Article

Eosinophilic Inflammation and Its Association with Small Airway Dysfunction in COPD Patients by Biomass.

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Abstract: COPD is a chronic inflammatory disease characterized by progressive airflow obstruction. Tobacco smoking is the main cause of COPD (COPD-TS), but chronic exposure to biomass smoke (COPD-BS), mainly wood smoke, is the second risk factor; both are considered to cause different phenotypes of COPD. COPD-BS is more eosinophilic than COPD-TS. The objective of the present study was to evaluate the serum level of interleukins involved in eosinophil maturation, recruitment, and survival, and their association with small airway obstruction, measuring cytokines by multiplex test (Bio-Plex) and evaluating the central airway resistance with impulse oscillometry (IOS), comparatively in patients with COPD due to biomass and smoking. The results showed that IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin were increased in COPD-BS related to COPD-TS. Resistance parameters showed that R5, X5, AX (area reactance), and R5-R20 were significantly higher in COPD-BS than in the COPD-TS group ($p < 0.05$). R20 was not different between the groups. These data suggest that the cytokines involved in the effect of eosinophils on airway inflammation in COPD-BS were increased compared with COPD-TS, which appears to be related to a predominance of peripheral airway obstruction in patients with COPD-BS more than in COPD-TS.

Keywords: airways obstruction; biomass smoke; COPD; cytokines; eosinophils; impulse oscillometry; tobacco smoking

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a systemic inflammatory disease that causes progressive obstruction of airflow in the lungs. Tobacco smoking (TS) is the main cause of COPD (COPD-TS), but chronic exposure to biomass smoke (COPD-BS), mainly wood smoke, is the second risk factor [1]. A growing body of research indicates that there are significant differences between COPD-BS and COPD-TS, including the clinical and functional features of the disease. This evidence has led some experts to propose COPD-BS as a distinct phenotype from COPD-TS [2].

It is important to mention that COPS patients generally have greater small airway impairment compared to COPD-TS patients [3]. Several studies have focused on evaluating the damage to the small airways in COPD-BS since it has been demonstrated both from the histological and functional points of view [3,4].

Airway inflammation in COPD-TS is usually neutrophilic in nature, although it is more likely to be eosinophilic in COPD-BS. Therefore, it is plausible that the differences in both types of COPD can be explained, at least partially, by the differences in the local and systemic inflammatory profile and the polarization towards a Th2 response. Two

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studies analyzed the inflammatory profile of COPD-BS and COPD-TS; found that patients with COPD-BS had higher markers of Th2 response compared to patients with COPD-TS [5,6]. However, unlike previous studies, Golpe et al did not find this predominant Th2-type inflammation in COPD-BS [7]. In this way, eosinophils are crucial inflammatory and immune effector cells, which under certain conditions, are recruited to the lungs, mainly to the airway tissues during COPD exacerbations; although, its full physiological role is partially known.

Therefore, the evidence that could support a predominance of eosinophilic inflammation in patients with COPD-BS is limited and heterogeneous. We hypothesize that women with COPD-BS will have increased airway resistance, associated with eosinophilic inflammation and some cytokines responsible for these effects, compared to women with COPD-TS. Consequently, the purpose of this study was to evaluate the serum level of the different cytokines involved in eosinophil maturation, recruitment, and survival, as well as their association with small airway dysfunction, quantifying the cytokine profile by multiplex cytokines (Bio-Plex) and assessment of central airway obstruction with pulse oscillometry (IOS), comparatively between women with COPD exposed to BS and TS.

2. Materials and Methods

2.1. Study population

The Science Bioethics Committee of the Ismael Cosío Villegas National Institute of Respiratory Diseases Ismael Cosío Villegas (INER) approved this cross-sectional study, a reference center for respiratory diseases in Mexico City, dedicated mainly to the care of people without health insurance. The participants were recruited from the INER COPD clinic, from a cohort that was followed up periodically. Twenty-five women with COPD-BS and 25 with COPD-TS were recruited, with a diagnosis of COPD confirmed by spirometry. The diagnosis of COPD was established by post-bronchodilator lung function tests, such as FEV1/FVC <70%. COPD was classified based on exposure history. Our analysis was restricted to women because women in Mexico tend to be more exposed than men to BS because they prepare food, as a domestic role; therefore, it is very difficult for men to develop COPD from this cause [4]

