

# Pattern of antiendothelial cell antibodies in patients with chronic obstructive pulmonary disease

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**Background** Autoimmune mechanisms have been recently recognized as being partly involved in the pathogenesis of chronic obstructive pulmonary disease (COPD). Circulating autoantibodies have been detected in patients with COPD.

**Objectives** The aim of this study was to estimate the level of antiendothelial cell antibodies (AECA) in COPD patients in the various GOLD stages.

**Patients and methods** A comparative study assessed the level of AECA in three groups. COPD patients without cor pulmonale (18), with cor pulmonale (12), and the control group (10), from Beni-Suef University Hospital. Each group underwent background questionnaires and BMI measures. Enzyme-linked immunosorbent assay was used to measure the level of AECA in serum (ng/ml). Right ventricular systolic pressure (mm Hg) was measured in COPD patients with cor pulmonale.

**Results** The studied groups consisted of male patients (age: 50–80 years). BMI was significantly lower (20.36) in the COPD group with cor pulmonale. Spirometry forced expiratory volume and forced expiratory volume/forced vital capacity ratios showed significantly lower levels among the COPD patients without cor pulmonale. AECA showed a lower level in the control group (26.25) compared with the COPD without cor pulmonale (57.87) and much lowered when

compared to cor pulmonale group (71.47). The level of AECA was higher in the patients in third (74.78) and fourth stages (79.10) compared with those in the second stage (58.75).

**Conclusion** There is a much higher level of AECA in COPD patients with cor pulmonale and in advanced stages in comparison with patients without cor pulmonale and at early stages, and a significant positive correlation was found between AECA levels and right ventricular systolic pressure.

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**Keywords:** antiendothelial cell antibodies, chronic obstructive pulmonary disease, pulmonary hypertension

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## Introduction

Chronic obstructive pulmonary disease (COPD) is a multicomponent disease in which airway inflammation plays an imperative role. According to the European Respiratory Society/American Thoracic Society, a biomarker refers to 'any molecule or material (e.g. cells, tissue) that reflects the disease process' [1]. Biomarkers can be categorized as either markers of the disease severity, lung function, prognosis, and inflammatory phenotype (during clinical stability) or markers of the exacerbation etiology and prognosis or both, [2] and recently also biomarkers that predict the COPD risk in healthy people (without asthma or COPD diagnosis) [3]. A novel COPD phenotype has been characterized by persistent systemic inflammation, based on 5 classic circulating inflammatory biomarkers, namely C-reactive protein, IL-6, IL-8, fibrinogen and TNF- $\alpha$ . An increased level of all-cause mortality was found to be associated with this phenotype. The role of COPD biomarkers in clinical practice was highlighted in this study [4]. Circulating antiendothelial cell antibodies (AECA) recognize various antigenic determinants on human endothelial cells. Although their target antigen and precise pathogenic role remain unclear, they bind to endothelial cell membrane antigens and induce

endothelial cell damage, which leads to vascular injury [5]. Recently, autoimmune mechanisms have been recognized as being partly involved in the pathogenesis of COPD. Circulating autoantibodies have been detected in patients with COPD [5,6]. We aimed to investigate the presence of AECA in patients with COPD. Investigations from the viewpoint of autoimmune mechanisms, including AECA, may provide important information toward a better understanding of COPD and a new therapeutic strategy for this disease.

## Aim of the work

The aim of this work was to study the pattern of AECA in COPD patients and to determine whether it is related to the degree of severity of COPD.

## Patients and methods

The study included 30 COPD male patients and 10 controls, who were chosen from the attendants

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of the chest department of Beni-Suief University Hospital.

Controls were selected from healthy age and sex matched individuals, without COPD history from April 2014 to January 2015.

#### Study design and setting

The included patients were classified into three groups:

- Group 1: COPD patient without cor pulmonale.
- Group 2: COPD patients with cor pulmonale.
- Group 3: control group.

