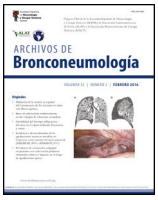
Arch Bronconeumol. 2018;54(8):414–419

Sociedad Española de Neumología y Cirugía Torácica SEPAR

ARCHIVOS DE Bronconeumología

www.archbronconeumol.org



Original Article

Alveolar and Bronchial Nitric Oxide in Chronic Obstructive Pulmonary Disease and Asthma–COPD Overlap☆

Bernardino Alcázar-Navarrete,[a,b,c,*] Francisca Castellano Miñán,[a] Pablo Santiago Díaz,[a] Oliverio Ruiz Rodríguez,[a] Pedro J. Romero Palacios[b]

[a] AIG de Medicina, Hospital de Alta Resolución de Loja, Agencia Sanitaria Hospital de Poniente, Loja, Granada, Spain
[b] Departamento de Medicina, Facultad de Medicina, Universidad de Granada, Granada, Spain
[c] Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain

ARTICLE INFO

Article history:
Received 8 September 2017
Accepted 1 February 2018
Available online 10 July 2018

Keywords:
COPD
Nitric oxide
Alveolar nitric oxide
Bronchial nitric oxide

ABSTRACT

Introduction: Exhaled nitric oxide (F_{ENO}) measurements differentiate COPD phenotypes from asthma–COPD overlap (ACO). To date, no study has been conducted to determine whether alveolar and bronchial components differ in this group of patients.
Methods: This was an observational cross-sectional study recruiting ambulatory COPD patients. F_{ENO} was measured, differentiating alveolar (C_{ANO}) from bronchial (J_{awNO}) components using a multiple-flow technique. C_{ANO} and J_{awNO} values were compared between eosinophilic COPD patients (defined as ≥300 eosinophils/μL in peripheral blood test, or ≥2% eosinophils or ≥3% eosinophils), and a linear regression analysis was performed to determine clinical and biological variables related to these measurements.
Results: 73 COPD patients were included in the study. Eosinophil counts were associated with increased values of C_{ANO} and J_{awNO} (for the latter only the association with ≥300 or ≥3% eosinophils was significant). C_{ANO} was also associated with CRP, and J_{awNO} with smoking.
Conclusions: Patients with COPD and ACO characteristics show increased inflammation in the large and small airways. C_{ANO} and J_{awNO} are associated with clinical and biological variables.
© 2018 SEPAR. Published by Elsevier España, S.L.U. All rights reserved.

Óxido nítrico alveolar y bronquial en la enfermedad pulmonar obstructiva crónica y el solapamiento de asma y EPOC (ACO)

Palabras clave:
EPOC
Óxido nítrico
Óxido nítrico alveolar
Óxido nítrico bronquial

RESUMEN

Introducción: La medición del óxido nítrico en el aire exhalado diferencia fenotipos de pacientes con EPOC del solapamiento de asma y EPOC (ACO). Hasta el momento no se ha estudiado si existen diferencias entre los componentes alveolar y bronquial del F_{ENO} en este grupo de pacientes.
Métodos: Estudio observacional transversal realizado en consultas externas de Neumología, incluyendo a pacientes con diagnóstico de EPOC a los que se les realizó una determinación del óxido nítrico en aire exhalado – F_{ENO}– diferenciando en esta medida el componente alveolar –C_{ANO}– y el de vía aérea central –J_{awNO}–, y realizando las mediciones a distintos flujos. Se compararon los valores de C_{ANO} y J_{awNO} entre los pacientes con eosinofilia (definidos como aquellos pacientes con ≥300 eosinófilos/μL en sangre periférica, o bien ≥2% eosinófilos o ≥3% eosinófilos) y se realizó un análisis de regresión lineal para estudiar las variables clínicas y biológicas que se asociaban a estas mediciones.

☆ Please cite this article as: Alcázar-Navarrete B, Castellano Miñán F, Santiago Díaz P, Ruiz Rodrígueza O, Romero Palacios PJ. Óxido nítrico alveolar y bronquial en la enfermedad pulmonar obstructiva crónica y el solapamiento de asma y EPOC (ACO). Arch Bronconeumol. 2018;54:414–419.

* Corresponding author.
E-mail address: balcazar@telefonica.net (B. Alcázar-Navarrete).

1579-2129/© 2018 SEPAR. Published by Elsevier España, S.L.U. All rights reserved.

