Project Title

Chronic obstructive pulmonary disease (COPD) related to wood smoke: an analytical sociodemographic, clinical, functional, imaging, and biomarkers profile characterization in comparison with tobacco smoke COPD.

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1. TITLE

Chronic obstructive pulmonary disease (COPD) related to wood smoke: an analytical sociodemographic, clinical, functional, imaging, and biomarkers profile characterization in comparison with tobacco smoke COPD.

2. SUMMARY

Around 40 percent of the world population, that is 2.8 billion people, continues using solid fuels (charcoal and biomass: wood, dung, crop) for cooking or home heating (1-3). Chronic exposure to biomass fuel smoke, particularly wood smoke, has been identified as a significant risk factor for various respiratory conditions, including respiratory infections in children, chronic bronchitis, asthma, and chronic obstructive pulmonary disease (COPD) (4-7). These conditions are of great global concern, given their high prevalence, substantial morbidity and mortality rates (8).

Of particular interest is the distinction between wood smoke-induced COPD (WS-COPD) and the more well-known tobacco smoke-induced COPD (TS-COPD), as shown in an increasing number of studies (9-23). Notably, WS-COPD exhibits some unique characteristics, such as a higher prevalence in women and a predominant impact on the airways, resulting in significantly greater degree of bronchial inflammation but milder or no emphysema (9, 10). Moreover, women with WS-COPD tend to be older and shorter in stature than those with TS-COPD, and they exhibit a more favorable lung function trajectory with a slower decline in forced expiratory volume in one second (FEV1) (9, 10, 19, 24, 25). These findings suggest that WS-COPD may involve different exposure patterns, smoke composition, and underlying pathophysiological mechanisms (10). Prior research has suggested potential distinctions in WS-COPD, including increased bronchial hyperresponsiveness, a likely predominance of the T2 inflammatory pattern, and variations in associated biomarkers. These findings lend support to the notion of distinct pathophysiological pathways in WS-COPD (9, 10, 15, 16, 26). It is reasonable to postulate that the greater involvement of inflammatory airways and the lower rate of emphysema in

WS-COPD, as compared to TS-COPD, may be rooted in distinct etiological, pathogenic, and pathophysiological foundations.

However, there is a significant lack of information about the demographic and clinical characteristics of patients with WS-COPD derived from population-based studies, about its functional and imaging consequences, and about its biomarkers profile in the sputum in comparison with TS-COPD. In addition, there is a paucity of information on the impact of the combined exposure to wood and tobacco smoke concerning the risk of COPD in the general population and its clinical features in people with COPD.

This project, titled 'Chronic Obstructive Pulmonary Disease Caused by Wood Smoke: An Analytical Sociodemographic, Clinical, Functional, Imaging, and Biomarkers Profile Characterization in Comparison with Tobacco Smoke COPD,' aims to address these gaps in knowledge by drawing from three distinct and complementary studies:

Study 1. *"COPD related to wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study"* **(Finished, submitted and** accepted **by the *International Journal of Tuberculosis and Lung Disease*, a Q1 journal** for Medicine**; Authors: Carlos A. Torres-Duque**, Claudia Jaramillo, Andrés Caballero, Nadia Juliana Proaños-Jurado, Maria J Pareja-Zabala, Joan B Soriano, Mauricio González-García Fundación Neumológica Colombiana – Universidad de La Sabana **(Attached the** acceptation **E-mail**. **Current citation**: ***Int J Tuberc Lung Dis. 2024*: http://dx.doi.org/10.5588/ijtldopen.24.0004).**

Study 2. *Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease* (**Accepted and published by the *International Journal of Chronic Obstructive Pulmonary Disease*, a Q1 journal); Authors:** Ángela M. Giraldo-Montoya, **Carlos A. Torres-Duque**[¥], Luis Fernando Giraldo-Cadavid, Maria Eugenia Laucho-Contreras, Angélica González-Flórez, Ana María Santos, Eduardo Tuta-Quintero, Bartolomé R. Celli and Mauricio González-García. Fundación Neumológica Colombiana – Universidad de La Sabana (¥I am the co-first and corresponding author**). Final citation:** *Int J Chron Obstruct Pulmon Dis. 2024:19:1-10*. **doi: 10.2147/COPD.S439064. PMID: 38179428; PMCID: PMC10763680**

Study 3. *Small airways disease in COPD associated to biomass exposure* (published. in Rev Invest Clin 2019;71:70-8; Authors: Alejandra Ramírez–Venegas. **Carlos A. Torres-Duque,** Nicolás Eduardo Guzmán- Bouilloud, Mauricio González-Garcia. Raul H Sansores).

As follows, I present the detailed project.

3. PROJECT DESCRIPTION

3.1. RESEARCH QUESTION AND JUSTIFICATION

Research question: Are the sociodemographic, clinical, functional, imaging and biomarker profile characteristics of wood smoke COPD different from those of tobacco smoke COPD?

Hypothesis: The sociodemographic, clinical, functional, imaging and biomarker profile characteristics of wood smoke COPD are different from those of tobacco smoke COPD

Answering these questions is relevant because preliminary evidence shows that the chronic respiratory disease (mainly COPD) caused by wood smoke (WS-COPD) in humans is different (more inflammatory compromise of the airways with mild or not emphysema) from that caused by tobacco smoke (TS-COPD), but a better and differential characterization of WS-COPD is missing and it is not clear what is the underlying mechanisms explaining such differences (9-13, 21). So, we currently diagnose and treat the patients with WS-COPD in the same way to TS-COPD. However, in view of the predominating inflammatory airway compromise, anti-inflammatories, such as inhaled corticosteroids, can be expected to play a more important role.

Some authors have proposed that biomass-induced COPD, including WS-COPD, might constitute a distinct COPD phenotype (12). However, considering the differing etiologies (wood smoke vs. tobacco smoke) and inhalation patterns, as well as the diverse clinical, functional, and tomographic characteristics reported in some studies, it might be more

appropriate to categorize the chronic respiratory disease caused by wood smoke as a separate nosological condition, potentially deserving unique approaches to its prevention and treatment (10, 24, 27).

In addition, biomass smoke exposure (including wood smoke exposure) could be currently the biggest risk factor for COPD globally (28). Around 40% (2.8 billion people) of the world population continue using biomass, mainly wood, as their main domestic fuel (1, 3). So, answering the research question will allow us to better understand and face a significant worldwide problem of public health. The model of respiratory pathologic responses to the exposure to wood smoke could be more similar to that generated by exposure to environmental pollution than tobacco smoke and could also guide research in this field.

3.2. STATE OF THE ART

***Solid and biomass fuels, including wood, are the most important global environmental risk factor*.** Around 40% of the world's population, over 2.8 billion people, particularly in developing countries, still use solid fuel, whether coal or biomass (wood, vegetable remains and dung), for cooking or heating their homes (1, 2, 29). In some countries, these fuels are the main source of energy for over 70% of the rural population. In countries where migration from rural areas to cities is high, the population of urban dwellers over the age of 40 years frequently has a significant history of exposure to biomass fuels. One example is Colombia, where 39% of the population over 40 years of age living in the five main cities had cooked with wood for more than 10 years before relocating (30).

Biomass fuels are usually burnt in open fires and inefficient traditional cookstoves, often in poorly ventilated cooking spaces. Women who are customarily responsible for cooking, and their young children, are most exposed to the resulting high levels of air pollutants released including carbon monoxide (CO) and particulate matter (PM) (29).

It has been estimated that household air pollution (HAP) from cooking cause around 4 million premature deaths (31, 32), with the most recent estimates from WHO reporting 4.3 million

deaths for 2012 (33). HAP is responsible for nearly 5% of the global disease burden (expressed as disability-adjusted life-years (DALYs), making it globally the single most important environmental risk factor (3).

Exposure to biomass fuels smoke, including wood smoke, as a risk factor for respiratory diseases. The chronic exposure to smoke derived from burning biomass fuels, including wood smoke, is recognized as a risk factor for respiratory infections in children, chronic bronchitis, asthma and chronic obstructive pulmonary disease (COPD) (4-7, 34). All these pathologic conditions are highly prevalent and cause huge morbidity and mortality worldwide (8). Four systematic reviews and meta-analyses confirm that individuals chronically exposed to solid fuels at home have a higher risk of developing COPD (5-7, 35).

***Wood (biomass) smoke related COPD is different from tobacco smoke related COPD*.** An increasing number of studies have suggested differences between the COPD caused by wood smoke (WS-COPD) and the well-known COPD caused by tobacco smoke (TS-COPD) (9-21, 26, 36-38). Interestingly, in comparison with TS-COPD, WS-COPD mainly affects the airways, causing more significant inflammatory changes of the bronchial tree, with mild or no emphysema (9, 10). Some specific differences are:

<u>*Demographic differences*</u>: WS-COPD is more common in women, probably because they are more often responsible for cooking, but also because a higher susceptibility depending on the female sex (9, 39, 40). Women with WS-COPD are shorter in height, with a higher body mass index (BMI) than women with TS-COPD (13, 16, 17, 19, 41-43). Since most women with WS-COPD are of a rural origin, and most of those with TS-COPD are from urban conglomerations, differences in height and BMI may be due to ethnic and environmental reasons that require investigation. Moreover, women with WS-COPD are older, suggesting that patients with this type of exposure need more time to develop the disease or are diagnosed later (13, 16, 41, 43).

<u>*Clinical differences*</u>: Some studies have shown that the frequency of respiratory symptoms (cough, expectoration, and dyspnea) and chronic bronchitis is high in subjects exposed to

biomass smoke (5, 6, 42). Regarding the physical examination, González-García found more frequent rhonchus and wheezing in WS-COPD (42).

Differences in lung function: Compared with TS-COPD, obstruction in WS-COPD is milder, both overall and after adjusting for age (12, 13, 17, 19, 36, 41, 43) and the decline in forced expiratory volume in 1 second (FEV1) is smaller and more homogeneous than in TS-COPD (19). Some studies show that carbon dioxide arterial pressure (PaCO2) is higher (lower ventilation) and oxygen arterial pressure (PaO2) and oxygen arterial saturation (SaO2) are lower in WS-COPD than in TS-COPD (12, 19, 42, 43). The lower oxygenation rates observed in WS-COPD may be explained in part by hypoventilation. It remains to be determined whether this behavior is related with a higher BMI in these patients, most of whom are women over 50 years of age.

Normal or mild alteration of the diffusing capacity (DLCO) and DLCO/alveolar volume (DLCO/VA) ratio has been observed in WS-COPD compared to TS-COPD, in which these parameters are significantly reduced (13, 42). This finding correlates with the lower grade of emphysema found on computed tomography (CT) in patients with WS-COPD (12, 13, 44) and occurs at all levels of COPD severity (13, 42).

Gonzalez-Garcia et al. found that women with WS-COPD had greater bronchial hyperresponsiveness than women with TS-COPD (16). This finding correlates with the higher frequency of the asthma-COPD overlap phenotype described by Golpe *et al*. in biomass-related COPD, although this difference disappeared after adjusting by sex (17). Considering the predominant role that inhaled corticosteroids may have in patients with asthma-COPD overlap syndrome, these medications could be expected to have a better impact on WS-COPD than on TS-COPD.

