SERBIAN ARCHIVES OF MEDICINE Est.1872

Address: 1 Kraljice Natalije Street, 11000 Belgrade, Serbia
☎ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

Paper Accepted* **ISSN Online 2406-0895**

Original Article / Оригинални рад

Danielius Serapinas[1,†], Ruta Nutautiene[2], Ruta Pukinskaite[1], Daiva Bartkeviciene[3], Diana Barkauskiene[4], Raimundas Sakalauskas[4]

Association of alpha-1 antitrypsin level and lung function in patients with chronic obstructive pulmonary disease

Повезаност нивоа алфа-1 антитрипсина и плућне функције код болесника са хроничном опструктивном болести плућа

[1] Mykolas Romeris University, Vilnius, Lithuania
[2] Kaunas State Hospital, Kaunas, Lithuania
[3] Department of Obstetrics and Gynecology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[4] Department of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Received: August 9, 2016
Revised: July 17, 2017
Accepted: July 20, 2017
Online First: August 1, 2017
DOI: https://doi.org/10.2298/SARH160809148S

† **Correspondence to:**
Danielius SERAPINAS
Mykolas Romeris University, Ateities 20, Vilnius LT 08303, Lithuania
E-mail: **dserapinas@gmail.com**

Association of alpha-1 antitrypsin level and lung function in patients with chronic obstructive pulmonary disease

Повезаност нивоа алфа-1 антитрипсина и плућне функције код болесника са хроничном опструктивном болести плућа

SUMMARY

Introduction/Objective Alpha-1 antitrypsin deficiency is well established inherited risk factor for chronic obstructive pulmonary disease (COPD), however alpha-1 antitrypsin level may result in different lung function reduction.

The aim of our study was to evaluate possible associations of alpha-1 antitrypsin level and lung function in COPD patients with different alpha-1 antitrypsin phenotypes.

Methods Serum alpha-1 antitrypsin concentration from patients (n=1167) with COPD, defined according to the GOLD criteria, were analysed by nephelometry, alpha-1 antitrypsin phenotype was determined by means of isoelectric-focusing.

Results In COPD patients without alpha-1 antitrypsin deficiency (MM) a significant negative association of lung function (FEV_1) with serum alpha-1 antitrypsin (r=-0,511, P<0.05) and CRP concentrations (r=-0.583, P<0.05) was detected; moreover the level of alpha-1 antitrypsin positively correlated with CRP concentration (r=0.667, P<0.05).

Conclusions In patients without alpha-1 antitrypsin deficiency, detected negative association of alpha-1 antitrypsin level with FEV_1 and positive association with CRP level defined an importance of alpha-1 antitrypsin for lung function in COPD patients.

Keywords: chronic obstructive pulmonary disease; alpha-1 antitrypsin; lung function

САЖЕТАК

Увод/Циљ Недостатак алфа-1 антитрипсина је добро познат наслеђен фактор ризика за хроничну опструктивну болест плућа (ХОБП). Међутим, низак ново алфа-1 антитрипсина може узроковати различита смањења плућних функција.

Циљ нашег рада био је да се процени могућа повезаности нивоа алфа-1 антитрипсина и плућне функције код болесника с ХОБП са разним фенотипама алфа-1 антитрипсина.

Методе рада: Концентрација серумског алфа-1 антитрипсина код болесника са ХОБП (n=1167) у складу с ГОЛД критеријумима је анализирана користећи нефилометрију, а фенотип алфа-1 антитрипсина одређен је изолелекричним фокусирањем.

Резултати Код ХОБП болесника без дефицита алфа-1 антитрипсина (MM) детектована је значајна негативна повезаност плућне функције (FEV_1) са серумслим алфа-1 антитрипсина (r=-0,511, p<0.05) и ЦРП концентрацијом (r=-0.583, П<0.05), осим тога, алфа-1 ниво био је у позитивној корелацији са концентрацијом ЦРП (r=0.667, p<0.05).

Закључак Код ХОБП болесника без дефицита алфа-1 антитрипсина детектована је значајна негативна повезаност са FEV_1 и позитивна повезаност са ЦРП нивоом доказала је значај алфа-1 антитрипсина као показатеља системске инфламације.

