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Women with COPD from biomass smoke have reduced serum levels of biomarkers of angiogenesis and cancer, with EGFR predominating, compared to women with COPD from smoking

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Abstract

The main causes of COPD are smoking (COPD-TS) and exposure to biomass smoke (COPD-BS), considered as different phenotypes. The association of COPD-TS with lung cancer (LC) is well established, but not in COPD-BS. Thus, we studied the serum concentration of cytokines that participate in inflammation, angiogenesis, and tumor progression, used frequently as LC biomarkers, in women with COPD-BS compared with COPD-TS (n = 70). Clinical and physiological characteristics and the serum concentration (multiplex immunoassay) of 16 cytokines were evaluated. The analysis revealed that women with COPD-BS were shorter and older, and had lower concentrations of 12 serum cytokines: 6 proinflammatory and angiogenic IL-6Rα, PECAM-1, leptin, osteopontin, prolactin, and follistatin; and 6 that participate in angiogenesis and in tumor progression FGF-2, HGF, sVEGFR-2, sHER2/neu, sTIE-2, G-CSF, and SCF. Notably, there was a significant increase in sEGFR in women with COPD-BS compared to women with COPD-TS. PDGF-AA/BB and sTIE-2 did not change. These findings suggest that women with COPD-BS have markedly decreased proinflammatory, angiogenic, and tumor progression potential, compared to women with COPD-TS, with sEGFR as the predominant mediator, which might reflect a differential pattern of inflammation in women exposed to BS, favoring the development of chronic bronchitis.

Keywords

Angiogenesis, biomass smoke, cytokines, COPD, sEGFR, tobacco smoking

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Introduction

Chronic obstructive pulmonary disease (COPD) is a pulmonary and systemic inflammatory process that causes progressive obstruction of the airflow in the lungs. The main risk factor for developing COPD is tobacco smoking (COPD-TS),[1] although exposure to biomass smoke (COPD-BS) has been suggested as one of the secondary causes, occurring mainly in developing countries associated with a low socioeconomic profile.[2] Currently these two forms of COPD are considered as distinct pathophysiological phenotypes. The clinical, pathophysiological, diagnostic, and prognostic factors, have been characterized, from both the genotypic and endotypic point of view.[2] Thus, it is important to highlight that BS exerts a physiopathogenic effect primarily in the airways, which leads to the development of chronic bronchitis more than pulmonary emphysema, prevailing the GOLD stages I–II; contrary to TS, whose effect is mainly in the lung parenchyma more than airways, thus inducing emphysema more frequently than chronic bronchitis, with a preponderance of GOLD stages III–IV.[1,2]

Given the two phenotypes of COPD, it is interesting to assess the participation of the different molecules involved in its physiopathogenesis; such is the case of the serum cytokines that intervene in inflammation and angiogenesis in COPD, as well as in tumor progression.

Tumor progression involves cell survival, proliferation, adhesion, and migration, characteristics of cancer diseases; but especially focused on lung cancer (LC); fundamentally because LC is one of the main comorbidities associated with the development of COPD, but basically with smoking, which has been widely documented.[3,4]

Worldwide, LC associated with smoking represents the main cause of death across genders, accounting for 14% of all new cancers, with one in every four deaths due to cancer. The two forms, small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), account for 85% and 15% of LC, respectively. In turn, NSCLC is classified into two subtypes, adenocarcinoma (ADC) and squamous cell carcinoma (SCC), which comprise 70% and 30% of cases, respectively.[5] Likewise, smokers with COPD are twice as likely to develop LC compared to smokers without COPD; therefore, LC is a frequent cause of death in patients with COPD-TS.[3,4] Worse still, when patients develop both LC and COPD together, their survival prognosis is lower, even lower than that of patients with LC without COPD.[4]

Angiogenesis is the formation of new capillary blood vessels from pre-existing ones, operating in vascular endothelial cells, stimulated by several physiological mediators.[4,6] Angiogenesis is involved in COPD in small airways and lung parenchyma, in combination with the epithelial-mesenchymal transition and remodeling of the extracellular matrix.[6] The pathogenic angiogenic mediators in COPD-TS have been widely documented,[3,4] however, those involved in COPD-BS are only incipiently known.[2]

Taking into consideration that angiogenesis is a common factor in the pathophysiology of both COPD and LC, it seems highly probable that there are molecular links that interconnect the two diseases, especially based on the interrelated comorbidity.[3,4] In this sense, it is relevant to know whether in women with COPD-BS there is a similar profile of mediators of inflammation and angiogenesis-related COPD-TS, which could eventually explain the potential of developing LC secondary to BS exposure, as occurs with COPD-TS. Consequently, the objective of this work was to determine whether some proinflammatory serum cytokines that could eventually participate as mediators of angiogenesis and tumor progression are similar in women with COPD-BS and women with COPD-TS.

Methods

Study population

The study was conducted following the Declaration of Helsinki. The protocol was approved by the Scientific, Bioethics, and Biosecurity Committees at INER (protocol INER: **B15-15**), in Mexico City. Thus, were selected 140 women with the clinical diagnosis of COPD, 70 women had COPD-BS and 70 COPD-TS. From May 2015 to May 2017, at the COPD clinic of the INER, in a regularly studied cohort.

