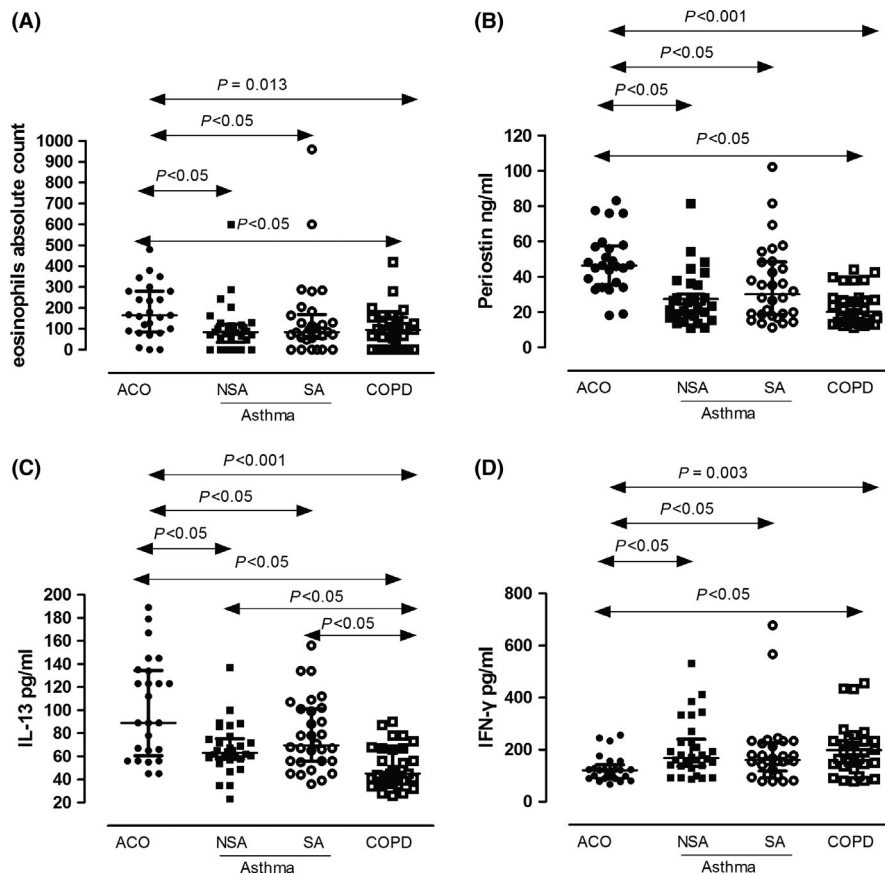


FIGURE 1 Eosinophil absolute count (A), periostin (B), IL-13 (C) and IFN- γ (D) values in all study groups. For data, see Table S2

with ACO, but only one of them showed a close relationship with ACO in the regression analysis. IFN- γ is a cytokine that is critical for innate and adaptive immunity. The impaired concentration of airway assessed IFN- γ observed in the current study may partially explain the high incidence of exacerbations that characterize the ACO in most clinical studies. As for sputum cells, our study did not reveal any differences in sputum eosinophils. This is somehow different compared to a previous study where sputum eosinophils were higher in both asthma and ACO patients. This may reveal the responsiveness of sputum eosinophilia to ICS intervention.⁹ In this prospective follow-up, we decided not to measure inflammatory indices at baseline, and this may be considered as a limitation. However, our primary outcome was the diagnostic stability, and the unbiased evaluation of the underlying airway and systemic inflammation. Additionally, the use of "control" groups of asthma and COPD represents an advantage in the whole process, since they are newly diagnosed and properly treated.

Summarizing our findings, we showed that defining ACO by the criteria used in our study lacks diagnostic stability in the 1-year follow-up towards the direction of nonoptimally treated asthma, and this must be considered in future clinical trials, as well as in the assessment of this phenotype in everyday clinical practice. Our results suggest that a firm diagnosis may not be feasible in a single evaluation of a patient and appropriate follow-up needs to be arranged in all patients with characteristics compatible with ACO. Furthermore, it seems that optimally treated patients with ACO

showed a persistent T2-high inflammatory process, predominantly characterized by increased blood eosinophils and serum periostin, whereas the observed IFN- γ impairment is an additive mechanism in terms of the disease immune process.