Кључне речи: хронична опструктивна болест плућа; алфа-1 антитрипсин; функција плућа

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent and costly disease characterized by progressive airflow limitation, related to an abnormal inflammatory response of the lung to long-term tobacco smoke or inhalation of toxic gases [1]. Lung inflammation is further amplified by oxidative stress and proteolytic damage by proteinases [2-3]. There is increasing data of systemic inflammation in patients with COPD [4-7]. Thus changes of inflammatory markers can be evaluated in the lungs and in serum affecting gas diffusion and lung function [3-5].

The best described inherited risk factor for COPD is alpha-1 antitrypsin (AAT) deficiency. Primary AAT function is to inhibit neutrophil elastase [6-8]. In severe AAT deficiency, anti-elastase protection in the lung interstitium and alveolar zone is decreased to about 15–20% of normal limits, similar to the decrease in serum levels [9-12]. The majority of AAT deficiency cases (96 %) have a PI*ZZ phenotype. The remaining belongs to PI*SZ, PI*MZ and other especially rare deficiency

phenotypes [9]. AAT is a rare disorder because it is worldwide underdiagnosed; more than 80% of AAT deficiency patients remain unrecognized [10].

The potential role of systemic inflammation in the pathogenesis of lung function decline in COPD patients with different AAT phenotypes has not yet been well established. The aim of our study was to evaluate possible associations of AAT level and lung function parameters in patients with COPD with different alpha-1 antitrypsin phenotypes.

The aim of our study was to evaluate possible associations of alpha-1 antitrypsin level and lung function in COPD patients with different alpha-1 antitrypsin phenotypes

METHODS

Sample sources and subjects selection

The study content was approved by the Lithuanian Bioethics Committee. 1167 patients with COPD were included in the study at the Department of Pulmonology and Immunology, Medical Academy, LUHS and gave their informed consent.

Only patients who met the GOLD [1] spirometric criteria for COPD: 1) ratio of post-bronchodilator forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) less than 0.7 and 2) FEV_1 less than 80% of the predicted value, were included in the study. Patients with chronic or acute inflammation were excluded from the study. After an appropriate physical examination, data on the symptoms of the patient and the diagnosis of COPD was also collected. Smoking history was also calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (years x cig. per day/20).

Sample collection and evaluation

Blood samples were taken in serum tubes, clotted at normal room temperature for 35-65 min and centrifuged for 15 min (4000 rpm). Then, samples were frozen at -70ºC for further analysis. The serum levels of AAT were determined by nephelometry using commercial kits (Dade Behring Marburg GmbH, Germany) according to the instructions of manufacturer. AAT phenotyping was carried out by means of isoelectric-focusing (LKB Multiphor II and LKB Macrodrive 5 Constant Power Supply, Amarcham Pharmacia Biotech, Piscataway, NJ, USA), as previously described [13]. Analysis of CRP in serum was done using standard assays (IBL Co, Germany).

Statistical analysis

Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations (SD). Differences of quantitative data were assessed by Kruskal-Wallis *H* test. Correlation between variables was determined by Spearman correlation test. A *p* value of less than 0.05 was considered significant. Statistical analysis was performed with the SPSS 20.0 program.

RESULTS

Demographic description of studied 1167 COPD patients are shown in table 1. Eighty-two percent of the patients were current (57%) or former (25%) smokers of 22.1±12.2 pack-years, and 18% never smokers.

Table 1. General data of study individuals.

Variable	Values
Age (years)	64±12
Male/female	834 (71%) / 333 (29%)
Smoking status	
Smokers	660 (57%)
Ex-smokers	294 (25%)
Never-smokers	213 (18%)

Phenotype distribution were: 1,076 (92.2%) PI*MM, 40 (3.4%) PI*MZ, 39 (3.3%) PI*MS, 1 (0.1%) PI*SS, 3 (0.3%) PI*SZ and 8 (0.7%) PI*ZZ.

The mean AAT serum level (g/l) was of 1.58±0.43. As expected, we found significant differences in AAT serum concentrations between groups ($p < 0.05$) (Figure 1). The PI*ZZ group showed a markedly lower AAT blood level (0.40±0.34) relative to the other AAT phenotype groups.

