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Severity of COPD and its relationship with IL-10

Bruna S.A. Silva[a,b,*], Fábio S. Lira[c], Dionei Ramos[d,e], Juliana S. Uzeloto[e], Fabrício Eduardo Rossi[f], Ana Paula C.F. Freire[e], Rebeca N. Silva[e], Iara B. Trevisan[e], Luis Alberto Gobbo[a,b,e], Ercy M.C. Ramos[d,e]

[a] Skeletal Muscle Assessment Laboratory (LABSIM), São Paulo State University (UNESP), School of Technology and Sciences, Department of Physcial Education, Presidente Prudente, SP, Brazil
[b] Post Graduation Program in Motricity Sciences, São Paulo State University (UNESP), School of Technology and Sciences, Presidente Prudente, SP, Brazil
[c] Exercise and Immunometabolism Research Group, Department of Physical Education, São Paulo State University (UNESP), School of Technology and Sciences, Presidente Prudente, SP, Brazil
[d] Department of Physical Therapy, São Paulo State University (UNESP), School of Technology and Sciences, Presidente Prudente, SP, Brazil
[e] Post Graduation Program in Physical Therapy, São Paulo State University (UNESP), School of Technology and Sciences, Presidente Prudente, SP, Brazil
[f] Immunometabolism of Skeletal Muscle and Exercise Research Group, Department of Physical Education, Federal University of Piauí (UFPI), Teresina, PI, Brazil

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ABSTRACT

Background: The present study was designed to compare inflammatory and metabolic responses according to severity of airflow among patients with COPD and to verify the relationship between pulmonary function, body composition, metabolic and inflammatory profile.

Methods: Fifty-one patients with mild to very severe COPD were recruited and divided according lung function in Mild-moderate (GOLD 1–2) n = 21; Severe (GOLD 3) n = 25 and Very severe (GOLD 4) n = 5. Patients were submitted to assessments of lung function (spirometry), functional exercise capacity (6-min walk test), body composition (Octopolar bioelectrical impedance), metabolic profile (glucose, triglycerides, total cholesterol, HDL-cholesterol and albumin (colorimetric assay)) and inflammatory profile (cytokines: IL-6, IL-10, TNF-α and IL-15 (ELISA)).

Results: We found that patients in GOLD 3 group had lower levels of IL-10, triglycerides, visceral fat area, and higher IL-6 and IL-6/IL-10 ratio when compared to GOLD 1–2 patients. Additionally, GOLD 1–2 group presented negative correlation between TNF-α and HDL cholesterol ($p = .01$) and positive correlation between IL-15 and FEV_1/FVC ($p = .01$), while GOLD 3 group showed positive correlation between IL-6 and IL-10 ($p < .01$), IL-6 and total cholesterol ($p < .01$) and negative correlation between IL-10 and HDL-cholesterol ($p = .01$).

Conclusion: Our findings suggest that patients with severe COPD can exhibit compromised "inflammatory status", characterized by higher IL6, IL-6/IL-10 ratio and lower IL-10 concentration. Furthermore, IL-10 seems to be an interesting cytokine to be investigated in this kind of patients.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is characterized by persistent airflow limitation that is usually progressive over time. It is associated with enhanced chronic inflammatory responses in the airways and the lungs and is the fourth leading cause of death in the world, representing an important problem for public health [1–3]. Pulmonary modifications are frequently observed in patients with COPD, including changes in body composition [4,5], skeletal muscle dysfunction [6], cardiovascular disease [7], depression [8], osteoporosis [9], reduced exercise tolerance [10] and systemic inflammation [11].

