Cytokine profile in the sputum of subjects with post-tuberculosis airflow obstruction and in those with tobacco related chronic obstructive pulmonary disease

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Guiedem Elise
Universite de Yaounde I

Elise Guiedem
guiedemelise86@yahoo.fr Corresponding Author
ORCiD: https://orcid.org/0000-0002-5976-7375

Eric Walter Pefura-Yone
Universite de Yaounde I

George Mondinde Ikomey
Universite de Yaounde I

Céline Nkenfou
Universite de Yaounde I

Martha Mesembe
Universite de Yaounde I

Yivala Mispa Mbanyamsig
Universite de Yaounde I

Chende Hycenta Bih
University of Stellenbosch

Jacobs Brendon Greame
University of Stellenbosch

Novel Njweipi Chegou
University of Stellenbosch

Marie Claire Assoumou Okomo
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Abstract

Background: Previous studies have shown that tuberculosis (TB) is a risk factor for chronic airflow limitation. Chronic obstructive pulmonary disease (COPD) is recognized as the result of chronic inflammation, usually related to noxious particles. Post-TB airflow obstruction (post-TB/AFO) and tobacco-related COPD (COPD/tobacco) have the same functional pathway characterized by persistent airflow limitation. We sought to compare the profile of 29 cytokines in the sputum of subjects with post-TB/AFO and those with COPD/tobacco.

Results: The forced expiratory volume in the first second (FEV1) and FEV1/forced vital capacity (FEV1/FVC) ratio were lower in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup. The stages of the disease were more advanced in COPD/tobacco patients. Among the cytokines, IL-1α, IL-1β, MIP-1β, sCD40L and VEGF levels were higher in COPD patients, compared to the controls with p values of 0.003, 0.0001, 0.03, 0.0001 and 0.02 respectively. When the two COPD subgroups were compared, IL-1α, IL-6, TNF-α and IL-8 levels were higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup with p-values of 0.031, 0.05, 0.021 and 0.016 respectively.

Conclusion: COPD/tobacco is more severe than post-TB/AFO. The pathogenesis of post-TB/AFO appears to involve the cytokines IL-1RA, IL-1α, IL-1β, IL-17, GRO and sCD40L, while COPD/tobacco involves more cytokines.

Background

Chronic obstructive pulmonary disease (COPD) is a respiratory disease characterized by progressive airflow obstruction that is not fully reversible, with combined emphysema and chronic obstructive bronchitis [1]. It is a major cause of morbidity and mortality worldwide and is gradually increasing. It can lead to abnormal inflammation in the airways. In the clinical diagnosis of COPD, the forced expiratory volume in the first second /forced vital capacity (FEV1/FVC) ratio is less than 70% and the severity of the disease is evaluated according to four clinical stages: Stage I (mild) with FEV1 ≥ 80%, Stage II (moderate) with 50% ≤ FEV1 < 80%, Stage III (severe) with 30% ≤ FEV1 < 50% and Stage IV (very severe) with FEV1 < 30% [2].

Cigarette smoking is known as the main risk factor for COPD [2]. The defense system of the
respiratory airways includes the mucociliary carpet, which cleans respiratory airways, and the epithelial tight junctions, which form a physical barrier between tissues and airway space. This barrier can be broken by chronic exposure to cigarette smoke, which irritates the lung wall by damaging epithelial cells and tissue. Alveolar macrophages and epithelial cells produce TNF-α. In turn, TNF-α stimulates migration of monocytes/macrophages and neutrophils to the airway epithelium. Macrophages and epithelial cells are induced to produce IL-8, GRO, MCP-1 and MIP-1 α. IL-8 and GRO also stimulate the migration of neutrophils and T cells to the airway epithelium. Both TNF-α and IL-8/GRO cause degranulation of neutrophils and respiratory bursts with the production and release of free radicals that cause matrix and epithelial damage. The derived products amplify the production of the mediators of inflammation [3]. MCP-1 and MIP-1 stimulate the influx of monocytes/macrophages. Alternatively, TNF-α can also cause epithelial damage and death, goblet cell metaplasia and/or mucus hypersecretion. TNF-α can stimulate the expression of epidermal growth factors that orchestrate epithelial repair. IL-8/GRO and MCP-1 could be directly involved in epithelial repair [4]. The damage induced by cigarette smoke leads to the limitation of respiratory flow that induces emphysema and chronic bronchitis that are peculiar to COPD.

Although smoking is the main cause of COPD, the same functional syndromes, characterized by persistent airflow obstruction, are increasingly observed in patients with previous pulmonary tuberculosis (TB) who are non smokers. Some studies have shown that TB is a risk factor for COPD, with COPD observed in approximately 7.6% of patients who previously had TB [5, 6, 7]. Other studies have identified post-TB airflow obstruction in patients who have a similar syndrome, with a history of pulmonary TB and have never smoked.

Post-TB airflow limitation is also common after TB treatment [8]. Inflammation of the bronchial endothelium leads to localized or generalized bronchial obstruction, hypertrophy of the submucous glands and smooth muscles, pulmonary fibrosis and edema of airways mucosa, with increased secretion of mucus [9]. The results of these events increase airway resistance to airflow [10]. The destruction of the parenchyma affects lung compliance by increasing the tendency of small pathways to collapse, the main characteristic of COPD [11].
Knowledge of the inflammatory characteristics of COPD and/or post-TB AFO could help to better adapt the anti-inflammatory treatment of patients, and thus reduce the frequent phenomenon of corticoresistance. The purpose of this study was to determine the profile of cytokines in COPD patients, with a history of smoking compared to those with a history of TB.

Results

Demographic characteristics
A total of 150 participants were recruited: 90 COPD patients and 60 clinically healthy persons for the control group. The COPD patients consisted of 50 smokers (COPD/tobacco) and 40 patients who had previously TB (COPD/post-TB).  

Spirometric characteristics
In the COPD/tobacco patients, FEV1 ranged from 20.3% to 64.60% and in the COPD/post-TB patients, it ranged from 30% to 79%. In the control group, FEV1/FVC ranged from 70% to 97%. The FEV1/FVC ratio of COPD/tobacco patients ranged from 35% to 68% and in the COPD/post-TB subgroup, it ranged from 35% to 74.35%. The FEV1 and FEV1/FVC ratio was lower in the COPD/tobacco subgroup than in the COPD/post-TB subgroup with p-values of 0.014 and 0.033 respectively. As a result, the stage of COPD was more advanced in COPD/tobacco patients compared to COPD/post-TB patients with a p-value of 0.032 (Table I and Table II).

