Sestrin 2 levels are associated with emphysematous phenotype of COPD

Leonidas Angelakis¹, Andriana I. Papaioannou², Evgenia Papanathanasiou², Argiro Mazioti³, Maria Kallieri², George Papatheodorou⁴, George Patentalakis¹, Georgios Hillas⁵, Spyridon Papiris², Nikolaos Koulouris¹, Stelios Loukides², Petros Bakakos¹*

¹ 1st University Department of Respiratory Medicine, National and Kapodistrian University of Athens, Athens, Greece, ² 2nd University Department of Respiratory Medicine, National and Kapodistrian University of Athens, Greece, ³ Radiology Department, “Mediterraneo” Hospital, Athens, Greece, ⁴ Research Laboratory, «401» General Military Hospital of Athens, Athens, Greece, ⁵ 5th Respiratory Medicine Department, “Sotiria” Hospital, Athens, Greece

* petros44@hotmail.com

Abstract

Sestrins (Sesns) are a family of highly conserved stress-inducible proteins and various stresses have been shown to strongly up-regulate them. Sestrin 2 (Sesn2) deficiency has been shown to partially suppress pulmonary emphysema. The aim of this study was to evaluate Sesn2 levels in COPD patients and its possible associations with the presence of emphysema and blood eosinophils. All patients underwent lung function testing and high-resolution computed tomography (HRCT) of the chest. The presence of emphysematous lesions in >15% of the pulmonary parenchyma was considered as significant emphysema. Sixty-seven patients were included in the study. 40/67 patients were characterized as having significant emphysema. Patients with significant emphysema had higher levels of Sesn2 (ng/ml) [median (IQR) 6.7 (2.7, 10.3 vs 1.09 (0.9, 1.9), p < 0.001] and significantly lower % and absolute blood eosinophil counts (cells/μL) compared to patients without emphysema [1 (0, 2) vs 4 (2, 4) p<0.001 and 62 (0, 110) vs 248 (180, 300), p<0.001 respectively]. Sesn2 presented a significant positive correlation to the score of emphysema in HRCT (r_s = 0.87, p<0.001) and similar positive but weaker correlation to FRC (r_s = 0.27, p = 0.024). Negative correlations were observed between Sesn2 and either the % of blood eosinophils and/or the absolute blood eosinophil count (cells/μL) compared to patients without emphysema [1 (0, 2) vs 4 (2, 4) p<0.001 and 62 (0, 110) vs 248 (180, 300), p<0.001 respectively]. Sesn2 levels above 1.87 ng/ml showed a high diagnostic performance for the presence of significant emphysema in HRCT with an AUC 0.93, 95% CI (0.85, 0.98), p<0.001. Sesn2 could serve as a potential biomarker of emphysema.

Introduction

Chronic obstructive pulmonary disease (COPD) presents with persistent respiratory symptoms (such as cough, sputum production and dyspnea) and airflow limitation that is attributed to airway and/or alveolar abnormalities [1] Emphysema represents the most well recognized...
anatomic change in the lung parenchyma of COPD patients [2]. High resolution computed
tomography (HRCT) has proven to be a reliable tool for the evaluation of structural changes
within the lung caused by a variety of respiratory diseases including COPD [3]. Two distinct
types of lung changes occur in COPD patients, airway remodeling and emphysema. Airway
remodeling can be partly reversible while emphysema correlates with destroyed lung tissue
and is considered irreversible. HRCT imaging can discriminate COPD patients into emphy-
sema-dominant and airway-dominant phenotype [4].

Sestrins (Sesns) consist of a family of highly conserved stress-inducible proteins and various
stresses such as hypoxia, oxidative stress and DNA damage seem to up-regulate them [5]. Sestrin
2 (Sesn2) is thought to reduce oxidative stress [6–9]. First by rescuing the peroxidase activity
of overoxidized peroxiredoxins and secondly by activating the transcription factor NRF2 (nuclear
factor erythroid 2-related factor 2), which is a potent antioxidant gene inducer. It has been
reported that although sestrins are molecules which attenuate ageing and suppress development
of many age-related diseases such as myocardial infarction, muscle atrophy, diabetes, and
immune dysfunction, they have an probable deleterious role in the development of COPD [10].
In mouse models of COPD, Sesn2 knock down resulted in suppression of the development of
emphysema while Sesn2 null animals were less susceptible to the development of COPD when
exposed to cigarette smoke [9]. Furthermore, it has been reported that Sesn2 is accumulated in
COPD smokers indicating an association between Sesn2 expression and COPD development [7].

The aim of the present study was to examine circulating Sesn2 levels in COPD patients and
examine possible correlations between Sesn2 levels and the extent of emphysema as estimated
using HRCT. As secondary outcomes we further examined whether Sesn2 levels are associated
to lung function parameters and blood eosinophils.

