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ORIGINAL RESEARCH

Systemic Cytokine Profiles of CD4+ T Lymphocytes Correlate with Clinical Features and Functional Status in Stable COPD

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Aims: To evaluate the expressions of intracellular cytokines in CD4+ T lymphocytes and to investigate the correlation between biomarker expressions and clinical and functional characteristics of stable COPD patients.

Patients and Methods: Peripheral blood was collected from 36 COPD patients, and the expression of cytokines (IL-8, IL-13, IL-17, IL-6, IL-2, IL-10, and TNF-α) in T lymphocytes CD4 + was investigated. In addition, lung function, dyspnea symptoms, quality of life, vital signs, body composition, level of physical activity, peripheral muscle strength, and functional capacity were assessed.

Results: Individuals with greater bronchial obstruction present a higher proportion of CD4 + IL-2 + lymphocytes compared to individuals with less severe bronchial obstruction. We found a positive correlation between the expression of the cytokines IL-13, IL-17, IL-6, IL-2, IL-10, and TNF-α in CD4+ T lymphocytes. In addition, we found a positive correlation between CD4+ IL-10+ T lymphocytes and lower limb muscle strength and a negative correlation between CD4+ IL-8+ T lymphocytes and peripheral oxygen saturation and steps per day.

Conclusion: Systemic CD4+IL-2+, IL-8+, and IL-10+ T lymphocytes presented a correlation with clinical characteristics and functional status in stable COPD.

Keywords: chronic obstructive pulmonary disease, inflammation, phenotype, flow cytometry

Introduction

Chronic obstructive pulmonary disease (COPD) has a high prevalence worldwide and is associated with high morbidity and mortality.[1] COPD is a heterogeneous disease characterized by airflow limitation due to abnormalities in the airways and lung parenchyma with persistent respiratory symptoms. It is mainly caused by inhalation of harmful particles and/or gases, such as tobacco smoking.[2] With the progression of the disease, the original pulmonary inflammation becomes systemic causing profound changes in immune cell functions.[3]

Peripheral blood mononuclear cells (PBMC) comprise monocytes and lymphocytes (T, B and NK), important circulating cell populations that act as sensors and effectors of metabolic and inflammatory stresses.[4] This action is orchestrated by immune signals conducted through cytokines in patients with COPD.[5] Studies have shown increased signaling of proinflammatory cytokines in the plasma of patients with COPD, which directs and perpetuates chronic low-grade inflammation in this population, with consequent systemic damage.[5,6]

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Besides plasma cytokine concentrations, intracellular analysis in specific cell populations may provide an even more detailed view of the complex chain of events occurring in the inflammatory process.[7] Among the cell populations responsible for immune function in COPD, T lymphocytes, specifically, play an important role in stimulating or attenuating antibody production by B lymphocytes, or directly acting on antigens or infected cells, phagocytizing them.[8,9] Clusters of differentiation (CD)4+ T helper 1 and 17 cells have been shown to accumulate in the lungs of patients with stable COPD, producing interferon-γ (IFN-γ) and increasing inflammatory cells in the lungs.[10,11] Although some investigations have been conducted, the response of CD4+ T cells in COPD is not yet fully understood.[12–14]

In addition, previous studies have suggested that changes in the expression of cytokines may be linked to the individual's ability to respond to physical and psychological stressors. That is, the inflammatory profile may present associations with clinical and functional aspects.[15,16] However, the findings on systemic inflammation biomarkers and clinical characteristics of COPD are contradictory.[15–21]

In this way, it is essential to investigate the cell line responsible for synthesizing cytokines that orchestrate inflammation in stable COPD.[22] In addition, exploring correlations not previously investigated between the intracellular inflammatory profile and clinical characteristics and functional status, may contribute to the management of this heterogeneous group of patients, with more promising results and better outcomes.[23]

Thus, the aims of the present study were to evaluate the expression of intracellular cytokines in CD4+ T lymphocytes and to investigate the relationship between the biomarker expression and clinical and functional characteristics of stable COPD patients.

Patients and Methods

Participants

This is a cross-sectional study, which included individuals with COPD recruited from medical clinics and outpatient clinics between 2018 and 2020. All volunteers were diagnosed according to the GOLD criteria (the presence of a post-bronchodilator $FEV_1/FVC<0.70$ and/or dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors for the disease).[2] The inclusion criteria were: absence of exacerbation in the three months prior to collection, absence of infectious and autoimmune diseases, and not presenting respiratory diseases other than COPD. Seven patients who refused to perform blood collection and sixty-three patients whose samples were lost during processing were excluded from the analyses of the present study (Figure 1). This study was performed in accordance with the Declaration of Helsinki. This human study was approved by the Research Ethics Committee of São Paulo State University Presidente Prudente, São Paulo, Brazil – approval: 77909317.2.0000.5402. All adult participants provided written informed consent to participate in this study.

