Comparative analysis of COPD associated with tobacco smoking, biomass smoke exposure or both

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Abstract

Background: Exposure to noxious gases and particles contained in both tobacco smoking (TS) and biomass smoke (BS) are well recognized environmental risk factors for chronic obstructive pulmonary disease (COPD). COPD is characterized by an abnormal inflammatory response, both in the pulmonary and systemic compartments. The differential effects of TS, BS or their combined exposure have not been well characterized yet. This study sought to compare the lung function characteristics and systemic inflammatory response in COPD patients exposed to TS, BS or their combination.

Methods: Sociodemographic, clinical and lung functional parameters were compared across 49 COPD patients with a history of smoking and no BS exposure (TS COPD), 31 never-smoker COPD patients with BS exposure (BS COPD), 46 COPD patients with a combined exposure (TS + BS COPD) and 52 healthy controls (HC) who have never been exposed neither to TS or BS. Blood cell counts, C-reactive protein (CRP), fibrinogen and immunoglobulin E (IgE) levels were quantified in all four groups.

Results: TS + BS COPD patients exhibited significantly lower oxygen saturation than the rest of groups (p < 0.01). Spirometry and diffusing capacity were significantly higher in BS than in TS or TS + BS patients. CRP levels were significantly higher in TS COPD patients than in BS COPD group (p < 0.05), whereas fibrinogen was raised in COPD patients with a history of smoking (TS and TS + BS) when compared to control subjects (p < 0.01). Finally, COPD patients with BS exposure (BS and BS + TS groups) showed higher IgE levels than TS and HC (p < 0.05).

Conclusions: There are significant physiological and inflammatory differences between COPD patients with TS, BS and TS + BS exposures. The latter had worse blood oxygenation, whereas the raised levels of IgE in BS exposed patients suggests a differential Th2 systemic inflammatory pattern triggered by this pollutant.

Keywords: COPD, Biomass smoke, Immunoglobulin E, Oxygen saturation, Systemic inflammation, Indoor pollution
and pulmonary fibrosis and less emphysema than TS COPD patients [8–10].

The evidence of different pathologic features in COPD patients exposed to BS or TS underpin the existence of a third potential phenotype which remains largely uncharacterized: COPD patients with a significant exposure to both TS and BS (TS + BS COPD). Given the toxicity of both kinds of smoke, a higher severity of pulmonary damage could be expected in double-exposed subjects. Following this line of thought, Dogan et al. [11] compared lung histopathological alterations among rats exposed exclusively to TS, those exclusively exposed to BS and a group with combined exposure to TS and BS. They found an increased severity in perivascular inflammation, as well as higher parenchymal infiltration, number of alveolar macrophages and vascular wall thickness in lungs of rats exposed to both BS and TS than in those only exposed to BS [11]. In turn, with data from the PREPOCOL study [12], Torres-Duque et al. [13] compared some demographical and functional parameters among 114 COPD patients exposed to wood smoke, 81 COPD patients exposed to TS and 264 COPD patients exposed to both types of smoke, reporting significant decreases in FEV₁ and FEV₁/FVC values in patients with a combined exposure [13]. In both studies, the authors suggested that a combined exposure to TS and BS could have a noxious additive effect in COPD patients and could increase the frequency of COPD symptoms.