Demographic, anthropometric, and clinical data were collected, including the smoking history (pack-year), which was obtained multiply the number of packs of 20 cigarettes smoked per day by the number of years the person has smoked, and the cumulative exposure to biomass, that was expressed as hour-years, and was calculated by multiplying the number of years cooking with wood stoves by the average daily hours spent in the kitchen, through a clinical interview and a standardized version in Spanish of the American Thoracic Society questionnaire [8,9], supplemented with additional questions directly related to cooking fuels. Firewood was the fuel used for cooking in all patients with COPD-BS; none were smokers. Women smokers had > 10 pack-years in their smoking habit. Patients with a history of other chronic lung diseases such as asthma, tuberculosis or bronchiectasis, or any other non-pulmonary diseases were excluded. COPD-BS patients were matched one-on-one with COPD-TS subjects by FEV1% status. COPD patients were clinically stable and free of exacerbations for at least 6 weeks before the study.

2.2. Pulmonary function tests

Pre- and post-bronchodilation spirometry was performed in all women following the procedures recommended by the American Thoracic Society/European Respiratory Society, with a dry rolling seal volume spirometer (Sensormedics, Yorbalinda, CA, USA). Forced expiratory volume in the 1st second FEV_1% (% predicted), forced vital capacity

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(FVC) and its ratio (FEV_1/FVC) were estimated. FEV1 and FVC were expressed as a percentage of the predicted value (FEV_1%, FVC%) according to Mexican standard reference equations [8,9].

2.3. Impulse oscillometry

Impulse oscillometry (IOS) is a non-invasive technique alternative to spirometry, to quantify airway obstruction, based on the use of sound waves, superimposed on normal tidal breathing during respiration, using transducers that measure the pressure and flow during exhalation for individual frequency, allowing to calculate of the impedance (the vector sum of resistance plus reactance) of the respiratory system, especially in the airways. IOS was performed before spirometry using the digital system MS-IOS (Jaeger, Würzburg, Germany), following the ERS protocols [10]. Subjects sat comfortably upright and were asked to wear a nose clip and apply manual compression to the face to minimize cheek vibration and air leakage, which was coupled to an antibacterial filter. During calm tidal breathing with cheeks supported by a research collaborator and nose clip in place, three IOS measurements were taken, each lasting 30 s, and performed 1 min apart to obtain respiratory resistance in R5 and R20. R5, the total resistance of the respiratory system, measured at 5 Hz, and central resistance, R20, measured at 20 Hz). The difference between R5 and R20 (R5-R20), the resonance frequency (Fres), and the reactance curve of the bass area between Fres and 5 Hz (AX) were recorded. The 5 Hz reactance (X5) results were also determined separately during inspiration and expiration using the IOS software [10]. Acceptability criteria for the recordings included the lack of visually detected artifacts and a coherence (ie, correlation between input and output signals) of at least 0.6 at 5 Hz and 0.9 at 10 Hz.

2.4. Blood samples

Eosinophils were counted by routine complete blood count, expressing their number of cells/ μL. Serum was obtained from whole blood in all women; For each sample, 5 mL of whole blood was collected in tubes free of anticoagulants (BD Vacutainer, Becton, Franklin Lakes, NJ, USA), the samples were collected in the morning with at least 8 h of fasting. The samples were incubated in a vertical position for 1 h at room temperature and then centrifuged at 5000xg for 15 min at room temperature to obtain the serum, which was kept at -20 °C until analysis.

2.5. Measurement of serum cytokines

Serum cytokine profiling was achieved using a Bio-Plex Pro Human Cytokine Group 27-Plex assay (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to the manufacturer's instructions, using the serum cytokine samples. serum without any dilution. This human panel included growth factors and chemokines. Specifically, basic fibroblast growth factor (FGF-2 or bFGF), eotaxin [eotaxin-1, CCL11], G-CSF (granulocyte colony-stimulating factor), GM-CSF (granulocyte-macrophage colony-stimulating factor), IFN-γ (interferon gamma), IP-10 (interferon-gamma induced protein-10), MCP-1 (chemoattractant protein-1), interleukin-(IL)-1β, IL-1ra (interleukin-1 receptor antagonist, IL-1RA/IL-1Rα/IL-1Rα1), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL- 15, IL-17, MIP-1α (macrophage (inflammatory protein-1 alpha), MIP-1β (macrophage inflammatory protein-1 beta), RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), PDGF-BB (platelet-derived growth factor B subunit), TNF-α (tumor necrosis factor-alpha) and VEGF (vascular endothelial growth factor).