Concentrations of cytokines in participants
The detected cytokines in sputum were anti-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-17 and TNF-α), chemokines (MCP-1, IL-8, MIP-1α, MIP-1β, GRO, IP-10 and sCD40L) and growth factors (VEGF, G-CSF and GM-CSF). Other cytokines such as IFN-α, IFN-γ, IL-10, IL-12, MDC, PDGF, IL-15, IL-2, IL-4, IL-7 and RANTES were not detected in the sputum of either the COPD patients or the control group. Table III shows the mean (± SD) concentrations of cytokines in sputum in the three groups: in patients with COPD, the levels of cytokines such as IL-1RA, IL-1α, IL-1β, MIP-1β, sCD40L and VEGF were statistically higher compared to the control group with p-values all lower at 0.05. IL-6 and TNF-α were higher in smokers. GRO concentration was higher in COPD/post-TB patients. Levels of IL-17 and GM-CSF were higher in COPD patients, but with no statistically significant difference. There was also no statistically significant difference in cytokine concentrations such as G-CSF, MIP-1α and IP-10.
Concentration of IL-1RA

In the control group, the maximum concentration of IL-1RA was 1590 pg/mL with an average of 637.7±191.3 pg/mL. In the COPD/tobacco subgroup, the concentration of IL-1RA ranged from 147.0 pg/mL to 4686 pg/mL with a mean of 1720 ±491.1pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-1RA was 109.3 pg/mL and the highest was 6566 pg/mL with a mean of 1945 ± 873.1). No statistically significant difference in IL-1RA concentration was found between the COPD/tobacco and COPD/post-TB subgroups (p = 0.8). IL-1RA concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.014) and higher in the COPD/post-TB subgroup compared to the control group (p = 0.014) (Figure 1).

Concentration of IL-1α

In the control group, the maximum concentration of IL-1α was 1726 pg/mL with an average of 539.1 ± 198.6 pg/mL. In the COPD/tobacco subgroup, the IL-1α concentration ranged from 81.06 pg/mL to 3937 pg/mL with a mean of 1555 ± 360.7 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-1α was 88.94 pg/mL and the highest was 1914 pg/mL with a mean of 821.2 ± 205.7 pg/mL. No statistically significant difference in IL-1α concentration was found between the COPD/tobacco and COPD/post-TB subgroups (p = 0.8). IL-1α concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.006) and higher in the COPD/post-TB compared to the control group (p = 0.029) (Figure 2).

Concentration of IL-1β

In the control group, the minimum concentration of IL-1β was 0.01 pg/mL and the maximum was 34.36 pg/mL with a mean of 9.410 ± 3.781 pg/mL. In the COPD/tobacco subgroup, the concentration of IL-1β ranged from 1.1 pg/mL to 1531 pg/mL (mean =296.4 ± 143.5 pg/mL). In the COPD/post-TB subgroup, the lowest IL-1β concentration was 0.58 and the highest was 578.7 pg/mL (mean = 137.9 ± 65.25 pg/mL). No statistically significant difference in IL-1β concentration was found between the COPD/tobacco and COPD/post-TB subgroups (p = 0.390). IL-1β concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.025) and higher in the COPD/post-TB subgroup compared to the control group with (p = 0.048) (Figure 3).

Concentration of IL-6

In the control group, the minimum concentration of IL-6 was 0.0 pg/mL and the maximum was 54.14 pg/mL (mean = 11.64 ± 2.90 pg/mL). In the COPD/tobacco subgroup, the IL-6 concentration ranged from 0.44 pg/mL to 83.27 pg/mL (mean = 16.23 ± 7.768
pg/mL). In the COPD/post-TB subgroup, the lowest IL-6 concentration was 0.0 pg/mL and the highest was 63.07 pg/mL with a mean of 137.9 ± 65.25 pg/mL. IL-6 concentration was higher in the COPD/tobacco than in COPD/post-TB subgroup (p = 0.049), and higher in the COPD/tobacco subgroup compared to the control group (p = 0.033). No statistically significant difference in IL-6 concentration was found between the COPD/post-TB subgroup and control group (p = 0.665) (Figure 4).

**Concentration of IL-17**

In the control group, the minimum concentration of IL-17 was 0.0 pg/mL and the maximum concentration was 4.27 pg/mL (mean = 1.421± 0.23 pg/mL). In the COPD/tobacco subgroup, the maximum concentration of IL-17 was 5.230 pg/mL with a mean of 1.54 ± 0.2650 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-17 was 1.33 pg/mL and the highest concentration was 4.06 pg/mL with a mean of 2.412 ± 0.41pg/mL. The difference in IL-17 concentration between the COPD/tobacco and the COPD/post-TB subgroups was not statistically significant (p = 0.053). IL-17 concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.041) and higher in the COPD/post-TB subgroup compared to the control group (p = 0.045) (Figure 5).

**Concentration of TNF-α**

In the control group, the minimum concentration of TNF-α was 0.0 pg/mL and the maximum concentration was 26.01 pg/mL (mean = 1.880 ± 0.31 pg/mL). In the COPD/tobacco subgroup, the maximum concentration of TNF-α was 43.01 pg/mL (mean = 7.112 ± 4.542 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of TNF-α was 0.0 pg/mL and the highest was 5.14 pg/mL (mean = 1.110± 0.5649 pg/mL). TNF-α concentration was higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup (p = 0.021) and higher in the COPD/tobacco subgroup compared to the control group (p = 0.048). Not statistically significant difference was found between the COPD/post-TB subgroup and the control group (p = 0.126) (Figure 6).
**Concentration of IL-8**

In the control group, the concentration of IL-8 varied from 7.5 pg/mL to 541.4 pg/mL with a mean of 135.5 ± 30.93 pg/mL. In the COPD/tobacco subgroup, the concentration of IL-8 ranged from 4.40 pg/mL to 2250 pg/mL with an average of 493.1 ± 244.9 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-8 was 1.2 pg/mL and the highest was 630.7 pg/mL with an average of 103.0 ± 68.58 pg/mL. IL-8 concentration was higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup (p = 0.016) and higher in the COPD/tobacco subgroup compared to the control group (p = 0.009). No statistically significant difference in IL-8 concentration was found between the COPD/post-TB subgroup and the control group (p = 0.714) (Figure 7).