Resultados: Participaron en el estudio 73 pacientes con EPOC. Los criterios de eosinofilia utilizados se asociaban a incrementos de los valores de C_{ANO} y de J_{awNO} (en este último caso solo los criterios ≥300 eosinófilos y ≥3% eosinófilos). C_{ANO} se asoció al recuento de eosinófilos y PCR, y J_{awNO} se asoció a tabaquismo y recuento de eosinófilos.
Conclusiones: Los pacientes diagnosticados de EPOC y que tienen características de ACO muestran mayor inflamación a nivel bronquial y de vía aérea pequeña. C_{ANO} y J_{awNO} se relacionan con variables clínicas y biológicas.

© 2018 SEPAR. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

Chronic obstructive pulmonary disease (COPD) is defined as chronic, irreversible airflow limitation, which is usually progressive and associated with an abnormal inflammatory reaction mainly caused by smoking.[1,2] This characteristic inflammatory response in COPD is mediated by macrophages, neutrophils, and cytotoxic T-cells (CD9+), and is accompanied by structural changes that can cause narrowing of the airways, changes in the arteries, pulmonary parenchymal emphysema, or combinations of all three.[3] These structural changes begin in the early stages of the disease,[4] especially in the small airways. In some patients, the inflammatory response is mediated primarily by Th2 lymphocytes and eosinophils, generating clinical symptoms that share features with bronchial asthma. Although the characteristics have not been clearly defined, this syndrome is currently known as asthma–COPD overlap (ACO).[5–7] One of the biological factors distinguishing COPD patients with this phenotype is their eosinophil count in peripheral blood,[8] but so far, no optimal cut-off points have been clearly established.

The measurement of nitric oxide in exhaled air as a marker of airway inflammation has advanced greatly in recent years, to the extent that several equations are now available that separate the alveolar or distal airway component (C_{ANO}) from the central bronchial component (J_{awNO}).[9–11] Both components have been widely studied in patients with bronchial asthma: the alveolar component (C_{ANO}) has been shown to be higher among patients with more severe asthma, suggesting greater inflammation in this region.[12,13] Measuring C_{ANO} can also help identify patients who are likely to improve with the use of inhaled corticosteroids (ICS)[14] and patients who present an increased risk of comorbidity.[15]

Different types of COPD patients can be identified by measuring nitric oxide in exhaled air (F_{ENO}).[16,17] This variable is also associated with the presence of eosinophils in sputum,[18,19] a typical finding in ACO. F_{ENO} levels are also a good predictor of response to the use of ICS.[20,21] Studies differentiating the alveolar fraction from the bronchial fraction of F_{ENO} in COPD patients have shown that inflammation is distal in some cases,[22] a phenomenon also observed in patients with severe asthma.

In a cross-sectional study conducted in Spain, elevated F_{ENO} levels were found in patients defined as having ACO, with an optimal cut-off of 20 parts per billion (ppb) for the diagnosis of ACO.[16]

However, the differences between the alveolar and bronchial components and the clinical and biological variables associated with inflammation in either of the territories have not been studied in depth.

The aim of this study is to determine differences in F_{ENO} levels between the alveolar and bronchial compartments in patients fulfilling biological criteria for ACO, and whether these differences are associated with other clinical or biological variables which might determine whether inflammation occurs more in a particular territory (C_{ANO} and J_{awNO}).

Materials and Methods

Study design

This was an observational, cross-sectional study to evaluate the differences in the production of C_{ANO} and J_{awNO} and the relationship of these variables with clinical variables in a consecutive series of COPD patients who performed complete lung function testing (measurement of lung volumes and diffusion) in our respiratory outpatient clinic. The study was performed between November 2014 and May 2015.

Study population

The study population comprised adult patients over 40 years of age, smokers, or non-smokers with an accumulated pack-year index of at least 10, and a diagnosis of COPD according to national and international guidelines and recommendations.[1] Exclusion criteria were the presence of any respiratory disease other than COPD that might significantly affect the examination (including a history of bronchial asthma), a history of COPD exacerbation in the 4 weeks before the test, inability to perform the study procedures or complete the questionnaires, and participation in any other clinical trial or research study.