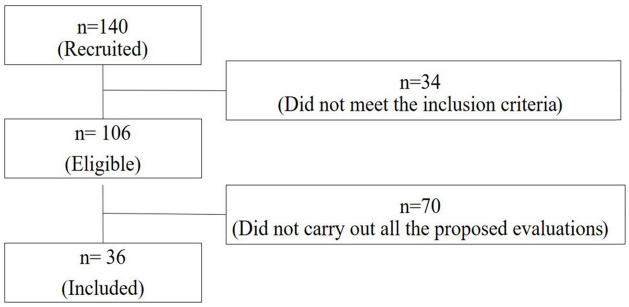

Figure 1 Flowchart of sample selection.

Procedures

Participants were familiarized with the study protocol and equipment before the experimental day. All assessments were performed in a room with a controlled temperature (24±2°C). Participants were assessed for dyspnea, quality of life, and vital signs in a resting sitting position. For assessment of lung function, body composition, peripheral muscle strength, and functional exercise capacity tests, individuals were instructed to arrive at the laboratory a minimum of 2 h after a light meal and were asked to abstain from caffeinated beverages and alcohol for at least 12 h before the test sessions. Individuals were instructed to fast for 12h prior to collection of peripheral blood for inflammation marker analyses.

The lung function, inflammation markers in peripheral blood, dyspnea, quality of life, vital signs, body composition, level of physical activity, peripheral muscle strength, and functional exercise capacity were evaluated. The procedures performed are listed below.

Lung Function

Lung function was verified by spirometry, to confirm the diagnosis of COPD,[2] using a portable spirometer MIR – Spirobank version 3.6. Data were interpreted based on the

methods proposed by Neder and collaborators who investigated the Brazilian population.[24]

Lymphocyte and Cytokine Staining

A 5 mL sample of peripheral venous blood was collected from the antecubital vein in a heparinized tube. The sample was diluted 1:1 in culture medium (RPMI 1640 Medium-Sigma Aldrich) and monensin (6.6 µL) and incubated for 4 hours at 37°C and 5% CO_2. The samples were divided into four analysis tubes (200 µL of sample per tube) and the red blood cell population was lysed (2mL of Lyse solution, per tube, with incubation for 10 minutes in the dark at room temperature). The tubes were centrifuged (500 × *g* for 5 minutes), the supernatant discarded, and the cells washed with filtered saline before being centrifuged again (500 × *g* for 5 minutes). CD3+ (10 µL Anti-CD3 FITC) and CD4+ (2 µL Anti-CD4 PERCP-CY5.5) were added to all tubes, which remained in incubation for 15 minutes at 4°C. In sequence, the cells were washed twice with filtered saline. Two hundred fifty microliters of Cytofix/Cytoperm® (Golg fixation/permeabilization kit) were added per tube and the samples remained for 20 minutes at 4°C under incubation. After two washes with 1mL Perm/Wash (Golg fixation/permeabilization kit), the samples were resuspended with 100µL of Perm/Wash, stained with monoclonal antibodies specific for the cytokines of interest (Anti-interleukin (IL) −8 PE, IL-13 APC, IL-17 PE, IL-6 APC, IL-2 APC, IL-10 PE, tumor necrosis factor alpha (TNF-α) APC), and then incubated for 30 minutes at 4°C. Next, the samples were washed twice with Perm/Wash and resuspended in 500µL of filtered saline for later reading on the flow cytometer. All reagents used in the analyses were obtained from Becton Dickinson, San Diego/CA.

Flow Cytometry Analysis

The FACSCalibur® 4-color cytometer (Beckton Dickinson) was used to acquire and analyze the samples, standardizing a total of $3x10^5$ events collected per tube. The lymphocyte identification strategy was based on the parameters of size (forward scatter) versus cell granularity (side scatter), following the characterization of the auxiliary T lymphocyte CD3+/CD4+ phenotype. The cell population of interest (gate) was selected to evaluate the expression of intracytoplasmic cytokines stained with specific monoclonal antibodies (Anti-IL-8, IL-13, IL-17, IL-6, IL-2, IL-10, TNF-α). Isotypic controls and fluorescence minus one (FMO) were used to distinguish non-specific fluorescences. The analyses were performed using CellQuetPRO® and FlowJo® software, with the results expressed in percentage values (Figure 2).

Dyspnea and Quality of Life

The sensation of dyspnea was assessed using the Medical Research Council (MRC) dyspnea scale,[25] and quality of life by the Chronic Respiratory Questionnaire (CRQ), validated for the Portuguese language.[26]

Vital Signs

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the left upper limb, with an analog sphygmomanometer and a stethoscope. Heart Rate (HR) and peripheral oxygen saturation (SpO_2) values were verified using a pulse oximeter (G-Tech Portable Oled Graph, Beijing, China).