Women had a clinical and functional diagnosis of COPD, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), which classifies COPD in four grades, according to the airflow limitation and severity of the symptoms.[1] The diagnosis of COPD was established by post-bronchodilator pulmonary function tests, including the percent predicted forced expiratory volume in the first second (FEV1%P), percent predicted FVC (FVC%P), and FEV1/FVC ratio <70%. Firewood was the only fuel used by COPD-BS, who came from low-income rural

and suburban regions in Mexico. Firewood was the fuel used for cooking in all COPD-BS.

Demographic, anthropometric, and clinical data were collected; as well as specific questionnaires on biomass exposure, where they were asked how many hours they were exposed to BS and the number of years of exposure; while smokers were asked how many cigarettes they smoked per day and for how many years. From these data, it was to calculate the tobacco index (pack-years = cigarettes smoked per day multiplied by the number of years smoking/20), and the biomass exposure index (hours-years = average number hours of exposure to BS per day multiplied by the number of years of exposure to BS. Besides, it was asked if they were an active smoker and if had been exposed to both factors, BS and TS.

Patients with COPD-TS were active smokers or quit smoking for less than 1 year before being enrolled in the study, and were not exposed to BS. None of the patients with COPD-BS was a smoker or had been exposed to TS. The COPD patients were clinically stable and without exacerbations for at least 6 weeks earlier the study.

The women did not have nodules suspicious for malignancy on simple chest CT scan, or pulmonary artery systolic pressure (PSAP > 40 mmHg) in the transthoracic echocardiogram. None of the recruited patients reported cardiovascular disease or type II *diabetes mellitus*.

Women with a history of chronic lung conditions such as asthma, tuberculosis, bronchiectasis, or any other non-pulmonary disease, and women with a history of exposure to both BS and TS, were excluded

Pulmonary function tests

The pulmonary function test applied to all women to diagnose COPD was pre- and post-bronchodilator spirometry. The diagnosis and classification of COPD were established, according to the exposure history following the procedures recommended by the American Thoracic Society/European Respiratory Society,[1] and the standard reference for Mexicans.[7] FEV_1%P, FVC%P, and FEV_1/FVC ratio were measured, using a Sensormedics dry seal spirometer (Yorba, Linda, CA, USA).

Blood samples

For each sample, 5 mL of whole blood was collected in anticoagulant-free tubes (BD Vacutainer, Becton, Franklin Lakes, NJ, USA), samples were collected in the morning with at least 8 h of fasting. The samples were incubated upright for 1 h at room temperature, for later centrifuging at 5000×g for 15 min at room temperature to obtain the serum, which was kept at –20°C until analysis.

Measurement of serum cytokine

Samples were assayed using the Bio-Plex Pro™ Human Cancer Biomarker Panel 1, 16-plex kit (171-AC500M; BioRad Inc., Hercules, CA, USA), which includes 16 magnetic bead-based assays to specifically measure interleukin 6 receptor alpha chain (IL-6Rα), platelet endothelial cell adhesion molecule 1 (PECAM-1), leptin, osteopontin, prolactin, follistatin, fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), soluble epidermal growth factor receptor (sVEGFR-1 and sVEGFR-2), soluble human epidermal growth factor 2 (sHER-2/neu), granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), platelet-derived growth factor-AB/BB (PDGF-AB/BB), soluble angiopoietin 2 receptor (sTIE-2), and soluble epidermal growth factor receptor (sEGFR); the assay followed the manufacturer's instructions. Data were analyzed using the Bio-Plex® 200 system. Cytokine concentration was expressed as median (interquartile range) of ng/ml of serum.

Statistical analysis

The normality of the data was evaluated using the Kolmogorov-Smirnov test, where it was detected that the serum concentrations of the tumor factors were mostly asymmetrical data so that all statistical comparisons of these (Student's t-test) were performed with logarithmically transformed data.

Continuous demographic and clinical data (gender, age, height, BMI, and tumor factors) were expressed as mean ± SD, while those of the serum concentration of cytokines were transformed with Ln (x) to adjust to the normal distribution, and expressed as Ln (natural logarithm) in ng/ml, median (interquartile range). All data were compared by Student's t-test. All analyses were performed in GraphPad Prism (v. 6.1, GraphPad Software, Inc., San Diego, CA, USA). A value of $P < 0.05$ was considered statistically significant.

Results

Characteristics of women in the study

The clinical data of women with COPD are shown in Table 1. The women in the COPD-BS group were

Table 1. Anthropometric, clinical, and physiological characteristics of the women in the study (n = 70).

Group Variable	COPD-TS Characteristics	COPD-BS
Age (years)	66.2 ± 7.79	73.77 ± 7.46*
Height (cm)	155.14 ± 7.92	146.24 ± 5.28*
Weight (kg)	63.49 ± 13.52	57.78 ± 11.68
BMI (kg/m^2)	6.52 ± 3.13	28.5 ± 5.28
Exposure characteristics		
Tobacco index (pack-years)	7.28 ± 11.43	0
Biomass smoke index (hour-years)	0	361 ± 177
Physiological characteristics		
FEV_1%P	52.40 ± 19.92	58.40 ± 14.80
FVC%P	79.49 ± 20.86	83.10 ± 16.62
FEV_1/FVC ratio	53.38 ± 12.62	56.87 ± 12.11
BODE index	3.45 ± 2.86	2.91 ± 1.94
GOLD grades	Case number (%)	
I	5 (7.14)	7 (10)
II	27 (38.57)	41 (58.57)
III	23 (32.86)	16 (22.86)
IV	15 (21.43)	6 (8.57)