Study variables

Clinical variables: for each patient, data were collected on their respiratory disease, including time since onset, toxic habits, comorbidities, baseline dyspnea measured according to the modified Medical Research Council (mMRC) scale, COPD Assessment Test (CAT®) questionnaire, and history of exacerbations in the previous year (classified as moderate if treated with systemic corticosteroids and/or antibiotics in an outpatient setting, and severe in the case of admission > 24 h to a hospital or emergency department).

Blood tests: before lung function tests were performed, peripheral blood was obtained for the determination of absolute eosinophil counts and percentages, and for the measurement of C-reactive protein (CRP).

Measurement of the alveolar (C_{ANO}) *and bronchial* (J_{awNO}) *component of nitric oxide in exhaled air:* before lung function tests were performed, patients performed 3 F_{ENO} maneuvers at 50 mL/s (F_{ENO50}) followed by additional determinations at 100 mL/s, 200 mL/s, and 350 mL/s (at least 2 each) in order to obtain C_{ANO} and J_{awNO} levels, according to international guidelines.[23,24] An NO chemiluminescence analyzer (HypAir F_{ENO}®, Medisoft, Belgium) was used.

Lung function variables: patients performed spirometry at baseline and after inhaling salbutamol 400 μg in accordance with national and international guidelines.[25,26] Lung volumes and diffusing capacity of the lung for carbon monoxide were also determined according to applicable recommendations.[27,28] All measurements were performed on the same lung function testing equipment (MasterLab, Jaeger GmbH, Germany).

Ethical aspects

The study was conducted in compliance with the principles of the Declaration of Helsinki. Patients who were invited to participate signed informed consent forms. The study was approved by the reference clinical research ethics committee. All patient records generated in the database were confidential and handled in compliance with the Personal Data Protection Law 15/1999 of 13 December 1999.

Statistical analysis

A descriptive statistical analysis was made of the study variables, using absolute and relative frequencies in the case of qualitative variables. Depending on whether distribution was normal or non-normal (applying the appropriate normality test for the sample size), quantitative variables were summarized by mean, standard deviation (Md ± SD), and range (minimum and maximum) or P50 (P25 - P75] (median, interquartile range), respectively.

Quantitative variables were compared by study groups using the Student's *t*-test for independent samples or the Kruskal–Wallis H (the appropriate test was selected from previously obtained results). Post hoc tests were conducted in the case of ANOVA; otherwise, the Mann–Whitney *U*-test with the Bonferroni correction was used. To determine the relationship between C_{ANO}, J_{awNO}, and the clinical variables, a multiple linear regression analysis was performed with C_{ANO} and J_{awNO} as dependent variables, respectively, using the forward stepwise method. The level of statistical significance was set at a *p*-value of 0.05. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 24.0.

Results

Characteristics of the study population

Ninety-five patients were invited to participate, 73 of whom finally completed all procedures. Fig. 1 is a STROBE diagram of the study participants.

Baseline patient characteristics are shown in Table 1. In summary, this is a series of patients with predominantly severe COPD, mean FEV_1 < 50%, and a high burden of symptoms and exacerbations in the previous year. About 25% of the study population were active smokers.

Table 2 shows patient characteristics according to the eosinophil count criteria for defining ACO (≥2% eosinophils, ≥3% eosinophils, or ≥300 cells/μL). Clinical parameters did not differ among the groups according to the criterion for defining ACO, except for nitric oxide measurements (F_{ENO50}, C_{ANO}, and J_{awNO}). Figs. 2 and 3 show C_{ANO} and J_{awNO} levels, respectively, using different eosinophil cut-off points for the definition of ACO (2%, 3%, and > 300 eosinophils, respectively). In all cases, C_{ANO} levels were higher for patients defined as having ACO according to the eosinophil count, while differences were greater when the criteria of >3% and >300 eosinophils were used. J_{awNO} levels were higher only in those patients with ACO defined by >3% or >300 eosinophils. The use of more restrictive criteria (>300 eosinophils and >3%) did not improve the detection of differences between patients classified as ACO and other patients.

Multiple linear regression analyses aimed at evaluating the associations between C_{ANO} and J_{awNO} and different clinical, biological, and lung function variables are shown in Tables 3 and 4, respectively. Variables associated with alveolar inflammation (C_{ANO}), and which predicted its finding, were CRP and total eosinophil count in peripheral blood, with an R^2 of 0.412. Variables that showed the closest association with bronchial inflammation (J_{awNO}), and which predicted its finding, were smoking, number of moderate exacerbations in the previous year, and eosinophil count in peripheral blood, with an R^2 of 0.477. Appendix B (additional material) depicts C_{ANO} and J_{awNO} values compared with the predicted values according to these models (expressed as regressions of the standardized predicted value).