CONFLICTS OF INTEREST

Konstantinos Kostikas was a Novartis employee until 28 October 2018. Georgios Patentlakis is currently a BI employee but, at the time that the study was conducted, worked at 1st Department of Respiratory Medicine, National and Kapodistrian University of Athens. None of the other authors have any conflicts to disclose.

Georgios Patentlakis¹

Petros Bakakos¹

Anastasia Papaportfyriou¹

Georgios Papatheodorou²

Georgios Hillas³

Konstantinos Kostikas⁴

Spyros Papiris⁴

Nikolaos Koulouris¹

Stelios Loukides⁴ 

¹1st Department of Respiratory Medicine, Medical School of National and Kapodistrian University of Athens, "Sotiria" Hospital of Chest Diseases, Athens, Greece

²Clinical Research Unit, 401 General Army Hospital, Athens, Greece

³5th Department of Respiratory Medicine, "Sotiria" Hospital of Chest Diseases, Athens, Greece

⁴2nd Department of Respiratory Medicine, Medical School of National and Kapodistrian University of Athens, "Attikon" Hospital, Athens, Greece

Correspondence

Stelios Loukides, 2nd Department of Respiratory Medicine, Medical School of National and Kapodistrian University of Athens, "Attikon" Hospital, Smolika 2 16673, Athens, Greece.

Email: loukstel@med.uoa.gr

ORCID

Stelios Loukides  <https://orcid.org/0000-0002-4278-9922>

REFERENCES

1. Global Initiative for Asthma 2017: Updated from Global Strategy for Asthma Management and Prevention. Workshop Report 2017. Available at www.ginasthma.com
2. Postma DS, Rabe KF. The asthma-COPD overlap syndrome. *N Engl J Med*. 2015;373(13):1241-1249.

3. Cosío BG, Pérez de Llano L, Lopez Viña A, et al. Th-2 signature in chronic airway diseases: towards the extinction of asthma-COPD overlap syndrome? *Eur Respir J*. 2017;49(5):1602397.
4. Gao J, Iwamoto H, Koskela J, et al. Characterization of sputum biomarkers for asthma-COPD overlap syndrome. *Int J Chron Obstruct Pulmon Dis*. 2016;11:2457-2465.
5. de Llano LP, Cosío BG, Iglesias A, et al. Mixed Th2 and non-Th2 inflammatory pattern in the asthma-COPD overlap: a network approach. *Int J Chron Obstruct Pulmon Dis*. 2018;13:591-601.
6. Izuhara K, Arima K, Ohta S, Suzuki S, Inamitsu M, Yamamoto K. Periostin in allergic inflammation. *Allergol Int*. 2014;63(2):143-151.
7. Watz H, Tetzlaff K, Wouters EF, et al. Blood eosinophil count and exacerbations in severe chronic obstructive pulmonary disease after withdrawal of inhaled corticosteroids: a post-hoc analysis of the WISDOM trial. *Lancet Respir Med*. 2016;4(5):390-398.
8. Denlinger LC, Phillips BR, Ramratnam S, et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. *Am J Respir Crit Care Med*. 2017;195(3):302-313.
9. Kitaguchi Y, Komatsu Y, Fujimoto K, Hanaoka M, Kubo K. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. *Int J Chron Obstruct Pulmon Dis*. 2012;7:283-289.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.13872