Data are expressed as the mean ± SD. BMI, body mass index; BODE index, composite prognostic marker that predicts mortality in COPD patients, including BMI, airflow obstruction, dyspnea score and exercise capacity utilizing the 6-min walk distance (6MWD); COPD-BS, chronic obstructive pulmonary disease from exposure to BS; COPD-TS, COPD from exposure to tobacco smoke; FEV1%P, forced expiratory volume in the first second (% predicted); FVC%P, forced vital capacity (% predicted); GOLD, global initiative for chronic obstructive lung disease; hour-years = average number of hours of exposure to BS daily multiplied by the number of years of exposure to BS; pack-years = cigarettes smoke by day multiplied by the number of years smoking / 20; pack-years = cigarettes smoke by day multiplied by the number of years smoking / 20. *$P < 0.01$.

older and shorter than those in the COPD-TS group. The mean exposure to BS was 361 ± 177 h-years, and the accumulated average tobacco consumption was 37.28 ± 11.43 pack-years. The FEV1%P, FVC%P, and FEV1/FVC ratio (Table 1). Of the women with COPD-BS, 48 were in GOLD stage I–II and 22 in stage III–IV; of the women with COPD-TS, 32 were in GOLD stage I–II and 38 in stage III–IV.

Serum cytokine levels in women with COPD

Comparing the 16 serum cytokines evaluated among women with COPD-BS related to COPD-TS, it was observed that 13 cytokines showed significant differences. Six of these, which act mainly as pro-inflammatory and angiogenic factors, showed significantly lower concentration in the COPD-BS related to COPD-TS group: IL-6Rα, PECAM-1, leptin, osteopontin, prolactin, and follistatin (Table 2, Figure 1(A) to (F)). Likewise, six cytokines that act as mediators of angiogenesis and function as tumor progression factors showed a significant decrease: FGF-2, HGF, sVEGFR-2, sHER2/neu, G-CSF, and SCF, also in the COPD-BS comparatively with the COPD-TS group (Table 2, Figure 2(A) to (F)). Two cytokines, PDGF-AA/BB and sTIE-2, did not show significant changes between groups (Table 2, Figure 3(A) and (B)). VEGFR-1 was not detected. Finally, and importantly, the only cytokine that showed an increase in the COPD-BS group compared to the COPD-TS group was sEGFR (Table 2, Figure 3(C)).

Discussion

Our objective was to determine whether 16 serum cytokines that participate in inflammation, angiogenesis, tumors progression and metastasis, show similar levels in women with COPD-BS compared with COPD-TS. Thus, in COPD-BS were decreased 12 cytokines: six that participate as proinflammatory and angiogenic factors, IL-6Rα, PECAM-1, leptin, osteopontin, prolactin, and follistatin; and six that participate as angiogenic and tumors progression factors, FGF-2, HGF, sVEGFR-2, sHER2/neu, sTIE-2, G-CSF, and SCF. Notably, sEGFR was the only increased in women with COPD-BS.

Regarding the decrease in the 12 cytokines, it is pertinent to mention that several studies of patients with COPD-TS showed that for most of the cytokines

Table 2. Serum concentration of cytokines IL-6Rα, PECAM-1, leptin, osteopontin, prolactin, and follistatin, FGF-2, HGF, sVEGFR-2, sHER2/neu, sTIE-2, G-CSF, SCF decreased in women with COPD-BS compared to women with COPD-TS, sEGFR increased, and PDGF-AA/BB and sTIE-2 did not change (n = 70).

	Angiogenic factor (ng/ml)	COPD-TS	COPD-BS	P-value
Proinflammatory factor	IL-6Rα	5.838 (1.527–3.93)	2.242 (0.82–4.089)	0.0001
	PECAM-1	1.976 (0.878–3.0)	1.378 (0.516–2.716)	0.0001
	Leptin	2.119 (−1.402 to 4.373)	1.542 (−1.141 to 3.480)	0.001
	Osteopontin	2.257 (−0.544 to 4.685)	1.529 (−0.817 to 3.982)	0.0001
	Prolactin	2.225 (−1.608 to 4.117)	1.409 (−2.479 to 4.101)	0.0001
	Follistatin	0.448 (−3.487 to 0.164)	−0.744 (−3.81 to 0.700)	0.01
Tumor progression factor	FGF-2	−1.99 (−4.145 to −0.308)	−2.17 (−4.165 to −0.441	0.01
	HGF	0.746 (−0.901 to 2.246)	0.328 (−2.136 to 1.937)	0.0001
	sVEGFR-2	2.346 (0.119–3.754)	1.222 (−0.898 to 3.249)	0.0001
	sHER-2/neu	2.042 (0.894–3.582)	1.601 (−0.476 to 3.031)	0.0001
	G-CSF	−1.905 (−4.395 to −0.433)	−2.672 (−5.86 to −1.324)	0.0001
	SCF	−1.280 (−3.275 to 0.4461)	2.007 (−4.536 to −0.839)	0.0001
	PDGF-AB/BB	−0.0216 (−2.974 to 1.735)	−0.234 (−2.951 to 1.681)	N.S.
	sTIE-2	2.589 (1.811–3.585)	2.593 (−0.776 to 4.362)	N.S.
	sEGFR	2.534 (−0.384 to 4.205)	9.067 (0.891–24.22)	0.0001

Data are presented as Ln of ng/ml, median (interquartile range), comparing women with COPD-BS and COPD-TS. COPD-BS, chronic obstructive pulmonary disease from BS exposure; COPD-TS, COPD from smoking; FGF-2, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; IL-6Rα, interleukin-6 receptor alpha chain; PDGF-AB/AA, platelet-derived growth factor-AB/AA; PECAM-1, platelet endothelial cell adhesion molecule-1; sEGFR, soluble epidermal growth factor receptor; sHER-2/neu, soluble human epidermal growth factor 2; SCF, stem cell factor; sTIE-2, soluble angiopoietin-2 receptor; sVEGFR-2, soluble vascular endothelial growth factor receptor 2. P-values are indicated in each line.