Table 1
Characteristics of study population.

Variable	Value (*n* = 73)
Age, years	68.0 ± 10.6
Sex (M/F) n (%)	63/10 (86.3%/13.7%)
Active smoking, n (%)	21 (28.8%)
Lung function	
FEV1% predicted	47.5 ± 15.8
FVC% predicted	77.2 ± 17.2
FEV1/FVC	47.7 ± 10.5
TLC% predicted	128.4 ± 19.1
RV% predicted	228.6 ± 58.1
IC/TLC	16.07 ± 5.8
DLCO% predicted	42.1 ± 16.3
Severity (BODE)	
Median (IQR)	4 (2–5)
BODE > 2, *n* (%)	53 (72.6)
GOLD 2017	
GOLD A, *n* (%)	4 (5.5)
GOLD B, *n* (%)	25 (34.2)
GOLD C, *n* (%)	4 (5.5)
GOLD D, *n* (%)	40 (54.8)
Mean reversibility, %	9.01 ± 7.84
Exacerbations, previous year	
Moderate exacerbations/year	1.82 ± 1.37
Severe exacerbations/year	0.17 ± 0.38
Usual treatment	
LAMA, *n* (%)	68 (93.1)
LABA, *n* (%)	72 (98.6)
ICS, *n* (%)	55 (75.3)
Concomitant disease	
Ischemic heart disease, *n* (%)	13 (17.8)
Depressive syndrome, *n* (%)	7 (9.6)
Heart failure, *n* (%)	8 (11.0)
DM type 2, *n* (%)	6 (8.2)
COTE index, median (IQR)	1 (0–6)
CAT score	22 (15–27)
Blood tests	
Total eosinophils, 10^{-3}/μL	0.215 ± 0.095
Eosinophils, %	2.51 ± 1.17
CRP, mg/L	0.70 ± 0.94
Fibrinogen, mg/dL	435.9 ± 133.6

BODE: body mass index, obstruction, dyspnea, and exercise tolerance; CAT: COPD assessment test; CRP: C-reactive protein; DLCO: diffusing capacity of the lung for carbon monoxide (expressed as a percentage of the predicted value); DM: diabetes mellitus; FEV_1% predicted: peak expiratory flow in 1 second (expressed as a percentage of the predicted value); FVC: forced vital capacity (expressed as a percentage of the predicted value); IC: inspiratory capacity; ICS: inhaled corticosteroids; IQR: interquartile range; LABA: long-acting β adrenergic agonists; LAMA: long-acting muscarinic antagonists; RV: residual volume (expressed as a percentage of the predicted value); TLC: total lung capacity (expressed as a percentage of the predicted value).

Discussion

The results of this study show that COPD patients who meet one of the proposed ACO criteria (elevated eosinophil count in peripheral blood) showed differences in nitric oxide production in the alveolar territory (C_{ANO}) or in the bronchial territory (J_{awNO}), depending on the threshold considered for eosinophil counts, while values were similar if the eosinophil criteria applied were ≥300 cells/μL or ≥3%.

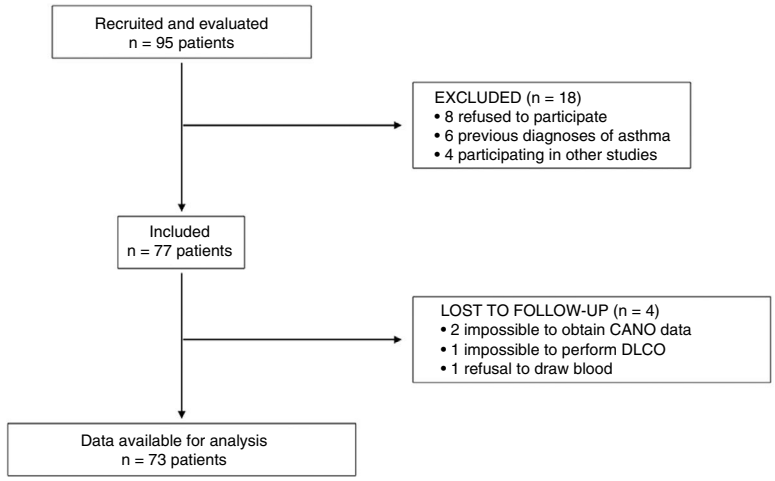


Figure 1. STROBE diagram of study participants.