**Concentration of MIP-1β** In the control group, the minimum concentration of MIP-1β was 0.0 pg/mL and the maximum concentration was 389.4 pg/mL with a mean of 81.65 ± 20.27 pg/mL. In the COPD/tobacco subgroup, the concentration of MIP-1β ranged from 18.20 pg/mL to 780.9 pg/mL (mean = 181.1 ± 63.57 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of MIP-1β was 9.630 pg/mL and the highest was 872.9 pg/mL (mean = 224.1 ± 103.4 pg/mL). No statistically significant difference in MIP-1β concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.71), but it was higher in the COPD/tobacco subgroup compared to the control group (p = 0.012) and without statistically significant difference between the COPD/post-TB subgroup and control group (p = 0.16) (Figure 8).

**Concentration of GRO** In the control group, the concentration of GRO varied from 0.0 pg/mL to 13518 pg/mL with a mean of 2563 ± 1617 pg/mL. In the COPD/tobacco subgroup, the concentration of GRO ranged from 0.0 pg/mL to 4850 pg/mL with a mean of 2563 ± 1617 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of GRO was 132.9 pg/mL and the highest was 105133 pg/mL (mean = 1721 ± 977.7 pg/mL). No statistically significant difference in GRO concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.37) and between the COPD/tobacco subgroup compared to control group (p = 0.404), but it was higher in the COPD/post-TB sub-group compare to control group (p = 0.028) (Figure 9).
Concentration of sCD40L

In the control group, the minimum concentration of sCD40L was 0.0 pg/mL and the maximum concentration was 2.600 pg/mL with a mean of 0.286 ± 0.164 pg/mL. In the COPD/tobacco subgroup, the concentration of sCD40L ranged from 0.0 pg/mL to 8.570 pg/mL with a mean of 1.506 ± 0.441 pg/mL. In the COPD/post-TB subgroup, the lowest sCD40L concentration was 0.0 pg/mL and the highest was 4.760 pg/mL with a mean of 1.958 ± 0.384 pg/mL. No statistically significant difference in sCD40L concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.462). The difference between the COPD/tobacco subgroup and the control group was not statistically significant (p = 0.058), but the sCD40L concentration was higher in the COPD/post-TB subgroup compared to the control group (p = 0.001) (Figure 10).

Concentration of VEGF

In the control group, the concentration of VEGF varied from 0.0 pg/mL to 1280 pg/mL with a mean of 332 ± 155.8 pg/mL. In the COPD/tobacco subgroup, the concentration of VEGF ranged from 33.63 pg/mL to 1214 pg/mL (mean = 435.2 ± 130.2 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of VEGF was 0.0 pg/mL and the highest was 1143 pg/mL (mean = 333.9 ± 136.5). No statistically significant difference in VEGF concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.59), but the concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.035) and not statistically significant difference between the COPD/post-TB subgroup and the control group (p = 0.926) (Figure 11).

Concentration of other cytokines:

No statistically significant differences between the control group and the subgroups were found in the concentrations of other cytokines such as MCP-1, MIP-1α, IP-10, G-CSF and GM-CSF using the Student T-test.

Correlation between clinical stage, cells and cytokine levels in sputum The concentration of
GRO and cells varied in the same direction with a statistically significant association between GRO and monocytes. A positive correlation was found between sCD40L and cells such as neutrophils and monocytes (r = 0.458 and r = 0.24 respectively) with p-values of 0.0001 and 0.042 respectively. The more advanced the clinical stage was, the higher the IL-1α and IL-1β concentrations were, with correlation coefficients of 0.473 and 0.466 respectively and p-values below 0.0001. Statistically significant positive correlations were also found between neutrophils levels and cytokines such as IL-1α and IL-1β. The more advanced the clinical stage was, the higher the concentrations of MIP-1α and IL-8 were (Table IV).

Discussion
We analyzed the spirometric characteristics, cell profile and cytokine profile in patients with a history of tobacco (COPD/tobacco subgroup) and with a history of previous TB (COPD/post-TB subgroup) and in healthy person who served as a control group. This is significant and relevant because we observed important differences.

Concerning spirometric characteristics, FEV1 and FEV1/FVC were lower in the COPD/tobacco subgroup than in the COPD/post TB subgroup; therefore, subjects in the COPD/tobacco subgroup were at a more advanced stage of the disease. These results suggest that the disturbances and irritations caused by smoking are more pronounced than those induced by TB. In addition, the treatment of TB eliminates mycobacterial infection and the damage associated with the mycobacterium itself is reduced, while in smokers, smoking continues to cause damage until the development of COPD.

Cytokines of the IL-1 family intervene in the inflammatory process; IL-1α, IL-1β and IL-1RA were assayed. Levels of IL-1α were statistically higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup. The study found through association tests that IL-1α was strongly correlated with the clinical stage of the disease. Since the stage of COPD was more advanced in the COPD/tobacco subgroup, this could justify the greater presence of IL-1α in smokers compared to COPD/post-TB patients. IL-1RA, IL-1α and IL-1β were higher in the COPD/tobacco and COPD/post-TB subgroups, in comparison to the control group, with statistically significant differences. The results for
post smoking COPD were similar to those found by Fernando et al [12] that showed a strong IL-1β expression following exposure to tobacco smoke. In a study by Rusznak et al [13], they noted significant secretion of IL-1β by epithelial cells cultured from COPD/tobacco patients. Since there is bronchial hyperactivity during COPD, IL-1RA is increased in patients, most likely with the aim of reducing the effect of high concentrations of IL-1α and IL-1β at the origin of this hyperactivity, hence the high concentrations in COPD/post-TB and in COPD/tobacco patients.

IL-1RA (IL-1 receptor antagonist) is a cytokine inhibitor present in all cells that express IL-1 (more particularly by monocytes/macrophages) and has an anti-inflammatory role [14]. No study has shown the function of IL-1α in the inflammatory process associated with exposure to cigarette smoke in vivo, in stable COPD or during exacerbations. The function of IL-1α was previously investigated in mice, which indicated a high level of IL-1α four days after exposure to cigarette smoke, during stable COPD and during exacerbations [15]. IL-1α may promote neutrophils infiltration into the airway. The study performed by Fernando et al (2011) showed a significant correlation between IL-1α and IL-1β levels and chemokine in the sputum of mice with COPD. IL-1α and IL-1β could therefore promote the production of other pro-inflammatory cytokines.