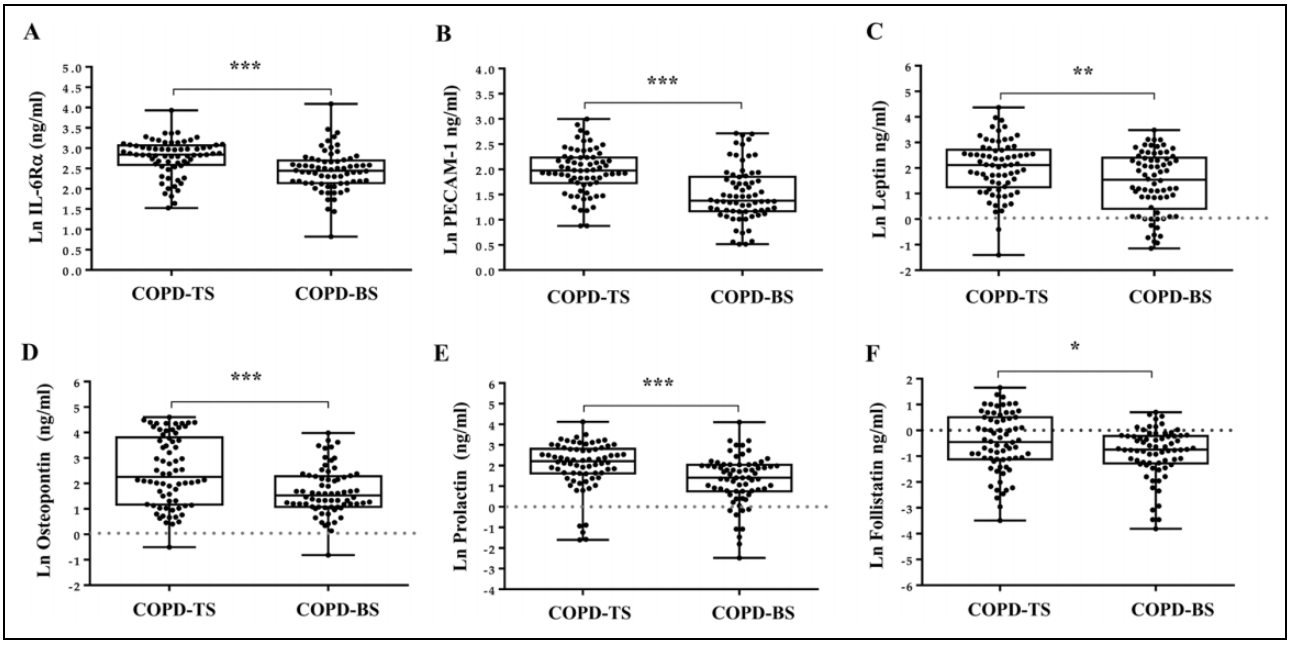

Figure 1. IL-6Rα, PECAM-1, leptin, osteopontin, prolactin, and follistatin, which participate as proinflammatory and angiogenic factors, showed lower serum concentrations in women with COPD-BS than women with COPD-TS. Bars represent Ln of ng/mL, median (interquartile range) (n = 70). (A) IL-6Rα, (B) PECAM-1, (C) leptin, (D) osteopontin, (E) prolactin, and (F) follistatin. COPD-BS, chronic obstructive pulmonary disease from BS exposure; COPD-TS, COPD from smoking; IL-6Rα, interleukin-6 receptor alpha chain; PECAM-1, platelet endothelial cell adhesion molecule 1. *P < 0.01, **P < 0.001, ***P < 0.0001.