Imaging and histological differences: some studies have found that patients with WS-COPD have less emphysema and more airway changes (bronchial thickening and fibrosis, bronchiectasis, and atelectasis) than patients with TS-COPD on both chest radiographs and histological studies (12, 13, 17, 44, 45). Recently, a significant and greater compromise of

small airways disease has been described in biomass COPD (18, 20, 21). These morphological differences can be related with a less compromised DLCO and probably with the findings of greater bronchial hyperresponsiveness and more frequent asthma phenotype in WS-COPD.

Differences in pulmonary hypertension: A recent study found that pulmonary hypertension (PH) on echocardiography was more common in patients with WS-COPD than in those with TS-COPD (46). In a previous study, on the basis of radiographical evaluations, suggested the same in patients with severe COPD (42). Sandoval et al showed a high rate of PH among individuals exposed to wood smoke compared to those with TS-COPD (47). The origin of PH in WS-COPD patients does not appear to be related solely with hypoxic pulmonary vasoconstriction, but also to direct effects caused by the inhaled substances or indirect inflammatory-mediated effects (48).

Possible reasons for differences between chronic obstructive pulmonary disease due to wood smoke and chronic obstructive pulmonary disease due to tobacco smoke. It is reasonable to expect that the greater airway inflammatory involvement and the lower rate of emphysema in WS-COPD compared to TS-COPD have an etiological, pathogenic and pathophysiological basis. However, there is very little information available to explain the reasons for these differences.

The composition of wood smoke, which contains hundreds of chemical compounds and particulates) is just as complex (49, 50), as that of cigarette smoke. Wood combustion is generally incomplete, generating greater concentrations of certain substances such as CO, benzene, and polycyclic hydrocarbons, such as benzopyrene, compared to cigarette smoke (49). Practically 100% of the particulate material in cigarette smoke is less than 2.5 μm in size (51). This proportion is nearer 90% in wood smoke; the remaining 10% of particles are between 2.5 and 10 μm in size. The role of this distribution of particle size in the greater airway compromise and more common development of anthracofibrosis in WS-COPD has not been determined.

Differences in inflammatory profile and pathophysiological ways. It is expected that the greater airway inflammatory involvement and the lower rate of emphysema in biomass COPD, including WS-COPD, compared to TS-COPD have an etiological, pathogenic and pathophysiological basis. However, although there is a growing information about the pathogenic mechanisms in COPD due to biomass smoke exposure, it is not clear what are the reasons explaining its differences with TS-COPD. Some studies have focused on looking for differences in the type of inflammation and the proteolytic activity, and recently the gene expression.

Ortiz-Quintero et al. (22) and Silva et al. (52) recently reviewed the pathogenic mechanisms involved in biomass COPD. As in TS-COPD, many of these mechanisms are related with inflammatory activation and oxidative stress, whereas no obvious significant differences in the mechanisms involved in the generation of respiratory injury in WS-COPD were identified. Although the lower rate or absence of emphysema in WS-COPD might suggest less proteolytic activity against exposure to biomass smoke, a recent study found no differences in this respect when comparing exposure to biomass smoke and to cigarette smoke (48).

The exposure to biomass smoke might pulmonary macrophages and mononuclear and polynuclear cells to generate numerous inflammatory mediators, including interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 2 (MIP2) and tumoral necrosis factor (TNF) (52, 53). These could generate a second wave of mediators that include enzymes, such as matrix metalloproteinase 9 (MMP-9) and matrix metalloproteinase 12 (MMP-12) involved in proteolysis and tissue remodeling typical of COPD. A recent study explored differences in chemokine and cytokine concentrations among biomass-COPD versus TS-COPD and exposed controls without COPD. The authors identified CCL27 and CXCL13 as putative, plausibly homeostatic/protective biomarkers for biomass COPD (85).

Golpe *et al*. found that serum IL-6, IL-8, IL-5 were significantly higher in TS-COPD patients than in biomass COPD without differences in serum IL-13, periostin, surfactant protein-P, TNF-α, IgE, erythrocyte sedimentation rate, C-reactive protein and fibrinogen (26). The level

of exhaled nitric oxide (FeNO) was higher in biomass COPD (39.0±14.6 ppb) than in TS-COPD (27.6±16.3 ppb); although the difference did not reach the statistical significance level, it was borderline (p: 0.056) and it could be related to a small sample size (26).

A study by Solleiro-Villavicencio *et al*., done in women with COPD and healthy controls, found that IL-4 and T_H2 cells were significantly higher in biomass COPD than in TS-COPD (15). Frequency of T_H17 cells in patients with TS-COPD was significantly higher than in patients with biomass COPD. They suggested that a T_H2 cytokine inflammatory profile could predominate in biomass COPD (15). Although the majority the authors have not found differences in blood eosinophils counts between biomass COPD and TS-COPD, Fernandes *et al*., using a cutoff of ≥3%, found more frequent sputum eosinophilia in biomass COPD than in TS-COPD (23). In the same way of the responses T_H2, Olloquequi *et al*. found higher levels of total IgE in patients with biomass smoke COPD than in TS-COPD (54).

It seems clear that the development and clinical course of COPD depend on an interaction between genetic and environmental factors. The gene regulation and expression are fundamentally involved in the pathophysiology of COPD and it is known that microRNAs (miRNAs) participate in the control of post-transcriptional regulation in TS-COPD. Recently, Velasco-Torres *et al.* have described the differential role of miR-34a (downregulated) (55) and of the axis miR-22 - histone deacetylase activity (HDAC4) – IL-17 (56). This axis has been linked to the development of emphysema in rats. Serum miR-22-3p was downregulated in biomass COPD-BS relative to COPD-TS. In contrast, the concentration of HDAC4 was higher in biomass and exhibited a significant positive correlation with DL_{CO}% (56). This mechanism could be involved in the lower expression of emphysema in WS-COPD. In summary, inflammation in biomass COPD, including WS-COPD, could be different from that in TS-COPD with a possible predominance of TH2 profile, and the lower generation of emphysema could be related to a particular and different response to biomass smoke.

On the other hand, some authors have suggested that differences between WS-COPD and TS-COPD may be determined in part by differences in the patterns of exposure (9).

In summary, there have been reported different demographic and clinical characteristics of patients with WS-COPD, but the information, particularly from population-based studies, is still scarce, and the pathophysiological mechanisms involved in WS-COPD remain unclear. It seems that the inflammatory activation in the airway could be different, and of a greater magnitude, and that proteolytic activity induces less emphysema. The great magnitude of the exposure to biomass fuels worldwide and the potential differences of preventive and treatment interventions for patients with WS-CPOD justify this study.

3.3. THEORETICAL FRAMEWORK

Around 40 percent of the world population, that is 2.8 billion people, continues using solid fuels (charcoal and biomass: wood, dung, crop) for cooking or home heating (1-3). The chronic exposure to smoke derived from burning biomass fuels, including wood smoke, is recognized as a risk factor for respiratory infections in children, chronic bronchitis, asthma and chronic obstructive pulmonary disease (COPD) (4-7). All these pathologic conditions are highly prevalent and cause huge morbidity and mortality worldwide (8).

The evidence consistently supports significant differences between WS-COPD and TS-COPD, mainly due to greater inflammatory airway compromise and a much lower or absent degree of emphysema in WS-COPD. Both WS-COPD and TS-COPD are covered under the term COPD because both cause persistent airflow limitation and wood and cigarette smoke can be grouped under the heading of noxious particles or gases.

However, considering the reported differences and the fact that wood smoke and tobacco smoke are not the same, it is reasonable to propose that WS-COPD be considered a distinct disease, rather than a new COPD phenotype. Additionally, recognition that exposure to wood smoke may be associated with radiological, functional and histological manifestations that differ from those described under the definition of COPD, such as pulmonary infiltrates, restrictive patterns and particulate deposits in the lung, may be taken as yet another argument for separating it into a different nosological entity.

Irrespective of whether WS-COPD is considered a new phenotype of COPD or a distinct entity, the most important consideration is how it affects prognosis and treatment. It can be presumed that the pathophysiological mechanisms of WS-COPD are different, and that a different approach to its treatment may be need. In view of the predominating airway compromise, anti-inflammatories, such as inhaled corticosteroids, could be expected to play a more important role. Further research is required on the pathophysiological mechanisms and treatment of disease caused by wood smoke. Better understanding of these differences could be applied to the considerable number of cases of COPD unrelated to cigarette or wood smoke, and disorders due to occupational and environmental air pollution which are classified under COPD could be better characterized.

Taking into account these considerations, this study is aimed to do an analytical sociodemographic, clinical, functional, imaging, and biomarkers profile characterization of COPD caused by wood smoke in comparison with tobacco smoke COPD

3.4. RESEARCH QUESTION

Are the sociodemographic, clinical, functional, imaging and biomarker profile characteristics of wood smoke COPD different from those of tobacco smoke COPD?

3.5. HYPOTHESIS

The sociodemographic, clinical, functional, imaging and biomarker profile characteristics of wood smoke COPD are different from those of tobacco smoke COPD.

3.6. OBJECTIVES

General objective

To compare the sociodemographic, clinical, functional, imaging, and biomarkers profile characteristics of the chronic obstructive pulmonary disease (COPD) caused by wood smoke (WS-COPD), a type of biomass smoke, with those of the COPD caused by tobacco smoke (cigarette smoking) (TS-COPD).

Specific objectives

1. To describe the **sociodemographic characteristics** (age, sex, height, weight, body mass index, and educational level) of WS-COPD and to compare it with those of TS-COPD.

2. To describe the **clinical characteristics** (respiratory symptoms: cough, phlegm, dyspnea) of WS-COPD and to compare it with those of TS-COPD.

3. To describe the **functional characteristics** (spirometry: FEV_1, FVC, FEV_1/FVC; diffusion capacity: DLCO) of WS-COPD and to compare it with those of TS-COPD.

4. To describe the **imaging characteristics** (high-resolution computed tomography) of WS-COPD and to compare it with those of TS-COPD.

5. To describe the **biomarkers profile in induced sputum** (chemokine ligand 5 [CCL5], metalloproteinase 9 [MMP-9], interleukin-8 [IL-8], chemokine ligand 16/ hemofiltrate CC chemokine 4 [CCL16/HCC-4], and vascular endothelial growth factor [VEGF-1]) of WS-COPD and to compare it with those of TS-COPD.

6. To analyze the effect of the **combined exposure to wood and tobacco smoke** on the risk of COPD in the general population and on the clinical and functional characteristics in COPD patients in comparison with those exposed to only wood smoke or only tobacco smoke.

3.7. MATERIAL AND METHODS

To approach the research question, I propose three complementary studies: two original studies and a critical review.