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Data were analyzed using the Bio-Plex 200® system (Bio-Rad Laboratories, Inc. Richmond, CA, USA). Cytokine concentrations were expressed as median (interquartile range) in pg/ml.

2.6. Correlation between IOS and inflammatory cytokines

To analyze whether there is a relationship between the serum concentration of inflammatory cytokines and small airway dysfunction (SAD), the level of cytokines was correlated with the difference between R5-R20, to obtain the correlation coefficient.

2.7. Statistical analysis

Continuous demographic and clinical data (gender, age, height, BMI) were expressed as means ± SD and were compared by Student's t-test. While those of the serum concentration of cytokines with asymmetric distributions were described by the median and interquartile range and analyzed by the Kolmogorov-Smirnov test. All analyses were performed using the GraphPad Prism (v. 6.1, GraphPad Software, Inc., San Die-go, CA, USA). A value of $P < 0.05$ was considered statistically significant. Additionally, the correlation between IOS and cytokines was calculated. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Characteristics of women in the study.

The clinical data of the women with COPD are shown in Table 1. Women in the COPD-BS group were older, shorter, heavier, and with a higher BMI than those with COPD-TS. The average exposure to BS was 366 ± 30 hours-year, while in TS it was a mean cumulative tobacco consumption of 26.4 ± 8 pack-years. We found no differences between the groups in the FEV1 values. Furthermore, the eosinophil count was higher in COPD-BS compared to COPD-TS (Table 1).

Table 1. Anthropometric, clinical, and physiological characteristics of women in the study.

	COPD-TS	**COPD-BS**
Age (years)	69.4 ± 7	73 ± 6*
Height (cm)	153.9 ± 8	147.6 ± 8*
Weight (Kg)	58.8 ± 12	68.8 ± 11*
Smoking history (pack-years)	26.4 ± 8	0
Cumulative exposure to biomass smoke (hour-years)	0	366 ± 3
BMI (Kg/m^2)	26.7 ± 5	29.2 ± 3*
FEV_1%	68.8 ± 5	69.9 ± 4
FEV_1/FVC ratio	58.1 ± 3	59.1 ± 4
Eosinophil count (cells/ml)	223.5 ± 49.5	311.5 ± 79.2***

Data are expressed as means ± SD. Abbreviations: BMI, body mass index; COPD-BS, chronic obstructive pulmonary disease by exposure to biomass smoke; COPD-TS, COPD by tobacco smoking; FEV1%P forced expiratory volume in the 1st second (% predicted); FVC, forced vital capacity; FEV1/FVC ratio, forced expiratory volume in the 1st second (% predicted)/forced vital capacity ratio. pack-year, was obtained multiply the number of packs of 20 cigarettes smoked per day by the number of years the person has smoked;

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hour-years, was calculated by multiplying the number of years cooking with wood stoves by the average daily hours spent in the kitchen. Statistical analysis was performed using Student's t-test. * $p < 0.05$. *** $p < 0.0001$.

3.2. Serum cytokine levels in women with COPD.

Comparative data for the serum concentration of inflammatory mediators between the study groups are shown in Table 2. Of the 27 cytokines tested in the serum, statistical analyzes indicated that the concentration levels of only eight of these showed differences between the groups. of COPD, all of which were higher in COPD-BS-related women COPD-TS: IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin (table two). The graphical comparative data of the serum concentration of inflammatory mediators between the study groups are shown in Fig. 1.

Table 2. Comparative data of the serum concentration of cytokines in the serum of women with COPD.