On another front, although COPD is primarily a disease of the lungs and airways, it also has several extra-pulmonary manifestations and comorbidities. Indeed, systemic inflammation is a hallmark of COPD and it may be involved in cardiovascular complications in COPD patients [14]. Accordingly, increased numbers of inflammatory cells have been reported not only in small airways and lung parenchyma of COPD patients [15–17], but also in peripheral blood [18, 19]. Moreover, several molecular inflammatory markers are also increased in blood of COPD patients. One of the most widely studied is C-reactive protein (CRP), an acute-phase reactant secreted by the liver during inflammatory processes, which is increased in both stable and exacerbated COPD patients [20]. This biomarker has also proved to predict mortality outcomes in subjects with COPD [21]. COPD patients show higher blood levels of other inflammatory markers, such as interleukine (IL)-6, IL-8, tumor necrosis factor (TNF)-α and fibrinogen when compared to control subjects [22, 23]. Regarding the last one, it has been shown that fibrinogen is associated with disease severity and can also predict risk of exacerbations and mortality in COPD patients, thus highlighting its importance in predicting clinical outcomes [24, 25]. Notwithstanding, the role of other classical biomarkers of inflammation in COPD remain elusive. This is case of immunoglobulin E (IgE), which is mostly associated with asthma-COPD overlap syndrome (ACOS) [26], although it has also been shown to be increased in non-atopic COPD patients [27]. In spite of the importance of systemic inflammatory biomarkers in the understanding of COPD, data on systemic inflammation profile in BS COPD is scarce and no study has assessed these biomarkers in TS + BS COPD patients so far.

The purpose of the present study is to elucidate whether there are differences in clinical, functional and systemic inflammatory markers among TS, BS and TS + BS COPD patients.

Methods

Study population

We studied 126 COPD patients and 52 healthy volunteers recruited at Hospital Regional de Talca, Talca, Chile. The Ethics Committees of Maulean Health Service and Universidad Autónoma de Chile approved the study and written informed consent was obtained from all the subjects.

COPD patients were recruited at the Respiratory Service of the Hospital, where they attended to undergo diagnostic tests after suspected COPD or for COPD monitoring visits. Control subjects were recruited in parallel at the same ward through a volunteer recruitment program. After clinical characterization and risk factors assessment, subjects were classified into four groups: 49 TS COPD patients (ex-smokers with no BS exposure), 31 BS COPD patients (never-smokers with BS exposure), 46 TS + BS COPD patients (subjects with a combined exposure to TS and BS) and 52 controls, consisting in subjects with no record of any specific illness and with neither smoking history nor biomass smoke exposure.

Diagnostic evaluation of subjects was conducted according to GOLD criteria [1] and medical history was considered standardizing clinical information. Standard procedures [28] and equipment (Masterlab; Jaeger, Würzburg, Germany) were used to assess pulmonary function in all patients, including measurements of forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC) and carbon monoxide diffusing capacity of the lung (DLCO). Oxygen saturation was measured in all patients by pulse-oximetry (Ohmeda TuffSat, Soma Technology, Connecticut, USA). Body mass index (BMI) was calculated using the current weight and the tallest height of each study participant. Dyspnea was determined using the modified Medical Research Council scale (mMRC) and exercise capacity was determined with the distance walked in 6 min test (6MWT). Health-related quality of life and symptom burden in patients with COPD was quantified using the COPD assessment test (CAT). A Body-mass, airflow Obstruction, Dyspnea and Exercise (BODE) score was assigned to each COPD
Results

Demographic and clinical findings
Table 1 presents the main clinical and functional characteristics of study subjects. The Chi-square test revealed significant differences in gender proportions between control subjects and smoking COPD groups (TS and TS + BS) as well as between TS COPD and BS COPD ($p < 0.05$). The four groups had similar age. TS and BS exposures were similar in TS COPD and BS COPD and TS + BS COPD, respectively. HS completed a significantly higher number of schooling years as compared to COPD patients. Furthermore, TS COPD patients showed a significantly higher average of schooling years when compared to BS-exposed groups. HC also had a significantly higher BMI value than COPD patients. There were no significant differences in the number of exacerbations in the previous year among COPD groups.

Lung function
COPD patients had a significantly reduced FEV$_1$ and FEV$_1$/FVC than HC. These parameters were significantly higher in BS than TS or double-exposed patients (Table 1). Likewise, BS COPD patients also showed a higher DL$_{CO}$ than other COPD groups. Interestingly, oxygen saturation was significantly reduced in TS + BS COPD patients when compared to the rest of groups ($p < 0.01$). Finally, no differences among COPD groups were found in 6 MW test, mMRC, CAT and BODE index.