While IL-1α and IL-1β are IL-1 receptor agonists, IL-RA functions as a competitive antagonist of IL-1 receptors. IL-1RA blocks the binding of IL-1α and IL-1β on their receptor (IL-RI), preventing the activation of IL-1RI and thus inhibiting the biological actions of IL1α and IL-1β. In humans, clinical trials of IL-1RA recombinants have been performed in persons with rheumatoid arthritis with notable observations of the decline of macrophages and lymphocytes [16]. Anakira which is a recombinant human IL-1RA, is a molecule that has been used in controlled clinical trials and is effective and well tolerated in the treatment of human-specific disorders of rheumatoid arthritis [17]. A therapeutic strategy using IL-1RA recombinants may also be considered in the treatment of COPD/tobacco and COPD/post-TB patients as is already the case in persons with rheumatoid arthritis. The higher IL-6 in COPD/tobacco subjects compared to COPD/post-TB subjects could be related to two facts: either IL-6 does not significantly interfere with post-TB airflow obstruction (there was no difference between the COPD/post-TB subgroup and the control group in the level of IL-6), or this result could be linked to the
fact that most COPD/post-TB subjects were at a less advanced clinical stage (the results showed a positive correlation between the clinical stage and the concentration of IL-6 although it was not statistically significant). Suleyman et al [18] found that the clinical stage of COPD was correlated with the concentration of IL-6. IL-6 acts by binding to a cell surface receptor, IL-6R. The receptor-cytokine complex then binds to the glycoprotein (gp) 130 (surface molecule) and the cell is activated. The inhibition of gp 130 blocks the effects of IL-6. Studies targeting IL-6 through the use of IL-6 and IL-6R antibodies have been conducted previously [19, 20]. Considering the risks of anaphylactic reactions after repeated treatments with murine anti-IL-6 in humans [21], an alternative strategy to block IL-6 is to target IL-6R since IL-6R inhibition suppresses the formation of the IL6-IL6R complex that binds to gp 130 at the origin of cell activation. The use of the recombinant anti human monoclonal antibody (human IL-6R) IgG1 has shown very favorable results with clinical improvements and has decreased inflammation in rheumatoid arthritis [22]. Interruption of IL-6 transduction signals with IL-6 or IL-6R antibodies may be a way to reduce inflammation in COPD patients, particularly in smokers. Levels of TNF-α were statistically significantly higher in the COPD/tobacco subgroup. Generally, the secretion of TNF-α in COPD is induced by tobacco smoke and maintained by the chronic inflammation process [23]. Since *Mycobacterium tuberculosis* is no longer present in COPD/post-TB patients, there is no real inducing element of TNF-α, which could justify its low concentration in COPD/post-TB patients. TNF-α has multiple pro inflammatory actions. TNF-α stimulates the migration of monocytes/macrophages and neutrophils into the epithelial airways. Macrophages and induced epithelial cells produce GRO, MIP-1 and IL-8. TNF-α and IL-8 cause degranulation of neutrophils and shortness of breath with the production of free radicals that causes epithelial and matrix damage. In addition, TNF-α has also been reported to have a direct effect on epithelial cells, being capable of inducing hypersecretion of mucus, resulting in cell death and emphysematous lesions and contributing to the deterioration of the clinical state seen in COPD correlated with weight loss [24]. Some diseases such as rheumatoid arthritis and chronic colitis characterized by hyperinflammation have been treated with TNF-α antagonists with significant reduction in inflammation [25]. Therapies based on TNF-α antagonists and TNFR antagonists (anti-TNF-α or anti-TNF receptors) may be considered as more specific and effective
means of weakening inflammation and epithelial remodeling in COPD patients with a history of smoking.

IL-17 concentrations in both subgroups were higher compared to the control group. During post-smoking COPD, IL-17 plays a role in T cell proliferation, activation of fibroblasts, endothelials and epithelial cells, induces release of cytokines (IL-6, GM-CSF) and activation of neutrophils [26]. IL-17 is involved in the proliferation of T lymphocytes, activation of fibroblasts, endothelial and epithelial cells. IL-17 induces the release of IL-6 and IL-8. The fact that the IL-17 concentration is high in the COPD/post TB subgroup could be justified by the fact that this cytokine also plays an important role in post-TB AFO as in COPD related to tobacco. Studies have been conducted on the use of monoclonal antibodies directed against IL-17 (ixekizumab) or the IL-17 receptor IL-17RA in the treatment of psoriasis with decreased infiltration of lymphocytes and epidermal hyperplasia [27, 28, 29]. However, no study on the use of these antibodies has been conducted in respiratory diseases. Regarding MIP-1β, a higher concentration was noted in the COPD/tobacco subgroup compared to the control group. MIP-1β is a chemokine that induces lymphocyte migration and recognizes CCR5 receptors in T cells and macrophages. Some studies have shown that MIP-1β is essentially produced by macrophages. Our results showed a higher concentration among the smoker subgroup certainly because these patients were at a more advanced stage of the disease (the lesions caused by the destruction of the airways were more severe), and this could be related to emphysema that characterizes COPD. MIP-1β plays an important role in the IgG immune complex induced by acute lung injury [30]. This chemokine was not strongly expressed in the COPD/post-TB subgroup; this could be explained by the fact that in this subgroup most patients were at a less advanced stage of the disease; so emphysematous damage should be lower compared to smokers. The high expression of IL-8 in smokers has certainly been induced by cigarette smoke, and is maintained by chronic intoxication of the airways as is the case in the studies conducted by Huang, Becker and colleagues. In addition, studies report that myeloperoxidase and elastase released by activated neutrophils amplify the production of IL-8, which would justify its high concentration in COPD/tobacco patients who have a higher number of
neutrophils. Our recent study on the same population showed that the neutrophil count was higher in post-smoking COPD compared to the post-TB form [31]. GRO is an important chemotactic mediator of neutrophils, endothelial cell adhesion and degranulation. Its chemotactic role in neutrophils has been demonstrated by some studies based on the animal model [32]. It would play the same role as IL-8 in tobacco related COPD. The concentration of GRO was rather high in the COPD/post-TB subgroup. Since this chemokine is high in the sputum of patients with a history of TB, it could be the chemotactic marker of neutrophils in post-TB AFO such as IL-8 in the post smoking COPD form.