#### Inclusion criteria

- (1) Age (>40 years).
- (2) Met the Global GOLD criteria for COPD: the latest Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification.
  - (1) Stage I: Mild forced expiratory volume (FEV<sub>1</sub>)/forced vital capacity (FVC)<0.70
    - (a) FEV<sub>1</sub>≥80% predicted.
  - (2) Stage II: Moderate FEV<sub>1</sub>/FVC<0.70
    - (a) 50%≤FEV<sub>1</sub><80% predicted.
  - (3) Stage III: Severe FEV<sub>1</sub>/FVC<0.70
    - (a) 30%≤FEV<sub>1</sub><50% predicted.
  - (4) Stage IV: Very severe FEV<sub>1</sub>/FVC<0.70
    - (a) FEV<sub>1</sub><30% predicted or FEV<sub>1</sub><50%.
    - (b) Attended Chest Department, Beni-Suief University.

Informed consent was obtained from all individuals before participation.

#### Exclusion criteria

- (1) Major debilitating mental or physical illness that would interfere with participation.
- (2) Refusal of participation.
- (3) COPD with coexisting autoimmune disease, vacuities, and collagen diseases.

#### Data collection

Each patient was subjected to the following:

- (1) Thorough history taking and full clinical examination.
- (2) Laboratory examination:
  - (a) Complete blood picture.
  - (b) Renal and liver function tests.
- (3) Radiological examination: Chest radiography (posteroanterior and lateral view).
- (4) Pulmonary function tests.

Measurements were obtained for FEV<sub>1</sub>% of predicted, the ratio between FEV<sub>1</sub>, and FVC, and the results were included in the statistical analysis as indices for an obstructive pattern of respiration.

#### (5) Calculation of BMI

The method used for the estimation of the BMI was the weight–height index according to the following equation [BMI=weight (kg)/height (m<sup>2</sup>)]. BMI results were interpreted according to WHO classification as follows:

- (a) BMI below 18.5: Underweight.
  - (b) BMI 18.5–24.9: Normal weight.
  - (c) BMI 25.0–29.9: Overweight.
  - (d) BMI >30.0: Obese.
- (6) Enzyme-linked immunosorbent assay (ELISA).

ELISA was used to detect AECA (ng/ml) in the serum. ELISA involves detection of an analyte in a liquid sample by a method that continues to use liquid reagents during the ‘analysis’ that stays liquid and remains inside a reaction chamber or well that keeps the reactants contained; ELISA separates some components of the analytical reaction mixture by adsorbing certain components onto a solid phase that is physically immobilized. In ELISA, a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated, and washed, followed by some optical change in the final liquid in the well from which the quantity of the analyte is measured. The qualitative ‘reading’ is usually based on the detection of the intensity of transmitted light by spectrophotometry, which involves quantitation of transmission of some specific wavelength of light through the liquid.

#### (7) Echocardiography.

Echo machine vivd-5 GE (Providian Medical Equipment, USA) was used to study all participants. Patients underwent a detailed M-mode, two-dimensional, color Doppler, and CW Doppler imaging analysis performed on resting patients according to the recommendation of the American Society of Echocardiography (2005). The peak tricuspid regurgitant jet velocity was measured using the Bernoulli equation to calculate right ventricular systolic pressure (RVSP) after adding atrial pressure. The pulmonary artery systolic pressure (PASP) is equal to RVSP, provided there is no pulmonary outflow tract obstruction or stenosis.

### Statistical analysis

Data were statistically described in terms of mean, SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was performed using the Mann-Whitney *U*-test for independent samples. For comparing categorical data, the  $\chi^2$ -test was performed. The exact test was used instead when the expected frequency was less than 5. Correlations between different study variables were determined using the Spearman Rank correlation test. Accuracy was represented in terms of sensitivity and specificity. *P* values less than 0.05 were considered statistically significant. All values will be analyzed using the software statistical Package of Social Science (SPSS; Providian Medical Equipment, USA) 15, for Window-Evaluation.

### Ethical review

Informed consent was obtained from all patients.