Designs. Its **design** are as follows:

1. **Study 1.** An analytical, cross-sectional, population-based study aimed to assess the COPD risk associated with wood smoke exposure, characterize individuals with WS-COPD in comparison to TS-COPD, and examine the effects of combined wood and tobacco smoke exposure in both the general population and COPD subjects. Study entitled ***"COPD related to wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study"*** (Finished, submitted and **accepted** by the ***International Journal of Tuberculosis and Lung Disease*, a Q1 journal for Medicine; Authors: Carlos A. Torres-Duque**, Claudia Jaramillo, Andrés Caballero, Nadia Juliana Proaños-Jurado, Maria J Pareja-Zabala, Joan B Soriano, Mauricio González-García Fundación Neumológica Colombiana – Universidad de La Sabana **(Attached the acceptation E-mail. Current citation: *Int J Tuberc Lung Dis. 2024*: http://dx.doi.org/10.5588/ijtldopen.24.0004).**

2. **Study 2.** An analytical cross-sectional study aimed to compare the biomarker profiles in induced sputum, including chemokine ligand 5 (CCL5), metalloproteinase 9 (MMP-9), interleukin-8 (IL-8), chemokine ligand 16/ hemofiltrate CC chemokine 4 (CCL16/HCC-4), and vascular endothelial growth factor (VEGF-1), among WS-COPD, TS-COPD, and a healthy control group. Study entitled **"*Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease*"** (**Accepted and published** by the ***International Journal of Chronic Obstructive Pulmonary Disease*,** a **Q1** journal); **Authors:** Ángela M. Giraldo-Montoya, **Carlos A. Torres-Duque[¥],** Luis Fernando Giraldo-Cadavid, Maria Eugenia Laucho-Contreras, Angélica González-Flórez, Ana María Santos, Eduardo Tuta-Quintero, Bartolomé R. Celli and Mauricio González-García**.** Fundación Neumológica Colombiana – Universidad de La Sabana **(**[¥]I am the co-

first and corresponding author**)**. **Final citation**: ***Int J Chron Obstruct Pulmon Dis. 2024:19:1-10***. doi: 10.2147/COPD.S439064. PMID: 38179428; PMCID: PMC10763680.

3. **Study 3.** A critical review of small airway disease in WS-COPD (a type of biomass-COPD) which included clinical, functional and imaging characteristics. Study **already published entitled** "*Small airways disease in COPD associated to biomass exposure"* (Alejandra Ramírez–Venegas. **Carlos A. Torres-Duque**, Nicolás Eduardo Guzmán-Bouilloud, Mauricio González-Garcia, Raul Sansores. **Rev Invest Clin** 2019;71:70-8;**)**.

Methods. The detailed methods for each of these three studies are presented as follows:

3.7.1. Study 1, Methods. *"COPD related to wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study":*

3.7.1.1. Design and general characteristics of the sample: We used information obtained in the PREPOCOL Study (30), a random cross-sectional, population-based study conducted in urban areas of five Colombian cities (Barranquilla, Bogotá, Bucaramanga, Cali, and Medellín). Detailed information about sample size and standardization of measurements is provided in the original article (30). In summary, subjects were selected by a probabilistic, two-stage clustered sampling technique. After signing an informed consent, civilian adults of both genders, aged 40 years and older, that performed a high-quality forced spirometry and answered a respiratory symptoms and risk factors questionnaire, were included. General demographic, socioeconomic, clinical, and spirometry variables were collected. All STROBE requirements for observational studies were considered.

3.7.1.2. Questionnaire and spirometry: We used a Spanish version of the Standardized Respiratory Questionnaire for epidemiologic studies of the American Thoracic Society (ATS-DLD-78A) with additional questions on WS exposure: Have you ever used wood for cooking habitually? If yes, for how many years? What type of fuel do you currently use for cooking? (Including wood as an option). Habitual use was defined as most days of the week. A spirometry before and after a bronchodilator, according to ATS recommendations

(MicroLoop; Micro Medical; Rochester, Kent, UK) (57), was performed. Predicted values were calculated using the Crapo reference values (58).

3.7.1.3. Definitions: COPD was defined by a post-bronchodilator forced expiratory volum3.7.1.3e in the first second (FEV_1) / forced vital capacity (FVC) ratio (FEV_1/FVC) < 0.70. Chronic bronchitis (CB) was defined by an affirmative answer to the question: Have you ever had cough and expectoration for three or more months a year for at least two consecutive years? Cough and phlegm were considered present if an affirmative answer was obtained for the following questions, respectively: a) Do you usually have cough? b) Do you usually bring up phlegm from your chest, not from the back of your nose?

3.7.1.4. Groups according to exposure to wood or tobacco smoke: All participants (N=5,539) and subjects with COPD (N=494) were separated in three groups: 1) Exposed to WS and non-exposed to TS (WS group); 2) Exposed to TS non-exposed to WS (TS group); and, 3) Exposed to both WS and TS (MS group). For grouping participants according to exposures, we used the following cutoffs: exposed to WS: ≥10 years of exposure (30) and exposed to TS: ≥10 pack/year. Participants with COPD were included in MS group, if they met the cutoff thresholds for both exposures. For comparative analyzes, subjects with COPD not exposed to WS or TS were excluded (N = 35).

3.7.1.5. Statistical analysis: The analysis addressed the COPD prevalence and the main characteristics of each group according to exposure to WS, TS or MS: age, sex, educational level, height, body mass index (BMI), symptoms (cough, phlegm, dyspnea, wheezing), FVC, FEV_1, FEV_1/FVC, reversibility and severity of the airflow limitation according to percentage of the predicted postbronchodilator FEV_1 (FEV_1 % predicted) (GOLD criteria) (59). Descriptive statistics are presented as mean and standard deviation for parametric continuous variables. Differences were evaluated by χ^2 and ANOVA. A P value <0.05 was considered for statistical significance. Following a univariate analysis, a logistic regression model was constructed using the variables that showed $p< 0.1$ in the univariate analysis. Interactions between WS and TS exposures were explored. The statistical software SigmaStat 3.2 (USA) was used.

3.7.1.6. Ethical Issues. The study was approved by the Institutional Ethics Committee of Fundación Neumológica Colombiana. The protocol followed the guidelines established by the Declaration of Helsinki and meets the criteria defined by resolution No. 008430 of October 4, 1993, which establishes the scientific, technical, and administrative standards for health research in Colombia. This study was classified in the category of minimal risk in which a respiratory questionnaire and a spirometry were requested. An informed consent was completed and signed by all the participants. The study complied with good clinical practice guidelines. Strict confidentiality of the information was guaranteed.

3.7.1.7. Funding Information. The original study was supported by an unrestrictive grant from Boehringer-Ingelheim (BI). BI did not participate in the design, development, analysis, and presentation of the results of the study. The COPD related to wood smoke Study was supported by Universidad de La Sabana (GRANT: MED-202-2015).

3.7.2 Study 2, Methods. *"Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease"*

3.7.2.1. Design and participants. This is an analytical cross-sectional study comparing the local inflammatory response as expressed by biomarkers measured in the induced sputum in women with WS-COPD, TS-COPD and in healthy subjects. The study was performed in a tertiary health care institution dedicated to respiratory health in Bogotá, Colombia, and was approved by the Institutional Review Board. All participants completed written informed consent.

The COPD participants were prospectively and consecutively recruited according to the following inclusion criteria: women older than 40 years, diagnosis of COPD confirmed by a post bronchodilator spirometry $FEV_1/FVC < 0.70$ and exposure to only wood smoke (WS-COPD) or only tobacco smoke (TS-COPD). Patients were considered as having WS-COPD if they had a history of exposure to wood smoke ≥ 10 years (30) without tobacco smoke exposure (no current smoker and pack-year index [PYI] <1) or as having TS COPD if they had a history of exposure to tobacco smoke with a PYI ≥ 10 without wood smoke exposure.

We excluded participants with a history of pulmonary diseases different from COPD (history of asthma, interstitial disease, pulmonary hypertension, bronchiectasis, etc.), pharmacological immunosuppression, simultaneous exposure to wood and tobacco smoke, contraindications, or inability to produce induced sputum or spirometry. They could not have a respiratory infection or exacerbation during the eight weeks prior to the sample collection. The control group consisted in healthy women older than 40 years who did not have any respiratory symptoms or disease, had no exposure to wood or tobacco smoke and had normal spirometry.

3.7.2.2. Procedures

Clinical evaluation, questionnaire, and spirometry. All patients received a standardized clinical evaluation by a pulmonologist, including the ATS-DLD-78 standard respiratory questionnaire (60), adapted for wood smoke exposure (30), a complete physical examination, pre and post bronchodilator spirometry and chest-x-ray. Spirometry was performed and interpreted according to the American Thoracic Society (ATS)/ European Respiratory Society (ERS) standards (61).

***Sputum induction and processing*.** The sputum was induced in all participants following existing protocols (62). All subjects received four inhalations of albuterol 100 μg, subsequently they were nebulized with 5% hypertonic saline solution. The biological material was undergone to filtration and centrifugation to remove any contaminating particles. A fluidizing solution was added, passed through a 48 micron filter, to avoid the presence of sediment, and the filtrate was collected in an Eppendorf tube, allowed to stand for 5 minutes, then cytocentrifuged at 750 g (3000 rpm for 4 minutes) and stored in cryovials at a temperature of -80 ° C (63).

Biomarkers measurement. The cytokines and chemokines were selected based on previous studies and their potential role in COPD pathogenesis. MMP-9, IL-8, VEGF-1, CCL16/HCC-4 and CCL5 were chosen because they have been widely studied in COPD, and some related to poor prognosis and development of emphysema (MMP-9 and CCL5). The enzyme-linked immunosorbent assay (ELISA) technique was performed for each biomarker in a total of 440 tests for the 88 patients and 40 tests for the technique standardization. The samples were

processed using Abcam human ELISA Kits in vitro (Cambridge, MA, USA). This assay employs an antibody specific for different molecules, in this case MMP-9 (ab 100610), IL-8 (ab 214030), VEGF-1 (ab 222510), CCL5 (ab 174446) and CCL16/HCC-4 (ab100532), which were measured simultaneously according to manufacturer's recommendations. Concentrations were determined by spectrophotometry iMark Microplate absorbance reader-1681135 (Biorad, Hercules, California, USA), for reading at 450 nm (64).

3.7.2.3. Statistical analysis. The quantitative variables were described as average $\pm$ standard deviation (SD) or as median and interquartile range (IQR), depending on their normal or non-normal distribution, respectively. We used the Shapiro-Wilk test to determine if the variables had normal distribution. The qualitative variables were described by means of absolute frequencies and proportions.

The comparison of the cytokine levels among the different groups was performed by the Kruskal Wallis test, with a maximum of two post hoc analyzes using the Mann Whitney U test, because the distribution of the variables was asymmetric.

Sample size was determined by estimating differences between means, using the information from previous TS-COPD and WS-COPD publications, and estimating the potential differences. Such publications have shown a standardized effect size ranging from 1.0 to 1.6 for MMP-9, IL-8, VEGF and CCL5 when comparing TS-COPD to healthy controls (65-67). Assuming that the differences between TS-COPD and WS-COPD would be smaller than between TS-COPD and healthy controls we estimated a standardized effect size of 0.75 for the differences between TS-COPD and WS-COPD, a confidence of 95% and a power of 80%, therefore a sample size of 90 subjects was calculated (30 per group of COPD and controls).

Statistical significance was set at a $P<0.05$ at to two tails, except in the post-hoc analyzes, in which the Bonferroni correction was applied and a P value of less than 0.025 was required (a maximum of two post hoc analyzes were made by family of tests). The Bootstrap method was used to increase the precision in the estimation of confidence intervals. The data was analyzed in the statistical package Stata version 16 (Stata Corp LLC, College Station, TX, US).

