Microalbuminuria and High-Sensitivity C-Reactive Protein as Potential Biomarkers of Systemic Inflammation in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

There isn't yet a clear definition for systemic inflammation in COPD (chronic obstructive pulmonary disease), but its recognition has been based on studies that show an increase in the plasma concentration of various inflammatory markers, such as the c-reactive protein (CRP), and in recent years, also the microalbuminuria has been suggested. The purposes of this work were to determine the microalbuminuria and CRP as potential biomarkers of systemic inflammation. We enrolled patients with stable COPD and non-COPD smokers diagnosed through spirometry; older than 40 years without AHT (arterial hypertension) or diabetes type I or II, between October 2017 and March 2019. In both groups, a venous blood sample was collected to determine high-sensitivity CRP and 3 urine samples were taken to determine microalbuminuria, calculating the mean value. At least two out of three determinations between 30 and 300 mg/g of urine creatinine were considered to be significant albuminuria. The high-sensitivity CRP was considered positive with a value $\geq$ 5 mg/L. Of the 47 analyzed patients, a mean albuminuria of 13.91 ± 5.04 was obtained in the COPD group, in comparison with 2.50 ± 0.36 in the control group. Also, the high-sensitivity CRP mean values were compared, showing 5.06 ± 2.24 in COPD patients and 2.46 ± 0.51 in the control group. Both variables showed non-statistically significant differences between the study groups (p = 0.058 for mean albuminuria and p = 0.330 for high-sensitivity CRP).

Key words: Microalbuminuria; CRP; Systemic inflammation; Biomarkers; COPD

Introduction

The inflammation pattern of chronic obstructive pulmonary disease (COPD) includes neutrophils, macrophages and lymphocytes (mainly CD8). These cells release inflammatory mediators which attract cells from the blood flow that amplify the inflammatory process and induce structural changes. That process is even more amplified by oxidative stress and excess of proteases in the lung[1].

Most patients with COPD have chronic concomitant diseases related to the same risk factors: tobacco, aging and inactivity, which cause a greater impact on prognosis and quality of life. Inflammatory mediators on blood flow may trigger or worsen other diseases present in these patients, such as: heart failure, ischemic heart disease, arterial hypertension (AHT), osteoporosis, normocytic anemia, diabetes and metabolic syndrome[2].

However, there isn't yet a clear definition of systemic inflammation in COPD. Its recognition has been based on studies showing an increase in the plasma concentration of various inflammatory markers: TNFα (tumor necrosis factor α), IL-6 (interleukin 6), IL-8 (interleukin 8), C-reactive protein (CRP), fibrinogen and leukocytes in patients with stable COPD, compared to the same parameters in a normal population[3].

The release of these inflammatory proteins hurts the endothelium, inducing a severe failure of blood microflow[4]. The vascular endothelium, acting as a semi-permeable membrane, increases its permeability whenever there is imbalance. Consequently, there is endothelial dysfunction that facilitates an abnormal filtration of proteins[5, 6].

In recent years, it has also been suggested that microalbuminuria could be a predictor of such endothelial dysfunction. The glomerulus, as an extension of the vascular endothelium, is also hurt during the systemic inflammatory response, with severe consequences in local hemodynamic factors and the diameter of the pores, thus resulting in protein filtration[7].

Basing on prior history, microalbuminuria and high-sensitivity CRP could be important indicators to predict systemic damage associated with COPD[8].

Primary objective

To determine the microalbuminuria as potential biomarker of systemic inflammation in patients with stable COPD compared to non-COPD smokers.

Secondary objective

To consider the high-sensitivity C-reactive protein (CRP) as another possible indicator associated with this disease.

Materials and methods

Analytic, observational, cross-sectional study. We used as a sample a group of patients who spontaneously went to the outpatient offices of the Pulmonology Department of the Hospital Tránsito Cáceres de Allende and signed the informed consent (version 1.3 – 2017 approved by the Institutional Ethics Committee on Adult Health Research - Ministry of Health of the Province of Córdoba; attached as annex), during the period between October 2017 and March 2019.