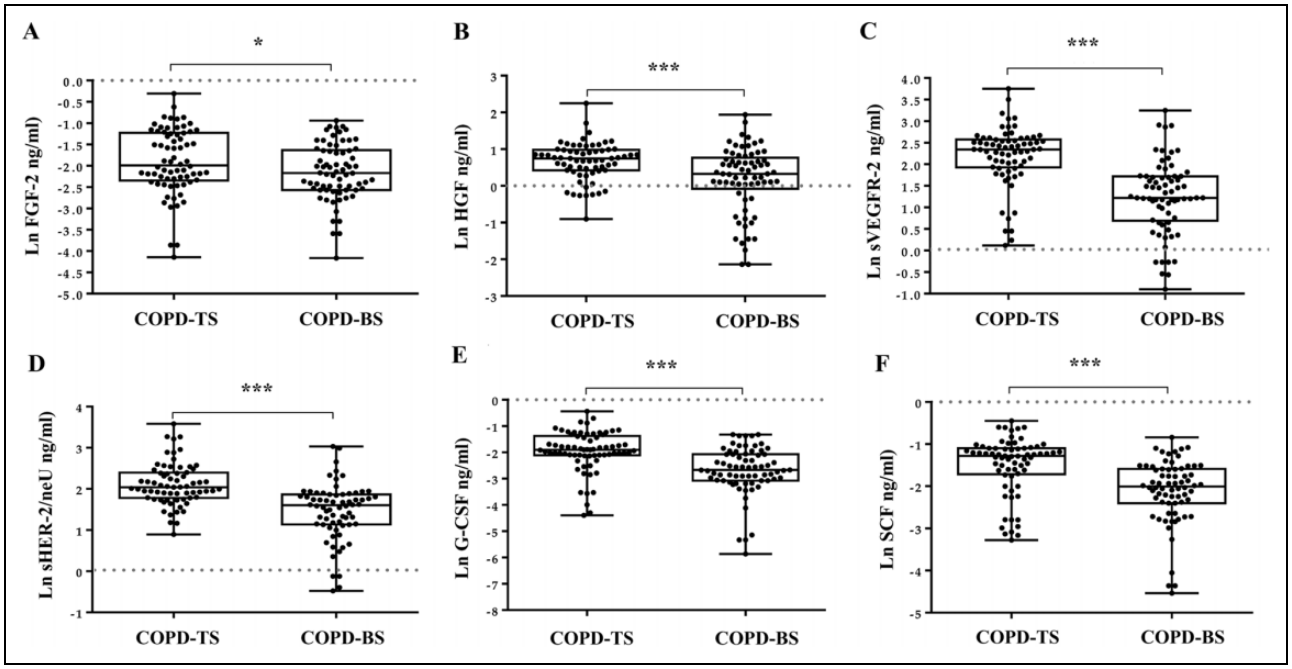

Figure 2. FGF-2, HGF, sVEGFR-2, sHER-2/neu, G-CSF, and SCF, which participate as angiogenic and tumor progression factors, showed lower serum concentrations in women with COPD-BS than women with COPD-TS (n = 70). Bars represent Ln of ng/mL, median (interquartile range). (A) FGF-2, (B) HGF, (C) sVEGFR-2, (D) sHER-2/neu, (E) G-CSF, and (F) SCF. COPD-BS, chronic obstructive pulmonary disease from exposure to BS; COPD-TS, COPD from smoking; FGF-2, fibroblast growth factor; HGF, hepatocyte growth factor; sVEGFR-2, soluble vascular endothelial growth factor receptor 2; SHER-2/neu, soluble human epidermal growth factor 2; G-CSF, granulocyte colony. *P < 0.01, ***P < 0.0001.

that were observed with low serum levels in COPD-BS, decreased levels were found in patients with COPD-TS when compared to normal subjects,[5,8–23] which was associated with increased angiogenesis and the development of LC,[3,4] so we will explain their relevance in this context.

sIL-6 participates in inflammation, angiogenesis, tumors promotion, progression, and metastasis in lung and other organs;[5,11] being the level of circulating IL-6 a prognostic marker of poor survival in advanced NSCLC. Increased serum levels of IL-6 are associated with the severity of COPD-TS and the prevalence of cachexia.[5]

PECAM-1 can induce the release of TIMP-1 from the endothelium into the tumor microenvironment, exacerbating angiogenesis, leukocyte migration, activation of integrins, and proliferation of lung tumor cells.[9]

Serum leptin has been associated with the severity and exacerbation of COPD and the development of LC.[9]

Osteopontin activates receptors associated with signaling pathways involved in the immune response, migration, angiogenesis, tumor progression and metastasis in LC.[10]

Prolactin participates in human T cell-mediated immunity; its plasma level increases in patients with NSCLC, and contributes to the LC progression and metastasis, modifying the pulmonary metabolism.[11]

Follistatin participates in the apoptosis of tumor cells secreted by ADC cells into the plasma, and may be beneficial for the survival of these cells by neutralizing the action of activin A.[12]

FGF-2 is the most potent inducer of angiogenesis in airways and lungs, it acts synergistically with VEGF,[13] Tie-1, Tie-2, and erythropoietins.[14] FGF-2 is actively involved in the development of various cancers, including LC. FGF-1, FGF-2, and FGFR1 are overexpressed in the lungs of COPD-TS compared to healthy smokers.[15] Furthermore, FGF-2 is actively secreted by the majority of NSCLC cell lines, stimulating their proliferation in an autocrine manner.[16]

HGF is overexpressed in type II cells and neutrophils, while HGF and HGFR in ADC and bronchoalveolar carcinoma cells (a subtype of ADC), in response to tobacco smoke.[17]

VEGFR-2 concomitantly with VEGF is overexpressed in endothelial cells of arteries muscular pulmonary, associated to their enlargement and