3.7.2.4. Ethical issues. The study was approved by the Institutional Ethics Committees of Fundación Neumológica Colombiana (approval number 201505-21008) and the University of La Sabana (School of Medicine Research Committee, Proceedings 337-2015). The protocol followed the guidelines established by the Declaration of Helsinki and meets the criteria defined by resolution No. 008430 of October 4, 1993, which establishes the scientific, technical, and administrative standards for health research in Colombia. This study was classified in the category of minimal risk, in which a sputum sample and a spirometry were requested. An informed consent was completed and signed. The study complied with good clinical practice guidelines. Strict confidentiality of the information was guaranteed as described in the procedure's manual.

3.7.2.5. Funding information. This research was founded by University of La Sabana (Grant: MED-202-2015)

3.7.3. Study 3, Methods. *"Small airways disease in COPD associated to biomass exposure"*

3.7.3.1. Methods. This is a critical review of literature about the respiratory effects, mainly in the small airways, described in patients with chronic obstructive pulmonary disease (COPD) caused by the chronic indoor (intra-domiciliary) exposure to biomass fuels, particularly to wood smoke. We also presented some of the described differences between the WS-COPD and the TS-COPD. This review was already published (*Ramírez-Venegas A, **Torres-Duque CA**, Guzmán-Bouilloud NE, González-García M, Sansores RH. Small airways disease in COPD associated to biomass exposure. Rev Invest Clin. 2019;71:70-8*).

We used a semi-structured search using Mesh and common terms related to the topic of interest (COPD, biomass, wood smoke, exposure, indoor air pollution, emphysema, chronic bronchitis, small airways, chronic airflow obstruction, bronchiolar disease, anthracofibrosis, anthracosis). In addition to present some of the described differences between WS-COPD and TS-COPD, we suggest that part of the explanation of the greater inflammatory compromise of airways, including small airways, and lower emphysema in WS-COPD could be related to the wider range of particle size of wood smoke and the different pattern of

inhalation of biomass (usually wood) and tobacco smoke. As the usual indoor exposure to biomass smoke is in domestic activities like cooking, individuals breathe using a consistent tidal volume pattern. This type of inhalation pattern probably reduces the exposure of the distal air space (alveolar zone) beyond the small airway, leading to an airway predominant damage, both in the central compartment with antracofibrosis commonly encountered in the airway of subjects exposed to wood smoke (68-71), sometimes accompanied by bronchial stenosis, and in the small airway that functions as the final part of the funnel, where an important inflammatory reaction takes place followed by remodeling of the small airway (20, 21).

Conversely, cigarette smokers usually smoke in a two-phase pattern: first, the smoke is drawn into the mouth without direct inhalation into the lungs, then there is a pause, and finally, the smoke is inhaled into the lungs with an additional volume of air. The average inhalation volumes have been measured at nearly 25% of vital capacity; this corresponds to close to twice the average tidal volume (72). The larger inhalation volume in tobacco smokers may allow the smoke to reach more deeply into the lungs and may increase the deposition in distal air space leading to an emphysema-predominant damage.

Using 65 references, the article presents some of the described characteristics of WS-COPD (biomass COPD) in comparison with TS-COPD emphasizing on the small airway affectation (21), including the imaging characteristics.

3.8. RESULTS

Results Study 1. *"COPD related to wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study"*.

Prevalence and risks according to exposures. A total of 5,539 participants were included. Table S1.1 shows the distribution of the overall population and the COPD subjects by sex, city of residence and risk factors. Sixty percent of the population was exposed to WS: 30.9% (n=1,713) only to WS and 29.8% (n=1,650) to both WS and TS. There were not significant

differences between the cities neither in the frequency of the exposure to WS nor in the combined exposure to both WS and TS.

The overall prevalence of COPD was 8.9% (n=494). According to exposures, the prevalence of COPD was significantly higher in people exposed to both WS and TS (16.0%) than in people exposed to TS only (7.8%) or in those exposed to WS only (6.7%) (p<0.001). Similarly, the prevalence of CB was significantly higher in people exposed to both WS and TS (9.0%) than in people exposed to TS only (5.2%) or WS only (4.2%) (p<0.001).

After adjustment by age, sex, smoking, educational level, occupational exposures and history of tuberculosis, exposure to WS 10 or more years was a risk factor for COPD in both women and men (overall: OR: 1.50, 95% CI: 1.22–1.86 [p:<0.001], women: OR: 1.84, 95% CI: 1.31–2.60 [p:<0.001], men: OR: 1.53, 95% CI: 1.08–2.18 [p:0.017]). Figure S1.1 shows how the prevalence of COPD significantly increases according to the duration of exposure to WS, reaching 23.2% of prevalence in those exposed 30 years or more. Table S1.2 shows that the unadjusted and adjusted odds ratios (OR) for COPD were significantly higher in those exposed to both WS and TS than those exposed to only WS or TS. No statistical interactions were identified between WS and TS.

Characteristics of COPD participants according to exposure. Of the 494 participants with COPD, 35 (7.1%) had no history of exposure to WS or TS and, as mentioned in Methods Section, they were not considered for the comparative analyzes which were done in the 459 COPD participants (219 [47.8%] women) exposed to WS, TS or both. Table S1.3 presents the demographic, clinical and spirometry characteristics of COPD participants according to exposure. Those with WS-COPD were predominantly female and had significantly lower height (p<0.001) and higher body mass index (BMI) (=0.026) than TS-COPD. Women exposed to WS (WS-COPD and MS-COPD) were older (p=0.036) and they also had lower height and higher BMI (BMI) than women with TS-COPD (p<0.001). The educational level was significant lower in people with COPD exposed to WS (WS-COPD and MS-COPD) than in tobacco smoke COPD (p<0.001).

Combined exposure to wood and tobacco smoke in COPD. Out of 494 COPD individuals, 264 (53.4%) were exposed to both WS and TS (MS-COPD). The exposures to WS (median: 17.0 years [IQR: 10.0 - 30.0]) and to TS (median: 21.0 pack/years [IQR: 8.8 - 34.5]) of the MS group were not significantly different from those of the WS group (19.0 years [12.0 - 30.0]) (P=0.174) and the TS group (25.5 pack/years [17.6 - 41.5]) (P=0.091). COPD individuals exposed to both WS and TS (MS-COPD) were significantly older and had more frequently persistent cough (p=0.018) and persistent phlegm (p=0.001) than those with WS-COPD or TS-COPD (Table S1.3). Women exposed to WS (WS-COPD and MS-COPD) referred more frequently dyspnea (p=0.004). MS-COPD subjects, both in the total group and women only (p=0.003), had significantly lower post-bronchodilator FEV_1% and FEV_1/FVC% (p=0.002) than those exposed to only WS or TS (Table S1.3).

Study 1. Tables. *"COPD caused by wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study"*

Table S1.1. Demographic and clinical characteristics of participants

Variable	Total Subjects N=5.539	COPD N=494
City of residence		
Bogotá	1106 (20.0)	94 (19.0)
Bucaramanga	1103 (19.9)	87 (17.6)
Cali	1100 (19.9)	93 (18.8)
Barranquilla	1102 (19.9)	68 (13.8)
Medellín	1128 (20.4)	152 (30.8)
Age,		
<64 years	4108 (74.2)	205 (41.5)
≥64 years	1431 (25.8)	289 (58.5)
Sex		
Female	3701 (66.8)	244 (49.4)
Male	1838 (33.2)	250 (50.6)
Education level		
Secondary or higher	1752 (31.6)	87 (17.6)
Primary or none	3786 (68.4)	407 (82.4)
Wood and tobacco smoke exposure		
Wood smoke (WS)	1713 (30.9)	114 (23.1)
Tobacco smoke (TS)	1035 (18.7)	81 (16.4)
Wood and tobacco smoke (MS)	1651 (29.8)	264 (53.4)
No wood or tobacco smoke	1140 (20.6)	35 (7.1)
Self-report history of tuberculosis		
No	5477 (98.9)	478 (96.8)
Yes	62 (1.1)	16 (3.2)
Occupational exposure to VGDF		
No	3129 (56.5)	239 (48.4)

Yes	2410 (43.5)	255 (51.6)

COPD: chronic obstructive pulmonary disease. VGDF: vapors, gases, dust, and fumes.
Definition of groups by exposure: ***WS:*** *exposed to wood smoke ≥ 10 years and to tobacco smoke < 10 pack/year;* ***TS:****: exposed to tobacco smoke ≥ 10 pack/year and to wood smoke < 10 years;* ***MS:*** *exposed to wood smoke ≥ 10 years and to tobacco smoke ≥ 10 pack/year;* ***no wood or tobacco smoke****: exposed to wood smoke < 10 years and to tobacco smoke < 10 pack/year.*
Values as N (%).

Table S1.2. Unadjusted and adjusted odds ratios (OR) for COPD (airflow obstruction) by exposure

Exposure	OR (95% CI)	p-value
Unadjusted		
Wood smoke	2.25 (1.53 – 3.31)	<0.001
Tobacco smoke	2.68 (1.79 – 4.02)	<0.001
Wood smoke and tobacco smoke	6.01 (4.19 – 8.62)	<0.001
Adjusted*		
Wood smoke	1.61 (1.08 - 2.40)	0.021
Tobacco smoke	2.10 (1.38 - 3.18)	0.001
Wood smoke and tobacco smoke	2.99 (2.04 - 4.38)	<0.001

* Adjusted by sex, age, educational level, city of residence, self-reported history of tuberculosis and occupational exposure to VGDF (vapors, gases, dust and fumes).

Table S1.3. Demographic, clinical and spirometric characteristics in COPD groups, by exposure (N=459)

	WS-COPD N=114	TS-COPD N=81	MS-COPD N=264	p-value
Female sex, %	90 (78.9)[a,b]	32 (39.5)	97 (36.7)	<0,001
Age, years	64.3 ± 11.1	61.5 ± 10.9[c]	66.9 ± 10.7	<0.001
Weight, kg	61.3 ± 12.5	63.2 ± 13.3	63.3 ± 13.1	0.342
Height, cm	154.1 ± 8.5[a,b]	162,3 ± 8.1	159.6 ± 9.5	<0.001
BMI, kg/m^2	25.8 ± 4.8[a]	23.9 ± 4.0	24.9 ± 4.8	0.026
Education level, secondary or higher	15 (13.2)[a]	34 (42.0)[c]	23 (8.7)	<0.001
Persistent cough, %	19 (16.7)[b]	21 (25.9)	81 (30.7)	0.018
Persistent phlegm, %	19 (16.7)[b]	16 (19.8)[c]	90 (34.1)	0.001
Dyspnea, %	60 (52.6)	36 (44.4)	150 (56.8)	0.144
FVC post-bd, %pred	95.5 ± 19.8	95.5 ± 18.7	92.4 ± 18.1	0.204
FEV_1 post-bd, %pred	76.9 ± 19.0[b]	73.9 ± 18.4	71.7 ± 19.3	0.048
FEV_1/FVC post-bd, %	63.8 ± 6.3[b]	61.2 ± 7.9	60.5 ± 9.2	0.002

COPD: chronic obstructive pulmonary disease; WS: wood smoke exposed; TS: tobacco smoke exposed; MS: exposed to both wood and tobacco smoke; BMI: body mass index; FVC: forced vital capacity, FEV_1: forced expiratory volume in the first second; post-bd: post-bronchodilator.
Data are presented as mean ± standard deviation or N (%)
[a]p<0.05 between WS-COPD and TS-COPD; [b]p<0.05 between WS-COPD and MS-COPD; [c]p<0.05 between TS-COPD and MS-COPD

Study 1. Figures.