Inclusion criteria:

1. Patients diagnosed with stable COPD (defined according to GOLD [Global Initiative for Chronic Obstructive Lung Disease] by a post-bronchodilator FEV_1/FVC [forced expiratory volume in first second/forced vital capacity] quotient < 0.70, confirming the presence of persistent airflow limitation in patients with the appropriate symptoms and exposed to noxious stimuli, mainly tobacco smoke)[9]: stages 1 [mild], 2 [moderate], 3 [severe], and 4 [very severe] according to the GOLD classification of COPD severity[10], previously diagnosed through spirometry.
2. Age > 40 years.
3. Males and females.

Exclusion criteria:

1. Arterial hypertension (AHT).
2. Diabetes mellitus 1 and 2 (DM).
3. Exacerbated COPD, defined as acute worsening of respiratory symptoms that requires additional treatment; increased dyspnea, cough and sputum volume and purulence, in the last year[11].
4. Acute chronic kidney disease.
5. Urinary tract infection (UTI).
6. Gross hematuria.
7. Pregnant women.
8. Patients who had exercised and had fever at the time of the consultation.
9. Pathological urine sediment.

Also, a control group was created of male and female smokers, older than 40 years, without COPD (airflow obstruction previously discarded by spirometry), without AHT or DM type 1 or 2 or acute respiratory infection, plus the remaining exclusion criteria previously established.

The following information was confirmed from each patient: age, smoking load (packs/year); pathological personal history (according to medical records), and they all underwent a thorough pulmonary

physical examination including oxygen arterial saturation and body mass index (BMI). Blood tests (see laboratory variables) and urine samples were requested, taking into account the first morning urine of three determinations with a maximum interval of one week between each sample. Patients made 3 visits to the hospital: the first visit when they were enrolled, underwent the physical examination and blood extraction and delivered the first urine sample; then the second and third visits when they delivered the second and third urine samples, respectively. Data obtained from each patient were registered in a form.

The following variables were analyzed:
o Demographic variables: age (years), gender, height (meters), weight (kg).
o Clinical variables: systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) expressed in mmHg, calculated with the sphygmomanometer technique recommended by the VIII Joint Committee for AHT[12], BMI expressed in kg/m2, oxygen saturation by means of the portable Choicemmed MD300C pulse oximeter, and pre- and post- bronchodilator spirometry, performed with a Medgraphic spirometer according to the recommendations of the ATS (American Thoracic Society)[13], using mainly the FEV1 (% of the value previously mentioned) as a spirometric variable.
o Laboratory variables: complete cytological evaluation, high-sensitivity CRP, glycemia, urea, creatinine, MDRD (Modification of Diet in Renal Disease) (glomerular filtration), complete urine test and MAO (monoamine oxidase).

For determining the biochemical variables, we collected 10 ml of venous blood from the ulnar or radial veins.

Serum samples were centrifuged at 200 rpm for 10 minutes in a Giumelli centrifuge and processed in the COBA C 311 analyzer for determining the CRP (mg/L), particle-enhanced immunoturbidimetric test.

For the complete urine tests, we used SIEMENS Multistix reagent strips, Rolco 2080 centrifuge (1500 rpm, 5 minutes) and Labomed optical microscope (40x and 10x objectives). The urine samples were centrifuged at 200 rpm for 3 minutes and then processed in the COBA C 311 analyzer for the creatininuria and albuminuria dosage, the latter by immunoturbidimetric assay.

A patient was considered to have significant albuminuria when at least two to three determinations had urine creatinine values between 30 and 300 mg/g (< than 30 mg/g was considered normal). With regard to the high-sensitivity CRP, it was considered positive with a value ≥ 5 mg/L[14-17].

Statistical analysis

Quantitative results were expressed as mean ± standard error comparing all possible combinations of pairs of mean values by multivariate ANAVA.

Qualitative results were expressed as numbers (percentage) and analyzed with the Chi-Square Test.