Additionally, chronic systemic inflammation is associated with several risk factors and can be linked with different complications,

Abbreviations: Δ, delta variation; 6MWT, 6-minute walk test; BMI, body mass index; cm^2, centimeter squared; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; FEV_1/FVC, ratio between forced expiratory volume in one second and forced vital capacity; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; g, grams; GOLD, global initiative for chronic obstructive lung disease; GOLD 1, mild $FEV_1 \geq 80\%$ predicted; GOLD 2, moderate $50\% \leq FEV_1 < 80\%$ predicted; GOLD 3, severe $30\% \leq FEV_1 < 50\%$ predicted; GOLD 4, very severe $FEV_1 < 30\%$ predicted; HDL, high-density lipoprotein; IL-10, interleukin 10; IL-15, interleukin 15; IL-6, interleukin 6; kg, kilogram; mg/dl, milligram per deciliter; ml, milliliters; pg/ml, picogram per milliliters; TNF-α, tumor necrosis factor alpha

* Corresponding author at: São Paulo State University (UNESP), School of Technology and Sciences, Presidente Prudente, Roberto Simonsen Street, 305, Educational center, 19060-900, Presidente Prudente, SP, Brazil.
E-mail address: brunaspolador@gmail.com (B.S.A. Silva).

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including atherosclerosis, cachexia, and anorexia [12]. Systemic inflammation in patients with COPD has been the focus of discussion, since it may be the cause for the development of many disorders associated with the disease. Several studies have characterized low-grade chronic inflammation by elevated serum tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6 (cytokines pro-inflammatory)) [11,13–15]. Recently, ECLIPSE study [16] showed that only 30% of patients with COPD do not exhibit increased levels of pro-inflammatory cytokines. In addition, few studies have investigated anti-inflammatory cytokines, such as interleukin 10 (IL-10) and interleukin 15 (IL-15) [17,18] and the etiology of systemic inflammation in patients with COPD is still unknown.

Most physicians use a spirometric method to detect obstruction of airflow [1] and to determine the severity and progression of the disease. Studies [19–21] have demonstrated specific associations between clinical and morphological characteristics of COPD and its severity. However, it is not clear the relationship between the severity of the disease and the profile of metabolic and inflammatory markers. Therefore, the present study was designed to explore the inflammatory status of patients with COPD and to characterize them according to clinical practice. Thus, the aim of this study was to compare inflammatory and metabolic responses according to severity of airflow in patients with COPD and to verify the relationship between pulmonary function, body composition, metabolic and inflammatory profile.

2. Material and methods

The sample was composed of 51 clinically stable patients with mild to very severe COPD, classified according to internationally accepted criteria [1]. Patients were excluded if they were active smokers or had respiratory disorders other than COPD.

All individuals were informed beforehand of the objectives and procedures of the study and provided written consent to participate. All procedures were approved by the Research Ethics Committee (CAAE: 12492113.5.0000.5402) and followed the Resolution 466/12 of the Brazilian National Health Council. Patients were recruited by convenience sample and divided according to severity of disease to avoid potencial bias. The sample size of this study was based on the observation from a previous study that verified the relationship between IL-10 and forced expiratory volume in one second in patients with asthma-chronic obstructive pulmonary disease overlap syndrome and observed a Pearson correlation of ($r = 0.58$, $p < .001$) [22]. Also, we used a power of 0.80 and a type I error of 0.05 according suggested by Miot et al. [23], which it estimated that we would need 21 participants per group. Considering a dropout rate of 25–40%, we over-recruited the number of participants. The sample number and division are detailed in Fig. 1.

2.1. Study design

Patients were recruited and submitted to assessments that included: measurements of lung function (spirometry) [1,24–26], functional exercise capacity (6 min walk test, 6 MWT), body composition (Bioelectrical impedance), inflammatory (ELISA) and metabolic (colorimetric assay) profile. A brief description of the assessments is presented below.

2.2. Procedures

2.2.1. Pulmonary function

Spirometry was performed using a digital spirometer (MIR–Spirobank® version 3.6, Waukesha-Wisconsin/EUA) according to the guidelines for pulmonary function tests [24]. The interpretation of the results followed the recommendations of the American Thoracic Society and the European Respiratory Society [25] and the results were compared to data of the Brazilian population [26].