Systemic inflammatory markers
In order to determine whether the systemic inflammatory response was different among TS, BS and TS + BS COPD patients, blood levels of CRP, fibrinogen and immunoglobulin E were assessed (Fig. 1).

TS COPD patients showed a significantly higher level of CRP than BS COPD patients and control subjects ($7.56 \pm 2.68 \text{ vs } 3.42 \pm 1.90 \text{ and } 1.79 \pm 1.10 \text{ mg/l, respectively} \ (p < 0.05, \text{ Fig. 1a})$). Blood levels of fibrinogen were significantly increased in TS and TS + BS COPD patients when compared to control subjects ($392.22 \pm 106.38 \text{ and } 378.85 \pm 84.27 \text{ vs } 319.81 \pm 70.52 \text{ mg/dl} \ (p < 0.01, \text{ Fig. 1b})$). However, no significant differences in this biomarker were found between BS COPD and control subjects. Importantly, total IgE levels were significantly increased in BS and TB + BS COPD groups ($492.87 \pm 127.89 \text{ and } 454.19 \pm 140.69 \text{ UI/ml, respectively} \text{ as compared to TB COPD patients and control subjects (81.23 \pm 50.34 \text{ and } 60.57 \pm 33.76 \text{ UI/ml, respectively} \ (p < 0.05, \text{ Fig. 1c})$. On the other hand, there were no significant differences in erythrocytes, platelets and basophils among COPD groups (Table 2). However, COPD patients had higher numbers of neutrophils and lymphocytes than control subjects ($p < 0.01$). Furthermore, total leucocyte...
and monocyte counts were significantly higher in COPD patients with a history of smoking than in BS COPD and control subjects \((p < 0.05 \text{ and } p < 0.01, \text{ respectively, Table } 2)\). Finally, the neutrophil-to-lymphocyte ratio (NLR) was significantly higher in COPD patients than control subjects \((p < 0.01)\).

### Correlations of interest

Some significant correlations between clinical and haematological parameters were found when all COPD patients were considered as one group. An inverse correlation was found between this CRP and FEV\(_1\) (% of predicted) \((R = -0.19, p < 0.05)\). FEV\(_1\) was also inversely correlated with total leucocyte counts \((R = -0.19, p < 0.05)\), as well as with neutrophils \((R = -0.24, p < 0.05)\) and monocytes \((R = -0.27, p < 0.01)\) counts. Moreover, exercise capacity was inversely correlated with fibrinogen and IgE \((R = -0.59, p < 0.01 \text{ and } R = -0.63, p < 0.01, \text{ respectively})\) as well as with eosinophils \((R = -0.47, p < 0.05)\). Regarding BODE index, it was significantly correlated with fibrinogen \((R = 0.59, p < 0.01)\) and IgE \((R = 0.53, p < 0.05)\).

With respect to exposure to risk factors, significant correlations were found between pack-years and eosinophils \((R = 0.30, p < 0.05)\) and neutrophils \((R = 0.27, p < 0.05)\) counts, as well as with NLR ratio \((R = 0.26, p < 0.05)\). Moreover, the cumulative exposure to biomass smoke was significantly correlated with total blood leucocytes \((R = 0.69, p < 0.01)\), neutrophils \((R = 0.78, p < 0.01)\) and lymphocytes \((R = 0.55, p < 0.05)\) counts, in addition to NLR ratio \((R = 0.52, p < 0.05)\). Finally, no significant correlation was found between tobacco pack-years or biomass cumulative exposure and lung function parameters.

### Discussion

The results of this study show that there are significant clinical, functional and systemic differences between COPD patients exposed to TS, BS or both. In general, lung function was more severely impaired in COPD patients exposed to TS, who also showed higher levels of fibrinogen, circulating leucocytes and monocytes, whereas COPD patients exposed to BS had significantly higher levels of blood IgE. These results suggest different inflammatory responses to different inhaled pollutants in COPD.