A significance level of $p < 0.05$ was established for all the cases. All the tests were performed with the InfoStat program, version 2018e.

Results

Results were obtained from the sample consisting of 47 individuals, one group of stable COPD (n= 27) and non-COPD smokers from the control group (n= 20).

COPD-associated conclusive and inconclusive variables were taken into account and used for comparative analysis with the corresponding control group. Taking into account the inconclusive variables, as shown in Table 1, both groups were homogeneous regarding gender, anthropometric characteristics, white blood cells count (including absolute and relative values of eosinophils and neutrophils), glycemia and oxygen saturation (%). This information is particularly interesting: the analysis of the group of COPD patients yielded a mean of 97.07 ± 0.18 in comparison with the control group, with a value of 97.45 ± 0.15, not showing significant differences between the groups.

As regards the systolic and diastolic arterial pressure, we found statistically significant differences (**Table 1**).

TABLE 1. Behavior of inconclusive COPD-associated variables related to the control group

Variable	COPD	Non-COPD smokers	*p*
Gender (%)	51.8% Men	35% Men	P = 0.2
	48.1% Women	65% Women	
BMI (Kg/m^2)	26.44 ± 1.58	26.65 ± 1.32	P = 0.92
SAP (mmHg)	119.44 ± 2.30	110 ± 2.71	P = 0.01
DAP (mmHg)	77.78 ± 1.95	71.00 ± 2.04	P = 0.02
WBC (10e3/uL)	8646.67 ± 416.07	9073.50 ± 807.19	P = 0.61
Absolute eosinophils ($^0/_{00}$)	0.21 ± 0.03	0.17 ± 0.02	P = 0.34
Relative eosinophils (%)	2.02 ± 0.18	1.69 ± 0.19	P = 0.22
Absolute neutrophils ($^0/_{00}$)	6.89 ± 0.56	6.80 ± 0.80	P = 0.92
Relative neutrophils (%)	59.30 ± 1.26	62.48 ± 2.22	P = 0.19
Glycemia (mg/dl)	94.07 ± 2.66	94.80 ± 2.74	P = 0.85
Saturation (%)	97.07 ± 0.18	97.45 ± 0.15	P = 0.13

Gender and oxygen saturation in percentages (%); quantitative variables expressed as mean ± standard error (X ± EE). P (Fisher) Anava.

On the other hand, considering the COPD-related conclusive variables, **Table 2** shows age distribution, smoking load (expressed in packs/year) and FEV1, with heterogeneity between the groups.

TABLE 2. Behavior of conclusive COPD-associated variables related to the control group

Variable	COPD	Non-COPD smokers	*p*
Age (years)	57.52 ± 0.97	48.00 ± 1.86	P ≤ 0.0001
Packs/year (N° cigarettes x year/20)	47.06 ± 5.36	16.36 ± 2.73	P ≤ 0.0001
FEV$_1$(%)	68.44 ± 2.69	100.85 ± 1.67	P ≤ 0.0001
High-sensitivity CRP (mg/l)	5.06 ± 2.24	2.46 ± 0.51	P = 0.330
Mean albuminuria (mg/g)	13.91 ± 5.04	2.50 ± 0.36	P = 0.058

FEV $_1$ percentage. Quantitative variables expressed as mean ± standard error (X ± EE). P (Fisher) Anava.

The degree of airflow obstruction in patients with COPD is expressed in **Figure 1**. Most patients showed mild and moderate obstruction, compared to a low percentage of severe and very severe obstruction.