	COPD-TS	**COPD-BS**
IL-1ra	24.76 (23.11 ± 36.14)	40.39 (30.39 ± 59.76) ***
IL-2	0.51(0.31 - 0.55)	1.31 (0.76 - 1.54) **
IL-4	0.68 (0.60 - 0.77)	0.91 (0.83 - 1.17) **
IL-8	4.28 (2.82 - 4.93)	6.11 (4.80 - 7.69) **
IL-9	5.38 (4.25 - 6.96)	7.54 (6.14 - 9.63) **
IL-13	0.34 (0.20 - 0.68)	0.82 (0.68 - 0.94) **
IL-17	0.88 (0.56 - 1.71)	2.21 (1.71 - 2.88) ***
Eotaxin	20.04 (15.48 - 29.47)	38.15 (25.69 - 49.26) **

Data are expressed as the median (interquartile ranges). The statistical analysis was carried out by the Kolmogorov-Smirnov test. Abbreviations: COPD-BS, chronic obstructive pulmonary disease by exposure to biomass smoke; COPD-TS, COPD by tobacco smoking; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist, IL-1RA/IL-1Rα/IL-1Rα1. ** $p < 0.002$, *** $p < 0.0001$.

Graphical comparative data of serum concentration of inflammatory mediators between groups of study are shown in Fig. 1.

Figure 1. Serum concentration levels of cytokines in COPD-TS and COPD-BS women.

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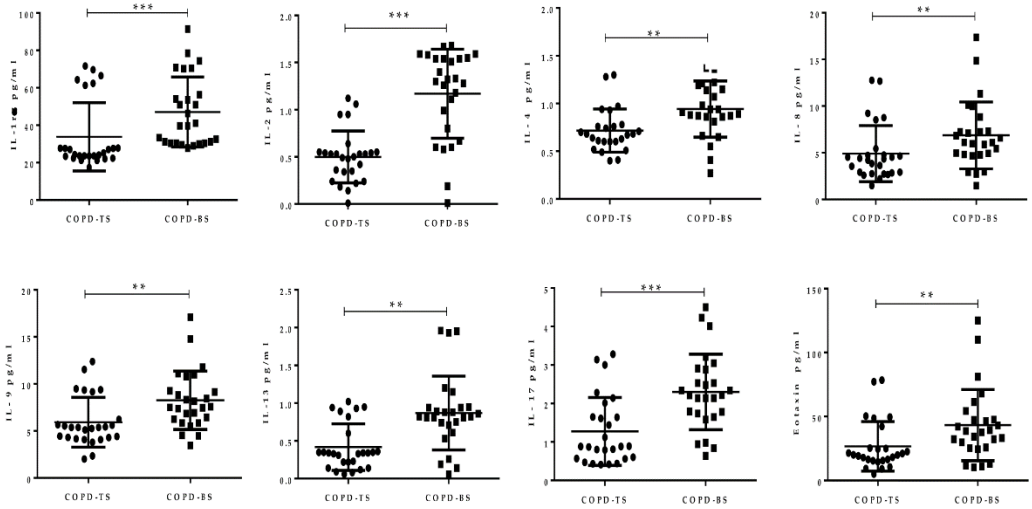

Individual data are expressed as points, indicating the median (interquartile range). Statistical analysis was performed using the Kolmogorov-Smirnov test. * $p < 0.5$. Abbreviations: COPD-BS, chronic obstructive pulmonary disease by exposure to biomass smoke; COPD-TS, COPD by tobacco smoking; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist, IL-1RA/IL-1Rα/IL-1Rα1. ** $p < 0.002$, *** $p < 0.0001$.

3.3. IOS analysis

The respiratory resistance parameters R5, AX (reactance area), and R5-R20, the most common indicator of small airway resistance in the COPD-BS group, were significantly higher than in the COPD-TS group, while X5 was significantly lower than in the COPD-TS group ($P < 0.05$), as shown in Table 3. There was no significant difference in R20 between the study groups. Although a similar level of obstruction was observed between the COPD groups quantified by spirometry, there may be a predominance of peripheral airway involvement in SB-COPD patients by the IOS analysis.

Table 3. Impulse Oscillometry (IOs) parameters between COPD groups

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IOS Parameter	COPD-TS	COPD-BS
R5 (kPa·s·L^{-1})	0.66 ± 0.33	0.83 ± 0.46*
R5%P (kPa·s·L^{-1})	185 ± 56	268 ± 90*
R20 ((kPa·s·L^{-1})	0.37 ± 0.10	0.38 ± 0.13
R20%P (kPa·s·L^{-1})	112.21 ± 35	113.54 ± 30
R5-R20 (kPa·s·L^{-1})	0.29 ± 0.25	0.48 ± 0.26*
X5 (kPa·s·L^{-1})	(-0.37) ± 0.32	-0.78) ± 0.23*
X5%P (kPa·s·L^{-1})	433 ± 190	640 ± 265.73*
AX (kPa·L^{-1})	3.8 ± 2.5	8.5 ± 3.5*