### Previous studies

To our knowledge, this is the first article comparing clinical, functional and blood parameters among TS COPD patients, BS COPD patients, COPD patients exposed to both risk factors and healthy subjects never exposed to either of them.

A previous study presented at the American Thoracic Society 2013 International Conference by Torres-Duque et al. [13], however, compared the effects of the combined exposure to TS and wood smoke in lung function of COPD patients. They reported significant decreases in FEV\(_1\) and FEV\(_1\)/FVC values in patients with a combined
exposure. Conversely, we found that FEV₁ and FEV₁/FVC values were similar between TS and TS + BS COPD group and significantly lower than in BS group. Since no data about amount of exposure to TS and BS was available in the study of Torres-Duque et al. [13], the differences in the double exposed group may be due to a higher exposure to TS and/or BS in these patients than in those with a single exposure. Moreover, the contradictory results between the study of Torres-Duque et al. and the present one could also be explained by the fact that they only considered wood smoke exposure, whereas we considered different sources of BS.

**Interpretation of findings**

Despite the Global Burden of Disease study reported 1.1 million COPD-related deaths for tobacco smoking and 850,000 to indoor pollution in 2010 [30], information about the characteristics of COPD patients with a combined exposure to both risk factors is virtually non-existent. The following novel observations emerge from our study:

First, although it is well established that lower socio-economic status and education level is associated with COPD [31–33], we observed that the number of years of scholarship was significantly reduced in BS COPD and TS + BS COPD patients when compared to TS COPD patients. This is likely related to the fact that the use of biomass as a source of energy is more widespread in rural areas, where also the access to education is more difficult, especially some decades ago.

Second, arterial oxygen saturation was significantly lower in double exposed COPD patients, suggesting an increased ventilation/perfusion mismatch in these patients [34]. Bearing in mind that both smoking [35] and exposure to some components of BS [36] have been related to a decrease in oxygen saturation, this result likely reflects an additive effect of both exposures, and it has important clinical implications, since an oxygen saturation cut-off value of ≤92% is considered when screening for respiratory failure in patients with COPD [37]. In spite of this, no other functional parameter was exclusively impaired in the TS + BS COPD group, albeit airflow limitation was more severe in TS COPD than in BS COPD patients, in keeping with some previous studies [4, 5, 7].

Third, CRP levels were significantly higher in TS than in BS COPD and HS. This contrasts with two previous studies reporting similar CRP levels in TS and woodsmoke exposed COPD patients [38, 39]. This discrepancy may be explained by the different techniques used in CRP quantification (ELISA and immunoturbidimetry) and the characteristics of the study populations. It’s worth to mention, however, that both in our study and in one of these previous ones [38] CRP levels were inversely related with FEV₁ values, as it has been reported in other TS COPD studies [40, 41]. Likewise, circulating levels of fibrinogen were also significantly higher in TS and TS + BS COPD patients than in HC, in keeping with some previous studies also reporting higher levels of fibrinogen in smokers [42, 43].
Fourth, circulating IgE levels were significantly higher in COPD groups with a BS exposure (BS and TS + BS) than in TS COPD and HC. Although it has been shown that particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs) contained in BS increase blood IgE levels [44–46], this is the first study reporting an increase of this inflammatory marker specifically in COPD patients exposed to BS. Since an increase of IgE production represents a typical feature of a Th2 response, this result suggests that BS could trigger a specific inflammatory Th2 response in COPD patients. In fact, Solleiro-Villavicencio et al. reported a higher frequency of Th2 cells and a higher concentration of IL-2 in blood of BS vs. TS COPD patients and HC [47]. Although quantification of lymphocyte subsets was not available in our study, it is noteworthy that cumulative BS was significantly correlated with total blood lymphocyte counts. On the other hand, the increased IgE levels observed in BS COPD patients could also underlie the higher prevalence of ACOS reported in COPD subjects exposed to BS [7]. Moreover, the transversal nature of the present study does not allow ruling out the possibility of other exposures capable of raising IgE levels, particularly in rural areas.