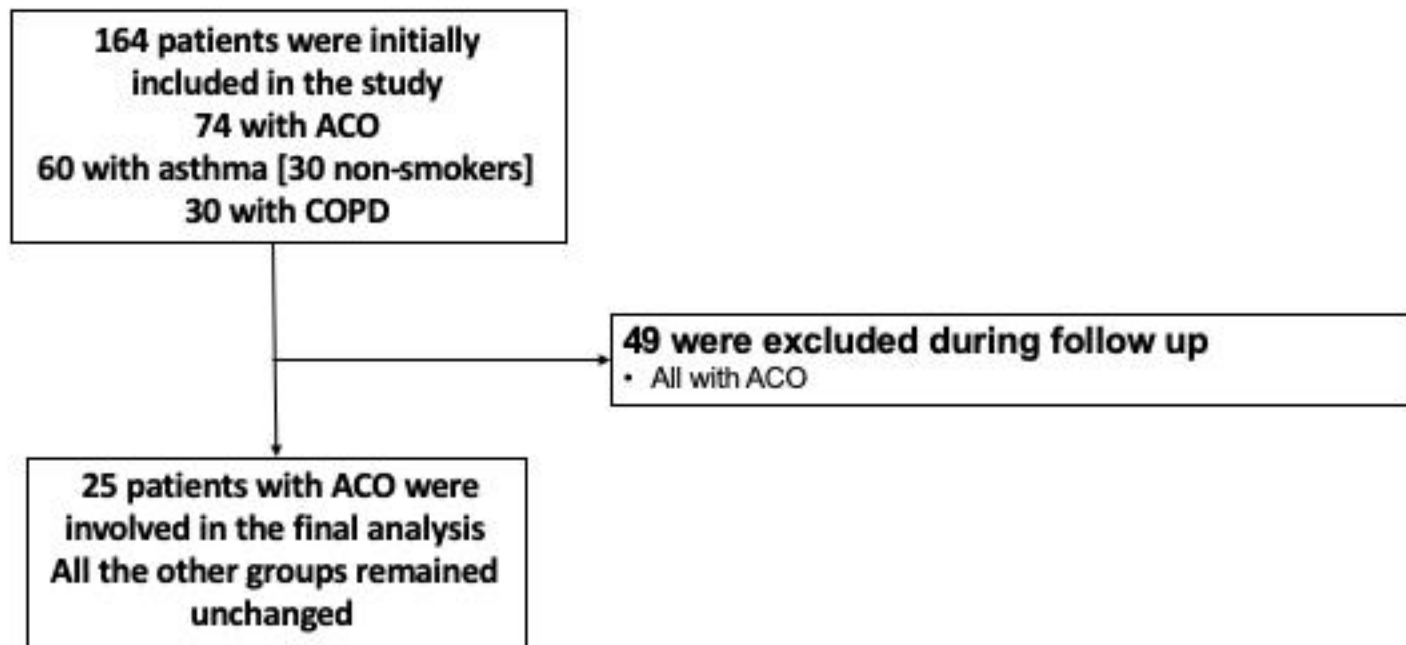
Dose escalation using carbamylated monomeric tree pollen drops is well tolerated in patients with allergic rhinoconjunctivitis and points towards clinical effects

To the Editor,

Pollen allergy is currently affecting about 40% of all allergic patients in Europe and the prevalence is increasing, with birch (*Betula*) being the most common tree pollen allergen.¹ In most cases, pollen allergy induces intermittent rhinoconjunctivitis, which can worsen into a persistent form and allergic asthma, if not treated properly.² Guidelines recommend allergen immunotherapy (AIT) for patients with allergic rhinoconjunctivitis regardless of concomitant asthma. The sublingual route of AIT, SLIT, showed to be generally effective, safe and well tolerated in patients with allergic rhinitis, conjunctivitis or both.³ Safety of SLIT can be improved with allergoids: in contrast to native allergens, polymerized allergoids have a lower allergenic potential, which reduces the risk of allergic reactions.⁴ Differently, carbamylated monomeric allergoids, which have selective amino acid substitution to limit IgE binding, are 25-80 times smaller than allergoids, making them suitable for SLIT. They are less susceptible to enzymatic degradation,^{5,6} enabling the remaining swallowed amount of extract to reach the gut-associated lymphoid tissue, exerting additional immune stimulation.^{4,5}

Here, we present the results of a phase IIa randomized, double-blind, placebo-controlled dose escalation trial to demonstrate the safety and feasibility of a dose of 50 000 UA per day of SLIT with tree pollen drops consisting of 50:50 carbamylated monomeric birch and alder pollen allergoid.