Data are expressed as means ± SD. Abbreviations: AX, reactance area; COPD-BS, chronic obstructive pulmonary disease by exposure to biomass smoke; COPD-TS, COPD by tobacco smoking; R5, resistance at 5 Hz [total]; R20, resistance at 20 Hz [central]; R5-R20, resistance at 5 Hz–resistance at 20 Hz [peripheral]; X5, reactance at 5 Hz; %P = percent of the predicted. The statistical analysis was carried out by Student's t-test. * $p < 0.05$.

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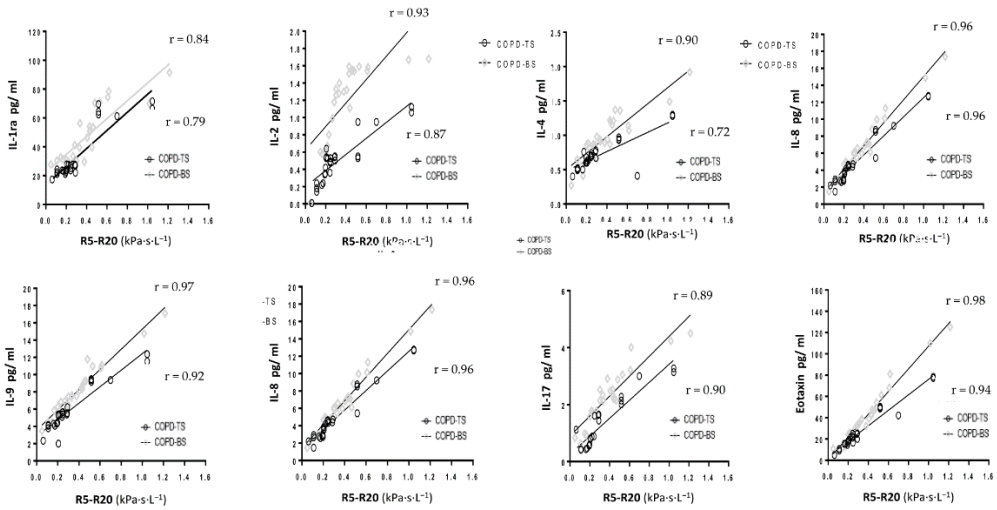
1.4. Correlation between IOS and inflammatory cytokines

To analyze whether there is an association between serum, the concentration of inflammatory cytokines with small airway dysfunction (SAD), and the difference between R5 and R20 (R5-R20). It was analyzed by evaluating the correlation coefficient between both variables, showing a positive correlation between IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin with R5 - R20 in both COPD groups, analyzed separately. $p < 0.01$ in all cases (Fig. 2).

Figure 2. Correlation analysis between serum concentration of IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin with R5-R20 (IOS).



COPD groups were analyzed independently. Individual data are expressed as points. Abbreviations: COPD-BS, chronic obstructive pulmonary disease by exposure to biomass smoke; COPD-TS, COPD by tobacco smoking; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist, IL-1RA/IL-1Rα/IL-1Rα1; R5-R2O, R5, the resistance of the respiratory system at 5 Hz- R20, the resistance of the respiratory system at 20 Hz. $p < 0.01$ in all cases.

4. Discussion

The main finding of this study is that women with COPD-BS compared to COPD-TS with similar FEV1% have greater eosinophilic inflammation, mainly those related to their migration, and the pro-inflammatory response, evidenced by high plasma concentrations of IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin; all of which showed a positive correlation with R5-R20 (airway resistance). As a second important finding, it was found that patients with COPD-BS present greater small airway dysfunction (SAD),

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as measured by IOS, compared with COPD-TS, resulting in a positive association between eosinophilic inflammation and SAD, which supports the fact that COPD-BS represents an eosinophilic inflammatory phenotype of the small airway [2, 6].

It could be considered a priori that the presence of these proinflammatory cytokines is associated with the Th2 response by activation of CD4+ Th2 lymphocytes, but also with eosinophilic inflammation and airway resistance, which is observed in the pathophysiology of patients. with COPD-BS [2, 6, 7]. Accordingly, we will consider the following facts.