Body Composition

In order to assess body composition, the electric bioimpedance Octopolar InBody 720 (Biospace, Seoul, Korea) was used. Skeletal muscle mass, body fat mass, and lean mass were evaluated. The data were electronically imported into Excel, using Lookin'Body software 3.0 (Biospace, Seoul, Korea).

Physical Activity Level

To analyze habitual daily physical activity, an Actigraph triaxial motion sensor, model GT3X (Actigraph LLC, Pensacola, FL) was used. The individuals were instructed to wear the equipment for seven days and remove it only when in contact with water (personal hygiene or water activities) and for night sleep. For data analysis, the specific software, ActiLife5 – Data Analysis Software by Actigraph, was used.

Peripheral Muscle Strength

Muscle strength was measured in the dominant hemibody using the Force Gauge® digital dynamometer, model FG-100kg (Tobarra, Spain) and the results were expressed in Newtons (N). The volunteers were instructed to perform elbow flexion, knee flexion, and extension movements.[27]

Functional Exercise Capacity

For the assessment of functional exercise capacity, participants performed the six-minute walk test (6MWT), according to the guidelines established by the American Thoracic Society.[28]

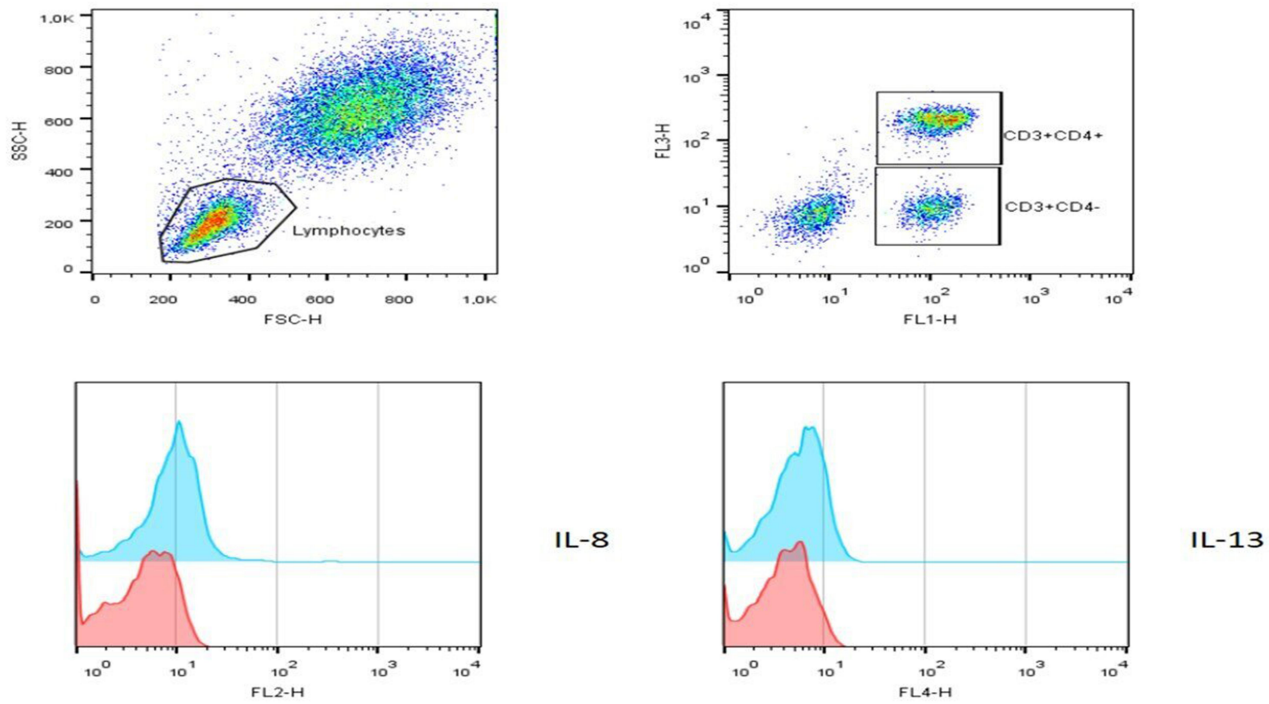

Figure 2 Characterization of intracytoplasmic cytokines in helper T lymphocytes. Peripheral blood cell suspension staining with specific monoclonal antibodies Anti-CD3, CD4 (surface) and IL-8, IL-13, IL-17, IL-6, IL-2, IL-10, TNF-α (cytoplasmic). Characterized CD3+/CD4+ T lymphocyte phenotype (dot plots), the expression of intracellular cytokines was evaluated as demonstrated in histograms (negative control - red; expression of cytokine - blue).