Table 2
Patient characteristics by ACO criterion.

	ACO defined by eosinophils > 300/μL			ACO defined by eosinophils ≥2%			ACO defined by eosinophils ≥3%		
	No(n = 61)	Yes(n = 12)	p-value	No(n = 23)	Yes(n = 50)	p-value	No(n = 59)	Yes(n = 14)	p-value
Age, years	67.1 ± 10.9	72.5 ± 8.5	0.113	68.8 ± 8.4	67.6 ± 11.6	0.651	67.9 ± 10.1	68.2 ± 13.2	0.921
Sex, M/F	51/10	12/0	0.131	18/5	45/5	0.175	49/10	14/0	0.097
Active smoking, n (%)	19 (31.1)	2 (16.7)	0.311	7 (30.4)	14 (28.0)	0.831	17 (28.8)	4 (28.6)	0.986
ICS, n (%)	45 (73.8)	10 (83.3)	0.482	15 (65.2)	40 (80.0)	0.173	45 (76.3)	10/71.4)	0.705
FEV1% predicted	45.9 ± 16.2	55.5 ± 10.7	0.055	46.7 ± 11.1	47.8 ± 17.6	0.772	46.4 ± 16.3	52.2 ± 12.9	0.217
BD response, %	8.5 ± 7.3	11.3 ± 9.9	0.262	9.1 ± 9.0	8.9 ± 7.3	0.903	8.7 ± 7.4	10.3 ± 9.5	0.494
Exacerbations, moderate	2.0 ± 1.3	1.5 ± 1.4	0.170	1.6 ± 1.3	2.1 ± 1.3	0.099	2.1 ± 1.3	1.4 ± 1.3	0.084
Exacerbations, severe	0.2 ± 0.4	0.0 ± 0.0	0.080	0.2 ± 0.4	0.1 ± 0.3	.215	0.2 ± 0.4	0.0 ± 0.0	0.054
F_{ENO50}, ppb	13.7 ± 5.5	24.3 ± 8.5	0.000	11.3 ± 3.5	17.7 ± 7.7	0.001	13.3 ± 5.1	24.2 ± 12.0	0.000
C_{ANO} ppb	9.2 ± 6.4	24.8 ± 12.9	0.000	8.0 ± 4.7	13.5 ± 10.8	0.024	8.7 ± 5.9	24.5 ± 12.0	0.000
J_{awNO} nL/min	29.0 ± 16.2	44.2 ± 15.8	0.004	27.9 ± 14.3	33.2 ± 18.0	0.217	28.0 ± 15.5	46.3 ± 15.5	0.000

BD: bronchodilator response; C_{ANO}: alveolar nitric oxide; F_{ENO50}: nitric oxide in exhaled air at 50 mL/s; J_{awNO}: bronchial nitric oxide.

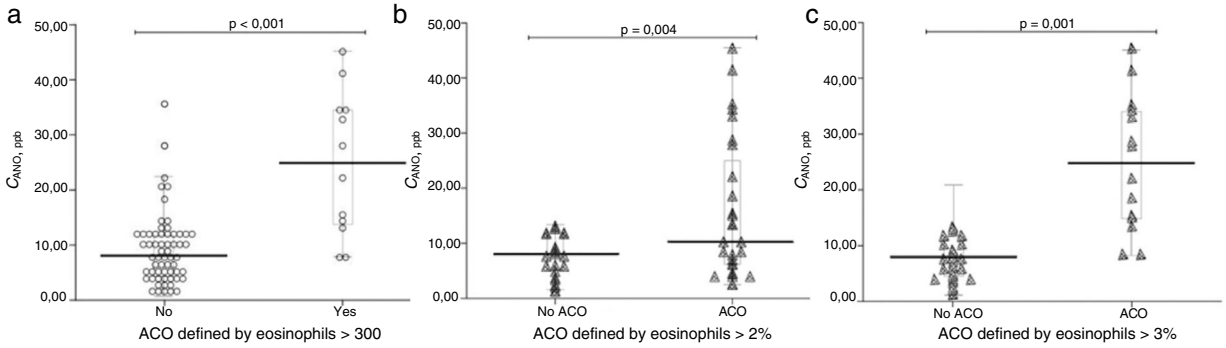


Figure 2. C_{ANO} values by ACO criterion. (a) ACO defined by ≥300 eosinophils/μL. (b) ACO defined by ≥2% eosinophils. (c) ACO defined by ≥3% eosinophils. Horizontal lines show the mean value, figures show the individual values, the box shows the IQ range, and the whiskers show the mean standard error.

