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T-cell profile and systemic cytokine levels in overweight-obese patients with moderate to very-severe COPD

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ABSTRACT

This study aimed to evaluate the immune profile of lean and overweight-obese COPD patients. Forty patients with moderate to very severe COPD were divided into lean group (n = 20; aged 62.00 ± 8.91 years; BMI 22.26 ± 1.65 kg/m^2) or overweight-obese group (n = 20; aged 65.40 ± 6.69 years; BMI 29.19 ± 3.55 kg/m^2). The cytokine profile (IL-2, IL-4, IL-6, IL-10, INF-γ, and TNF-α) was evaluated through the Cytometric Bead Array technique, and the expression of CD4, CD8, CD25, CD45ra, CD45ro, CD69, CD195(CCr5) and HLA-DR were evaluated in CD3+ T-cells. Overweight-obese COPD group had lower levels of IL-2 (p = 0.01) and higher INF-γ levels (p = 0.02) and IL-6 (p = 0.003) than lean COPD. Lean COPD patients had higher CD25+ (p = 0.01), CCr5 (p = 0.04) and HLA-DR (p = 0.007) expression on T cell surface compared to overweight-obese COPD participants. These changes are related to immune dysfunction of obesity, and excess of fat mass in COPD can be a key factor to low T-cells activation.

1. Introduction

Body mass loss is one of the main determinants of low functional capacity and poor quality of life in chronic obstructive pulmonary disease (COPD) (Wilson et al., 1989; Schols et al., 1998). Interestingly, the low body mass index (BMI) is a risk factor for mortality in severe COPD with poor ventilator capacity (Landbo et al., 1999; Prescott et al., 2002). In addition, high BMI (ranging from 25.0 to 35.0 kg/m^2) exerts a protective effect in mortality of COPD patients with poor pulmonary function (Landbo et al., 1999; Prescott et al., 2002). This obesity paradox is observed in others conditions, such in elderly individuals and chronic diseases (Poirier et al., 2006; Kim et al., 2016; Chittal et al., 2015). On the other hand, Poulain et al. (2008 showed that airflow obstruction was less severe in overweight-obese COPD individuals compared with normal weight COPD patients, despite the higher metabolic syndrome prevalence in the former. However, obesity induces an expiratory flow limitation and airway closure, causing hypoxemia from ventilation-perfusion mismatching (Salome et al., 2010). Moreover, obese COPD individuals also present lower forced vital capacity (FVC) compared to non-obese COPD, which may represents a restrictive abnormality (Baustista et al., 2011).

However, both COPD and obesity conditions are associated with a low-grade systemic inflammation as potential mechanism linking to increased rate of cardiometabolic diseases (Kanneganti and Dixit, 2012). Due to increased visceral fat mass, obesity stimulates many metabolic derangements and inflammatory disturbances such as insulin resistance, dyslipidemia and increased levels of proinflammatory tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) (Cildir et al., 2013). Adipocyte hypertrophy, macrophage and T-cells infiltration, and an increase in proinflammatory cytokines production are some of the most important events in immune dysfunction of obesity (Schipper et al., 2012). It is well known that T Lymphocytes, commonly identified by expressing CD3 on cell surface, have been implicated in obesity-related adipose tissue inflammation (Cildir et al., 2013; Schipper et al., 2012). Previous data reported that T-cells infiltrate into adipose tissue prior to macrophages and to contribute to the development of insulin resistance in obese individuals (Cildir et al., 2013; Chatzigeorgiou et al., 2012). Alongside studies assessing T-cells profile, data evaluating T-cells activation status or co-stimulatory molecules also were performed. In this sense, the higher frequency of CD25+, but not CD69+ or

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Human Leukocyte Antigen-DR (HLA-DR), on T-cell surface was reported in unhealthy obese individuals but not in metabolically healthy obese persons (Viardot et al., 2010, 2012).

Multiples studies showed a strong relationship between systemic inflammation, metabolic abnormalities and higher cardiovascular diseases prevalence in COPD (Poulain et al., 2008; Breyer et al., 2009; Van der Borst et al., 2011). In this way, tobacco smoking is the main risk factor for development of COPD, leading to pulmonary inflammation which induces pulmonary structural alterations, such as alveolar destruction and pulmonary remodeling (Sethi et al., 2012). While pulmonary inflammation have a key role in COPD pathophysiology, systemic inflammation is also present in COPD and it is specially related to COPD exacerbations and lung function decline (Stockley, 2009). In addition, the presence of abdominal fat mass or overweight-obesity status in COPD patients play a key role to induction of higher levels of TNF-α and IL-6 (Van der Borst et al., 2013; Skyba et al., 2010). For instance, the BODE index, a multidimensional system based on the body mass index, degree of airflow obstruction, dyspnea and exercise capacity, strongly correlate with C-reactive protein in COPD patients (Sarioglu et al., 2010). In addition, the higher T-cell activation, indicating through the higher expression of HLA-DR+ on T-cells, in COPD patients contribute to elevated cytokine levels found in these patients (Tan et al., 2014; Takabatake et al., 2000; Gadgil and Duncan, 2008). In this way, the association between COPD and obesity may represent a high risk for metabolic syndrome and cardiovascular diseases due to increases in inflammatory subclinical status (Poulain et al., 2008).