Statistical Analysis

To assess the normality of the data, the Shapiro–Wilk test was applied. Comparisons were performed using pulmonary function stratifications, according to the GOLD categorization.[2] GOLD subgroups 1 and 2 were combined, as well as GOLD subgroups 3 and 4, before being compared using the Student's *t*-test or Mann–Whitney test, according to the normality of the data. Correlation analyses were performed using the Pearson test for parametric data and the Spearman test for non-parametric data. To interpret the correlation coefficient, the following classifications were used: very strong ($r \geq 0.9$); strong ($0.9 > r \geq 0.7$); moderate ($0.7 > r \geq 0.5$); or weak ($r < 0.5$).[31] In addition, for significant correlation results ($p < 0.05$), linear regression was performed.

Results

A total of 36 volunteers with COPD were assessed in the study, 19 patients with FEV_1 (% of predicted) over 50% and 17 under 50%. GOLD 1 and 2 presented better lung function, SpO_2, steps per day, and functional exercise capacity than GOLD 3 and 4. Similar symptoms, quality of life, body composition, muscle strength, and functional capacity were observed among the GOLD subgroups. Characteristics of all participants are shown in Table 1.

Table 2 shows the cytokine expression of the sample. When comparing the proportion of TCD4 + lymphocytes that expressed cytokines, a 5.61% higher mean IL-2+ was observed in patients with more severe obstruction ($p = 0.03$). The proportions of IL-8 +, IL13 +, IL-17 +, IL-6 +, IL-10 +, and TNF-α + in T lymphocytes were not distinguished between patients with different degrees of airway obstruction ($p > 0.05$).

Figure 3 presents linear regression graphs of significant positive correlations between the expression of IL-13, IL-17, IL-6, IL-2, IL-10, and TNF-α in CD4+ T lymphocytes ($p < 0.05$). No significant correlations were observed in the analyses of IL-8 with the other cytokines.

Figure 4 presents the correlations between cytokine expression in CD4+ T lymphocytes and clinical and functional characteristics. Significant correlations were observed between IL-10 expression and muscle strength of knee flexors ($p=0.003$; $r=0.493$) and knee extensors ($p=0.014$; $r=0.413$) (Figure 4A). In addition, negative correlations were observed between IL-8 expression and steps per day ($p=0.043$; $r=-0.339$) and IL-8 and peripheral oxygen saturation ($p=0.031$; $r=-0.361$) (Figure 4B). Finally, a correlation

Table 1 Participant Characteristics

	Total Sample (n=36)	GOLD 1 and 2 (n=19)	GOLD 3 and 4 (n=17)	p	Mean Difference (95% CI)
Male/Female n (%)	19 (52.8)/17 (47.2)	9 (47.4)/10 (52.6)	10 (58.8)/7 (41.2)	0.49	
Age (years)	69.67±7.3	69.79±8.05	69.53±6.61	0.92	0.26 (−4.76 to 5.28)
Body weight (kg)	67.12±14.1	65.93±15.58	68.44±12.57	0.60	−2.51 (−12.17 to 7.16)
Height (m)	1.63±0.09	1.60±0.08	1.66±0.09	0.06	−0.06 (−0.11 to 0.00)
Lung function					
FEV_1 (% of predicted)	52.81±20.6	68.79±13.73	34.94±8.56	<0.001*	33.85 (25.99 to 41.71)
FVC (% of predicted)	72.42±18.8	82.68±15.15	60.94±15.8	<0.001*	21.74 (11.25 to 32.23)
FEV_1/FVC (%)	53.48±11.76	61.54±6.35	44.46±9.69	<0.001*	17.07 (11.58 to 22.56)
Dyspnea					
MRC	3 (2–4)	3.5 (2–4)	3 (2–5)	0.61	−0.24 (−1.17 to 0.69)
Quality of life					
CRQ	19.88±5.11	21.16±4.25	18.45±5.71	0.11	2.72 (−0.67 to 6.10)
Vital signs					
SBP (mmHg)	120.56±15.85	121.58±16.42	119.41±15.6	0.69	2.17 (−8.71 to 13.05)
DBP (mmHg)	80 (70–90)	80 (70–90)	80 (70–90)	0.56	−2.04 (−9.13 to 5.05)
HR (beats/minute)	74.5 (68.25–81.75)	74 (67–81)	75 (69.5–82.5)	0.24	−4.35 (−11.67 to 2.98)
RF (breaths/minute)	18 (16–20)	20 (16–20)	16 (15–20)	0.75	0.39 (−2.11 to 2.89)
SpO_2 (%)	94.39±3.24	96.05±1.58	92.53±3.62	<0.001*	3.52 (1.66 to 5.38)
Body composition					
Fat mass (kg)	24.12±9.56	23.69±10.6	24.58±8.61	0.79	−0.89 (−7.55 to 5.78)
Skeletal muscle mass (kg)	22.9±4.92	22.23±5.15	23.61±4.72	0.42	−1.37 (−4.78 to 2.03)
Lean mass (kg)	40.11±7.77	38.97±8.08	41.32±7.47	0.38	−2.35 (−7.71 to 3.01)
Physical activity level					
Steps per day	4080.5 (2606.93–5599.21)	4436.14 (2788.43–6112.71)	3093.71 (1356.29–4552.41)	0.03*	2381.37 (282.51 to 4480.23)
Muscle strength					
Elbow flexors (N)	87.4 (64.4–123.2)	82.9 (61.35–115.9)	104.6 (64.6–127.8)	0.66	−6.95 (−38.32 to 24.42)
Knee flexors (N)	124.63±43.5	121.36±40.37	128.09±47.58	0.65	−6.74 (−37.02 to 23.55)
Knee extensors (N)	162.2 (106.2–223.2)	166.7 (105.65–205.8)	155.8 (96.5–228)	0.64	13.75 (−45.17 to 72.67)
Functional exercise capacity					
6MWT distance (m)	439.56±123.35	480.58±113.13	393.71±121.05	0.03*	86.87 (7.55 to 166.20)