However, the preferential involvement of the GRO in post-TB AFO remains to be clarified. IL-8 and GRO act via their receptors, CXCR1 and CXCR2. Treatment with antagonists of CXCR2 and GRO or anti-IL-8 neutralizing antibody showed reduction of neutrophils, decreased alveolar damage and decreased associated mortality in endotoxinemia. In addition, a deficiency of CXCR2 in mice showed a disturbance of neutrophil influx and myeloperoxidase activity in skin wounds. Clinical trials in rheumatoid arthritis and psoriasis, with human anti-IL-8 antibodies or CXCR2 antagonists have been conducted. These molecules may be potential therapeutic agents for the treatment of COPD. However, IL-8 and GRO are involved in the stimulation of repair of epithelial wounds via CXCR2 according to Boer et al. [4]. An antagonistic therapy in COPD could prevent this repair. CXCR1 mainly expressed by neutrophils, is also expressed by macrophages, and CD8 T lymphocytes. A specific antagonist for CXCR1 can inhibit both respiratory fatigue (dyspnea) and degranulation of neutrophils. CXCR1 antagonists may be a more effective approach to reducing inflammation in COPD in patients with a history of smoking or a history of previous TB.

VEGF was strongly expressed in the sputum of COPD/tobacco patients compared to the control group, and not expressed in the COPD/post-TB subgroup. VEGF is a growth factor involved in angiogenesis (forming new preexisting vessel growths) in asthma. As in asthma, VEGF would be involved in the mechanism of bronchial vascular remodeling during COPD. Kanazawa et al [33] found an increased level of VEGF in the induced sputum of subjects with COPD. Kranenburg et al [34] showed that COPD was associated with increased VEGF expression in the bronchi and bronchial and alveolar epithelium.
Two studies conducted by Sichelstiel et al. [35] and Esmaeil et al. [36] found higher concentrations of VEGF in the sputum of COPD patients compared to healthy subjects. Calabrese et al., [37] found an association between increased bronchial vasculature and cellular expression of VEGF and that VEGF receptor blockade induced apoptosis of alveolar endothelial cells. The high pulmonary concentrations of VEGF in COPD, and more particularly in smokers, may therefore reflect two effects: either a regulatory effect upstream of the irritations caused by tobacco smoke, or an attempt to repair epithelial damage related to pathogenesis and emphysema in COPD [34].

Conclusion
The objective of the study was to compare the profile of certain cytokines in COPD/tobacco and COPD/post-TB patients. We reached the following conclusions: COPD related to tobacco use is more severe than the post-TB form. The pathogenesis of COPD in patients with a history of smoking involves several cytokines in the local pathway such as IL-1α, IL-1β, IL-6, IL-17, TNF-α, IL-8, MIP-1β and VEGF. In contrast the pathogenesis of the post-TB form seems to imply fewer inflammatory markers with IL-1RA, IL-1β, GRO and sCD40L as local marker. Considering more specific anti-cytokine therapies capable of targeting cytokines such as TNF-α, IL-6, IL-8, IL-1, MIP and GRO or a small group would be a probative approach to improving the management of COPD.

Methods

Study participants
Study participants were recruited from the Yaoundé Jamot Hospital (YJH) from February 2016 to July 2017. YJH is the main and biggest center in Cameroon specializing in the management of respiratory disorders. Participants constituted COPD patients and healthy persons who served as controls. COPD participants were chosen among patients consulting at the YJH and who had been diagnosed by a pneumologist. COPD patients comprised two sub-groups: patients with a history of smoking (COPD/tobacco) and patients with post-TB airflow obstruction (post-TB AFO or COPD/post TB). Participants were eligible for the study if they gave written informed consent for participation, including consent for HIV testing. Patients were excluded if they had active TB, or were physically or
mentally unable to perform a respiratory function test. The work received ethical clearance from the Cameroon National Ethical Committee of Research for Human Health (N° 2016/06/772/CE/CNERSH/SP). **Data and sample collection**

Once the pneumologist had diagnosed an eligible patient with COPD, the spirometric results of the patient were extracted from his/her medical record. The history of TB or smoking was also extracted from patients‘ medical records and confirmed by each patient. Spirometric measurements for the control group were done with the turbine pneumotachograph (SpiroUSB, Care fusion, Yorba Linda USA) following the American Thoracic Society standard to ensure that participants did not have any respiratory problems. Sputum samples were collected from each participant. Samples were transported at ambient conditions in a coolbox to the Center for the Study and Control of Communicable Diseases of the Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. Upon receipt in the laboratory, cell counts (neutrophils, macrophages and lymphocytes) were performed after staining of the smear with May-Grunwald Giemsa and read by light microscopy. After the cell counts cells, the sputum was liquefied by mixing with phosphate buffered saline. The sputum was centrifuged at 2000 rpm for 10 minutes, and the supernatant was aliquoted and stored at -80°C until cytokine analyses. The cytokine analyses were performed by the Luminex technique at Stellenbosch University, Cape Town, South Africa.

**Luminex multiplex immunoassay**

The concentrations of 29 cytokines including pro-inflammatory cytokines (interleukin: IL-1β, IL-1α, IL-2, IL-4, IL-6, IL-7α, IL-12p70, IL-12p40, IL-15 and IL-17A, tumor necrosis factor: TNF-α and TNF-β, and interferon: IFN-α and IFN-γ), chemokines (IL-8, growth regulated oncogene: GRO, interferon-inductible protein: IP-10, macrophage inflammatory protein: MIP-1α and MIP-1β, monocyte chemiotactic protein: MCP-1, regulated on activation normal T cells expressed and secreted: RANTES, soluble CD40 ligand: sCD40L and macrophage-derived chemokine: MDC); anti-cytokines (IL-10 and IL-1 receptor antagonist: RA) and growth factors (granulocyte-colony stimulating Factor: G-CSF, granulocyte macrophage-CSF: GM-CSF, platelet-derived growth factor: PDGF and vascular endothelial growth
factor: VEGF) were investigated in sputum samples from all study participants. Reagents were purchased from Merck Millipore, Billerica, Massachusetts, United States of America. Experiments were performed and read on the Bio-Plex platform (Bio-Rad), with the Bio-Plex Manager Software version 6.1 used for bead acquisition and analysis.