Of note, a previous study comparing IgE and fibrinogen between COPD patients exposed to TS and BS [39] reported similar blood levels of both biomarkers in both groups. We believe that this discrepancy can be explained by sample size, ethnic and sex differences [48] (75% of males in the cited study vs. 58% of males in the present one). Moreover, since the composition of BS depends on the type of fuel and the combustion conditions, BS exposure from different sources may lead to different clinical features.

Finally, our results showed significantly increased numbers of neutrophils, lymphocytes and NLR in all COPD groups vs. HC. This highlights a general a systemic inflammatory process mounted in response to the inhalation of noxious gases and particles in COPD, regardless of their specific type.

**Potential limitations**

The present study has several potential limitations. Firstly, the study population was relatively small. However, we believe that the rigorous characterization of subjects allowed establishing reliable comparisons among groups. Secondly, imaging and histopathological analyses which would have allowed the assessment of small airways thickening and emphysema were not available. Thirdly, patterns of exposure to BS and TS may have biased our results, since COPD patients with a smoking history were actually ex-smokers, whereas several BS exposed subjects reported a current use of biomass fuels. Fourthly, although subjects with a history of asthma and/or reversibility in post-bronchodilator FEV1 were excluded from our study, we cannot rule out the possibility of ACOS among our patients, since no unified definition or diagnostic criteria for ACOS exists. Finally, given that subjects exposed to BS exhibited a lower socioeconomic status and an ancestry characterization was not available, both environmental and genetic variables may have been a potential source of bias.

**Conclusions**

There is a synergistic damaging effect of the combined exposure to TB and BS. The systemic inflammatory response in these two types of COPD is, however, distinct. Given that BS and TS have many components in common, a further characterization at a molecular level would be necessary in order to elucidate which ones could be responsible of different inflammatory features, and in which concentrations.
Abbreviations
6MWT: Six minutes walking test; ACOS: Asthma-COPD overlapping syndrome; BMI: Body mass index; BODE: Body-mass, airflow Obstruction, Dyspnea and Exercise score; BS: Biomass smoke; CAT: COPD assessment test; COPD: Chronic obstructive pulmonary disease; CRP: C-reactive protein; DLCO: Carbon monoxide diffusing capacity; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; HC: Healthy controls; IgE: Immunoglobulin E; IL: Interleukine; mMRI: Modified Medical Research Council scale; NLR: Neutrophil-to-lymphocyte ratio; TNF: Tumor necrosis factor; TS: Tobacco smoke

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.

Authors’ contributions
Study design: JO, RSO, SJ, GV. Supplied samples: RSO, SJ, VP, EC. Generated data: JO, RSO, SJ. Analyzed data: JO. Manuscript writing: JO. Manuscript editing and critically reviewing: JO, SJ, VP, EC, GV, AA, RSO. JO had full access to all the data in the study, interpreted the data and prepared the manuscript independently, and had final responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study protocols were approved by the local ethics committees of the Universidad Católica de Chile, Av. Libertador Bernardo O’Higgins #340, 35420000 Santiago, Chile. 2Departamento de Salud Pública, Facultad de Medicina, Pontificia Universidad Católica de Chile, Av. Libertador Bernardo O’Higgins #340, 35420000 Santiago, Chile. 3Subdepartamento de Salud Laboral, Hospital Regional de Talca, 1 Norte #1990, 3460000 Talca, Chile. 4Instituto de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, 5 Poniente #1670, 3460000 Talca, Chile. 5Laboratorio de Salud Pública, Facultad de Medicina, Pontificia Universidad Católica de Chile, Av. Libertador Bernardo O’Higgins #340, 35420000 Santiago, Chile. 6Respiratory Institute, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) Universitat de Barcelona, Rosselló #149-153, 08036 Barcelona, Catalonia, Spain.

Consent of publication
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Competing interests
The authors declare that they have no competing interests.

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