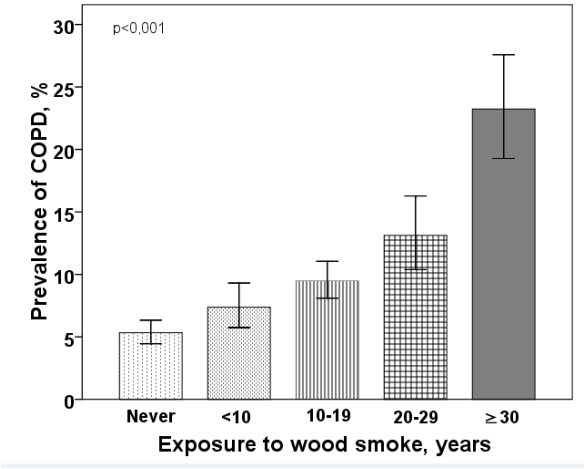

Figure S1.1. Prevalence of COPD according to years of exposure to wood smoke

Results Study 2. "*Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease".*

Population characteristics. A total of 88 women were included, 31 in the WS-COPD group, 29 in the TS-COPD group and 28 in the control group (Figure S2.1). Table S2.1 shows that patients with WS-COPD were slightly older, shorter in height and had a lower airflow limitation than those with TS-COPD without other significant differences, including the macroscopic features and weight of sputum (Table S2.1). Per definition, the control group had not airflow obstruction.

Inflammatory profile in COPD patients and differences between groups. Tables S2.2, S2.3 and S2.4 show the cytokine and chemokine levels comparing COPD with controls, the three study groups and WS-COPD with TS-COPD, respectively. In comparison to the controls, the WS-COPD and TS-COPD had higher levels of MMP-9 (P=0.004), CCL5 (P=0.002) and IL-8 (P<0.001), while no significant differences were found for VEGF-1 and CCL16/HCC-4 (P= 0.22 and 0.27, respectively) (Figure S2.2) (Tables S2.2 and S2.3).

Among COPD groups, CCL5 was significantly higher in TS-COPD compared with WS-COPD (p = 0.04) (Table S2.3, Table S2.4, Figure S2.2).

Correlations between airflow obstruction and biomarkers. There was a monotonic inverse correlation between the degree of obstruction in COPD and the levels of MMP-9, CCL5, IL-8 and VEGF-1, but not for CCL16/HCC-4 (Table S2.5).

Study 2. Tables. *"Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease".*

Table S2.1. Demographic and clinical characteristics of the women participating in the study

Characteristic	WS-COPD (N = 31)	TS-COPD (N = 29)	Control (N = 28)	P (WS vs TS)
Age, years	76 ± 8	68 ± 8	61 ± 13	<0.001[a]
Height, cm	149 ± 7	153 ± 7	151 ± 7	0.02[a]
BMI, kg/m^2	29.2 ± 4.8	27.7 ± 6.1	30 ± 4.4	0.29[a]
Pack/year Index	-	30 [43]	-	
Wood smoke exposure, years	20 [20]	-	-	
FEV_1 post β_2 (% predicted)	74% ± 19	67% ± 22	115% ± 19	0.19[a]
FEV_1/FVC post β_2	0.59 ± 0.08	0.52 ± 0.13	0.80 ± 0.05	0.02[a]
Treatment				
Inhaled corticosteroids	21 (67%)	19 (65%)	-	0.99[b]
Inhaled anticholinergics	26 (84%)	22 (76%)	-	0.65[b]
Inhaled LABA	23 (74%)	15 (52%)	-	0.12[b]
Long-term oxygen therapy[d]	9 (29%)	11 (41%)	-	0.65[b]
Classification of COPD by GOLD[e]				
I	13 (41%)	9 (31%)	-	0.54[b]
II	14 (45%)	11 (37%)	-	0.76[b]
III	4 (12%)	8 (27%)	-	0.27[b]
IV	0	1 (3%)	-	0.99[b]

Demographic, clinical, and functional variables are expressed as mean ± standard deviation; Pack/year Index and wood smoke exposure are expressed as median [interquartile range]; treatment and classification by GOLD are expressed as number and proportions: n (%).
WS-COPD: COPD due to wood smoke exposure; TS-COPD: COPD due to tobacco smoke exposure; BMI: Body mass index. Pack/year Index: number of cigarettes smoked per day multiplied by the number of years smoking and divided by 20; FEV_1: Forced expiratory volume in the first second; FVC: Forced vital capacity; β_2: Beta-2-agonist bronchodilator; LABA: long-acting β_2-agonist; GOLD: Global Initiative For Chronic Obstructive Lung Disease. [d]Oxygen utilization during more than 15 hours per day; [e]GOLD Classification for COPD: I mild: $FEV_1 \geq 80\%$ of predicted; II moderate: $FEV_1 \geq 50\%$ – <80% of the predicted; III severe: $FEV_1 \geq 30\%$ – <50% of the predicted; IV very severe: FEV_1 less than 30% of the predicted. [a]P value from Student's t-test (two-tailed). [b]P value from Fisher Exact-test (two-tailed).

Table S2.2. Biomarker levels between COPD and healthy controls

Biomarker	COPD (N = 60)	Control (N= 28)	Value of P[a]
MMP- 9 (ng/ml)	1,177 (4933)	364 (1931)	0.004
CCL5 (pg/ml)	1.62 (1.46)	1.1 (0.58)	0.002
IL-8 (pg/ml)	0.26 (0.11)	0.21 (0.02)	< 0.001
VEGF-1 (pg/ml)	0.06 (0.01)	0.05 (0.01)	0.082
CCL16/HCC-4 (pg/ml)	0.002 (0.0001)	0.002 (0.0001)	0.107

Data are presented as median (IQR [interquartile range])
[a] U-Mann Whitney Test, exact method, two-tailed. A P < 0,025 was considered significant because the Bonferroni correction was applied to the two post-hoc analyses per family of test. IQR: Interquartile range (75^{th} percentile-25^{th} percentile). COPD: Chronic obstructive pulmonary disease; MMP-9: matrix metalloproteinase 9; CCL5: chemokine ligand 5; CCL16/HCC-4: chemokine ligand 16/hemofiltrate CC chemokine 4; Il-8: Interleukin 8. Concentrations: pg/ml: picograms/millilitre, ng/ml: nanogram/millilitre.

Table S2.3. Concentration of biomarkers in women with COPD from wood smoke (WS), tobacco smoke (TS) and healthy controls.

Biomarker	WS-COPD (N=31)	TS-COPD (N=29)	Control (N=28)	P[a]	P[b]
MMP- 9 (ng/ml)	1,089 (4600)	1,843 (6,419)	364 (1,931)	0.013	0.29
CCL5 (pg/ml)	1.50 (0.79)	1.97 (2.0)	1.10 (0.58)	0.003	0.03
IL-8 (pg/ml)	0.28 (0.2)	0.25 (0.08)	0.21 (0.02)	< 0.001	0.11
VEGF-1 (pg/ml)	0.055 (0.01)	0.056 (0.01)	0.050 (0.01)	0.22	0.87
CCL16/HCC-4 (pg/ml)	0.002 (0.0001)	0.002 (0.0001)	0.002 (0.0001)	0.27	0.99

All variables are expressed as median (IQR: Interquartile range [75 percentile-25th percentile]). WS-COPD: COPD from exposure to wood smoke; TS-COPD: COPD from exposure to tobacco smoke; MMP-9: Matrix metalloproteinase 9; CCL5: chemokine ligand 5; CCL16/HCC-4: chemokine ligand 16/hemofiltrate CC chemokine 4; IL-8: Interleukin 8, VEGF-1: vascular endothelium-derived growth factor. Concentrations: pg /ml: picograms /millilitre; ng/ml: nanogram/millilitre. [a]P value from Kruskal-Wallis non-parametric test (two-tailed) comparing WS-COPD, TS-COPD, and controls; [b]P value from Robust Regression (two-tailed) comparing WS-COPD and TS-COPD and adjusting for potential confounders, that is variables associated with the exposure (WS-COPD/TS-COPD) and the outcome (biomarker): MMP-9 adjusted by FEV_1 (forced expiratory volume in one second) post-bronchodilator percent predicted, LABA (long-acting beta-agonist), statins, height, CCL5 adjusted by FEV_1 post-bronchodilator percent predicted, LABA, statins, IL-8 adjusted by FEV_1/FVC (forced vital capacity) post-bronchodilator, LABA, statins, VEGF-1 adjusted by FEV_1 post-bronchodilator percent predicted, LABA, statins, CCL16 adjusted by FEV_1 post-bronchodilator percent predicted, LABA, statins, age

Table S2.4. Biomarker levels between patients with TS-COPD and WS-COPD

Biomarker	TS-COPD (N = 29)	WS-COPD (N = 31)	P[a]
CCL5 (pg/ml)	1.97 (2.00)	1.50 (0.79)	0.04
IL-8 (pg/ml)	0.25(0.08)	0.28 (0.2)	0.18
MMP- 9 (ng/ml)	1,843 (6419)	1,089 (4600)	0.53
CCL16/HCC-4 (pg/ml)	0.002 (0.0001)	0.002 (0.0001)	0.93
VEGF-1 (pg/ml)	0.06 (0.01)	0.06 (0.01)	0.84

Data are presented as median (IQR [interquartile range])
TS-COPD: COPD from exposure to tobacco smoke; WS-COPD: COPD from exposure to wood smoke; [a]Mann-Whitney U Test, Exact method, two-tailed. Differences between TS-COPD and BS-COPD showed statistical significance for the CCL5. COPD: Obstructive pulmonary disease Chronicle; MMP9: Matrix metalloproteinase 9; CCL5: chemokine ligand 5; CCL16/HCC-4: chemokine ligand 16/ hemofiltrate CC chemokine 4; Il-8: Interleukin 8; VEGF-1: vascular endothelium-derived growth factor. Concentrations: pg/ml: picograms/millilitre, ng/ml: nanogram/millilitre

Table S2.5. Correlation between the levels of biomarkers and the degree of airflow obstruction

Biomarker	Spearman correlation coefficient (Rho)	95%CI (Rho)	P[a]
MMP-9 vs FEV_1 post β_2 (% pred)	-0.26	-0.46 to -0.04	0.016
CCL5 vs FEV_1 post β_2 (% pred)	-0.37	-0.56 to -0.15	0.001
IL-8 vs FEV_1 post β_2 (% pred)	-0.42	-0.61 to -0.21	<0.001
CCL16/HCC-4 vs FEV_1 post β_2 (% pred)	-0.12	-0.32 to 0.13	0.29
VEGF-1 vs FEV_1 post β_2 (% pred)	-0.22	-0.43 to -0.001	0.04

(Rho) Spearman correlation coefficient with 95% Bootstrap Confidence Intervals. 95%CI: 95% confidence interval of the Spearman correlation coefficient, [a] P value (two-tailed) is considered significant at $P < 0.05$; MMP9: matrix metalloproteinase 9; CCL5: chemokine ligand 5; CCL16/HCC-4: chemokine ligand 16/hemofiltrate CC chemokine 4; Il-8: Interleukin 8; FEV_1: Forced expiratory volume in the first second; β_2: beta-2-agonist bronchodilator; % pred: percentage of the predicted value. Concentrations: pg/ml: picograms/millilitre, ng/ml: nanogram/millilitre.