### Results

This was a comparative study conducted to assess the level of AECA in three groups: one group included COPD patients without cor pulmonale (18 patients); the second group included COPD patients with cor pulmonale (12 patients); and the third was the control group (10 patients). Patients were selected from the attendants of the Chest Department, Beni-Sueif University Hospital and controls were selected from the associated individuals, who were age and sex matched, and did not have a COPD history from April 2014 to January 2015. There were no statistical difference with regard to age. However, patients with COPD and core pulmonale had a low BMI when compared with other groups. FEV1% of predicted and FEV1/FVC% were statistically lower in group 2 than in group 1 and control group. The AECA level was higher in group 2 than in group 1 and control group. Group 1 was higher than control (Table 1 and Fig. 1).

The correlation was negative for the following characteristics: BMI, FEV1, and FEV1/FVC ratio. The results was significant with FEV1 ( $P=0.000$ ) and FEV1/FVC ratio ( $P=0.001$ ) and for RVSP ( $P=0.001$ ). It was nonsignificant for the BMI and age (Table 2 and Fig. 2).

### Discussion

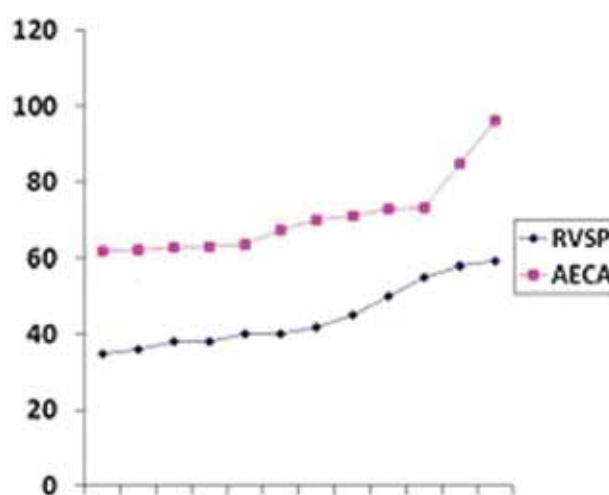
COPD is characterized by airflow limitation that is not fully reversible. The air flow limitation is usually progressive and associated with an abnormal

**Table 1** Statistical comparison between different groups with regard to age, BMI, FEV1, FEV1/FVC and AECA level

	COPD without core pulmonale (group 1)	COPD with core pulmonale (group 2)	Control group (group 3)	<i>P</i> -value
Mean age	61.76	61.60	62.80	0.699
BMI (kg/m <sup>2</sup> )	26.66	20.36	27.86	G1 vs. G20.007* G1 vs. G30.464 G2 vs. G30.001*
FEV1%	56.24	42.6	85.80	G1 vs. G30.001* G2 vs. G30.001* G1 vs G20.044*
FEV1/FVC%	61.90	58.33	67.2	G1 vs G30.001* G2 vs. G30.010* G1 vs. G20.029*
AECA (ng/ml)	57.87	71.47	26.25	G1 vs. G30.001* G2 vs G30.003* G1 vs G20.001*

AECA, antiendothelial cell antibody; BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity.  $P \geq 0.05$  (nonsignificant). \* $P < 0.05$  (significant). \*\* $P < 0.01$  (highly significant). \*\*\* $P < 0.001$  (extremely significant).

**Figure 1**



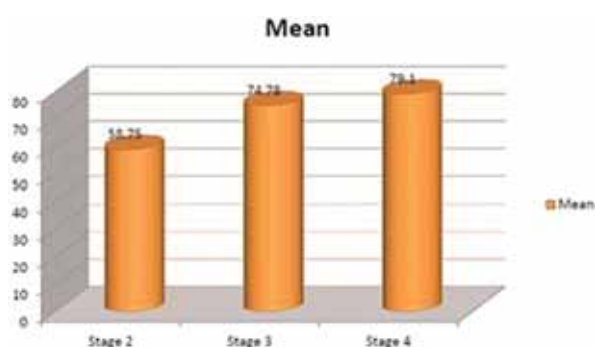
Correlation of AECA levels (ng/ml) and RVSP (mmHg) in COPD patients with cor pulmonale. Pearson correlation ( $r=0.913$ ;  $P=0.001$ ). AECA, antiendothelial cell antibody; COPD, chronic obstructive pulmonary disease; RVSP, right ventricular systolic pressure.

