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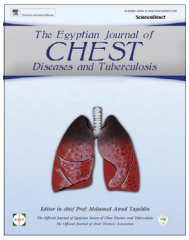

ORIGINAL ARTICLE

Circulating fibrocytes are an indicator of severity and exacerbation in chronic obstructive pulmonary disease

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KEYWORDS

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Abstract *Rationale:* Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation that is associated with an enhanced inflammatory response in the airways and the lung. The remodeling process in COPD is greatly under the influence of growth factors. Lung fibroblasts in COPD demonstrated alterations in its functional capacity that is mediated by TGF-β1, therefore, could play a role in the pathogenesis of COPD. Fibrocytes are bone marrow derived cells that migrate to the injured sites and differentiate into fibroblast-like cells.

Objectives: To test the hypothesis that assay of circulating fibrocytes may provide a biomarker for exacerbation and severity of COPD.

Methods: Fibrocytes were defined by flow cytometry and quantified in fifty male patients with stable COPD and during exacerbation. We investigated the clinical and prognostic value of fibrocytes by comparison with standard clinical parameters. Thirteen healthy subjects were selected as control.

Results: Fibrocytes were significantly elevated in stable COPD patients ($n = 25$), with a further increase during exacerbation ($n = 25$; $P < 0.001$ vs. control subjects $n = 13$). Correlation analysis between fibrocyte counts and mMRC score, 6-MWT, BODE index, arterial oxygen saturation, pre- and post-bronchodilator FEV_1/FVC, FEV_1, FVC and FEF25–75 showed a direct relationship in COPD patients. There was a direct correlation between fibrocytes with the mMRC score and the serum levels of TGF-β_1 only in COPD patients in exacerbations ($n = 25$).

Conclusions: Fibrocytes are an indicator of COPD exacerbation and might be useful as a clinical (bio) marker for disease progression.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world. It is

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a common preventable and treatable disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and co-morbidities contribute to the overall severity in individual patients [1]. Worldwide, the most commonly encountered risk factor for COPD is tobacco smoking; current concepts suggest that cigarette smoke leads to an abnormal inflammatory response in the lower respiratory tract that, in turn, leads to tissue damage and destruction [2]. The peri-bronchiolar fibrosis, which develops in the small airways, is believed to be a response to this injury [3]. There are several anatomic lesions that contribute to the reduced airflow found in COPD patients. These include accumulation of mucous secretions, peri-bronchiolar fibrosis, narrowing of small airways and destruction of alveolar walls, which is the defining characteristic of emphysema [4,5]. Destruction of alveolar walls is believed to result from tissue destruction in excess of the capacity of the lung to repair cigarette smoke – induced damage [5,6].

Fibroblasts are believed to be the major cells responsible for the production and maintenance of extracellular matrix (ECM). Alterations in fibroblast functional capacity, therefore, could play a role in the pathogenesis of pulmonary emphysema. Lung fibroblasts from patients with COPD demonstrated less activity in several *in vitro* measures associated with tissue repair. These appeared to be mediated by decreased sensitivity to transforming growth factor-β [6].

Fibrocytes were identified in 1994 as spindle-shaped cells that are likely bone marrow derived. They migrate to sites of tissue injury and can differentiate into fibroblast-like cells [7]. A unique feature of fibrocytes is that they are circulating in the blood stream and are capable of producing ECM components [8]. They express a variety of mesenchymal markers including collagen-1, the leukocyte marker CD45, and the hematopoietic stem cell marker CD34 [7], which is down-regulated with recruitment of the cell to the tissue [9]. The profibrotic cytokine transforming growth factor-β (TGF-β1) stimulates fibrocytes to express α-smooth muscle actin, a typical but nonspecific myofibroblast marker [10], supporting a potential role of these cells in myofibroblast differentiation. Although fibrocytes participate in wound repair, tissue regeneration, and angiogenesis in a positive manner [11,12], they may be negatively involved in the progression of several pulmonary diseases [13,14].

Treatment of COPD is now aimed at immediately relieving and reducing the impact of symptoms, as well as reducing the risk of future adverse health effects such as exacerbations [1]. In COPD, the most common medications prescribed include bronchodilators and glucocorticoids. Despite considerable research effort in defining the effects of glucocorticoid receptor activation in regulating leukocyte function, little is known about its role in modulating fibrocytes. Current evidence suggests that cellular responses to glucocorticoids in fibrocytes may differ from those observed in leukocytes [15].

We hypothesized that in COPD patients with exacerbations, there could be an increased number of circulating fibrocytes compared with stable COPD patients.

Therefore, the objective of our study was to quantify circulating fibrocytes in COPD patients during stable disease and in exacerbations, also to determine a possible biomarker role of fibrocytes in COPD, and finally to examine a potential contribution role of TGF-β_1 and treatment with glucocorticoids on fibrocytes.

Subjects and methods

A prospective study that was conducted at the Chest Department, Kasr El-Aini hospital, Cairo University. Fifty male patients with confirmed diagnosis of COPD (25 stable COPD patients and 25 COPD patients in exacerbation), and 13 healthy male volunteers with no history of smoking as a control group, were involved in the study. Diagnosis of COPD was based on the clinical history, physical examination and spirometric measurements (the presence of a post-bronchodilator $FEV_1/FVC < 0.70$) which confirms the presence of persistent airflow limitation according to the Global Initiative for Chronic Obstructive Lung Disease GOLD 2014 [1]. COPD exacerbation is defined according to GOLD 2014 by an acute worsening of the patient's condition from stable state and beyond normal day-to-day variations, which presents with worsened dyspnea; worsened sputum volume and/or change in its color; or any combination of these symptoms, and requires a change in regular medication [1].

Exclusion criteria were: patients with history of asthma, IPF, cystic fibrosis or active pulmonary tuberculosis. This study was approved by the Human Ethical Committee of Cairo University and all subjects gave informed consent.

The following data were collected:

Clinical data: Age and body mass index (BMI) were obtained for all groups. Smoking status, modified British Medical Research Council scale (mMRC) for dyspnea, presence of co-morbidities, number of previous exacerbations, and steroid therapy in COPD patients, were collected.

6-Minute Walk Test (6-MWT): 6-Minute Walk Distance and measurement of oxygen saturation using pulse oximetry (pre and post 6-MWT), were assessed for all groups. The test was performed according to American Thoracic Society guidelines [16]. For the exacerbation group, it was performed after one week.

Results of spirometry and grading of severity: Measurement of the forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, forced expiratory flow 25–75% (FEF25–75%) were obtained for all groups. The results of post-bronchodilator were obtained only for the COPD group. It was performed after one week for patients in exacerbation.

COPD grading according to the severity of airflow limitation (based on Post-Bronchodilator FEV_1) was as follows; GOLD 1(mild) $FEV_1 \geqslant 80\%$ predicted, GOLD 2(moderate) $50\% \leqslant FEV_1 < 80\%$ predicted, GOLD 3(severe) $30\% \leqslant FEV_1 < 50\%$ predicted, GOLD 4(very severe) $FEV_1 < 30\%$ predicted [1].

Calculation of the BODE index: BODE index (body mass index, airflow obstruction, dyspnoea, and exercise capacity) which is a multistage functional scoring system for COPD comprising an assessment of symptoms, a surrogate of the nutritional state, and exercise capacity together with the spirometric measure of airflow (FEV_1) [17]. This multidimensional grading system was shown to be superior over the FEV_1-based GOLD classification [18] for predicting hospitalization and the risk of death among patients with COPD [17,19] (Table 1).