As regards the variables that are the purpose of this study, we took into account the mean values for albuminuria and high-sensitivity CRP of the COPD group (n= 27), in comparison with the control group (n = 20) (**Table 2**). As for the albuminuria, since there were 3 determinations corresponding to 3 different urine samples, we calculated a mean value for those determinations that was analyzed together with the high-sensitivity CRP.
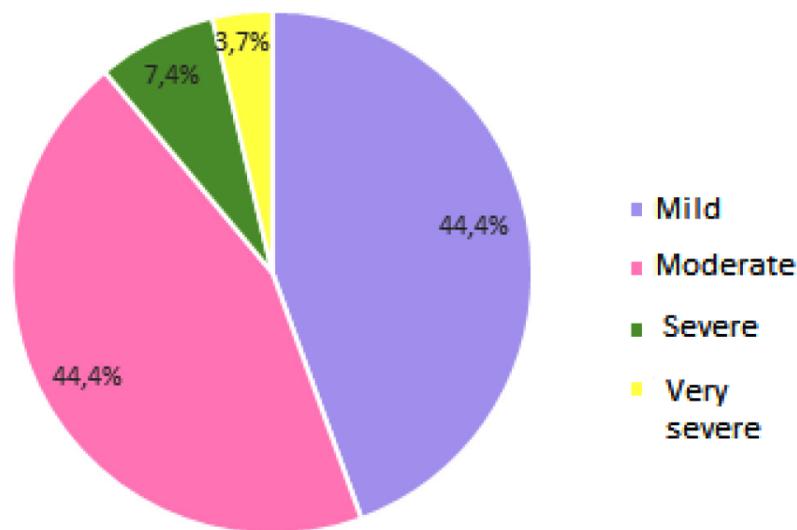
Figure 1. Spirometric patterns in COPD patients

Thus, a mean albuminuria of 13.91 ± 5.04 was obtained in the COPD group, compared to 2.50 ± 0.36 in the control group (**Figure 2**). Also, the mean values for high-sensitivity CRP were compared, with a value of 5.06 ± 2.24 in patients with COPD and 2.46 ± 0.51 in patients from the control group (**Figure 3**). Both variables showed non-statistically significant differences between the study groups (p=0.058 for mean albuminuria and p=0.330 for high-sensitivity CRP).
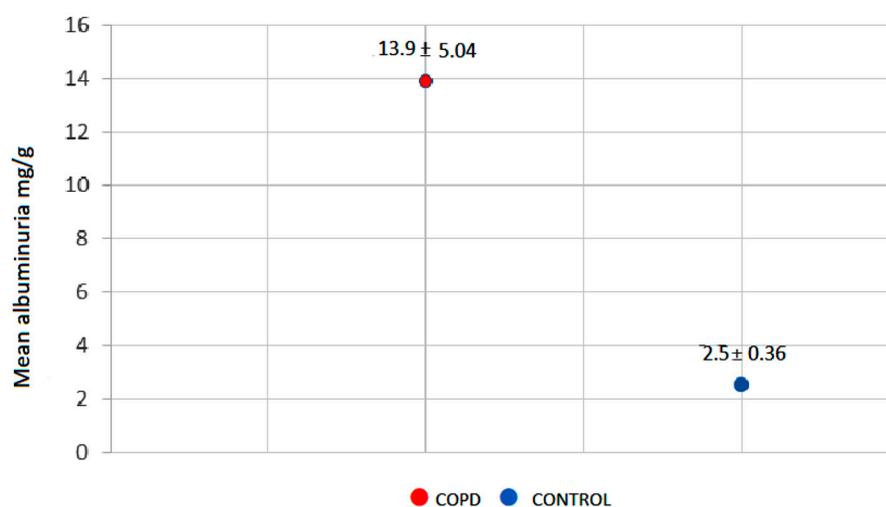

Figure 2. Comparison of mean albuminuria between the COPD group and the control group

Figure 3. Xomparison of high-sensitivity CRP between the COPD group and control group

Discussion

The fact that COPD has systemic effects in the form of structural and biochemical alterations in other organs apart from the lungs is an emerging phenomenon. It has been reported that the vascular endothelium is an important site where the systemic effects of inflammation occur; thus, the microalbuminuria is an indirect manifestation of the systemic inflammation effect on the renal endothelial permeability [18].

Various studies have suggested the microalbuminuria as a biomarker of COPD-associated systemic inflammation. Many of those studies considered not only the patients with stable obstructive disease, and didn't discard other diseases that cause microalbuminuria, such as AHT and diabetes mellitus[19-20-21].