Notes: Data expressed as mean ± standard deviation or median (range 25% −75%), according to normality. *p<0.05 when comparing the ratings by GOLD.
Abbreviations: n, sample size; %, percentage; kg, kilograms; m, meters; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; MRC, Medical Research Council dyspnea scale; CRQ, Chronic Respiratory Questionnaire; SBP, systolic blood pressure; DBP, diastolic blood pressure; mmHg, millimeters of mercury; HR, heart rate; RF, respiration frequency; SpO_2, peripheral oxygen saturation; N, newton; 6MWT, six-minute walk test.

Table 2 Expression of Cytokines in CD4+ T Lymphocytes in the Total Sample and in Subgroups Classified by GOLD

	Total Sample (n=36)	GOLD 1 and 2 (n=19)	GOLD 3 and 4 (n=17)	p	Mean Difference (95% CI)
IL-8 (%)	4.59 (1.82–23.22)	2.64 (1.5–22.47)	5.35 (2.82–26.28)	0.60	−3.59 (−17.42 to 10.24)
IL-13 (%)	10.14±4.46	9.34±4.74	11.04±4.09	0.26	−1.69 (−4.71 to 1.32)
IL-17 (%)	0.12 (0.06–0.22)	0.11 (0.06–0.28)	0.12 (0.06–0.2)	0.35	0.05 (−0.06 to 0.17)
IL-6 (%)	10.75±5.02	10.23±5.25	11.32±4.84	0.52	−1.09 (−4.52 to 2.35)
IL-2 (%)	21.18±7.72	18.53±6.94	24.15±7.66	0.03*	−5.61 (−10.55 to −0.67)
IL-10 (%)	0.14 (0.08–0.24)	0.14 (0.06–0.24)	0.14 (0.09–0.27)	0.44	−0.05 (−0.17 to 0.08)
TNF-α (%)	8.42±3.78	8.13±4.56	8.75±2.76	0.63	−0.62 (−3.21 to 1.97)

Notes: Data expressed as mean ± standard deviation or median (range 25% −75%), according to normality. *p<0.05 when comparing the ratings by GOLD.
Abbreviations: IL, interleukin; TNF-α, tumour necrosis factor alpha; %, percentual.