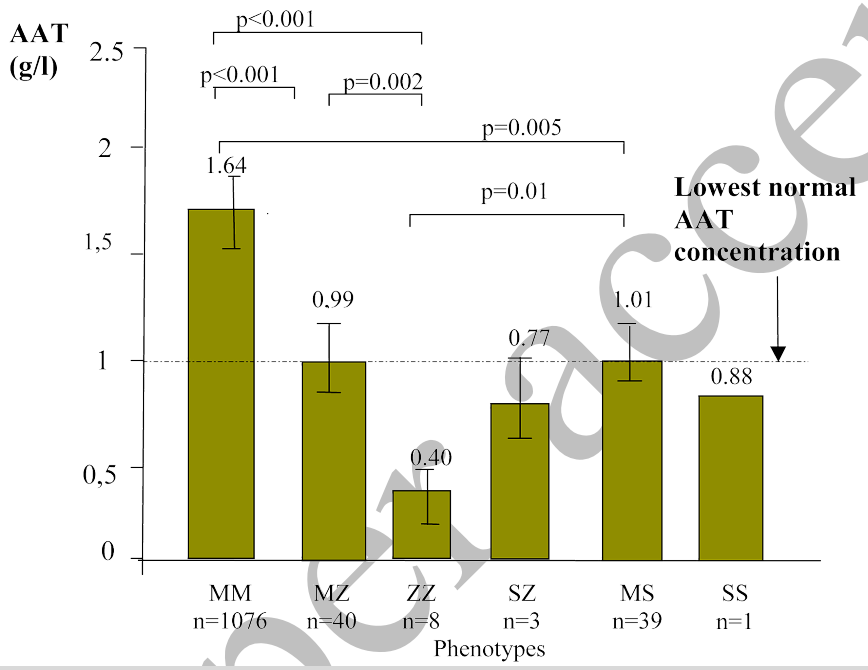

Figure 1. Serum alpha-1 antitrypsin (AAT) concentration in COPD patients with different AAT phenotypes.

While analysing lung function patients with AAT deficiency (PI*ZZ. PI*SZ. PI*SS.) were grouped to one group. These individuals with severe AAT deficiency showed poorer spirometric values FEV_1 (46±20, p<0,05) and FEV1/FVC (48±16, p<0,05) than PI*MM, PI*MS and PI*MZ patients (Table 2).

Table 2. Expresion of lung function and CRP concentration in COPD patients with different AAT phenotypes.

Variable	MM n=1076	MS n=39	MZ n=40	SS, SZ, ZZ n=12
FVC (% predicted normal)	74 ±19	75 ±15	73 ±15	75±17
FEV_1 (% predicted normal)	48±17	51±16	52±18	46±20
FEV_1/FVC (%)	54±11	56±11	57±12	48±16
CRP (mg/L)	9.6 ±0.7	10.2±0.8	11.3±1.2	9.3±1.5
COPD stage				
I	32 (3%)	2 (5%)	-	1 (8%)
II	538(50%)	21 (54%)	22 (55%)	3 (25%)
III	433 (40%)	12 (31%)	14 (35%)	5 (42%)
IV	73 (7%)	4 (10%)	4 (10%)	3 (25%)

We found statistically significant negative correlation between the AAT concentration and FEV_1 % pred. in PI*MM phenotype (r = -0.511, P<0.05) (Figure 2). While analysing correlation