In two similar studies carried out in women, higher levels of some of the inflammatory cytokines reported here were observed in COPD-BS compared to those with COPD-TS; specifically, IL-1ra, IL-4, IL-6, IL-8, and eotaxin, although higher levels of IP-10, RANTES, and VEGF were also observed. This supports the fact that a different inflammatory profile is present concerning COPD-TS. This could favor the eosinophilic inflammatory response and airway resistance in COPD-BS [11,12].

IL-1ra regulates the function of IL-1α/β [13], when it is decreased in asthma and COPD, the activity of IL-1α/β increases favoring the inflammatory process, concomitant with the turnover of the extracellular matrix due to the activity of fibroblasts in the airways; [14]. IL-1ra also activates eosinophils to produce IL-17, which induces CD4+ T cell differentiation and activation, to regulate the generation of various Th1 and Th2 cytokines in innate immune cells; but also to secrete MMP-9 which induces the release of IL-1β, independently of the inflammasome/caspase-1, which may be a link of eosinophils to the eosinophil/type-2 immune response and the neutrophil/type-1 immune response, which is commonly associated with a difficult-to-treat form of asthma [15,16].

IL-2 orchestrates immune responses to stimulate CD4+ and CD8+ effector T cell proliferation and differentiation, [17] and can downregulate Th2 cell responses to in-haled glucocorticoids (IGCs), promoting survival and survival. anti-inflammatory capacity in asthma and COPD, associated with eosinophilic inflammation, shed pathways driven by IL-2 and IL-13 [18].

Elevated levels of IL-4, IL-13, and IL-17 have been associated with increased secretion of Th2 cytokines, thereby increasing blood eosinophil counts to improve response to ICS therapy in COPD-BS [19], as suggested in two metastases. analyses and several studies, where elevated peripheral blood eosinophils are positively correlated with a greater likelihood of exacerbation reduction benefits for ICS in COPD-BS [5, 19, 20].

IL-4 and IL-13 are expressed in the respiratory epithelium and participate in mucus hypersecretion by goblet cells. IL-4 induces the differentiation of naive T helper cells to Th2 cells and induces class switching from B cells to IgE. Complementarily, IL-4-activated Th2 cells produce more IL-4 [21].

The increase in IL-13 in COPD-BS is consistent with the results obtained by Blow et al [7]. IL-13 regulates inflammation in allergies, modifying the responses of lymphocytes, myeloid cells, and non-hematopoietic cells to IL-13 and IL-17; but mediated by the overexpression of IL-1ra that induces the production of IL-13 [5]. IL-13 is an important factor mediating eosinophil recruitment to the lungs and is involved in epithelial and subepithelial remodeling and alveolar destruction in COPD, through the induction of MMP-12, demonstrating reversible obstruction of blood flow air or airway hyperresponsiveness in COPD, [22].

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IL-6 and IL-8 are involved in pulmonary and systemic inflammation, correlate with the severity and frequency of COPD exacerbation, and are associated with TGF-β1 secretion in a process of active remodeling of COPD. airways, which may promote airway obstruction [23]. For their part, eotaxin and RANTES participate in the blood chemotaxis of eosinophils by the CC-chemokine receptor 3 (CCR3) to the lungs [24].

IL-9 is mainly produced by Th9 cells (CD4+ T cells). IL9 is actively involved in asthma and bronchial hyperresponsiveness [25]. In a mouse model of COPD, IL-9 activated STAT3, induced oxidative stress, aggravated inflammation, and exacerbated lung damage [26]. Eosinophils secrete IL-8 and IL-9, which mediate predominantly th2 inflammation at sites of allergic inflammation, promoting mast cell growth and goblet cell metaplasia. Thus, the increased levels of these cytokines found in patients with WS-COPD and their presence in induced sputum could explain both histological changes and the high risk of suffering chronic bronchitis; cough, and phlegm in individuals exposed to biomass. for ≥ 3 months per year for at least 2 consecutive years [27, 28].