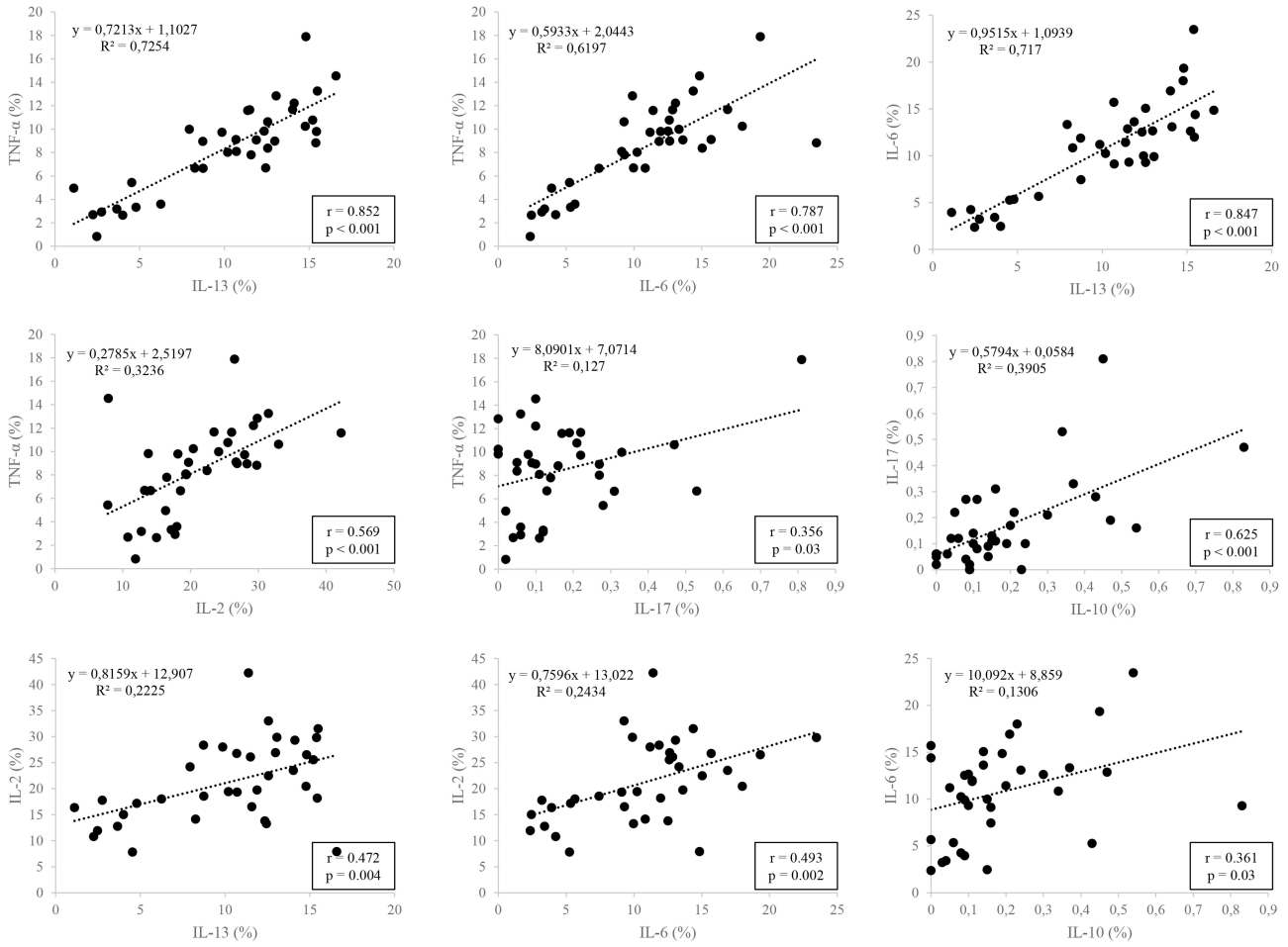

Figure 3 Graphical representation of linear regression between cytokine expressions in CD4+ T lymphocytes.

was also observed between the expression of IL-17 and FVC (% of predicted) (p=0.047; r=0.333) (Figure 4C).

Quality of life, body composition, and functional exercise capacity were not related to the proportion of lymphocytes expressing the evaluated cytokines (Supplementary Table).

Discussion

In the present study, individuals with more severe bronchial obstruction presented a higher proportion of CD4+ IL-2+ T lymphocytes. The study also demonstrated correlations between pro (IL-13, IL-6, IL-17, IL-2, and TNF-α) and anti-inflammatory (IL-10) cytokines in CD4+ T lymphocytes. Furthermore, to the best of our knowledge, this is the first study to show positive correlations between CD4+T IL-10+ T lymphocytes and muscle strength, and negative correlations between CD4+ IL-8+T lymphocytes and steps per day and peripheral oxygen saturation, as well as CD4+ IL-17+ T lymphocytes and forced vital capacity in patients with stable COPD.

Systemic inflammation in COPD has been extensively investigated.[30] Meta-analyses have confirmed that COPD patients, even when clinically stable, present high plasma concentrations of several factors such as leukocytes, C-reactive protein (CRP), IL-6, IL −8, fibrinogen, and TNF-α characterizing a condition of systemic inflammation.[31,32] Furthermore, many studies have explored the association of cytokines with the lung function of patients with stable COPD.[30,33–35]

Previous studies have shown correlations between plasma levels of TNF-α, IL-1β, IL-6, IL-10, IL-15, and IL-17 with the severity of bronchial obstruction.[33–35] Bradford et al, in a combined analysis of two large studies, including 2123 subjects from "COPDGene" and 1117 subjects from "SPIROMICS", with analysis of six cytokines (IL-2, IL-6, IL-8, IL-10, TNF-α, and IFN-γ), found an association of four biomarkers (IL-6, IL-8, and IL-10) with worse airflow obstruction.[30]

However, the inflammatory response is the result of complex connections of many cells and different molecules,