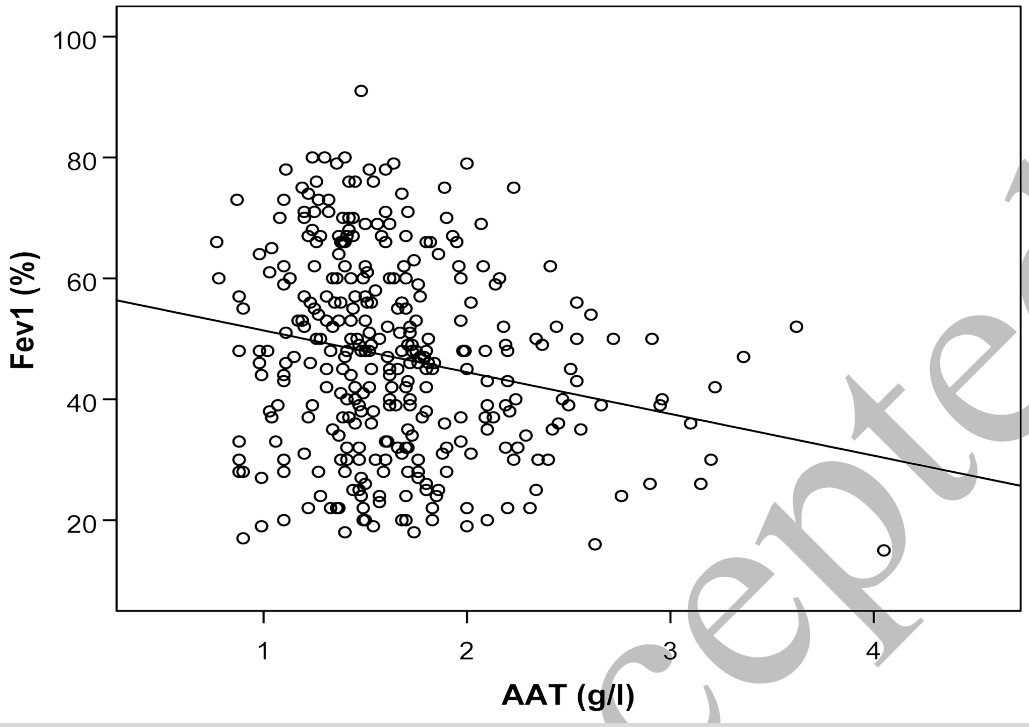

Figure 2. Correlation between AAT concentration and FEV_1 in COPD patients without AAT deficiency (r – Spearman correlation coeficient).

between AAT concentration and FEV_1 in COPD patients according to smoking status and gender we observer inverse correlation in smokers and ex-smokers , but not in non-smokers. In males this correlation was stronger than in females (Table 3). Patients with elevated CRP were excluded from calculations. In addition, we detected inverse correlations between CRP and FEV_1 also have been

Table 3. Correlation between AAT concentration and FEV_1 in COPD patients without AAT deficiency according to smoking status and gender.

Group	n	r	Gender	n	r
Smokers	n=617	r=-0.511 (p<0.05)	Males	n=415	r = -0.407 (p<0.05)
			Females	n=202	r = -0.332 (p<0.05)
Ex-smokers	n=249	r=-0.403 (p<0.05)	Males	n=179	r = -0.398 (p<0.05)
			Females	n=70	r = -0.178 (p>0.05)
Never smokers	n=210	r=-0.211 (p>0.05)	Males	n=160	r = -0.166 (p>0.05)
			Females	n=50	r = -0.152 (p>0.05)

r – Spearman correlation coeficient.

shown in COPD patients with PI*MM phenotype (r=-0.583, p<0.05). However in COPD patients with AAT deficiency we didn‘t find such correlation. In patients without AAT deficiency a significant positive association of blood AAT and CRP levels was detected (r=0.667, p<0.05).

DISCUSSION

The importance of the presented data is that circulating AAT inversely correlated with FEV_1 in COPD cases without AAT deficiency. Such relationship also have been showed before with healthy

individuals [14-15]. SAPALDIA project investigated associations of circulating AAT level with lung function in general population and detected negative correlation of serum AAT concentration with FEV_1 [14]. The amount of AAT that passively diffuses from the serum to the lung increases during an inflammation, which may be present in COPD [16]. This may show increased the need of AAT production to meet requirements of overcoming the release of various endogenous enzymes from inflammatory cells in the lungs, but its protective function may be overrun by the high level of secreted proteases [17]. However other studies have not found such relationship between serum AAT level and FEV_1% predicted value, in COPD patients [18]. Possibly many others mechanisms also might be important for pulmonary function, and not only for the inflammatory respond.