**Statistical analyses**

The data was analyzed using Graphpad Prism version 5 (GraphPad Software Inc., California, USA). Quantitative variables were presented as mean (± standard error) when the distribution was considered normal; if not they were represented by their median (± interquartile interval). A chi-square test was used to compare proportions. The Student’s t-test or Mann-Whitney U test was used to compare means or medians respectively. The Pearson’s chi squared test was used to test the association between the concentrations of cytokines and spirometric data. P-values ≤ 0.05 were considered significant.

**Declarations**

**List of abbreviations**

AFO: airflow obstruction; ATS: American Thoracic Society; CD: cluster differentiation; CI: confidence interval; COPD: chronic obstructive pulmonary disease; CSCCD: Center for the Study and Control of Communicable Diseases; FEV: forced expiratory volume in the first second; FVC: forced vital capacity; G-CSF: granulocyte-colony stimulating Factor; GM-CSF: granunocyte macrophage-CSF; GOLD: global initiative for obstructive lung diseases; IFN: interferon; Ig: immunoglobuline; IL: interleukin; IL-1RA: IL-1receptor antagonist; RA IP: interferon-inducible protein; MCP: monocyte chemotactic protein; MDC: macrophage-derived chemokine; MGG: May–Grunwald Giemsa; MIG: monokine induced by interferon-gamma; ml: milliliter; MMP: metalloproteinase matrix; PDGF: platelet-derived growth factor; RANTES: regulated on activation normal T cells expressed and secreted; sCD40L: soluble CD40 ligand TB: tuberculosis; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor; YJH: Yaoundé Jamot Hospital.

**Ethics approval and consent to participate**
The work received administrative approval from competent authorities at the YJH and ethical clearance from the Cameroon National Ethical Committee of Research for Human Health (N° 2016/06/772/CE/CNERSH/SP). All participants included in the study gave written informed consent for participation, including consent for HIV testing.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analyzed during this study are included in this article.

**Competing interests**

The authors declare that they have no competing interests.

**Source of funding**

The major source of funding was provided by each co-author.

**Authors’ contributions**

GE, being the principal investigator, conceived and designed the study, implemented sample collection, implemented the laboratory analysis and wrote the first draft of the manuscript. PYEW participated in the design of the study, implemented the clinical selection of participants and corrected the first draft of the manuscript. GMI, GBJ and NC participated in the design of the study and the laboratory implementation, supervised the study and participated in the writing of the article. MM brought some corrections to the draft of the manuscript. BHC and MMY had performed the laboratory analysis. NNC and GBJ participated in the design of the study, performed the laboratory analysis and improved the final version of this manuscript. OAMC participated in the design of the study, supervised the study and substantially revised the first draft of the manuscript. All the authors read and approved the final manuscript.

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**Authors' information**
1 Center for the Study and Control of Communicable Diseases (CSCCD), Faculty of Medicine and Biomedical Sciences, University of Yaounde 1, Yaoundé, Cameroon.

2 Pneumological Service, Yaounde Jamot Hospital, Yaoundé, Cameroon.

3 Chantal BIYA International Reference Centre for Research on HIV/AIDS Prevention and Management (CBIRC), Yaoundé, Cameroon.

4 DST-NRF Centre of Excellence for Biomedical Tuberculosis Research; and SAMRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa.

5 Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa.

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Tables

Table I: Sociodemographic and clinical data of all participants

|                  | COPD/post-TB | COPD/tobacco | Control | p     |
|------------------|--------------|--------------|---------|-------|
| Age              | 40 ± 2.1     | 63 ± 10.45   | 43 ± 12.38 | 0.0002 |
| Male             |              |              |         |       |
| Female           | 16 (40%)     | 42 (84%)     | 40 (66.7%) | 0.0002 |
|                  | 24 (60%)     | 8 (16%)      | 20 (33.3%) |       |
| FEV1             | 53.30% (± 17.21%) | 36.88% (± 14.95%) | 83 ± 6.56% | < 0.0001 |
| FVC              | 84.89 ± 15.1% | 72.97 ± 14.5% | 97.64 ± 4.15% | < 0.0001 |
| FEV1/FVC         | 62.79% (± 17.95%) | 50.54% (± 12.09%) | 85 ± 6.06% | < 0.0001 |
| Stage of disease | Stage I: 0   | Stage II: 0  | Stage I: 0 | 0.038 |
|                  | Stage II: 20 (50%) | Stage II: 11 (22%) |       |       |
|                  | Stage III: 15 (37.5%) | Stage III: 14 (28%) |       |       |
|                  | Stage IV: 5 (12.5%) | Stage IV: 25 (50%) |       |       |

Table II: Sociodemographic and clinical data of COPD patients

|                  | COPD/Post-TB | COPD/tobacco | p     |
|------------------|--------------|--------------|-------|
| Age              | 40 ± 2.1     | 63 ± 10.45   | 0.0023 |
| Male             |              |              |       |
| Female           | 16 (40%)     | 42 (84%)     | 0.0002 |
|                  | 24 (60%)     | 8 (16%)      |       |
| FEV1             | 53.30% (± 17.21%) | 36.88% (± 14.95%) | 0.015 |
| FVC              | 84.89 ± 15.1% | 72.97 ± 14.5% | 0.021 |
| FEV1/FVC         | 62.79% (± 17.95%) | 50.54% (± 12.09%) | 0.033 |
| Stage of COPD    | Stage II: 20 (50%) | Stage II: 11 (22%) | 0.038 |
|                  | Stage III: 15 (37.5%) | Stage III: 14 (28%) |       |
|                  | Stage IV: 5 (12.5%) | Stage IV: 25 (50%) |       |