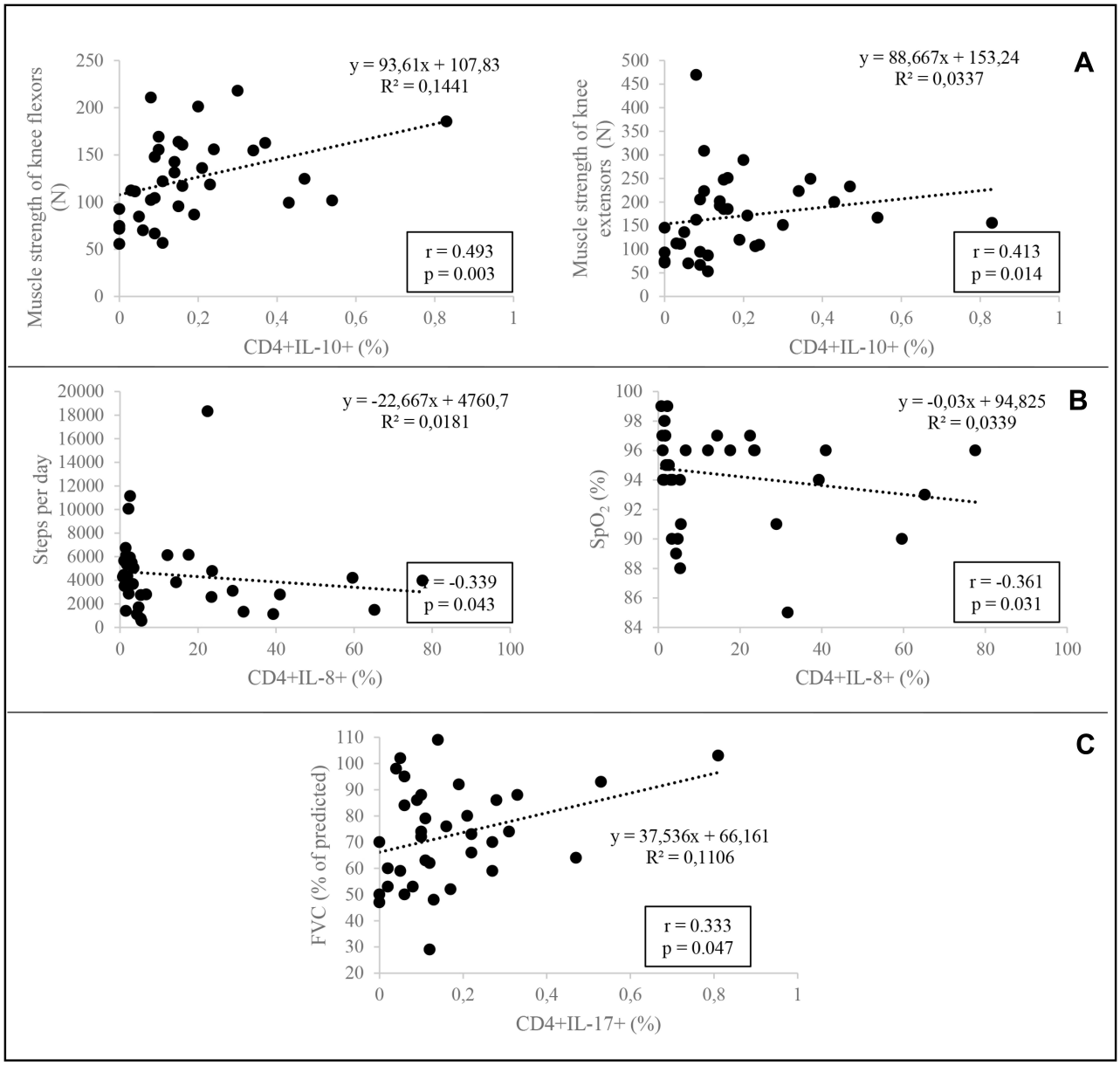

Figure 4 Graphical representation of linear regression between cytokine expressions in CD4+ T lymphocytes and clinical and functional characteristics. (**A**) presents the correlations between CD4+IL-10+ and muscle strength expression; (**B**) is between CD4+IL-8+ and steps per day and peripheral oxygen saturation; (**C**) is the correlation between CD4+IL-17+ and FVC (% of predicted).

which may justify some contradictions observed in the studies described above that evaluated cytokines at plasma levels. Knowledge of the cell line responsible for the synthesis of cytokines is essential for the precise definition of the chain of events that leads to the analysis of a biological function. In this way, intracellular analyses have emerged to explore the specific behavior of the expression of inflammation markers and, consequently, clarify the broad process that occurs in diseases.[36]

In the present study, we identified positive correlations in the proportion of CD4+ T lymphocytes that expressed IL-2, IL-6, IL-10, IL-13, IL-17, and TNF-α, in the total sample. Studies have shown that both TNF-α[37] and IL-2[38] promote inflammatory reactions and IL-6 seems to be linked to the pathological mechanisms of COPD.[39] The findings about IL-13 are conflicting, but in summary, this cytokine appears to be more associated with the mucus-producing cell metaplasia in COPD.[39] Furthermore, evidence indicates that IL-17 can induce the expression of innumerable pro-inflammatory cytokines, and that its production is controlled by regulatory cytokines, with emphasis on IL-10,[40] which may explain the correlation between them observed in the present study. These

associations could encourage future research on the exploration and understanding of intracellular mechanisms, since if not controlled, the signaling performed by this range of cytokines, by cells of the immune system, may amplify inflammatory responses.