Study 2. Figures. "*Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease".*

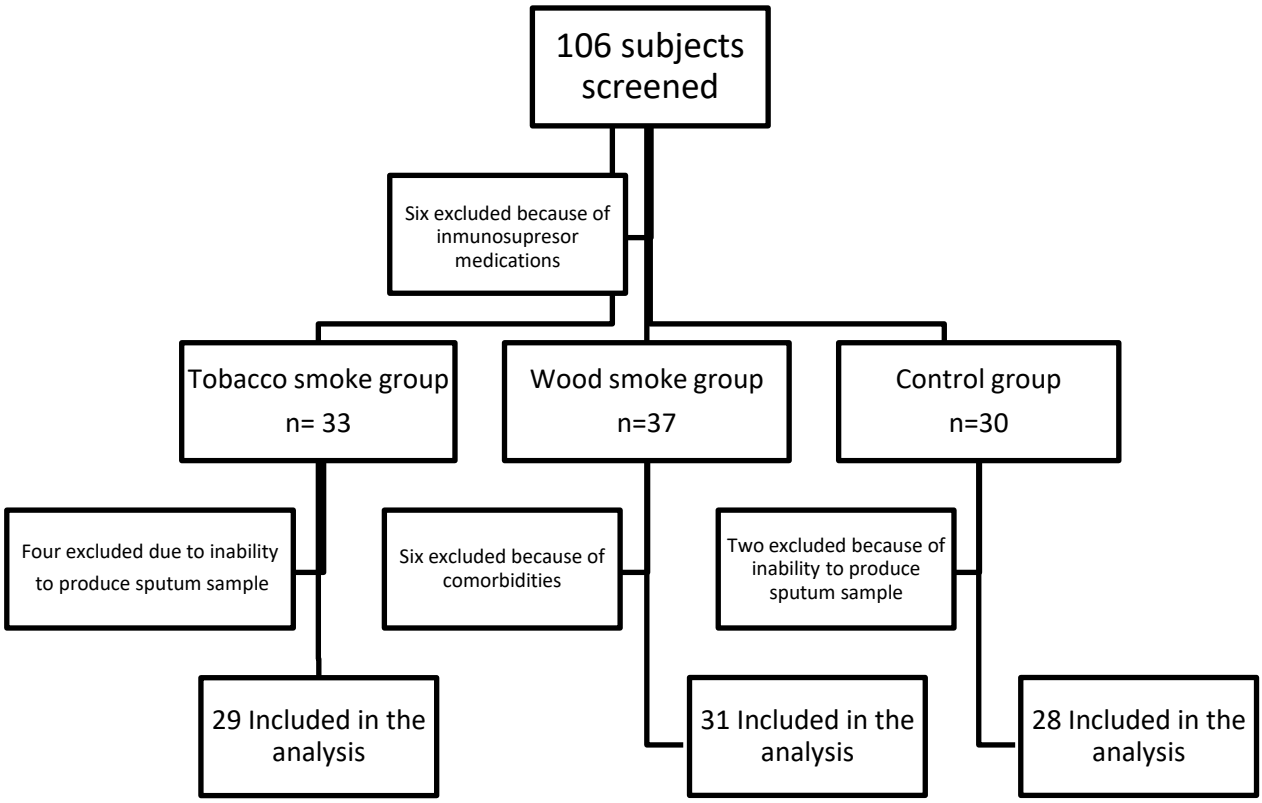

Figure S2.1. Flow diagram of subject recruitment.

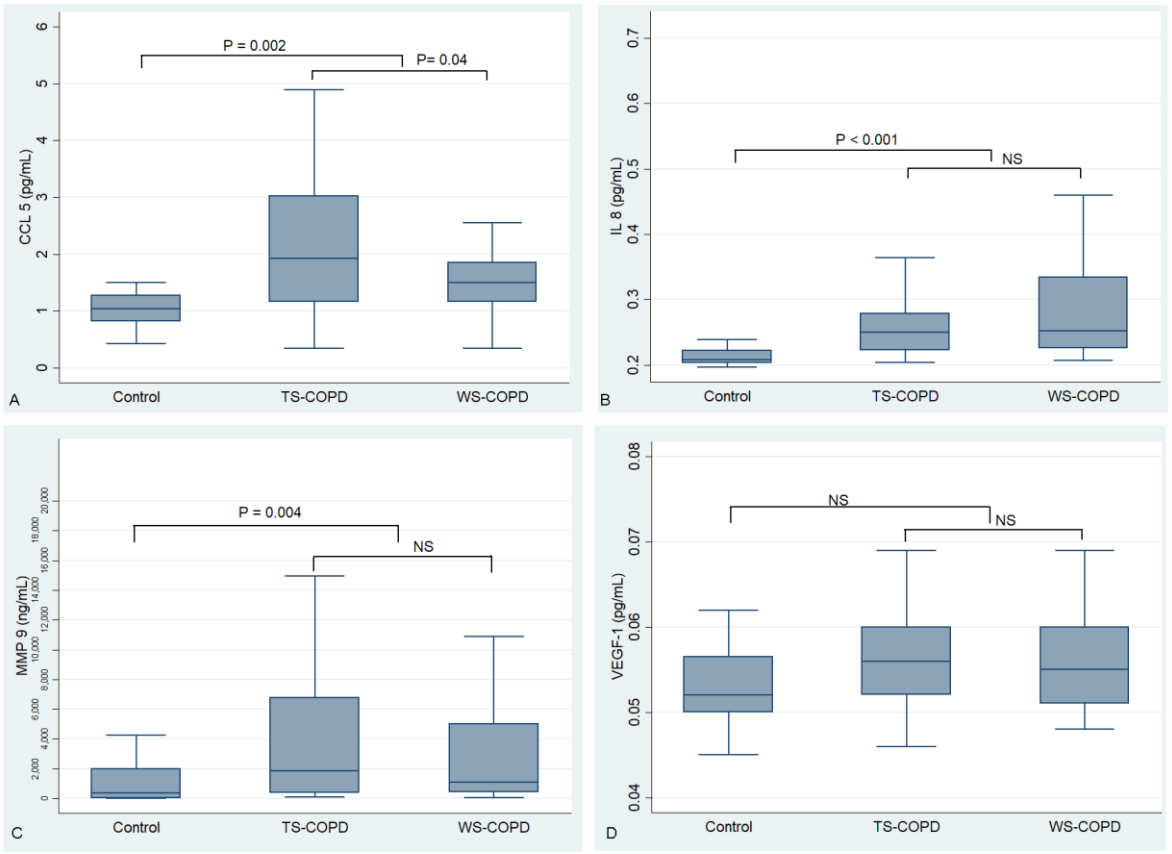

Figure S2.2: TS-COPD: COPD from exposure to tobacco smoke; WS-COPD: COPD from exposure to wood smoke; MMP-9: matrix metalloproteinase 9; CCL5: chemokine ligand 5; IL-8: Interleukin 8, VEGF-1: vascular endothelium-derived growth factor-1. Concentrations: pg /mL: picograms /millilitre, ng/mL: nanogram/millilitre. P values using two-tailed Mann-Whitney U Test, exact method.

Results Study 3. *"Small airways disease in COPD associated to biomass exposure".*

In the annexes, I am including the PDF of this study (*Ramirez-Venegas A,* ***Torres-Duque CA,*** *Guzman-Bouilloud NE, et al. Small airway disease in COPD associated to biomass exposure. Revista de investigacion clínica. 2019;71:70-78)*.

This article was a non-structured review of evidence about the affectation of the airways due to the intra-domiciliary chronic exposure to wood smoke used for cooking. Different from the tobacco smoke and tobacco exposure, the distribution of the size of particles is more heterogeneous in wood smoke and the pattern of inhalation is based on a usual tidal respiration. The chronic exposure causes an inflammatory compromise of both the large and the small airways. Central anthracofibrosis is more frequent in WS-COPD than in TS-COPD. Growing evidence confirms the significant affectation of small airways in WS-COPD the which could be detected by new techniques of imaging and functional tests. Remarkably, emphysema is mild or absent.

Different from TS-COPD, in WS-COPD the exposure to polluted air from wood burning usually starts very early in the life. The levels of pollutants inside homes burning biomass in unvented open fires are very high and women and children are exposed since the neonatal period and at all stages of life, during pregnancy and childhood and especially during adolescence when they begin to cook (49, 73). So, wood smoke exposure begins much earlier in life (*in utero* and from the neonatal period) than does active smoking (usually in the teenage years), thereby increasing the **risk for COPD and the consequent compromise of small and large airways** (74).

Airway damage in COPD from exposure to biomass smoke. A growing body of evidence supports that COPD caused by chronic indoor exposure to biomass smoke, in contrast to COPD due to cigarette smoke, is predominantly a disease of the airways with mild or minimum emphysema (9-13, 18, 20, 27). Although recent studies have focused on the small airways' damage in WS-COPD (18, 20), bronchial anthracofibrosis affecting also the central airways seems a trait more frequent and severe in WS-COPD than in TS-COPD, which could

cause bronchial stenosis (20, 68-70). In the following paragraphs, we present some of the evidence and characteristics of the airways' damage in WS-COPD.

Histological and tomographic findings. Pathological studies of samples obtained from bronchial and lung biopsies and from autopsies in persons chronically exposed to biomass smoke, with or without a diagnosis of COPD, revealed an important thickening of the bronchial wall, mainly of its basal membrane, squamous-cell metaplasia, goblet cell hyperplasia, peribronchiolar fibrosis, and bronchiectasis with a remarkable anthracotic pigment deposition in the bronchi and pulmonary interstitium (11, 20, 36, 37, 75). Among these findings, in the study by Rivera et al. (37) the autopsies of 10 women with WS-COPD and 10 women with TS-COPD showed greater remodeling and more fibrosis in the small airway in WS-COPD compared to TS-COPD. This is a clear evidence that the **damage to the small airway** is the main pathological feature in WS-COPD.

Importantly, these pathological changes are well correlated with the radiographic findings, whether in the chest X-ray or the computed tomography (12, 13, 36, 44). High-resolution computed tomography (HRCT) scans show peribronchial thickening, bronchial dilation, laminar subsegmental atelectasis, mosaic perfusion pattern, parenchymal bands, and no significant emphysema (12, 13, 36, 44). Using parametric response mapping, an imaging tool that allows the quantification of small airway disease and emphysema in COPD, Fernandes et al. confirmed the absence of important emphysema in patients with WS-COPD but, interestingly, suggested that these patients had a distinct pattern of small airway disease (18).