inflammatory response of the lungs to noxious particles or gases[7]. Recently, autoimmune

**Table 2 Correlation of the COPD patients' antiendothelial cell antibodies and age, BMI, spirometry FEV1, FEV1/FVC ratio and RVSP**

Characteristics	AECA value	
	R	P-value
Age	0.010	0.959
BMI (kg/m <sup>2</sup> )	-0.071	0.709
FEV1%	-0.687	0.000
FEV/FVC%	-0.579	0.001
RVSP (mmHg)	0.913	0.001

AECA, antiendothelial cell antibody; COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity; RVSP, right ventricular systolic pressure.

**Figure 2**

Statistical comparison of the stages of COPD patients with the antiendothelial cell antibodies (AECA, ng/ml). AECA, antiendothelial cell antibody; COPD, chronic obstructive pulmonary disease.

mechanisms have been recognized as being partly involved in the pathogenesis of COPD. Circulating autoantibodies have been detected in patients with COPD [5,6]. Endothelial cells (EC) are now considered as a dynamic, heterogeneous tissue that plays key roles in hemostatic balance and vessel tone regulation [8]. Antiendothelial cell (anti-EC) antibodies (AECAs) have been detected in a wide range of systemic inflammatory and/or autoimmune diseases, including primary and/or secondary systemic vasculitis [9]. In pulmonary arterial hypertension, pulmonary EC dysfunction is considered as a key player in the initiation and progression of the disease [10]. Investigations from the viewpoint of autoimmune mechanisms, including AECA, may provide important information toward a better understanding of COPD and a new therapeutic strategy for this disease. Endothelial dysfunction plays a central role in the development of pulmonary hypertension. Cor pulmonale is a maladaptive response to pulmonary hypertension [11]. The present study aimed to investigate differences in levels of AECA in non-COPD individuals and COPD patients in the various gold stages and to detect the development of pulmonary hypertension/ cor pulmonale in the severe

stage of COPD. Our results showed no significant difference in the mean age between the two studied groups: COPD without cor pulmonale and COPD with cor pulmonale that represents the selected sample. The sample showed a nearly equal mean elderly age for both groups. This result agreed with the studies that reported a decline of the lung function with age. Concerning the BMI, the BMI of the group of COPD with cor pulmonale was the least value among the studied groups with a significant difference. This low value could be associated with the severity of the disease. The results agreed with the literature reporting the association between a low BMI and poor prognosis of patients with COPD to be a common clinical observation, which varies with different stages of COPD [12]. Patients with low BMI are at an increased risk for developing severe COPD. Low BMI is also an independent negative determinant of survival in patients with COPD [12]. A cross-sectional study among men in India revealed a positive correlation between BMI and the severity of obstruction in COPD patients. The BMI of the patients decreased with severity of obstruction (GOLD staging), and this was statistically significant [13]. Another descriptive study in India detected a high prevalence of underweight among clinically stable COPD patients and further decrease in BMI with increasing severity of COPD [14]. The BMI was inversely related to the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity FEV<sub>1</sub>/FVC. More respiratory deaths were observed in the lowest BMI group even though they had a lower number of comorbidities. A meta-analysis study showed that for patients with COPD, being overweight or obese had a protective effect against mortality [15]. COPD is a systemic disease. Malnutrition in COPD is due to increased metabolic demands caused by basal oxygen consumption, release of cachexia producing cytokine-like tumor necrosis factor- $\alpha$ , interleukin-6, etc. Alternatively, other COPD patients have problems with obesity, often related to systemic corticosteroid use and inactivity [16]. The present study results revealed significantly lower levels of FEV1% and FEV1/FVC% ratio among the COPD patients; these results agreed with the GOLD classification of COPD [7]. Once the diagnosis of COPD is established, pulmonary function testing is useful to quantitatively monitor the course of the disease. Regarding AECA, the results revealed a significantly higher level among COPD patients and higher levels in the group with cor pulmonale. The results of our study could be explained. AECAs have been detected in a wide range of systemic inflammatory and/or autoimmune