The study ran from January to June 2018 in 5 centres in Germany. After having given written informed consent, 21 eligible patients (6 placebo and 15 actively treated patients) (Appendix S1: consort flow, Appendix S2: baseline characteristics) were randomized and treated for 70 days. Up-dosing was performed preseasonally within 8 days, starting with 2000 UA (units of allergen, Appendix S3) and increasing to 50 000 UA/day in 5 steps over 3 visits, with a short home interphase between the visits (Appendix S3). This was followed by a maintenance phase with 50 000 UA/day for 62 days. Safety and clinical tolerability of tree pollen sublingual drops were assessed through documentation of solicited (expected local reactions at the administration site according to Passalacqua et al⁷) adverse drug reactions (ADRs) and systemic allergic reactions.⁸ Clinical and immunological effects of the treatment were investigated through the proportion



On line supplement

Title

Asthma-Chronic obstructive pulmonary disease overlap: Diagnostic stability and inflammatory characteristics.

Methods

Patients

Patients were recruited from an open cohort of asthmatic patients who were followed in the asthma clinics of the 1st and 2nd Respiratory Medicine Departments of the University of Athens for at least 2 years. Diagnosis & treatment of asthma was based on GINA guidelines(1). Diagnosis & treatment of COPD was based on GOLD guidelines(2). We excluded patients with severe asthma, any other respiratory disease, active malignancy and those receiving immunosuppressants for any reason, in order to avoid any effect on inflammatory markers. The study was approved by the ethics committees of both hospitals and all subjects provided an informed consent (Number of approval 3746 & 6961/16-2-15).

Sputum induction

Sputum was induced using an ultrasonic nebulizer (DeVilbiss Co., Heston, UK) at the maximal saline output (4 ml/min), as previously described, using all the modifications for safe measurements according to the underlying asthma severity(3, 4). Sputum was processed using selected plugs as previously described(5). Cell counting was performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each sample. A sample was considered adequate when the patient was able to expectorate at least 2 ml of sputum and the slides contained <10% squamous cells on differential cell counting. Total cell count expressed as the number of cells $\times 10^6$ and the percentage (%) of sputum inflammatory cells were used for analysis. Sputum supernatants were kept at -70°C for further measurement of cytokines.

Blood measurements

Blood was drawn for the measurement of periostin and blood eosinophils (% and absolute counts).

Lung Function

Forced Expiratory Volume in 1st sec (FEV1) and Forced Vital Capacity (FVC), were measured using Master Screen Body (Viasys Healthcare, Jaeger, Hoechberg, Germany) according to the American Thoracic Society guidelines(6). All spirometries, either in the initial recruitment or/and during follow up, were conducted at a stable state pre and post bronchodilation. All values are post bronchodilation.

FeNO measurement

The fraction of exhaled nitric oxide (FeNO) was measured using a portable NO analyzer (NIOX MINO, Aerocrine; Sweden) as previously described(7).

Atopic Status

A positive skin prick test (mean wheal diameter of 3mm or greater) to any of twenty common aeroallergens (including mites, grasses, trees, fungus, domestic animals) guided by clinical symptoms was used to confirm atopy.

Mediator Assays

Serum periostin was measured by an enzyme-linked immunosorbent assay (R&D Minneapolis, Minnesota, USA, duoset Elisa development system, cat. DY3548). The lowest detection level of periostin is 0.975 ng/ml in diluted samples (1:25). The limit of detection was determined by adding 3 standard deviations (SD) to the mean value of 30 zero standard measurements. Interleukin (IL)-13 was measured using an enzyme-linked immunosorbent assay kit (ELISA, R&D systems, Minneapolis, Minnesota, USA with a

detection limit of 13,2 pg/ml, cat.D1300B). IL-8 & Interferon-(IFN)- γ were measured using an ELISA kit (R&D Systems, Minneapolis, Minnesota, USA) with detection limits of 3.5 pg/ml and 15.6pg/ml respectively.