It has been acknowledged that COPD is frequently associated with a certain degree of systemic inflammation. So, in patients undergoing stable stages of the disease, an increase in several systemic inflammation markers in the peripheral blood has been described: TNFα, IL-6, IL-8, C-reactive protein (CRP), fibrinogen, leukocytes and, in recent years, microalbuminuria[22].

In our area, it is one of the first studies to determine a relationship of inflammatory markers, in this case microalbuminuria and high-sensitivity CRP, between patients with stable COPD and non-COPD smokers. A previous work from Casanova et al showed a similar fact, suggesting that microalbuminuria could help identify a subgroup of COPD patients with increased cardiovascular risk and a potential adverse prognosis. In that study, COPD patients showed significantly higher levels of microalbuminuria compared to smokers without obstruction, as opposed to what could be observed in this study, probably due to a small sample size and the cross-sectional nature of the study.

As for the CRP, it showed a tendency to increase in the COPD groups compared to control groups. Since the CRP was not significant, our study couldn't show its positive association, just like it happened with microalbuminuria. It is important to underline that Casanova et al didn't take this biomarker into account, considering that microalbuminuria is related to cardiovascular events and death to a greater extent than the CRP. These findings weren't proven by our research. Perhaps they showed a larger albuminuria increase in their group of stable COPD patients by not discarding subjects with elevated numbers of AHT; in fact, they showed that patients with albuminuria had higher levels of systolic arterial pressure. They could determine that PO2 and systolic arterial pressure were significant predictors

of microalbuminuria levels. The microalbuminuria levels were inversely proportional to the PO_2, thus establishing a high prevalence associated with hypoxemia, another finding not proven by our work.

The main results of our study consist in showing a CRP tendency to increase (which was found to be moderately above the cut-off point) and higher mean albuminuria (despite the fact that the values were within normal limits, without a microalbuminuria range) in COPD patients compared to the control groups, which could show a tendency to a higher degree of inflammation in patients with obstructive disease. We think the data weren't statistically significant due to the reduced sample size.

Even though the potential of the COPD biomarkers is promising, at present there isn't any truly differing marker that allows us to accurately predict the development and progression of the disease, the onset of exacerbations, the response to a particular treatment or the risk of mortality[23]. More studies are necessary in order to determine the correlation of certain biomarkers with systemic inflammation in COPD patients.

To conclude, the determination of albuminuria didn't show significant differences between the groups. Even though a higher degree was evidenced in COPD patients compared to the control group, it wasn't within the microalbuminuria range, so it can't be considered as a biomarker of systemic inflammation in patients with stable COPD.

As for the high-sensitivity CRP, a tendency to increase was evidenced in COPD patients compared to the control group, but no significant difference was proven between the groups so as to consider it as another useful biomarker to predict associated systemic inflammation.