Table III: Sputum cytokine concentrations in patients and controls
| Cytokines                | COPD/post-TB | COPD/tobacco | Control      | p      |
|-------------------------|--------------|--------------|--------------|--------|
| **Anti-cytokines (pg/mL)** |              |              |              |        |
| IL-1RA                  | 1945 (±873.1) | 1720 (±491.1) | 637.7 ± 191.3 | 0.046  |
| **Pro-inflammatory cytokines (pg/mL)** |              |              |              |        |
| IL-1α                   | 821.2 ± 205.7 | 1555 ± 360.7  | 539.1 ± 198.6 | 0.03   |
| IL-1β                   | 137.9 ± 65.25 | 296.4 ± 143.5 | 9.410 ± 3.781 | <0.001 |
| IL-6                    | 9.966 ± 6.916 | 16.23 ± 7.768 | 11.64 ± 2.90  | 0.04   |
| IL-17A                  | 2.412 ± 0.41  | 1.54 ± 0.2650 | 1.421± 0.23   | 0.14   |
| TNF-α                   | 1.110± 0.5649 | 7.112 ± 4.542 | 1.880 ± 0.31  | 0.095  |
| **Chemokines (pg/mL)**  |              |              |              |        |
| MCP-1                   | 254.3 ± 101.4 | 657.8 ± 276.3 | 522.7 ± 69.00 | 0.08   |
| IL-8                    | 103.0 ± 68.58 | 493.1 ± 244.9 | 135.5 ± 30.93 | 0.023  |
| MIP-1α                  | 11.12 ± 6.6   | 10.20 ± 4.9   | 23.64 ± 6.2   | 0.1674 |
| MIP-1β                  | 224.1 ± 103.4 | 181.1 ± 63.57 | 81.65 ± 20.27 | 0.03   |
| GRO                     | 14047± 13020  | 1721 ± 977.7  | 2563 ± 1617   | 0.05   |
| IP-10                   | 420.8 ± 239.5 | 242.2 ± 123.9 | 848.4 ± 314.0 | 0.069  |
| sCD40L                  | 1.958 ± 0.384 | 1.506 ± 0.441 | 0.286 ± 0.164 | <0.0001|
| **Growth factors (pg/mL)** |              |              |              |        |
| VEGF                    | 333.9 ± 136.5 | 435.2 ± 130.2 | 332 ± 155.8   | 0.02   |
| G-CSF                   | 237.1 ± 46.4  | 592.3 ± 227   | 774.3 ± 207.9 | 0.68   |
| GM-CSF                  | 1.920 ± 0.35  | 592.3 ± 400.6 | 1.55 ± 0.35   | 0.34   |
### Table IV: Correlation between clinical stage, cells and cytokine levels in sputum

| Cytokines | FEV1   | FEV1/FVC | Clinical stage | Neutrophils | Lymphocyt |
|-----------|--------|----------|----------------|-------------|-----------|
| GRO       | r = 0.021, p = 0.870 | r = -0.048, p = 0.710 | r = -0.262, p = 0.061 | r = 0.458, p = 0.749 | r = -0.223, p = 0.863 |
| sCD40L    | r = 0.249, p = 0.026 | r = -0.332, p = 0.003 | r = 0.199, p = 0.079 | r = < 0.0001, p = 0.816 |
| IL-17     | r = 0.014, p = 0.899 | r = -0.012, p = 0.911 | r = 0.063, p = 0.582 | r = -0.058, p = 0.641 | r = -0.015, p = 0.894 |
| IL-1RA    | r = 0.153, p = 0.180 | r = -0.158, p = 0.167 | r = 0.207, p = 0.072 | r = 0.036, p = 0.776 | r = -0.084, p = 0.479 |
| IL-1β     | r = -0.490, p < 0.0001 | r = -0.576, p < 0.0001 | r = 0.473, p < 0.0001 | r = 0.478, p < 0.0001 |
| IL-1α     | r = -0.368, p = 0.0008 | r = -0.498, p < 0.0001 | r = 0.466, p < 0.0001 | r = 0.284, p < 0.0001 | r = 0.10, p = 0.394 |
| IL-6      | r = 0.015, p = 0.894 | r = -0.021, p = 0.854 | r = 0.103, p = 0.368 | r = 0.002, p = 0.990 | r = 0.119, p = 0.312 |
| MIP-1α    | r = 0.281, p = 0.012 | r = 0.189, p = 0.095 | r = 0.261, p = 0.021 | r = -0.184, p = 0.140 | r = -0.079, p = 0.503 |
| MIP-1β    | r = -0.125, p = 0.271 | r = -0.158, p = 0.162 | r = 0.157, p = 0.171 | r = -0.0004, p = 0.998 |
| TNF-α     | r = 0.299, p = 0.013 | r = 0.167, p = 0.173 | r = -0.134, p = 0.278 | r = -0.111, p = 0.407 |
| VEGF      | r = 0.023, p = 0.848 | r = 0.014, p = 0.908 | r = -0.085, p = 0.946 | r = -0.143, p = 0.290 | r = 0.142, p = 0.263 |
| IL-8      | r = -0.336, p = 0.0005 | r = -0.333, p = 0.0005 | r = 0.384, p < 0.0001 | r = 0.038, p = 0.753 | r = -0.107, p = 0.345 |

Figures
No statistically significant difference in IL-1RA concentration was found between the COPD/tobacco and COPD/post-TB subgroups ($p = 0.8$). IL-1RA concentration was higher in the COPD/tobacco subgroup compared to the control group ($p = 0.014$) and higher in the COPD/post-TB subgroup compared to the control group ($p = 0.014$).
In the control group, the maximum concentration of IL-1α was 1726 pg/mL with an average of 539.1 ± 198.6 pg/mL. In the COPD/tobacco subgroup, the IL-1α concentration ranged from 81.06 pg/mL to 3937 pg/mL with a mean of 1555 ± 360.7 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-1α was 88.94 pg/mL and the highest was 1914 pg/mL with a mean of 821.2 ± 205.7 pg/mL. No statistically significant difference in IL-1α concentration was found between the COPD/tobacco and COPD/post-TB subgroups (p = 0.8). IL-1α concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.006) and higher in the COPD/post-TB compared to the control group (p = 0.029).
Concentration of IL-1β In the control group, the minimum concentration of IL-1β was 0.01 pg/mL and the maximum was 34.36 pg/mL with a mean of 9.410 ± 3.781 pg/mL. In the COPD/tobacco subgroup, the concentration of IL-1β ranged from 1.1 pg/mL to 1531 pg/mL (mean = 296.4 ± 143.5 pg/mL). In the COPD/post-TB subgroup, the lowest IL-1β concentration was 0.58 and the highest was 578.7 pg/mL (mean = 137.9 ± 65.25 pg/mL).