The spirometric criterion for airflow limitation was a post-bronchodilator fixed ratio of $FEV_1/FVC < 0.70$. Classification of COPD severity followed the *Global Initiative for Chronic Obstructive Lung Disease* [1]: GOLD 1: Mild $FEV_1 \geq 80\%$ predicted; GOLD 2: Moderate $50\% \leq FEV_1 < 80\%$ predicted; GOLD 3: Severe $30\% \leq FEV_1 < 50\%$ predicted; and GOLD 4: Very Severe $FEV_1 < 30\%$ predicted.

2.2.2. Functional exercise capacity

Functional exercise capacity was assessed using the 6 MWT, according to the guidelines of the American Thoracic Society [27]. The test was conducted in a 30 m track by previously trained researcher. The participants were requested to walk as fast as possible during six minutes and, if necessary, they could stop and then retake the test. Encouragement phrases were used with the purpose of keeping the same walking pace throughout the test. At the end, the walking distance was measured.

2.2.3. Bioelectrical impedance analysis

Bioelectrical impedance analysis was performed using the Octopolar InBody 720 Composition Analyzer (Copyright®, 1996–2006, by Biospace Corporation, USA). First, the participant's age, gender and height were entered into the software. Then, the patients stood barefoot on the metal footplate and held the handles with relaxed arms. Once impedance was measured, values of fat mass (kg), muscle mass (kg) and visceral fat area (cm^2) of five different body sites (arms, legs, trunk and general overall set) were recorded. Anthropometric measurements were assessed by the same researcher throughout the study to minimize interpersonal errors. Patients were asked not to eat or drink two hours before the test, not to engage in moderate or vigorous exercise 24 h before the test, and not to consume alcohol beverages.

2.2.4. Blood sampling analyses

Blood samples (15 ml) were collected at rest (fasting) and were immediately allocated into three different tubes: 5 ml Vacutainer® tubes (Becton Dickinson, BD®, Juiz de Fora, MG, Brazil) containing EDTA for plasma separation and one 5 ml dry Vacutainer® tube for serum separation. The tubes were centrifuged at 3500*g* for 15 min at 4 °C, and plasma and serum samples were stored at −20 °C until analysis. The cytokines: IL-6 (range 0.30–2.30 Log-pg/ml), IL-10 (range 0.30–2.48 Log- pg/ml), TNF-α (range 0.60–2.70 Log- pg/ml), and IL-15 (range 1.30–3.40 Log- pg/ml) were analyzed using human ELISA Ready-Set-Go kits (eBioscience® Vienna, Austria). Glucose (mg/dl), triglycerides (mg/dl), total cholesterol (mg/dl) and high-density lipoprotein (HDL) cholesterol (mg/dl) were assessed using commercial kits (Labtest®, São Paulo, Brazil). Albumin measurement was analyzed by color change of Coomassie brilliant blue G-250 dye [28].

2.3. Statistical analysis

A statistical package (SPSS version 22.0, SPSS® Inc., USA) was used for data analysis. Normality of the data was assessed using the Kolmogorov-Smirnov test and results were described as mean ± standard deviation or as median (interquartile range 25–75%), according to data distribution. Categorical variables were analyzed using the chi-square test (For medicine intake GOLD groups were categorized into: GOLD 1–2 = 1 and GOLD 3 = 2, and drugs used: 0–1 drugs = category 1; 2–4 drugs = 2; and over 5 drugs = 3). The comparison between the groups GOLD 1–2 and GOLD 3 was performed using Student *t* test for parametric distributions or the Mann Whitney test for nonparametric distributions. For biochemical variables (cytokines) was performed *t*-test on log base scale. To determine the cut-off value for IL-10 concentration, receiver-operating characteristic (ROC) curves were constructed using percentile 50 and the area under the curves (AUC) determined. The relationship between variables was analyzed using Pearson's correlation coefficient (*r*) and the level of significance was set at $p < .05$.