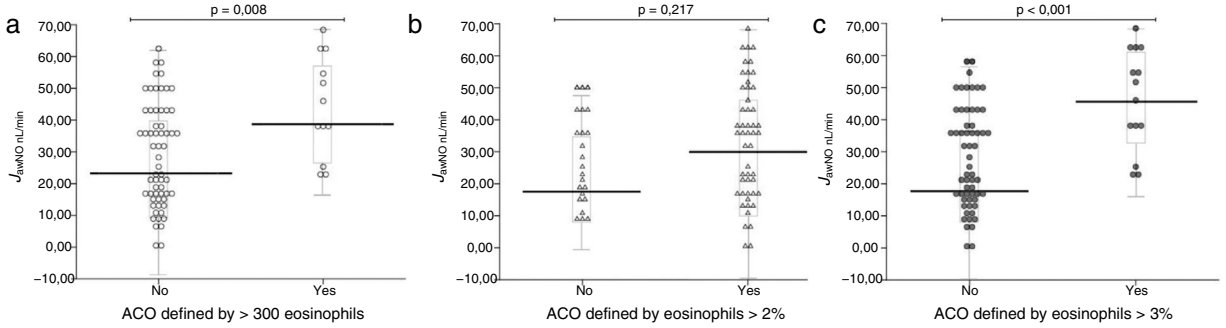


Figure 3. J_{awNO} by ACO criterion. (a) ACO defined by ≥300 eosinophils/μL. (b) ACO defined by ≥2% eosinophils. (c) ACO defined by ≥3% eosinophils.

Table 3
Multiple linear regression analysis with C_{ANO} as the dependent variable.

	Non-standardized coefficients		Standardized coefficients	*p*-value	95% confidence interval for B		Collinearity statistics	
	B	Standard error	Beta		Lower limit	Upper limit	Tolerance	VIF
(Constant)								
Tobacco use	−0.923	7.059		0.897	−15.191	13.344		
ICS	−2.573	2.895	−0.113	0.379	−8.424	3.277	0.755	1.324
FEV1%	2.255	3.145	0.097	0.478	−4.101	8.611	0.670	1.494
DLCO%	−0.050	0.103	−0.072	0.630	−0.257	0.158	0.552	1.813
CRP	0.158	0.087	0.237	0.076	−0.017	0.333	0.720	1.389
Eosinophils $\times 10^{-3}$, μL	1.525	1.114	0.166	0.043	0.727	3.776	0.821	1.217
Moderate and severe	57.573	11.299	0.602	0.000	34.736	80.410	0.869	1.151
ex., previous year	−1.487	0.999	−0.228	0.144	−3.506	0.531	0.519	1.926

Ex.: exacerbations; VIF: variance inflation factor.

Table 4
Multiple linear regression analysis with J_{awNO} as the dependent variable.

	Non-standardized coefficients		Standardized coefficients		95% confidence interval for B		Collinearity statistics	
	B	Standard error	Beta	Sig.	Lower limit	Upper limit	Tolerance	VIF
(Constant)								
Tobacco use	−5.466	14.676		0.712	−35.324	24.392		
ICS	15.757	6.190	0.376	0.016	3.163	28.351	0.695	1.439
FEV1%	10.496	7.127	0.228	0.150	−4.003	24.995	0.634	1.578
DLCO%	−0.312	0.258	−0.212	0.235	−0.837	0.213	0.494	2.026
CRP	0.257	0.186	0.206	0.176	−0.121	0.636	0.681	1.469
Eosinophils $\times 10^{-3}$, μL	−1.815	2.195	−0.114	0.414	−6.281	2.650	0.795	1.258
Moderate and severe	92.513	23.697	0.551	0.000	44.301	140.724	0.763	1.311
ex., previous year	−3.742	2.049	−0.316	0.077	−7.912	0.428	0.506	1.978

Ex.: exacerbations; VIF: variance inflation factor.