diseases, including primary and/or secondary systemic vasculitis [9]. Circulating AECA recognize various antigenic determinants on human endothelial cells. Recently, autoimmune mechanisms have been recognized as being partly involved in the pathogenesis of COPD [17]. Higher levels of circulating endothelial microparticles were found in 95% of healthy smokers with normal spirometry [18]. Circulating autoantibodies have been detected in patients with COPD [5,6]. In the study by Karayma *et al.* [5], data collected from 116 patients with COPD, whose condition was established on the basis of the Global Initiative for Chronic Obstructive Lung Disease criteria, were evaluated. Serum samples examined for AECA demonstrated that patients with COPD had a significantly higher prevalence and levels of AECA than a the reference population, suggesting that an autoimmune component associated with endothelial cell injury is involved in the pathogenesis of COPD. The significantly higher prevalence and levels of AECA among COPD cases with cor pulmonale than those without cor pulmonale could be attributed to disease severity. The level of AECA (ng/ml) was higher in the third (77.5) and the fourth stages (74.1) compared with the level among the patients in the second stage (59.5). This positive correlation explained by studies investigated the association of AECA with clinical and laboratory findings in patients with active systemic lupus erythematosus and reported that serum titers of AECA are elevated in patients with active systemic lupus erythematosus, especially with pulmonary hypertension. AECAs are more often found in patients with complications such as digital ischemia and pulmonary hypertension [19]. The patients with COPD exhibited significantly higher serum AECA concentrations than the reference group [5].

Lin *et al.* [20] concluded that autoimmunity constituents that induce endothelial cell lesions may participate in the pathogenesis of COPD; detection of serum AECA levels in COPD may have some clinical significance. In the present study, a positive correlation of AECA levels (ng/ml) and RVSP (mmHg) in COPD patients with cor pulmonale was detected; the results agreed with an Egyptian case control study by Labib *et al.* [21], which compared the level of AECA between COPD patients with cor pulmonale and those without cor pulmonale. The study involved 30 male COPD patients without cor pulmonale (group 1), 30 male COPD patients with cor pulmonale (group 2), and 30 male healthy controls (group 3). All participants underwent spirometric pulmonary function testing, echocardiography and measurement of AECA in the serum.

The study revealed a significantly higher level of AECA level in COPD patients with cor pulmonale compared with patients without cor pulmonale, with a positive correlation with the level of pulmonary artery systolic pressure, suggesting a role for AECA in the pathogenesis of pulmonary hypertension and development of cor pulmonale in these patients.

## Conclusion

A significantly lower BMI value was detected among the COPD patients compared with the control group and the BMI of the group of COPD with cor pulmonale was the least.

The level of AECA (ng/ml) was higher in the third and fourth stages compared with the second stage. A significant positive correlation of AECA levels (ng/ml) and RVSP (mmHg) was detected in COPD patients with cor pulmonale.

The level of AECA increases with COPD and its titer increases with the severity of the disease. AECA could be used as one of the biomarkers for COPD diagnosis and detection of severity.