We found a correlation between CD4+IL-17+ and FVC that was not observed in a previous study.[12]

In the present study, we observed a higher proportion of CD4+ IL-2+ T lymphocytes in individuals with more severe bronchial obstruction. It is known that IL-2 triggers the proliferation and differentiation of T cells, being crucial for the defense of pathogens.[41]

Corroborating our results, Knobloch et al, observed that patients with COPD with inflammation present more intense IL-2 + production by CD4 + lymphocytes, compared to healthy individuals and smokers. However, Knobloch et al point out that despite increased expression of IL-2, people with COPD may not respond with increased T cell proliferation.[42] Thus, despite the increase in IL-2 in the most severe COPD, there may not be a proliferative response of lymphocytes in the same proportion in these individuals, which compromises their defense system. In addition, the authors[42] hypothesize that bacteria partially block T-cell proliferation more intensely in COPD, and with greater intensity in patients with greater obstruction, resulting in immunosuppressive mechanisms.

Systemic manifestations in COPD are diverse and affect patients differently, making them a heterogeneous population,[43] in which individuals are distinguished in relation to biological, clinical, and functional characteristics.[44] In an attempt to identify subgroups of patients, in order to act more precisely, the correlation of biomarkers with clinical and functional characteristics of patients with stable COPD has been explored. However, studies that investigated inflammatory biomarkers using plasma analysis and clinical characteristics such as sex,[18] dyspnea,[17] fatigue,[17] pain,[17] and exacerbation risk[19] found no associations between these variables.

In the present study, we observed a positive correlation between the proportion of CD4+ IL-10+ T cells and the strength of the knee flexor and extensor muscles. It is known that IL-10 is considered an anti-inflammatory cytokine, as it has the ability to suppress the pro-inflammatory response and, therefore, limit tissue damage from the inflammatory process, contributing to homeostasis.[45] In addition, the benefits of muscle strength, specifically of the lower limbs, for COPD patients are widely known.[46,47] However, to the best of our knowledge, this is the first study to show a correlation between CD4+ IL10+ T cells and muscle strength in stable COPD. This finding reinforces the importance of peripheral muscles not only for functionality but also as a possible factor for suppressing the systemic inflammatory response.

In addition, negative correlations were observed between CD4+ IL-8 T lymphocytes and peripheral oxygen saturation and the mean number of steps taken per day. The cytokine IL-8 is one of the most active and widespread biological molecules, with a pro-inflammatory role, as it has chemotactic properties for immune cells.[37] In addition, IL-8 is also associated with increased mucus production and remodeling processes that affect the airways.[37] In an in vitro experiment, Dziurla et al showed that oxygen deprivation can facilitate inflammatory reactions,[48] which may explain the negative correlations found in the present study, that is, lower oxygen values in peripheral blood, and higher proportions of CD4+ T lymphocytes that express IL-8+. In addition, peripheral oxygen saturation showed a positive correlation with the mean number of steps taken per day ($p=0.001$; $r=0.534$), indicating a direct link between these variables.

It is clear that the cross-sectional nature of this research is limiting and does not allow us to clarify the relation between cause and effect. Furthermore, the investigation of a single cell population (CD4+ T lymphocytes) is not able to reveal the interaction of other populations throughout the systemic inflammation process that occurs in stable COPD. Therefore, future prospective research, involving several cells, is necessary to assist in understanding biomarkers of systemic inflammation in patients with stable COPD and their association with clinical and functional characteristics, to assist clinicians in decision-making.

The novel findings of this study are the profile of cytokines in CD4+ T lymphocytes and the correlation with clinical and functional characteristics in stable COPD. The main findings were a higher proportion of CD4+ IL-2+ T lymphocytes in patients with greater bronchial obstruction. In addition, the data indicate a link between IL-13, IL-6, IL-2, and TNF-α signaling in CD4+ T lymphocytes. Finally, there is a positive correlation between CD4+ IL-10+ T lymphocytes and lower limb muscle strength and a negative correlation between CD4+ IL-8+ T lymphocytes and peripheral oxygen saturation and steps per day. These findings may contribute to understanding of the role of systemic inflammation in the pathogenesis and progression of COPD and, particularly, provide guidance for clinical decisions.

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Disclosure

The authors report no conflicts of interest in this work.

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