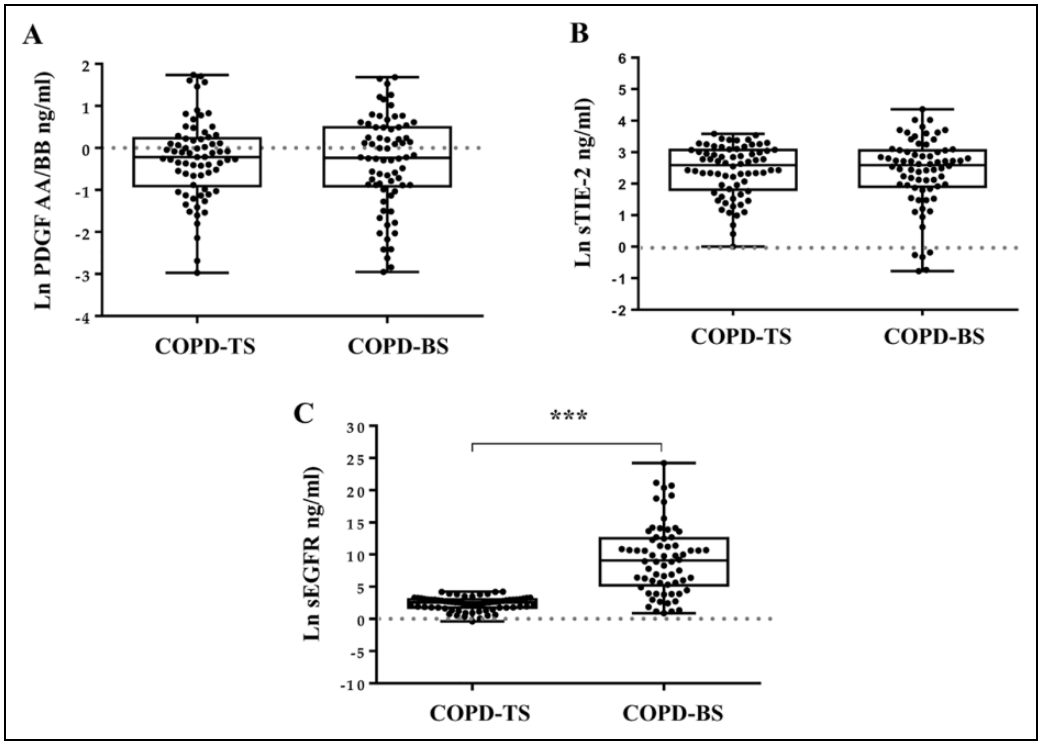

Figure 3. sEGF-2, which participate as angiogenic and tumor progression factors, exhibited higher concentration in the serum of women with COPD-BS compared to those with COPD-BS, while PDGF-AA/BB and sTIE-2 did not change ($n = 70$). Bars represent Ln of ng/mL, median (interquartile range). (A) PDGF-AA/BB, (B) sTIE-2, (C) sEGFR-2. Bars represent Ln of ng/mL (interquartile range). COPD-BS, chronic obstructive pulmonary disease from BS exposure; COPD-TS, COPD from smoking; PDGF-AB/AA, platelet-derived growth factor-AB/AA; sTIE-2, soluble angiopoietin-2 receptor; sVEGFR-2, soluble vascular endothelial growth factor receptor. ***$P < 0.0001$.

angiogenesis in COPD-TS with normal lung function compared to nonsmokers.[13,18] Furthermore, the VEGF1-3/VEGF-R system plays an important role in tumors growth and metastasis in LC and SCLC cell lines.[18]

HER-2, which is activated by the neuregulin-1 ligand, increases epithelial permeability in models of acute inflammatory lung injury induced by TS. HER-2 is secreted in cultured bronchial and epithelial cells from smokers;[19] additionally, serum sHER-2 increases more in patients with ADC than those with SCC, regardless of gender.[20]

Relative to G-CSF, it has been observed increased in the bronchoalveolar lavage fluid of COPD-TS patients,[21] associated with the presence of extreme leukocytosis, airways inflammation, systemic inflammation, right heart hypertrophy, and destruction of lung tissue in LC.[20,22]

Finally, SCF increased in patients with COPD-TS compared to controls, and it participates directly in metabolic tissue turnover in the airways and lung parenchyma, maintaining the deterioration of lung histoarchitecture.[23]

In a global context, considering the participation of these 12 cytokines in LC, including inflammation, angiogenesis, and tumors progression in COPD-BS women; and also taking into consideration the existence of mounting evidence that exposure to BS increases LC risk,[24] because of contains well-known carcinogenic substances, comprising benzene and benzopyrene which are also found in TS, that our findings might reflect a differential pattern of inflammation in women exposed to COPD-BS, such as has been reported previously.[25]

Concerning the high serum concentration of sEGFR in COPD-BS compared to COPD-TS, it is important to point out its possible relevance in the context of the LC, and the COPD phenotypes, because one of the main characteristics in COPD-BS is chronic mucus hypersecretion, which is closely associated with EGF-EGFR activity at the level of the airways.[1–3] sEGFR overexpressing in the epithelium

of the airways in COPD-TS, and has been associated with the development of LC due to changes in its gene regulation; but very important due to its mutations associated with the activation of cell transformation, which are the second most common oncogenic driver event in ADC and NSCLC.[26]

It is known that TS induces overexpression of the EGF/EGFR tyrosine kinase receptor (EGFR-TKI) cascade in the airways, resulting in goblet cell hyperplasia, and the subsequent synthesis and mucous hypersecretion by epithelial goblet cells and submucosal glands of the respiratory tract. MUC1, MUC2, MUC5AC, and MUC5B are the major mucins produced by TS.[27]

The level of EGFR expression is positively correlated with the level of goblet cell hyperplasia. Furthermore, there is an inverse association in increased goblet cells with decreased FEV1%P.[25] EGFR overexpression is also stimulated by ligands that bind to EGFR including TGF-alpha, VEGF, and TNF-α. Hypoxia-inducible transcription factor 1 (HIF-1) stimulated downstream of EGFR, which upregulates many genes, including VEGF, to induce mucus production. HIF-1 also binds to the MUC5AC promoter, increasing MUC5 AC expression, and promoting goblet cell hyperplasia.[27,28] Furthermore, the reactive oxygen species derived from neutrophils and oxidative stress caused by TS.[28]

This well-known mechanism for TS has not been established for BS, although goblet cells and bronchial mucus increase, it is not known which mucins are overexpressed. Possibly, BS can stimulate a mechanism similar to that described for TS in EGF-EGFR, but this has not yet been investigated.