However, to date, no data regarding the impact of overweight-obese condition on T-cell profile of COPD was found. Due to the aforementioned facts about the role of T-cells on inflammatory status in obesity, it is possible to hypothesize that excessive body mass in COPD can be a key factor to modifications in T-cells activation markers. In this line, the aim of the present study was to evaluate the immune profile of lean and overweight-obese COPD patients.

2. Methods

2.1. Patients

The study was approved by Institutional Ethics Committee (0302.0.005.000-08) and all participants read and signed an informed consent. We recruited 40 COPD patients of both gender: 20 overweight-obese subjects with BMI greater than 27.0 kg/m^2 (age 65.40 ± 6.69 years; BMI 29.19 ± 3.55 kg/m^2) and 20 matched lean patients with BMI less than 25.0 kg/m^2 but greater than 21.0 kg/m^2 (age 62.00 ± 8.91 years; BMI 22.26 ± 1.65 kg/m^2) from a pneumology ambulatory of the Irmandade Santa Casa de Misericórdia de Porto Alegre (Brazil). The diagnosis and severity of COPD were established by a pneumologist according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria and all participants were classified in stages 2–4 of severity (Vogelmeier et al., 2017). Obese and non-obese subjects were matched by age, gender and COPD severity in accordance with GOLD criteria. All patients recruited for this study were sedentary and not engaged into cardiopulmonary rehabilitation program of institution.

All participants were under clinical care with a pneumologist and presented their disease controlled, receiving standard medical treatment through bronchodilators. None of the patients were underweight or had exacerbation of the COPD in the previous 3 months. Antibiotic or oral/intravenous corticosteroids use in the last 4 weeks prior to the study was considered as exclusion criteria. Also, all individuals who had some disease of non-pulmonary origin (cardiovascular, neurological or skeletal muscle) that might interfere with the purpose of the study were excluded. Blood collection and clinical evaluations were performed in the morning (8:00–11:00).

2.2. Procedures

Body mass (kg) and height (meters) were determined by a semi-analytical scale (Welmy, Santa Barbara D'Oeste, Brazil), with capacity for 200 kg and a stadiometer attached (Welmy, Santa Barbara D'Oeste, Brazil) with accuracy of 0.1 kg and 0.005 cm, respectively. BMI (kg/m^2) was defined as body mass (kg) divided by the square of height (m^2).

Physical examinations and spirometric tests to assess lung function were made to define the severity of disease, and a manovacuometric test to assess respiratory muscle strength by measuring maximal inspiratory and expiratory pressures (MIP and MEP, respectively). The environment of the examinations was quiet and private, with constant temperature and humidity, the achievements of spirometry and manovacuometric followed the recommendations established by the American Thoracic Society (ATS)/European Respiratory Society (ERS) Statement on Respiratory Muscle Testing and ATS/ERS Task Force: Standardization of Lung Function Testing established by ATS and ERS (American Thoracic Society/European Respiratory Society, 2002; ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002).

2.3. Six minute walk test

The 6MWT was performed in a track 30 m long with measurements every 3 m demarcated on the ground as previously described (Dorneles et al., 2016). All patients underwent to 6MWT after the blood collection, to avoid the impact of acute exercise on the inflammatory profile of COPD (Dorneles et al., 2016). All subjects received verbal stimuli each minute to help them complete the test. We measured the heart rate and Peripheral oxygen saturarion (SpO2) by pulse oximetry (model 1001 Morrya Ipiranga, São Paulo, Brazil) and perceived exertion by the modified Borg scale (data not shown). The 6MWT followed the recommendations established by the American Thoracic Society/European Respiratory Society (2002).

2.4. Blood collection, cytokine analysis and immunophenotyping

Patients arrived to laboratory at 8:00–9:00 a.m. in fasting state to blood collection. 10 mL of venous blood from the antecubital vein were collected in heparinized tubes by a capacitated professional. The collected material was stored in heparinized tubes to perform the analysis of the cytokine profile through the Cytometric Bead Array technique (CBA) (Becton Dickinson, San Jose, CA, USA): Interleukin-2 (IL-2), Interleukin-4 (IL-4), IL-6, Interleukin-10 (IL-10), Interferon-gamma (IFN-γ) and TNF-α. Six populations of beads with distinct fluorescence intensities were conjugated with specific capture antibody for each cytokine, mixed to form the CBA and read in the FL3 channel of the FACSCalibur flow cytometer (Becton Dickinson).

Immunophenotyping was carried out using a direct immunofluorescence technique. Monoclonal antibodies specific for CD3, CD4, CD8, CD25, CD45ra, CD45ro, CD69, CD195 (CCr5) and HLA-DR conjugated with specific fluorochromes (PE, FITc or Cy-chrome) as well as the appropriate isotype controls, were purchased from Becton Dickinson (San Jose, CA, USA). We used a two-color staining method using monoclonal antibodies labeled with fluorescein isothiocyanate and antibodies labeled with phycoerythrin added to 12 × 75 mm test-tubes. Flow cytometry was performed using a FACSCalibur instrument and CellQuest software (Becton Dickinson, San Jose, CA, USA). Lymphocyte population was gated in basis of forward scatter (FSC-H) versus side scatter (SSC-H) and appropriated control antibodies as CD14 + CD45+. A minimum of 20,000 cells per gate were obtained with each sample. Data are reported as the peripheral frequency of CD3 + CD4+, CD3 + CD8+, CD3 + CD25+, CD3 + CD45ra+, CD3 + CD45ro+, CD3 + CD69+, CD3 + CCr5+ and CD3 + HLA-DR on lymphocyte gate.