Our results confirm the fact that COPD-BS patients have greater eosinophilic inflammation than COPD-TS patients; although, previous studies have been contradictory. Women with WS-COPD were found to have a greater bias toward the Th2 response [5], higher sputum eosinophil levels [29], and higher FeNO levels (39 ppm vs. 27 ppm) compared with patients with COPD-TS; a surrogate for eosinophilic inflammation in the airways [7]. On the other hand, when systemic eosinophilia was analyzed, Golpe et al. [7] and Olloquequi et al [6] did not find greater peripheral eosinophilia when comparing patients with COPD-BS and COPD-TS. In addition, Salvi and colleagues [30] reported a 16% increase in mean absolute eosinophils in patients with COPD-TS; however, it was not statistically significant (control 330.4 ± 319 vs 343.5 ± 408 in COPD-TS).

The cellular and molecular pathways that lead to eosinophilic airway inflammation have been poorly studied in COPD-BS. Thus, we explored some characteristics of eosinophil homeostasis in patients with COPD-BS and found that eosinophilic activity is mostly included, as well as the cytokines that participate in their migration in the proinflammatory state, and not those that participate in maturation. or survival therein. vascular bed. A known clinical characteristic of patients with COPD-BS, which presents increased bronchial hyperreactivity [31] and bronchial abnormalities, characterized histologically by significant thickening of the bronchial wall, mainly its basement membrane [32], squamous cell metaplasia, cells goblet hyperplasia, peribronchiolar fibrosis, and bronchiectasis, with marked deposition of anthracosis pigment in the bronchial, peribronchovascular, and pulmonary interstitium, important components of airway remodeling [3].

Finally, we would like to mention that our hypothesis proposing that women with COPD-BS, when compared to women with COPD-TS, could have greater airway resistance, associated with eosinophilic inflammation and some stimulant cytokines of these effects, was supported by the pieces of evidence shown here.

5. Conclusions

Our findings show that the eosinophils count and the cytokines IL-1ra, IL-2, IL-4, IL-8, IL-9, IL-13, IL-17, and eotaxin were increased with COPD by wood smoke, compared with those by tobacco smoking. All these cytokines showed a positive correlation with IOS analysis (R5-R20), similarly, a predominance of peripheral airway obstruction in patients with COPD-BS compared with COPD-TS was observed.

6. Patents

Not applicable.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization, Oliver Pérez-Bautista; Data curation, Oliver Pérez-Bautista, Rogelio Pérez-Padilla, Alejandra Ramírez-Venegas, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Formal analysis, Oliver Pérez-Bautista, Rogelio Pérez-Padilla, Alejandra Ramírez-Venegas, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Funding acquisition, Martha Montaño and Carlos Ramos; Investigation, Oliver Pérez-Bautista, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Methodology, Oliver Pérez-Bautista, Alejandra Ramírez-Venegas, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Project administration, Carlos Ramos; Resources, Oliver Pérez-Bautista, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Software, Oliver Pérez-Bautista, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Supervision, Carlos Ramos; Validation, Oliver Pérez-Bautista, Rogelio Pérez-Padilla, Alejandra Ramírez-Venegas, Martha Montaño and Carlos Ramos; Visualization, Oliver Pérez-Bautista, Rogelio Pérez-Padilla and Carlos Ramos; Writing – original draft, Oliver Pérez-Bautista, Rogelio Pérez-Padilla, Yadira Velasco-Torres, Martha Montaño and Carlos Ramos; Writing – review & editing, Oliver Pérez-Bautista and Carlos Ramos.

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Institutional Review Board Statement: The Science, Bioethics, and Biosafety Committees of the NATIONAL INSTITUTE OF RESPIRATORY DISEASES ISMAEL COSÍO VILLEGAS (INER) in Mexico City approved the study. The research was developed according to the guidelines of the Declaration of Helsinki and the Official Mexican Standard NOM-012-SSA3–2012, which establishes the criteria for the execution of research projects for human health. The protocol approved at INER was the B15-15 (entitled ´´Perfiles de expresión de micrornas en suero de pacientes con enfermedad pulmonar obstructiva crónica por humo de leña y humo de tabaco´´), on June 5, 2015.

Informed Consent Statement: All participants of the study were recruited at the COPD Clinic of INER, and a written and signed In-formed consent form was obtained from all subjects involved in the study.

Data Availability Statement: Data related to this study can be requested from Oliver Perez-Bautista (first author) or Carlos Ramos (corresponding author).

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Conflicts of Interest: The authors declare no conflict of interest. The funders (CONACyT) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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