Clinical findings. Individuals exposed to biomass smoke have a high risk of chronic bronchitis (cough and phlegm for ≥ 3 months per year for at least 2 consecutive years) (76, 77). With regard to the physical examination, rhonchus and wheezing are relatively frequent in WS-COPD (77). The high frequency of cough, expectoration, rhonchus, and wheezing is clearly indicating the **predominant damage to the airways in WS-COPD**.

Functional findings. Compared with TE-COPD, obstruction in WS-COPD is milder, both overall and after adjusting for age (12, 13, 17, 19, 41, 42). Normal or mildly altered diffusing capacity (DL_{CO}) and DL_{CO}/alveolar volume (DLCO/AV) ratio are consistently observed in

WS-COPD when they are compared to TE-COPD, in which these parameters are significantly reduced (13, 14). This finding correlates with the lower grade of emphysema found in HRCT in patients with WS-COPD (12, 13, 18, 20, 44) at all levels of COPD severity. This functional picture of decreased DL_{CO} with normal DLCO/AV has been described in cases with **significantly compromised small airways** with little emphysema (pseudophysiological emphysema) (78).

Women with WS-COPD have greater bronchial hyperreactivity than women with TS-COPD (16). Further, research is needed to determine if this correlates with the higher frequency of the asthma–COPD overlap phenotype observed in WS-COPD (17).

Clinical phenotypes in WS-COPD. Golpe et al (17) evaluated that the frequency of clinical phenotypes defined by the Spanish COPD guidelines in patients with COPD caused by biomass or tobacco smoke. The asthma–COPD overlap phenotype was more common in biomass COPD, but the difference disappeared after adjusting for sex. Like the findings discussed in the previous sections, they found a greater frequency of emphysema phenotype in TS-COPD. No difference was found in the frequencies of chronic bronchitis or exacerbator phenotypes (17).

Conclusion. The effect on the small airway of biomass, including smoke from burning wood, is as harmful as that caused by smoke in the lung parenchyma. Therefore, WS-COPD seems equally damaging than TS-COPD. The earlier exposure occurring in WS-COPD cause a direct impact on lung growth that could damage mainly the small airway. The histological and tomographic findings in WS-COPD show a more intense small airway damage in comparison with TS-COPD. However, people with WS-COPD, particularly women, have a higher baseline FEV_1 and the decline of FEV_1 seems lower and not as detrimental as that induced by tobacco. Even so, WS-COPD patients have significant symptoms and a worse quality of life than smokers.

3.9. DISCUSSION

The results of these three complementary studies show that COPD caused by the chronic exposure to wood smoke – WS-COPD-, is significantly different from the COPD caused by tobacco which is a condition better known and characterized. The three studies contribute to a better knowledge and understanding of the sociodemographic, clinical, functional, imaging, and biomarkers profile characterization of WS-COPD in comparison with tobacco smoke COPD.

Discussion Study 1. *"COPD related to wood smoke: characterization and effect of the combined exposure with tobacco in a population-based study".*

This population-based study, in addition to confirming that the exposure to wood smoke (WS) for 10 years or more is a risk factor of COPD in women, is one of the largest studies in showing that **this exposure is a risk factor also in men and the first one, as population-based study, in showing that people exposed to both WS and tobacco smoke (TS) (the combined exposure) have a significantly higher prevalence of COPD and CB than those exposed to only WS or TS**. We also found, as previously described, that people and women with WS-COPD are older and have significantly shorter height, higher BMI, and lower educational level than TS-COPD. Finally, our study showed that people with COPD exposed to both WS and TS (MS-COPD) have more frequently persistent respiratory symptoms (cough and phlegm) and significantly greater airflow limitation (lower post-bronchodilator FEV_1% and FEV_1/FVC%) than those exposed to only WS or TS. The analysis in women with COPD exposed to WS additionally showed more frequent occurrence of dyspnea.

These findings, both in the general population (higher prevalence of COPD and CB) and in those with COPD (worse clinical and functional outcomes), demonstrate an additive adverse effect of the combined exposure to WS and TS. According to the length of exposures, in many of our study participants the exposure to both types of smoke was simultaneous, but regrettably the questionnaires used did not allow to define a precise sequence, therefore

exposures could have occurred at different times. A significant proportion of the people included were born and lived for many years in rural areas or small towns before arriving in the mentioned cities where they did not continue using wood as fuel for cooking.

Although the information about the effect of the combined exposure, simultaneous or not, to biomass smoke (including WS) and TS is still scarce, some studies have shown findings in the same direction as our results (79, 80). In a cohort of smokers, Sood et al. found that self-reported WS exposure was independently associated with lower percent predicted FEV_1 and a higher prevalence of airflow obstruction and CB (79). These associations were stronger among current cigarette smokers. In the Lovelace Smokers Cohort, compared to subjects without WS exposure, subjects with WS exposure had a more rapid decline of FEV_1 and worse quality of life (80). Recently, Olloquequi et al. described significantly lower oxygen saturation in patients with COPD and combined exposure to biomass and tobacco smoke in comparison with patients exposed to only one of them (14). In a cohort of patients with COPD, which included a small proportion of patients exposed to biomass, López-Campos et al. found that patients with an additional factor to the tobacco exposure, mainly occupational, had more chronic sputum production, worse score in the COPD Assessment Test (CAT) and higher long-term oxygen therapy requirement, in comparison with patients exposed to tobacco only (81).

Although we did not find a statistical interaction, the findings of our study suggest synergistic and/or additive pathophysiologic mechanisms derived from their combined exposure, enhancing the risk for developing COPD, and the negative effects on airways and lungs. Sood et al. showed that wood smoke exposure interacted in a multiplicative manner with aberrant promoter methylation of the p16 or GATA4 genes, increasing the risk of lower percent predicted FEV_1 and COPD in smokers (79). Awji et al found that wood smoke enhances cigarette smoke-induced inflammation by inducing the aryl hydrocarbon receptor repressor in airway epithelial cells (82). The inflammatory pathways induced by biomass/wood smoke or by tobacco smoke could be, at least partially, different (22, 52, 83) with a Th2 cytokine profile in biomass COPD (14, 15) and could interact enhancing the inflammatory responses and the negative effects of the combined exposure.

As previously described in several studies (10, 11, 17, 19, 84), we found that people with WS-COPD are predominantly female and older, and have significantly shorter height and higher BMI than TS-COPD. Traditionally, in developing countries, cooking is an activity mainly done by women who also spent more time indoors, increasing their indoor household exposures. Something similar could happen in groups with low socioeconomic status in developed countries. This explains the predominance of women in WS-COPD and, in general, in COPD due to biomass. However, as mentioned, we highlight that exposure to WS ≥ 10 years was a risk factor for COPD also for men in our population-based study, probably due to poor household ventilation conditions and kitchen location, not evaluated in this study, leading to prolonged high concentrations of pollutants from WS throughout the home. This finding confirms the results of a previous meta-analysis that had shown that biomass smoke exposure is a risk factor for COPD also in men (35).

The older age observed in patients with COPD due to WS suggests a different pattern of exposure-response (84), in comparison with TS-COPD, with a probable longer exposure required for the development of COPD. Similarly, when adjusted by age, obstruction was milder in WS-COPD as described in other studies (10, 17, 82, 84), suggesting also a different (lower) decline of FEV_1 in patients with WS-COPD as it has been described (25). There is not a clear explanation for the lower height and higher BMI found in general people and women exposed to WS (WS-COPD and MS-COPD) in comparison with TS-COPD. The use of biomass fuels (including wood) for cooking is associated with low socioeconomic status, mainly in rural settings in developing countries. The prenatal exposure (mother's exposure) to biomass fuels has been associated with low-weight at birth (85) and this, in turn, with low height in adulthood. Lower height has been found in children from rural areas in developing countries (86). In addition, although we did not take information about race/ethnic origin of participants, in Colombia, indigenous ethnicity is more frequent in people from rural areas, in which the exposure to biomass fuels is more common, in comparison with people from urban areas where white and mixed races are more frequent. Andean Colombian indigenous groups have lower height and higher BMI than general Colombian population (87).

The higher frequency of cough, phlegm and CB in WS-COPD in comparison with TS-COPD has been described in some studies (10, 21). The innovative aspect of this study is that the frequency of these symptoms, and dyspnea in women, is even higher in those who have the combined exposure to both WS and TS. In addition, this is the first population-based study in concluding that COPD exposed to both wood smoke and cigarette smoke have greater obstruction (lower FEV_1 and lower FEV_1/ FVC) than COPD exposed to WS only or TS only. These clinical and functional findings could reflect the greater damage of the airways (inflammation, anthracofibrosis, peribronchial fibrosis and bronchial hyperresponsiveness) in COPD patients exposed to WS than those exposed to TS only (10, 14, 20, 21, 68, 85, 88), and reinforce the concept of higher risk of developing obstruction and of accelerated decline of FEV_1 in those with more than one airway disorder or risk factor due to additive or synergistic pathophysiologic pathways (22, 27, 57, 79, 81). It is not clear if the results of our study can be extrapolated to COPD caused by other types of biomass such as dung or plant residues, but it seems plausible.

This study has strengths, including a random population-based study with a high response rate and enough participants and subjects with COPD in each type of exposure, single or combined, allowing consistent conclusions about the effect of combined exposure and the characterization of people with COPD exposed to biomass. Questionnaires included specific questions about the exposure to wood smoke and spirometry met high quality standards. However, our research also has limitations worth discussing. Population studies based on questionnaires may yield information bias about past exposures and events, and the lack of a better structured questionnaire for estimating the intensity of exposures (ventilation conditions, location and separation of the kitchen and type of stove, for example), and their timing and sequence. However, the questions used in our study about the length of exposure (in years) and the type of fuel routinely used by people for cooking make the analysis and conclusions robust. As discussed, race/ethnicity, or the new use of e-cigarettes or heat-not-burn tobacco, could be further explored in future research.

The 2023 Report of the Global Strategy for Prevention, Diagnosis and Management of Chronic Obstructive Pulmonary Disease (GOLD) (59) and the 2022 Lancet Commission (89)

have highlighted the relevance of risk factors different from tobacco smoke and have posed five types (etiotypes) of COPD and the need for a better characterization and understanding of the other types of COPD different from TS-COPD. Our study presents relevant information about a type of biomass COPD due to indoor air pollution derived from WS and highlights the need for research on the underlying mechanisms of the combined exposures in COPD and the pathophysiological ways of the biomass COPD.

Conclusions

This population-based study found that wood smoke is associated with COPD, both in women and in men, and showed that the combined exposure to wood and tobacco smoke is associated with a higher prevalence of COPD and with worse clinical and functional outcomes in patients with COPD. It showed that COPD associated with wood smoke exposure has demographic, clinical and functional characteristics different from COPD associated to tobacco smoke. These findings suggest that different risk factors could have different patterns of exposure-response and could induce different pathophysiologic pathways, which could interact in an additive or synergistic way. The implications in preventive, diagnostic and therapeutic interventions need oriented research.