Detected low AAT level in PI*ZZ phenotype and the FEV_1 AAT ratio association may reflect a dual role of AAT molecule as a pulmonary disease marker. The impact of AAT on pulmonary function seems to be a conclusion of context-dependent (i.e. AAT phenotype) and contrasting protective and proinflammatory effects in lung lining. On the one hand, elevated blood AAT level can show a beneficial shift in the antiprotease-protease balance, the centre piece of the pathophysiological mechanism mediating the effect of most severe AAT deficiency on COPD. On the other hand, elevated blood AAT can also reflect low-grade inflammatory reaction in the lung [19-20]. Significantly higher AAT concentration was even reported for AAT deficient (PI*ZZ) patients with COPD compared to PI*ZZ patients without COPD, further supporting the hypothesis that AAT concentration may also reflect an ongoing proinflammatory reaction [21]. Thus our results support the hypothesis that reduction of lung function may be a consequence of the presence of inflammatory stimuli.

Consistent with these findings, we could show a positive relationship between AAT and CRP levels. High serum CRP concentrations in severe COPD individuals have been reported in other studies [5,13-14,23]. Gan and co-workers were the first to emphasize the importance of high CRP levels in COPD patients showing the inflammatory process in even stable disease cases [22]. Both CRP and AAT are acute-phase proteins. Several studies found CRP and AAT elevation in COPD patients [5,7,21,23], indicating, that inflammatory process is present in pathogenesis of disease. In addition, we find inverse correlations between CRP and FEV_1. Even in healthy individuals elevation of CRP concentration during time was connected with a steeper FEV_1 decline [23,24]. In these studies FEV_1 was also inversely associated with blood CRP level. CRP reflects total systemic inflammation in many diseases and has been shown to upregulate the production of inflammatory cytokines [7]. The reasons for the inverse association between reduced lung function and systemic inflammation are not fully undrestoodr, but several mechanisms may be involved. Firstly, reduced pulmonary function may be responsible for the observed systemic inflammatory process. Inflammatory pulmonary epithelial cells, have been shown to express small amounts of CRP and IL-6 [20,25,26]. So persistence of systemic inflammatory process, may result in damage to the airways, promoting decline in FEV_1 of COPD patients. These data show, that AAT has immunomodulating

capacity and acute increase in AAT level during various infectious and inflammatory states may enhance the magnitude of proinflammatory cells reaction to endotoxic materials and subsequently accelerate resolution of the inflammatory process.

CONCLUSION

We found that in patients without alpha-1 antitrypsin deficiency, detected negative association of alpha-1 antitrypsin level with FEV_1 and positive association with CRP level defined an importance of alpha-1 antitrypsin, as biomarker of systemic inflammation, for lung function in COPD. However associations are complex and understanding the reactions of various mediators will require appropriately designed further studies.

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease. GOLD 2017 Global Strategy for the Diagnosis, Management and Prevention of COPD. Executive Summary. [Accessed: July 2017.] Available from: http://goldcopd.org.
2. Wu YQ, Shen YC, Wang H, Zhang JL, Li DD, Zhang X, et al. Serum angiopoietin-like 4 is over-expressed in COPD patients: association with pulmonary function and inflammation. Eur Rev Med Pharmacol Sci. 2016; 20: 44–53.
3. Wang 1, Shumansky K, Sin DD, Man SP, Akhabir L Connett JE, et al. Associations of interleukin-1 gene cluster polymorphisms with C-reactive protein concentration and lung function decline in smoking-induced chronic obstructive pulmonary disease. Int J Clin Exp Pathol. 2015; 8: 13125–35.
4. Topic A, Prokic D, Stankovic I. Alpha-1-antitrypsin deficiency in early childhood. Fetal Pediatr Pathol. 2011; 30: 312–9.
5. López-Sánchez M, Muñoz-Esquerre M, Huertas D, Montes A, Molina-Molina M, Manresa F, et al. Inflammatory markers and circulating extracellular matrix proteins in patients with chronic obstructive pulmonary disease and left ventricular diastolic dysfunction. Clin Respir J. 2015; 00: 000–000 DOI: 10.1111/crj.12428.
6. Wannamethee SG, Shaper AG, Papacosta O, Lennon L, Welsh P, Whincup PH. Lung function and airway obstruction: associations with circulating markers of cardiac function and incident heart failure in older men-the British Regional Heart Study. Thorax. 2016; 71: 526–534.
7. Karadag F, Kirdar S, Karul AB, Ceylan E. The value of C-reactive protein as a marker of systemic inflammation in stable chronic obstructive pulmonary disease. Eur J Intern Med. 2008; 19: 104–8.
8. Bradley WP, Boyer MA, Nguyen HT, Birdwell LD, Yu J, Ribeiro JM, et al. Primary role for TLR-driven TNF rather than cytosolic immune detection in restricting Coxiella burnetii phase II replication within mouse macrophages. Infect Immun. 2016; 84: 998–1015.
9. American Thoracic Society/ European Respiratory Society Statement: standards for the diagnosis and management of individuals with alpha1-antitrypsin deficiency. Am J Respir Crit Care Med. 2003; 168: 818–900.
10. Greulich T, Vogelmeier CF. Alpha-1-antitrypsin deficiency: increasing awareness and improving diagnosis.Ther Adv Respir Dis. 2016; 10: 72–84.
11. Arias P, Kerner J, Christofferson M, Berquist W, Park KT. Misdiagnosis of α-1 antitrypsin phenotype in an infant with CMV infection and liver failure. Dig Dis Sci. 2014; 59(8): 1710–3.