BS predominantly induces effects in the airways, rather than in the lung parenchyma, which is why the development of chronic bronchitis is more common than emphysema, contrary to TS, which primarily affects the lung parenchyma, inducing emphysema.[2,3] In this sense, the presence of higher levels of sEGFR in women with COPD-BS is maybe related to the phenomenon of more abundant mucus, which could explain the prevalence of chronic bronchitis,[2,3] although this assertion, considered a priori, requires further analysis.

Taking the previously mentioned aspects in context, we can indicate that our findings suggest that in women with COPD-BS there is a cytokine profile that contributes to lower pro-inflammatory, angiogenic, and tumor progression potential than that observed in COPD-TS, with sEGFR as the predominant mediator, which in turn seems to contribute to the prevalence of airway damage, associated with mucus production in these women exposed to BS.

Conclusions

The concentration of serum cytokines involved in pro-inflammatory and angiogenic mechanisms that are promoters of tumor growth is decreased in women with COPD-BS compared to those with COPD-TS, with sEGFR being the only cytokine increased in COPD-BS concerning COPD-TS, which might reflect a differential pattern of inflammation in women exposed to COPD-BS, favoring the development of chronic bronchitis.

Limitations of the study

The limitations of this study are related to the heterogeneity of the study groups, given in terms of socioeconomic levels, ethnicity, environmental, and hereditary individual genetic susceptibility; because of the difficult to evaluate the effect of each factor in the pathophysiology of COPD phenotypes.[1,2] However, it has been documented in COPD cohorts similar to those studied here;[2,29] by multiple population-based studies, that lower, education, household income, and composite socioeconomic status index were all associated with higher odds of having COPD.2,30 Contrary, smoking women have a higher socioeconomic status index, with better social opportunities including education, household income, and medical service.[2,30]

Our analysis was performed on women since a domestic role is preparing meals, developing more damage in the small airways that men. However, it is pertinent to consider the sex differences in the development of LC and COPD due to exposure to BS and smoking.[2,29]

LC by Smoking is the world's leading cause of cancer death, especially in women, whose onset of the disease is at an earlier age than men (<50 years), being the age-adjusted incidence of LC higher in women than in never-smoker men. Interestingly, the LC death rate in non-smokers is 25% higher in men compared to women, suggesting distinct sex-based biology.[5,31,32]

Although the role of sex differences in LC risk is unclear, various evidence supports the notion that the epidemiology and biology of LC are distinct between the sexes including age, anatomy, histology, smoking pattern, exogenous and endogenous estrogens, viral

infection, and molecular alterations. Molecular alterations, including targetable and untargetable oncogene drivers, are more common in female LC patients, especially EGFR mutants. EGFR mutants are most frequent in lung ADC in women, occur in 21% of metastatic ADC and predict a 70% response rate to EGFR-TKI treatment, which women have increased survival advantage over men.[32] The smoking-related p53 mutations are more common in women, related to an increased susceptibility to tobacco carcinogen observed in smokers.[32]

Concerning to BS effect in LC, has been shown that the smoke derived from coal is a more potent lung carcinogen than the derived from wood, being the risk of LC greater in females compared to males, mainly causing ADC than squamous cell carcinoma and tumors of unspecified cell type, which suggest that in-home burning of both coal and wood smokers is consistently associated with an increased risk of LC.[33] Nevertheless, this study provides knowledge about potential physiopathological mechanisms that could denote a difference between COPD phenotypes.

Authors' note

All authors take responsibility for the content of the manuscript, including data and analysis.

Author contributions

MM, OPB, YVT, and CR were involved in conception and design. MM, OPB, YVT, GGA and CR were involved in analysis and interpretation. MM, OPB, YVT, GGA and CR were involved in acquisition of data. MM, YVT, and CR were involved in writing and revisions.

Availability of data and material

The data are available upon request.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval

The study was approved by the Science, Bioethics and Biosafety Committees of Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER) in Mexico City (protocol number INER: **B15-15**). All answered a written questionnaire and received and provided informed consent to participate in the study, agreeing to protocol approved by the Committees.

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References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). https://goldcopd.org/wp-content/uploads/2019/11/GOLD-2020-REPORT-ver1.0wms.pdf (2020, accessed 10 October 2020).
2. Camp PG, Ramirez-Venegas A, Sansores RH, et al. COPD phenotypes in biomass smoke-versus tobacco smoke-exposed Mexican women. *Eur Respir J* 2014; 43(3): 725–734.
3. Barreiro E, Bustamante V, Curull V, et al. Relationships between chronic obstructive pulmonary disease and lung cancer: *biological insights*. *J Thorac Dis* 2015; 8(10): E1122–E1136.
4. Eapen MS, Hansbro PM, Larsson Callerfelt AK, et al. Chronic obstructive pulmonary disease and lung cancer: underlying pathophysiology and new therapeutic modalities. *Drugs* 2018; 78(16): 1717–1724.
5. White JP. Cancer and cachexia: metabolic dysfunction creates the perfect storm. *Transl Cancer Res* 2017; 6(2): S280–S285.
6. Siegel RL, Miller KD, Fedewa SA, et al. Cancer statistics. 2017. *CA Cancer J Clin* 2017; 67(3): 177–193.
7. Pérez-Padilla JR, Regalado-Pineda J and Vázquez-García JC. Reproducibility of spirometry in Mexican workers and international reference values. *Salud Publica Mex* 2001: 43(2): 113–121.
8. Farahi N, Paige E, Balla J, et al. Neutrophil-mediated IL-6 receptor trans-signaling and the risk of chronic obstructive pulmonary disease and asthma. *Hum Mol Genet* 2017; 26(8): 1584–1596.
9. Abraham V, Cao G, Parambath A, et al. Involvement of TIMP-1 in PECAM-1-mediated tumor dissemination. *Int J Oncol* 2018; 53(2): 488–502.
10. Zhao H, Chen Q, Alam A, et al. The role of osteopontin in the progression of solid organ tumour. *Cell Death Dis* 2018; 9(3): 1–15.
11. Caponnetto S, Iannantuono GM, Barchiesi G, et al. Prolactin as a potential early predictive factor in