Statistical analysis

Categorical variables are presented as n (%), whereas numerical variables are presented as mean $\pm$ standard deviation (SD) or median (interquartile ranges) for normally distributed and skewed data, respectively. Normality of distributions was checked using Kolmogorov-Smirnov test. Comparisons between different study groups were performed using chi-square tests for categorical data, as well as One-way ANOVA or Kruskal-Wallis test for normally distributed or skewed numerical data, respectively, accompanied by appropriate post-hoc tests for multiple comparisons (Bonferroni and Dunn's, respectively). If an inflammatory parameter provides statistical significance in favour of ACO then a linear regression analysis was performed in a combined model for the whole study population using as co-variates each disease as a single entity, age, gender, BMI, atopy, and treatment regimens. All p-values <0.05 were considered statistically significant. Data were analysed using GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA, USA) and MedCalc 9 (MedCalcSoftware, Mariakerke, Belgium).

Results

Patients' recruitment and follow up is provided in Figure S1. From the initially recruited patients with ACO 49 normalized their lung function tests during follow up. 41 at the first visit after three months and the remaining 8 at the second visit after six months from the initial visit. Demographic characteristics are summarized in table S1. Groups were differentiated regarding smoking status, lung function, atopy and rate of exacerbations. Inflammatory characteristics are summarized in table S2. Blood eosinophils as absolute count, serum Periostin as well as sputum IL-13 were significantly higher in ACO and sputum INF- γ was significantly lower in ACO compared to the other three groups (for data see table S2).

Georgios Patentakis MD¹, Petros Bakakos MD¹, Anastasia Papaporfyriou MD¹, Georgios Papatheodorou PhD², Georgios Hillas MD³, Konstantinos Kostikas MD⁴, Spyros Papiris MD⁴, Nikolaos Koulouris MD¹ and, Stelios Loukides MD FERS⁴.

1. 1st Department of Respiratory Medicine, Medical School of National and Kapodistrian University of Athens, "Sotiria" Hospital of Chest Diseases, Athens, Greece.

2. Clinical Research Unit, 401 General Army Hospital, Athens, Greece

3. 5th Department of Respiratory Medicine, "Sotiria" Hospital of Chest Diseases, Athens, Greece.

4. 2nd Department of Respiratory Medicine, Medical School of National and Kapodistrian University of Athens, "Attikon" Hospital, Athens, Greece.

Correspondence:

Stelios Loukides, Smolika 2 16673, Athens, Greece, e-mail: loukstel@med.uoa.gr

Conflict of Interest

Konstantinos Kostikas was a Novartis employee until 28-10-2018. Georgios Patentlakis is currently a BI employee but at the time that the study was conducted worked at National and Kapodistrian University of Athens -1st Rep Med Dept. None of the other authors have any conflicts to disclose.

Authors Contributions:

Drs Patentlakis, Papaporfyriou and Hillas have performed the sputum inductions and cells measurements as well as the collection of the whole data.

Dr Papatheodorou performed the mediator measurements.

Prof Bakakos, Loukides and Kostikas recruited patients and confirmed their eligibility for entering the study. They also supervised every non and semi- invasive procedure. Prof Loukides was also responsible for the overall content of the manuscript as guarantor.

Prof Koulouris and Prof Papiris coordinated the whole study on behalf of the 2 departments.

References

1. Global Initiative for Asthma 2017: Updated from Global Strategy for Asthma Management and Prevention. Workshop Report 2017. Available at www.ginasthma.com.
2. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. *Eur Respir J* 2017;49(3).
3. Kips JC, Fahy JV, Hargreave FE, Ind PW, in't Veen JC. Methods for sputum induction and analysis of induced sputum: a method for assessing airway inflammation in asthma. *Eur Respir J Suppl* 1998; 26:9S-12S.
4. Grootendorst DC, van den Bos JW, Romeijn JJ, Veselic-Charvat M, Duiverman EJ, Vrijlandt EJ, et al. Induced sputum in adolescents with severe stable asthma. Safety and the relationship of cell counts and eosinophil cationic protein to clinical severity. *Eur Respir J* 1999;13(3):647-53.
5. Efthimiadis A, Spanevello A, Hamid Q, Kelly MM, Linden M, Louis R, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur Respir J Suppl* 2002; 37:19s-23s.
6. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171(8):912-30.
7. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: Comparison with the "gold standard" technique. *Chest* 2007;131(2):410-4.