No statistically significant difference in IL-1β concentration was found between the COPD/tobacco and COPD/post-TB subgroups (p = 0.390). IL-1β concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.025) and higher in the COPD/post-TB subgroup compared to the control group with (p = 0.048)
In the control group, the minimum concentration of IL-6 was 0.0 pg/mL and the maximum was 54.14 pg/mL (mean = 11.64 ± 2.90 pg/mL). In the COPD/tobacco subgroup, the IL-6 concentration ranged from 0.44 pg/mL to 83.27 pg/mL (mean = 16.23 ± 7.768 pg/mL). In the COPD/post-TB subgroup, the lowest IL-6 concentration was 0.0 pg/mL and the highest was 63.07 pg/mL with a mean of 137.9 ± 65.25 pg/mL. IL-6 concentration was higher in the COPD/tobacco than in COPD/post-TB subgroup (p = 0.049), and higher in the COPD/tobacco subgroup compared to the control group (p = 0.033). No statistically significant difference in IL-6 concentration was found between the COPD/post-TB subgroup and control group (p = 0.665).
In the control group, the minimum concentration of IL-17 was 0.0 pg/mL and the maximum concentration was 4.27 pg/mL (mean = 1.421 ± 0.23 pg/mL). In the COPD/tobacco subgroup, the maximum concentration of IL-17 was 5.230 pg/mL with a mean of 1.54 ± 0.2650 pg/mL.

In the COPD/post-TB subgroup, the lowest concentration of IL-17 was 1.33 pg/mL and the highest concentration was 4.06 pg/mL with a mean of 2.412 ± 0.41 pg/mL. The difference in IL-17 concentration between the COPD/tobacco and the COPD/post-TB subgroups was not statistically significant (p = 0.053). IL-17 concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.041) and higher in the COPD/post-TB subgroup compared to the control group (p = 0.045)
In the control group, the minimum concentration of TNF-α was 0.0 pg/mL and the maximum concentration was 26.01 pg/mL (mean = 1.880 ± 0.31 pg/mL). In the COPD/tobacco subgroup, the maximum concentration of TNF-α was 43.01 pg/mL (mean = 7.112 ± 4.542 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of TNF-α was 0.0 pg/mL and the highest was 5.14 pg/mL (mean = 1.110± 0.5649 pg/mL). TNF-α concentration was higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup (p = 0.021) and higher in the COPD/tobacco subgroup compared to the control group (p = 0.048). Not statistically significant difference was found between the COPD/post-TB subgroup and the control group (p = 0.126).
In the control group, the concentration of IL-8 varied from 7.5 pg/mL to 541.4 pg/mL with a mean of $135.5 \pm 30.93$ pg/mL. In the COPD/tobacco subgroup, the concentration of IL-8 ranged from 4.40 pg/mL to 2250 pg/mL with an average of $493.1 \pm 244.9$ pg/mL. In the COPD/post-TB subgroup, the lowest concentration of IL-8 was 1.2 pg/mL and the highest was 630.7 pg/mL with an average of $103.0 \pm 68.58$ pg/mL. IL-8 concentration was higher in the COPD/tobacco subgroup compared to the COPD/post-TB subgroup ($p = 0.016$) and higher in the COPD/tobacco subgroup compared to the control group ($p = 0.009$). No statistically significant difference in IL-8 concentration was found between the COPD/post-TB subgroup and the control group ($p = 0.714$) (
In the control group, the minimum concentration of MIP-1β was 0.0 pg/mL and the maximum concentration was 389.4 pg/mL with a mean of 81.65 ± 20.27 pg/mL. In the COPD/tobacco subgroup, the concentration of MIP-1β ranged from 18.20 pg/mL to 780.9 pg/mL (mean = 181.1 ± 63.57 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of MIP-1β was 9.630 pg/mL and the highest was 872.9 pg/mL (mean = 224.1 ± 103.4 pg/mL). No statistically significant difference in MIP-1β concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.71), but it was higher in the COPD/tobacco subgroup compared to the control group (p = 0.012) and without statistically significant difference between the COPD/post-TB subgroup and control group (p = 0.16)
In the control group, the concentration of GRO varied from 0.0 pg/mL to 13518 pg/mL with a mean of 2563 ± 1617 pg/mL. In the COPD/tobacco subgroup, the concentration of GRO ranged from 0.0 pg/mL to 4850 pg/mL with a mean of 2563 ± 1617 pg/mL. In the COPD/post-TB subgroup, the lowest concentration of GRO was 132.9 pg/mL and the highest was 105133 pg/mL (mean = 1721 ± 977.7 pg/mL). No statistically significant difference in GRO concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.37) and between the COPD/tobacco subgroup compared to control group (p = 0.404), but it was higher in the COPD/post-TB sub-group compare to control group (p = 0.028).
In the control group, the minimum concentration of sCD40L was 0.0 pg/mL and the maximum concentration was 2.600 pg/mL with a mean of 0.286 ± 0.164 pg/mL. In the COPD/tobacco subgroup, the concentration of sCD40L ranged from 0.0 pg/mL to 8.570 pg/mL with a mean of 1.506 ± 0.441 pg/mL. In the COPD/post-TB subgroup, the lowest sCD40L concentration was 0.0 pg/mL and the highest was 4.760 pg/mL with a mean of 1.958 ± 0.384 pg/mL. No statistically significant difference in sCD40L concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.462). The difference between the COPD/tobacco subgroup and the control group was not statistically significant (p = 0.058), but the sCD40L concentration was higher in the COPD/post-TB subgroup compared to the control group (p = 0.001)
In the control group, the concentration of VEGF varied from 0.0 pg/mL to 1280 pg/mL with a mean of 332 ± 155.8 pg/mL. In the COPD/tobacco subgroup, the concentration of VEGF ranged from 33.63 pg/mL to 1214 pg/mL (mean = 435.2 ± 130.2 pg/mL). In the COPD/post-TB subgroup, the lowest concentration of VEGF was 0.0 pg/mL and the highest was 1143 pg/mL (mean = 333.9 ± 136.5). No statistically significant difference in VEGF concentration was found between the COPD/tobacco and the COPD/post-TB subgroups (p = 0.59), but the concentration was higher in the COPD/tobacco subgroup compared to the control group (p = 0.035) and not statistically significant difference between the COPD/post-TB subgroup and the control group (p = 0.926)