References

1. Kumar V, Abbas A, Fausto N. Robbins y Cotran. Patología estructural y funcional. 7ma edición. Barcelona (España): Elsevier Saunders; 2009 (cap. 15 El Pulmón) p: 723-4.
2. Alvar Agustí MD, Claus Vogelmeier MD, Celli B MD, et al. Global Initiative for Chronic Obstructive Lung Disease. U.S National Library of Medicine, Bethesda, USA. 2018. 12-15, Chapter 1: definition and overview, pathology, pathogenesis and pathophysiology.
3. Casanova Macario C, de Torres Tajes JP, Córdoba Lanús E. EPOC: inflamación bronquial y sistémica. Arch Bronconeumol. 2010; 46(Supl 4): 9-15
4. Resendiz-Hernández J, Camarena A. Mecanismos inmunológicos de la respuesta inflamatoria en el EPOC, NCT. 2010; 69(4): 210-7.
5. Kumar V, Abbas A, Fausto N. Robbins y Cotran. Patología estructural y funcional. 7ma edición. Barelona (España): Elsevier Saunders; 2009. 519-520: Cap 11; Vasos sanguíneos.
6. Badimón L, Martinez-gonzález J. Disfunción endotelial. Rev Esp Cardiol Supl. 2006; 6: 21-30.
7. Alegre J, Alles A, Angerosa M. Implicancia de la Proteinuria en el Diagnóstico y Seguimiento de la Enfermedad Renal Crónica. Sociedad Argentina de Nefrología. Documento de Consenso. 2015. 1-22
8. Ford E, Cunningham TJ, Mannino DM. Inflammatory markers and mortality among US adults with obstructive lung function. Respirology. 2015; 20(4): 587-3.
9. Alvar A, Claus V, Celli B, et al. Global Initiative for Chronic Obstructive Lung Disease. 2019;(4).Chapter 2: diagnosis and initial assessment.
10. Casas A, Sansores R, Tokumoto A. Recomendaciones para el diagnóstico y tratamiento de la enfermedad pulmonar obstructiva crónica. Asociación Latinoamericana de Tórax. Edición 1; 2011.Capítulo 2: Curso clínico, diagnóstico, espirométrico y estratificación de la gravedad. 12-18
11. Alvar A MD, Claus V MD, Celli B MD, et al. Global Initiative for Chronic Obstructive Lung Disease. U.S National Library of Medicine, Bethesda, USA. 2018. 100-2, Chapter 5: Management of exacerbations
12. Gómez-León Mandujano A, Morales López S, Álvarez Díaz CJ. Técnica para una correcta toma de la presión arterial en el paciente ambulatorio. 2016; 59: 49-55.
13. García-Río F, Calle M, Burgos F, Casan P, et al. Espirometría. Arch Bronconeumol. 2013; 499): 388-401.
14. Alegre J, Alles A, Angerosa M, et al. Implicancia de la Proteinuria en el Diagnóstico y Seguimiento de la enfermedad renal crónica. Sociedad Argentina de Nefrología. Documento de consenso. 2016. 8-18.
15. Calabia E. Medida de la función renal. Evaluación del cociente microalbuminuria. Valor de la tira reactiva y del examen del sedimento urinario. Indicaciones para solicitar ecografía renal. Nefrología. 2004; 24 (Supl 6): 35-46.
16. Figueroa Casas J, Schiavi E, Mazzei J. Recomendaciones de la EPOC en la Argentina. Medicina (Buenos Aires). 2012;72 (Supl 1): 2-15.
17. Gorosito M, Santamaría R, Alcázar R. Documento de la Sociedad Española de Nefrología sobre las guías KDIGO para la evaluación y el tratamiento de la enfermedad renal crónica. Nefrología 2014; 34 (Supl 3): 302-16.

18. Agrawal A, Garg R, Sahu D, Kumar M. Study the association of COPD with early endotelial dysfunction and its impact on cardiovascular system by estimating urinary albumin creatinine ratio. Lung India. Pubmed. 2017; 34: 138-43.

19. Saldias F, Díaz O, Dreyse J, C et al. Etiología y biomarcadores de inflamación sistémica en las exacerbaciones leves a moderadas de la enfermedad pulmonar obstructiva crónica. Rev Med Chile. 2012; 140: 10-8.

20. Jhon M, Hussain S, Prayle A, Simms R, Cockcroft JR, Bolton CE. Tarjet renal damage: the microvascular associations of increased aortic stiffness in patients with COPD. John et al. Respiratory Research. 2013; 14: 31.

21. Kaysoydu E, Arslan S, Yildiz G, Candan F. Factors Related to Microalbuminuria in patients with Chronic Obstructive Pulmonary Disease. Adv Clin Exp Med. 2014; 235): 749-55.

22. Morales A, Dreyse J, Diaz O, Saldias F, Carrasco F, Lisboa C. Marcadores de inflamación sistémica en pacientes ex fumadores con enfermedad pulmonar obstructiva crónica en etapa estable. Rev med Chile. 2010; 138: 957-64.

23. Izquierdo Alonso JL. Futuro de los marcadores biológicos en la EPOC. Arch Bronconeumol. 2017; 53(10): 541-2.