Our findings are in line with the previous study published by our group and show that F_{ENO50} may be an accessible marker for the detection of ACO.[16] Our results contribute additional, clinically useful data, and define in more detail the contribution of eosinophil levels in peripheral blood.

Measuring F_{ENO} and differentiating the alveolar and bronchial components is an approach that has been developed mainly in the field of bronchial asthma, in which it has previously shown utility in the evaluation of disease control and for predicting the future risk or adjustment of inhaled treatment.[29–31]

Fewer studies have been published on determinations of C_{ANO} and J_{awNO} in COPD patients, and the results have been less convincing,[32–34] although until now, no analyses have been based on the criteria for defining ACO, i.e., eosinophils in peripheral blood or bronchodilator response. Our findings in the group of patients without biological characteristics of ACO are comparable with series reported by other authors.[32,33] Similarly, we found a relationship between smoking and low J_{awNO} values, also noted in another study, suggesting that smoking influences the production of bronchial but not alveolar nitric oxide. In our study, the COPD patient group showed no differences with respect to alveolar and bronchial inflammation (C_{ANO} and J_{awNO}) from the patient group with no signs of ACO.

Our study has limitations: it is cross-sectional, it was conducted in a single center, and few patients were included, due to the technical demands of the test procedure. However, the series size is similar to that of other studies.[32] Moreover, in order to eliminate other possible confounding factors, we excluded patients previously diagnosed with bronchial asthma, so the results obtained are not applicable to all patients with ACO. We should also mention that not all patients are classified as ACO due to their eosinophil count. Another weakness of our study is that some individuals were active smokers and some were receiving treatment with ICS, which may alter C_{ANO} and J_{awNO} levels.

From the perspective of clinical utility, the use of the variables included in the linear regression models of this study could help clinicians identify patients or patient groups who may have greater bronchial or alveolar Th2 inflammation,[35] and who therefore require an inhalation system or molecule that deposits preferentially in the bronchi or the alveoli. This would individualize treatment and reduce the risk of side effects.[36]

In summary, we present evidence that F_{ENO} production in COPD patient differs depending on eosinophil levels in peripheral blood, particularly with regard to nitric oxide produced by peripheral airways and its associated clinical variables, factors that are easy for the clinician to identify.

Conflict of interest

The authors state that they have no conflict of interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.arbres.2018.02.006.

References

1. Vogelmeier CF, Criner GJ, Martínez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Informe 2017 de la iniciativa global para el diagnóstico, tratamiento y prevención de la enfermedad pulmonar obstructiva crónica: Resumen ejecutivo de GOLD. Arch Bronconeumol. 2017;53:128–49.
2. Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, et al. Guía española de la enfermedad pulmonar obstructiva crónica (GesEPOC) 2017, Tratamiento farmacológico en fase estable. Arch Bronconeumol. 2017;53:324–35.
3. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. N Engl J Med. 2009;360:2445–54.
4. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004;350:2645–53.
5. Miravitlles M. Diagnosis of asthma–COPD overlap: the five commandments. Eur Respir J. 2017:49.
6. Miravitlles M, Alcázar B, Alvarez FJ, Bazús T, Calle M, Casanova C, et al. What pulmonologists think about the asthma–COPD overlap syndrome. Int J COPD. 2015:10.