Discussion Study 2. *"Sputum biomarkers in wood and tobacco smoke etiotypes of chronic obstructive pulmonary disease".*

This study, focused on selected sputum biomarkers in women with COPD caused by the exposure to two different risk factors (wood and tobacco smoke), had several novel findings. First, TS-COPD patients have significantly higher sputum levels of CCL5 than women with WS-COPD, a previously unreported observation with potential pathobiological significance. Second, there was a monotonic inverse correlation between the level of sputum biomarkers in COPD patients and the degree of airflow obstruction, suggesting a biological plausibility to our findings. Third, women with COPD, whether caused by tobacco smoke or wood smoke exposure, have higher levels of the inflammatory markers IL-8, MMP-9 and CCL5 than

healthy controls, supporting a common generic role of these cytokines in the genesis of these two etiotypes of COPD.

Different from TS-COPD, WS-COPD is consistently characterized by the presence of mild or total absence of emphysema (9, 10, 12, 38, 88). Our finding of a higher sputum level of CCL5 in TS-COPD than in WS-COPD could be related to the different expression of emphysema in these two subtypes of COPD, which is supported by previous studies. The CCL5 is a chemokine that attracts neutrophils and eosinophils and that has been thought to be involved in the pathogenesis of TS-COPD (90-93). In comparison with subjects without COPD, Di Stefano et al. found that the numbers of CCL5+ cells in the submucosa of patients with stable TS-COPD were 2 to 15 times higher than any other chemokines and also an increased expression of extracellular matrix-binding receptors on neutrophils (90). Similarly, Costa et al. showed that CCL5 was increased in sputum from TS-COPD patients compared with nonsmokers (94) while Grumelli et al. found a high percentage of CD4+ and CD8+ T lymphocytes that expressed CCR5 (the receptor for CCL5), a marker of T helper 1 cells, in patients with TS-COPD that were currently smoking vs ex-smokers (92). Interestingly, polymorphisms of CCL5 gene have been associated with the emphysema expression, with 28G allele genotype inversely associated with computed tomography score of emphysema (91). In addition, Kratzer et al. found an increase of CCL5 in a rat model of second-hand smoke induced emphysema (93). These observations are consistent with the higher sputum level of CCL5 in TS-COPD than in WS-COPD found in our study and could help explain the difference in the high degree of emphysema present in patients with TS-COPD and the low levels of emphysema observed in patients with WS-COPD. Our findings differ from those of Falfan-Valencia et al. who compared healthy women exposed to TS or WS and found that the serum CCL5 levels were higher in the individuals exposed to WS exposed vs. TS exposed (95). However, this study, which did not include COPD patients, measured the CCL5 in serum and not in sputum and the local and systemic responses could differ in the same individuals (96).

We did not find differences between WS-COPD and TS-COPD in the other tested cytokines: IL-8, MMP-9, CCL16/HCC-4 and VEGF. However, both groups had higher levels of the assessed biomarkers than non-COPD control group. The increased levels of cytokines have

been consistently observed in patients with stable TS or WS COPD compared to control groups (14, 83), but the described inflammatory profiles have exhibited wide heterogeneity, varying according with the population studied and the method used. The increased levels of IL-8 observed in the induced sputum (97) of COPD patients could be related to the increased Th1 and Th17 responses described as part of the COPD pathogenesis. MMP-9 is a gelatinase that promotes neutrophil chemotaxis and mediate inflammation, contributing to the develop of emphysema; multiple studies had shown an inverse correlation between MMP-9 and FEV_1 (98). In line with these previous findings, the present study confirms the presence of persistent lung inflammation with higher levels of most of the measured biomarkers in COPD, both in the WS or TS exposure.

One study showed that the sputum levels of VEGF-1 were linked to the chronic bronchitis or emphysema phenotype of TS-COPD (high in chronic bronchitis and low in emphysema) (99) and other one that its serum levels were higher in the group with COPD compared with the controls, but without differences between biomass COPD and TS-COPD (95). In our study, there was a trend towards increased sputum levels of VEFG-1 in the COPD group (Supplement Table E1) in comparison with the control group without differences between the COPD groups exposed to biomass or tobacco. Although CCL16/HCC-4 levels have been described as being high in COPD (100), we did not find elevated levels of this cytokine in patients with COPD compared to the controls, nor differences between WS-COPD and TS-COPD.

A second important finding of our study is that of a monotonic inverse correlation between the level of sputum biomarkers in COPD patients and the degree of airflow obstruction. Moreover, when we compared the obstruction severity with the levels of CCL5, we observed a moderate, but significant, negative correlation between the FEV_1 and the CCL5 levels (Table 3), corroborating that CCL5, at least in part, could mediate a deleterious response on the natural history in COPD patients (90). We also found an inverse correlation between the levels of MMP-9, IL-8, VEGF-1 and FEV_1%. This relationship suggests a potential

etiological role of the cytokine itself or of the mechanisms responsible for the cytokine generation and its relationship with chronic airflow obstruction. Previous studies have described that the levels of TNF-α, IL-1β and IL-6 are directly proportional to the post-bronchodilator FEV_1 % (101). A recent study focused on biomarker-based clustering of patients with COPD showed that the degree of airflow limitation was comparable between clusters, indicating a limited value of relating airflow limitation in predicting systemic levels of the biomarkers and viceversa (102).

Our third finding was that women with COPD, whether caused by tobacco smoke or wood smoke exposure, had higher levels of IL-8, MMP-9 and CCL5 in sputum than healthy controls, confirming previous observations that have documented similar results and indicating the activation of inflammatory and lung damage mechanisms in COPD (67, 100, 103, 104).

Our study has several strengths. First, its prospective inclusion of strictly selected women with stable COPD and healthy controls, thus eliminating the usual gender bias, the clear classification of the two types of causes and the selection of representative inflammatory, lung damage and vascular compromise biomarkers. However, there were also some limitations. First, the study was cross-sectional, reflecting a moment in a patient history, thereby not allowing the establishment of a temporal relationships or causal assumptions. Second, we had a relatively small sample size, which does not permit to completely exclude a role in COPD pathogenesis for those cytokines that showed a trend but did not reach the level of statistical significance. Finally, although only a small selected group of biomarkers were tested, they represent some of the best studied biomarkers that have shown some relationship to COPD.

In conclusion we found higher levels of CCL-5 in the sputum of women with TS-COPD than in WS-COPD. This finding could be of importance to help explain a pathophysiological relation between the higher frequency of emphysema in TS-COPD compared to WS-COPD. We also found a significant elevation of the sputum levels of CCL-5, MPP-9 and IL-8 in women with either tobacco or wood smoke related COPD compared to a healthy control group. The levels of such cytokines correlated inversely with the degree of airflow obstruction, providing some plausible common role in both etiotypes of COPD. More studies

are required to clarify the role of such cytokines to explain the similarities and differences between TS-COPD and WS-COPD.

Discussion Study 3. "*Small airways disease in COPD associated to biomass exposure".*

This article non-structured review of evidence about the affectation of the airways due to the intra-domiciliary chronic exposure to wood smoke used for cooking showed that the chronic exposure causes an inflammatory compromise both the large and the small airways. Growing evidence confirms the significant affectation of small airways in WS-COPD the which could be detected by new techniques of imaging and functional tests. Remarkably, emphysema is mild or absent. Different from the tobacco smoke and tobacco exposure, the composition and distribution of the size of particles is more heterogeneous in wood smoke and the pattern of inhalation is based on a usual tidal respiration. These characteristics could explain that central anthracofibrosis is more frequent in WS-COPD than in TS-COPD, although recent studies have focused on the small airways' damage in WS-COPD.

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The effect on the small airway of biomass, including smoke from burning wood, is as harmful as that caused by smoke in the lung parenchyma. Therefore, WS-COPD seems equally damaging than TS-COPD. The earlier exposure occurring in WS-COPD cause a direct impact on lung growth that could damage mainly the small airway. The histological and tomographic findings in WS-COPD show a more intense small airway damage in comparison with TS-COPD. However, people with WS-COPD, particularly women, have a higher baseline FEV_1 and the decline of FEV_1 seems lower and not as detrimental as that induced by tobacco. Even so, WS-COPD patients have significant symptoms and a worse quality of life than smokers.

4. GENERAL CONCLUSION

The exposure to biomass smoke, frequently wood smoke, is the biggest risk factor for COPD. Almost 3 billion people in the world continue using biomass fuels. WS-COPD is a frequent condition still not-well characterized. With these three studies we add novel information about the sociodemographic, clinical, functional, and imaging characteristics of patients with WS-COPD in comparison with TS-COPD and about of the effect of the combined exposure to both wood and tobacco smoke.

These studies found that the exposure to wood smoke (WS) for 10 years or more is a risk factor of COPD not only in women, but it is one of the largest studies in showing that this exposure is a risk factor also in men and the first one, as population-based study, in showing that people exposed to both WS and tobacco smoke (TS) (the combined exposure) have a significantly higher prevalence of COPD and CB than those exposed to only WS or TS. We also found that people and women with WS-COPD are older and have significantly shorter height, higher BMI, and lower educational level than TS-COPD.

Innovatively the first study showed that people with COPD exposed to both WS and TS (MS-COPD) have more frequently persistent respiratory symptoms (cough and phlegm) and significantly greater airflow limitation (lower post-bronchodilator FEV_1% and FEV_1/FVC%) than those exposed to only WS or TS. The analysis in women with COPD exposed to WS additionally showed more frequent occurrence of dyspnea.

The second study**,** focused on selected sputum biomarkers, showed that women with WS-COPD have significantly lowers sputum levels of CCL5 than women with TS-COPD, a previously unreported observation with potential pathobiological significance. We also found a monotonic inverse correlation between the level of sputum biomarkers in COPD patients and the degree of airflow obstruction. Finally, we found that women with COPD, whether caused by tobacco smoke or wood smoke exposure, have higher levels of the inflammatory markers IL-8, MMP-9 and CCL5 than healthy controls, supporting a common generic role of these cytokines in the genesis of these two etiotypes of COPD.

The third study, a non-structured review of evidence about the affectation of the airways due to the intra-domiciliary chronic exposure to wood smoke used for cooking, showed that the chronic exposure causes an inflammatory compromise both the large and the small airways. Growing evidence confirms the significant affectation of small airways in WS-COPD the which could be detected by new techniques of imaging and functional tests. Remarkably, emphysema is mild or absent.

The studies being part of this thesis contribute to the knowledge about the chronic disease caused by wood smoke, which in some cases could be named COPD (WS-COPD). Long-term indoor exposure to wood smoke affects mainly women and produces a disease significantly different from that caused by tobacco smoke with inflammatory compromise of airways (bronchitis, bronchial anthracofibrosis and bronchiolitis [small airways disease]) and mild or not emphysema. The lower level of CCL5 in comparison with TS-COPD suggests a different pathophysiological pathway which could be related with some different composition of the wood smoke and/or the pattern of inhalation of WS. The combined exposure to WS and TS increase the risk of COPD and produces more symptoms and greater airflow obstruction than the isolated exposure to WS or TS.

The results of these studies could improve the diagnosis and management of WS-COPD. Chronic exposure to WS will continue being a huge problem of public health for long time that justify to strength the research on this field, particularly on the pathophysiology of WS-COPD, the impact of the combined exposure to both wood and tobacco smoke and the potential interventions for prevention and treatment of respiratory diseases caused by household air pollution derived from biomass exposure, including WS-COPD.

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