## Recommendations

More focus is required on the relation between the AECA level and pulmonary hypertension secondary to COPD. Assessment of the AECA level before and after COPD therapy is necessary.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

- 1 Cazzola M, MacNee W, Martinez FJ, Rabe KF, Franciosi LG, Barnes PJ, *et al.* Outcomes for COPD pharmacological trials from lung function to biomarkers. *Eur Respir J* 2008; **31**:416–469.
- 2 Koutsokera A, Stolz D, Loukides S, Konstantinos K. Systemic biomarkers in exacerbations of COPD: the evolving clinical challenge. *Chest* 2012; **141**:396–405.
- 3 Rosenberg SR, Kalhan R. Biomarkers in chronic obstructive pulmonary disease. *Transl Res* 2012; **159**:228–237.
- 4 Agustí A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, *et al.* Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PLoS One* 2012; **7**:e 37483.
- 5 Karayama M, Inui N, Suda T, Nakamura Y, Nakamura H, Chida K. Anti-endothelial cell antibodies in patients with COPD. *Chest* 2010; **38**:1303–1308.
- 6 Feghali-Bostwick CA, Gadgil AS, Otterbein LE, Pilewski JM, Stoner MW, Csizmadia E, *et al.* Autoantibodies in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008; **177**:156–163.
- 7 Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global strategy for the diagnosis, management and prevention of COPD*; 2013. Available at: <http://www.goldcopd.org>. [Last accessed 2015 Jan].

- 8 Sandoo A, Jet JCS, Zanten V, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. *Open Cardiovasc Med J* 2010; **4**:302–312.
- 9 Guilpain P, Mouthon L. Antiendothelial cells autoantibodies in vasculitis-associated systemic diseases. *Clin Rev Allergy Immunol* 2008; **35**:59–65.
- 10 Humbert M, Montani D, Perros F, Dorfmuller P, Adnot S, Eddahibi S. Endothelial cell dysfunction and cross talk between endothelium and smooth muscle cells in pulmonary arterial hypertension. *Vascul Pharmacol* 2008; **49**:113–118.
- 11 Shujaat A, Minkin R, Eden E. Pulmonary hypertension and chronic cor pulmonale in COPD. *Int J Chron Obstruct Pulmon Dis* 2007; **2**:273–282.
- 12 Benton MJ, Wagner CL, Alexander JL. Relationship between body mass index, nutrition, strength, and function in elderly individuals with chronic obstructive pulmonary disease. *J Cardiopulm Rehabil Prev* 2010; **30**:260–263.
- 13 Mitra M, Ghosh S, Saha K, Saha A, Panchadhyayee P, Biswas A, *et al.* Study of correlation between body mass index and GOLD staging of chronic obstructive pulmonary disease patients. *J Assoc Chest Physicians* 2013; **1**:58–61.
- 14 Sajal DE. Body mass index among patient with chronic obstructive pulmonary diseases. *Indian J Physiol Pharmacol* 2012; **56**:353–358.
- 15 Cao C, Wang R, Wang J, Bunjhoo H, Xu Y, Xiong W. Body mass index and mortality in chronic obstructive pulmonary disease: a meta-analysis. *PLoS ONE* 2012; **7**:e43892.
- 16 Yao H, Rahman I. Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease. *Toxicol Appl Pharmacol* 2011; **254**:72–85.
- 17 Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med* 2009; **360**:2445–2454.
- 18 Gordon C, Gudi K, Krause A, Sackrowitz R, Harvey BG, Strulovici-Barel Y, *et al.* Circulating endothelial micro particles as a measure of early lung destruction in cigarette smokers. *Am J Respir Crit Care Med* 2011; **184**:224–232.
- 19 Song J, Park YB, Lee WK, Lee KH, Lee SK. Clinical associations of anti-endothelial cell antibodies in patients with systemic lupus erythematosus. *Rheumatol Int* 2000; **20**:1–7.
- 20 Lin Y, Zhang Q, Ling X-F, Sun S-Q. Detection of serum anti endothelial cell antibodies (AECA) in COPD rats. *Eur Respir J* 2011; **38**:55:3891.
- 21 Labib S, Wagih K, Wagih Y, E I-Kilany W. Evaluation of ant endothelial cell antibodies in COPD patients, with and without cor pulmonale. *Egypt J Chest Dis Tuberc* 2014; **63**:589–596.