12. Wright TK, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ. Neutrophil extracellular traps are associated with inflammation in chronic airway disease. Respirology. 2016; 21: 467–75.

13. Pierce JA, Eradio BG. Improved identification of antitrypsin phenotypes through isoelectric focusing with dithioerythritol. J Lab Clin Med. 1979; 94: 826–31.

14. Garcia-Rio F, Miravitlles M, Soriano JB, Muñoz L, Duran-Tauleria E, Sánchez G, et al. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. Respir Res. 2010; 11: 63.

15. Senn O, Russi EW, Schindler C, Imboden M, von Eckardstein A, Brändli O, et.al. Circulating alpha1-antitrypsin in the general population: determinants and association with lung function. Respir Res. 2008; 25: 9:35.

16. Greulich T, Vogelmeier CF. Alpha-1-antitrypsin deficiency: increasing awareness and improving diagnosis. Ther Adv Respir Dis. 2016; 10: 72–84.

17. Lomas DA. Twenty years of polymers: a personal perspective on alpha-1 antitrypsin deficiency. COPD. 2013 Mar;10 Suppl 1: 17–25.

18. Higashimoto Y, Yamagata Y, Taya S, Iwata T, Okada M, Ishiguchi T, et al. Systemic inflammation in chronic obstructive pulmonary disease and asthma: Similarities and differences. Respirology. 2008; 13: 128–33.

19. Meyer KC, Rosenthal NS, Soergel P, Peterson K. Neutrophils and low-grade inflammation in the seemingly normal aging human lung. Mech Ageing Dev. 1998; 104: 169–81.

20. Wei J, Xiong XF, Lin YH, Zheng BX, Cheng DY. Association between serum interleukin-6 concentrations and chronic obstructive. pulmonary disease: a systematic review and meta-analysis. Peer J. 2015; 3: e1199.

21. Welle I, Bakke PS, Eide GE, Fagerhol MK, Omenaas E, Gulsvik A. Increased circulating levels of alpha1-antitrypsin and calprotectin are associated with reduced gas diffusion in the lungs. Eur Respir J. 2001; 17: 1105–11.

22. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 2004; 59: 574–80.

23. Shaaban R, Kony S, Driss F, Leynaert B, Soussan D, Pin I, et.al. Change in C-reactive protein levels and FEV1 decline: a longitudinal population-based study. Respir Med. 2006; 100: 2112–20.

24. Kony S, Zureik M, Driss F, Neukirch C, Leynaert B, Neukirch F. Association of bronchial hyperresponsiveness and lung function with C-reactive protein (CRP): a population based study. Thorax. 2004; 59: 892–6.

25. Jane M, Gould, Jeffrey N. Weiser. Expression of C-reactive protein in the human respiratory tract. Infect Immun. 2001; 69: 1747–54.

26. Lopez-Campos JL, Calero-Acuña C, Lopez-Ramirez C, Abad-Arranz M, Márquez-Martín E, Ortega-Ruiz F. et al. Implications of the inflammatory response for the identification of biomarkers of chronic obstructive pulmonary disease. Biomark Med. 2016; 10(2): 109–22.