metastatic non-small cell lung cancer patients treated with Nivolumab. *Oncology* 2017; 93(1): 62–66.
12. Chen F, Ren P, Feng Y, et al. Follistatin is a novel biomarker for lung adenocarcinoma in humans. *PLoS One* 2014; 9(10): e111398.
13. Laddha AP and Kulkarni YA. VEGF and FGF-2: promising targets for the treatment of respiratory disorders. *Respir Med* 2019; 156: 33–46.
14. Garcia-Lucio J, Argemi G, Tura-Ceide GO, et al. Gene expression profile of angiogenic factors in pulmonary arteries in COPD: relationship with vascular remodeling. *Am J Physiol Lung Cell Mol Physiol* 2016; 310(7): L583–L592.
15. Kranenburg AR, Willems-Widyastuti A, Mooi WJ, et al. Chronic obstructive pulmonary disease is associated with enhanced bronchial expression of FGF-1, FGF-2, and FGFR-1. *J Pathol* 2005; 206(1): 28–38.
16. Kuhn H, Köpff C, Konrad J, et al. Influence of basic fibroblast growth factor on the proliferation of non-small cell lung cancer cell lines. *Lung Cancer* 2004; 44(2): 167–174.
17. Chen JT, Lin TS, Chow KC, et al. Cigarette smoking induces overexpression of hepatocyte growth factor in type II pneumocytes and lung cancer cells. *Am J Respir Cell Mol Biol* 2006; 34(3): 264–273.
18. Matarese A and Santulli G. Angiogenesis in chronic obstructive pulmonary disease: a translational appraisal. *Transl Med UniSa* 2012; 3(6): 49–56.
19. Mishra R, Foster D, Vasu VT, et al. Cigarette smoke induces human epidermal receptor 2-dependent changes in epithelial permeability. *Am J Respir Cell Mol Biol* 2016; 54(6): 853–864.
20. Cosentino-Boehm AL, Lafky JM, Greenwood TM, et al. Soluble human epidermal growth factor receptor 2 (sHER2) as a potential risk assessment, screening, and diagnostic biomarker of lung adenocarcinoma. *Diagnostics (Basel)* 2013; 3(1): 13–32.
21. Tsantikos E, Lau M, Castelino CM, et al. Granulocyte-CSF links destructive inflammation and comorbidities in obstructive lung disease. *J Clin Invest* 2018; 128: 2406–2418.
22. Murakami K, Kudo Y, Kakihana M, et al. Granulocyte colony stimulating factor-producing lung cancer with severe anemia of inflammation. *Gen Thorac Cardiovasc Surg* 2018; 66: 415–418.
23. Bade G, Khan MA, Srivastava AK, et al. Serum cytokine profiling and enrichment analysis reveal the involvement of immunological and inflammatory pathways in stable patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2014; 9: 759–773.
24. Hosgood III HD, Boffetta P, Greenland S, et al. In-home coal and wood use and lung cancer risk: a pooled analysis of the International Lung Cancer Consortium. *Environ Health Perspect* 2010; 18: 1743–1747.
25. Olloquequi J, Jaime S, Parra V, et al. Comparative analysis of COPD associated with tobacco smoking, biomass smoke exposure or both. *Respiratory Res* 2018; 19: 1–8.
26. Harrison PT, Vyse S and Huang PH. Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. *Semin Cancer Biol* 2020; 61: 167–179.
27. Merikallio H, Kaarteenaho R, Lindén S, et al. Smoking-associated increase in mucins 1 and 4 in human airways. *Respir Res* 2020; 21: 1–15.
28. Ha EV and Rogers DF. Novel therapies to inhibit mucus synthesis and secretion in airway hypersecretory diseases. *Pharmacology* 2016; 97: 84–100.
29. Ramírez-Venegas A, Velázquez-Uncal M, Pérez-Hernández R, et al. Prevalence of COPD and respiratory symptoms associated with biomass smoke exposure in a suburban area. *Int J Chron Obstruct Pulmon Dis* 2018, 13: 1727–1734.
30. Grigsby M, Siddharthan T, Chowdhury MA, et al. Socioeconomic status and COPD among low-and middle-income countries. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 2497–2507.
31. Barnes PJ. Sex differences in chronic obstructive pulmonary disease mechanisms. *Am J Respir Criti Care Med* 2016; 193: 813–814.
32. Stabile LP and Burns TF. Sex-specific differences in lung cancer. In: Hemnes A (ed) *Gender, sex hormones and respiratory disease. respiratory medicine*. Cham: Humana Press, 2016, pp. 141–171.
33. Kurmi OP, Arya PH, Lam KBH, et al. Lung cancer risk and solid fuel smoke exposure: a systematic review and meta-analysis. *Eur Respir J* 2012; 40: 1228–1237.