7. Sin DD, Miravitlles M, Mannino DM, Soriano JB, Price D, Celli BR, et al. What is asthma? COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J. 2016;48:664–73.
8. Plaza V, Álvarez F, Calle M, Casanova C, Cosío BG, López-Viña A, et al. Consensus on the Asthma–COPD Overlap Syndrome (ACOS) Between the Spanish COPD Guidelines (GesEPOC) and the Spanish Guidelines on the Management of Asthma (GEMA). Arch Bronconeumol. 2017;53:443–9.
9. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. J Appl Physiol. 1998;85:653–66.
10. Paredi P, Kharitonov SA, Meah S, Barnes PJ, Usmani OS. A novel approach to partition central and peripheral airway nitric oxide. Chest. 2014;145:113–9.
11. Condorelli P, Shin H-W, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. J Appl Physiol. 2007;102:417–25.
12. van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. Eur Respir J. 2006;27:951–6.
13. Tulic MK, Christodoulopoulos P, Hamid Q. Small airway inflammation in asthma. Respir Res. 2001;2:333–9.
14. Thornadtsson A, Neerincx AH, Högman M, Hugen C, Sintnicolaas C, Harren FJM, et al. Extended nitric oxide analysis may improve personalized anti-inflammatory treatment in asthmatic children with intermediate F_E NO_{50}. J Breath Res. 2015;9:47114.
15. Corcuera-Elosegui P, Sardón-Prado O, Aldasoro-Ruiz A, Korta-Murua J, Mintegui-Aramburu J, Emparanza-Knorr JI, et al. Patrones inflamatorios en niños asmáticos basados en la determinación de óxido nítrico alveolar. Arch Bronconeumol. 2015;51:279–84.
16. Alcázar-Navarrete B, Romero-Palacios PJ, Ruiz-Sancho A, Ruiz-Rodriguez O. Diagnostic performance of the measurement of nitric oxide in exhaled air in the diagnosis of COPD phenotypes. Nitric Oxide—Biol Chem. 2016;54:67–72.
17. Donohue JF, Herje N, Crater G, Rickard K. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. Int J Chron Obstruct Pulmon Dis. 2014;9:745–51.
18. Chou K, Su K, Huang S, YH H, Tseng C, Su V, et al. Exhaled nitric oxide predicts eosinophilic airway inflammation in COPD. Lung. 2014;192:499–504.
19. Soter S, Barta I, Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. Inflammation. 2013;36:1178–85.
20. Antus B, Barta I, Horvath I, Csiszer E. Relationship between exhaled nitric oxide and treatment response in COPD patients with exacerbations. Respirology. 2010;15:472–7.
21. Feng J, Lin Y, Lin J, He S, Chen M, Wu X, et al. Relationship between fractional exhaled nitric oxide level and efficacy of inhaled corticosteroid in asthma–COPD overlap syndrome patients with different disease severity. J Korean Med Sci. 2017;32:439.
22. Brindicci C, Ito K, Resta O, Pride NB, Barnes PJ, Kharitonov SA. Exhaled nitric oxide from lung periphery is increased in COPD. Eur Respir J. 2005;26:52–9.
23. 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;171:912–30.
24. Horváth I, Barnes PJ, Loukides S, Sterk PJ, Högman M, Olin A-C, et al. A European Respiratory Society technical standard: exhaled biomarkers in lung disease. Eur Respir J. 2017;49:1600965.
25. García-Río F, Calle M, Burgos F, Casan P, del Campo F, Galdiz JB, et al. Espirometría. Arch Bronconeumol. 2013;49:388–401.
26. Miller MR. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
27. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26:511–22.
28. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J. 2017;49:1600016.
29. Saito J, Gibeon D, Macedo P, Menzies-Gow A, Bhavsar PK, Chung KF. Domiciliary diurnal variation of exhaled nitric oxide fraction for asthma control. Eur Respir J. 2014;43:474–84.
30. Petsky HL, Kew KM, Turner C, Chang AB. Exhaled nitric oxide levels to guide treatment for adults with asthma. Cochrane Database Syst Rev. 2016;9:CD011440.
31. Puckett JL, Taylor RW, Leu S-Y, Guijon OL, Aledia AS, Galant SP, et al. Clinical patterns in asthma based on proximal and distal airway nitric oxide categories. Respir Res. 2010;11:47.
32. Bazeghi N, Gerds TA, Budtz-Jørgensen E, Hove J, Vestbo J. Exhaled nitric oxide measure using multiple flows in clinically relevant subgroups of COPD. Respir Med. 2011;105:1338–44.
33. Gelb AF, Flynn Taylor C, Krishnan A, Fraser C, Shinar CM, Schein MJ, et al. Central and peripheral airway sites of nitric oxide gas exchange in COPD. Chest. 2010;137:575–84.
34. Amer M, Cowan J, Gray A, Brockway B, Dummer J. Effect of inhaled β2-agonist on exhaled nitric oxide in chronic obstructive pulmonary disease. PLOS One. 2016;11:e0157019.
35. Stockley J, Cooper B, Stockley R, Sapey E. Small airways disease: time for a revisit? Int J Chron Obstruct Pulmon Dis. 2017;12:2343–53.
36. Sonnappa S, Martin R, Israel E, Postma D, van Aalderen W, Burden A, et al. Risk of pneumonia in obstructive lung disease: a real-life study comparing extra-fine and fine-particle inhaled corticosteroids. PLOS One. 2017;12:e0178112.