Table S1: Demographic characteristics of the study participants

Variable	ACO n=25	Asthma smokers n=30	Asthma non- smokers n=30	COPD n=30
Age	60(55, 66)	59(52-67)	61(44-68)	62(53-68)
Gender(male) n(%)	10 (40)	13(43)	14(46)	14(46)
BMI kg/m²	26(23,30)	27(24-30)	28(25-31)	27(25-30)
Current smokers n (%)	10 (40)	14 (45)	0	18(60)
Pack years	40 (25, 60)	37(32-58)	0	69(50-76)
FEV₁ (%pred.)	62±17	88±10	88±17	57±16
FEV₁/FVC	62±6	73±8	74±7	56±9
Reversibility	17(12,26)	14(11,19)	16(11,20)	8(6,13)
Exacerbations in the previous year (n)	1.7 (1,2.5)	0.3(0-1)	0.4(0-0.9)	1.2(0.7,1.4)
Atopy n(%)	8(32)	12(40)	16(53)	1(3)
Treatment				
ICS	25	30	30	12
LABA	25	27	28	24
LAMA	15	4	1	30
LTRA	0	5	5	0

Normally distributed data are presented as mean±standard deviation (SD); skewed data are presented as median (interquartile ranges). Abbreviations: ACO= Asthma COPD phenotype, COPD= Chronic Obstructive Pulmonary disease, M=Male, BMI=Body mass index, FEV₁=Forced expiratory volume in one second, FVC=Forced vital capacity, ICS=inhaled corticosteroids, LABA=Long acting β_2 agonists, LAMA= Long acting muscarinic antagonists LTRA: Leukotriene receptor antagonists. N/D=not done.

Table S2: Inflammatory characteristics of the study participants

Variable	ACO n=25	Asthma smokers n=30	Asthma non- smokers n=30	COPD n=30	P value
FeNO ppb	19(13,25.5)	12(10,16)	16(12,21)	14(11-17)	0.156
Blood eosinophils %	2.8 (1,5)	1(0.75,2.5)	1(0,2)	2 (0,3)	0.289
Blood eosinophils absolute count	165 (85,280) ^{#, \$, ¶}	84 (54,169)	84 (36,122)	96 (0-145)	0.013
Periostin ng/ml	46(35,57) ^{#, \$, ¶}	30 (18,48) [¶]	22(17,35)	20(13,28)	<0.001
Sputum eosinophils %	1 (0,3)	2(1,6)	0	1(0,2)	0.302
Sputum neutrophils %	18(7,38)	44(37,58) ^{*, #}	26(18,39)	62(55,75) ^{*, #, \$}	<0.001
IFN- γ pg/ml	121(89,142) ^{#, \$, ¶}	160(118,226)	167(136,240)	177(134,275)	0.003
IL-8 pg/ml	456 (272,883)	666 (450,1014) ^{*, #}	452 (325,687)	1985 (1092,3006) ^{*, #, \$}	<0.001
IL-13 pg/ml	89 (60,134) ^{#, \$, ¶}	69 (57,101) [¶]	63 (57,75) [¶]	45 (36,67)	<0.001

Normally distributed data are presented as mean $\pm$ standard deviation (SD); skewed data are presented as median (interquartile ranges). Bold letters indicate significant p values among the four groups. Abbreviations: ACO= Asthma COPD phenotype, COPD= Chronic Obstructive Pulmonary disease, FENO=Fraction exhaled nitric oxide, IL=Interleukin, * Statistically significant difference compared to ACO; # statistically significant difference compared to non-smoking asthma; \$ statistically significant difference compared to smoking asthma, ¶ statistically significant difference compared to COPD.

FIGURE LEGENDS

Figure S1: Patients' recruitment and follow up during the study