Methods

Study design

In the present cross-sectional observational study, we have included consecutive patients with
COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) rec-
ommendations [1] which were treated in the outpatient clinics of the 1st and 2nd University
Respiratory Medicine Departments (“Sotiria” and “Attikon” University Hospital). Inclusion
criteria were:

- Previous COPD diagnosis
- Treatment for stable COPD for at least 6 weeks prior to the inclusion to the study
- Ability of the patient to perform a spirometry test
- Acceptance of the patient to participate to the study

Exclusion criteria were:

- Significant respiratory disease other than COPD, i.e coexistence of asthma
- Significant uncontrolled cardiovascular disease
- Current Malignancy or history of malignant disease
- Presence of eosinophilic lung diseases
- Previous (last 8 weeks) CODP exacerbation
- Treatment with systemic corticosteroids or antibiotics for any reason during the last 8 weeks
• Chronic respiratory failure requiring long term oxygen therapy
• Inability or unwillingness of the patient to collaborate with the study investigators

Demographic characteristics of the patients, including age, sex, BMI (expressed in kg/m²), comorbidities and smoking habit were recorded. All patients underwent physical examination, pulse oximetry, and pulmonary function tests. The diagnosis and severity of COPD was established by post-bronchodilator spirometry on stable condition according to GOLD recommendations. Patients’ treatment including inhaled corticosteroids was also recorded. Peripheral blood samples were collected from all patients and white blood cell and blood eosinophil counts were recorded. Pulmonary function tests and blood samples collection were always performed in the morning. The study protocol was approved by the local Ethics Committee (Scientific Board of Sotiria Hospital, approval no 3747/16-2-2015) and all patients provided written informed consent.

Lung function
Pulmonary function tests (PFTs) were performed using a commercially available system (Master Screen, Erich Jaeger GmbH, Wuerzburg, Germany) Forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), FEV₁/FVC ratio, and forced expiratory flow 25–75% (FEF25-75%) were recorded. COPD severity was evaluated by post-bronchodilator values (i.e. 30 minutes after the administration of 400 mg salbutamol with a spacer) according to GOLD recommendations. [1]. The single breath method was used for the assessment of the diffusing capacity for carbon monoxide (DL_{CO}) with the patient in the sitting position [11]. Lung function measurements were expressed as percentages of predicted values and were performed according to the American Thoracic Society (ATS) guidelines [12].

HRCT
All patients underwent HRCT using either a Somaton HiQ or a Somaton Plus scanner (Siemens, Erlanger, Germany). Scans were performed with 1–1.5mm section thickness and a 1–2 sec scanning time during breath holding at end inspiration. Films were assessed by two experienced radiologists in HRCT who were blinded to the functional, laboratory and clinical data of the patients. The degree of emphysema was calculated using a visual emphysema score [13]. Emphysema score was measured visually, as areas of low attenuation that contrast with the normal attenuation of surrounding lung parenchyma. A threshold HU value, as described in quantitative CT (QCT emphysema is defined as ≥5% of lung volume occupied by low attenuation areas ≤−950 Hounsfield units) was not applicable. All HRCT images in inspiration were evaluated and scored and the sum was divided by the slices, in order to measure total emphysema score. Briefly, emphysema was identified as areas of hypovascular low attenuation and was graded with a five-point scale taking into consideration the percentage of lung involved as follows: 0: no emphysema; 1: up to 25% of the lung parenchyma involved; 2: between 26–50% of lung parenchyma involved; 3: between 51–75% of the lung parenchyma involved; and 4 between 76–100% of lung parenchyma involved. Grades of the axial images of each lung were added and divided by the number of images evaluated to yield emphysema scores that ranged from 0 to 4 [13]. The scores of the two radiologists were added and divided by two and the mean value was used for the analysis. Emphysematous phenotype was defined by the presence of emphysematous lesions ≥15% of the pulmonary parenchyma (i.e. score ≥0.6) [14].

Sesn2 levels measurement
Peripheral blood samples were collected from each patient. Serum supernatant was collected after centrifugation at 3,500 x g for 10 min and stored at −80°C until measurement. Sesn2
levels were assayed by an Enzyme-linked Immunosorbent Assay Kit for Sesn2 (Cloud-Clone Corp., USA) according to the manufacturer’s instructions.

**Statistical analysis**

Categorical variables were presented as n (%), whereas numerical variables were presented as mean±SD or median (interquartile ranges) for normally distributed and skewed data respectively. Normality of distributions was checked using Kolmogorov-Smirnov test. Comparisons between groups were performed using chi-square tests for categorical data, as well as Mann-Whitney U-tests for numerical data, since the distribution was skewed. Correlations were performed using Spearman correlation coefficient. For the assessment of the diagnostic performance of Sesn2 for significant emphysema in HRCT, a receiver operating characteristic (ROC) curve was created. Area under the ROC curve (AUC) with 95% confidence intervals (CI) and their differences from 0.5 was calculated. Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) were calculated for the optimal cut-off value. All p-values <0.05 were considered statistically significant. Data were analysed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and Graphs have been created using GraphPad Prism 6 (GraphPad Software, Inc La Jolla CA USA).

**Results**

Sixty-seven consecutive patients with stable COPD were included in the study. Demographic, functional and inflammatory characteristics of the study participants are shown in Table 1.

**Sesn2 levels according to the presence of significant emphysema**

Patients with significant emphysema, as defined by the presence of emphysematous lesions in ≥15% of the pulmonary parenchyma had higher levels of Sesn2 (ng/ml) compared to those with emphysematous lesions in <15% of the pulmonary parenchyma [6.7 (2.7,10.3) vs 1.09 (0.9,1.9), p<0.001, Fig 1]. Patients with significant emphysema had significantly lower % and absolute blood eosinophil counts, higher levels of FRC compared to patients without emphysema. (Table 1). Sesn2 levels(ng/ml) did not differ between current and ex-smokers (2.67 (0.97,9.3) vs 2.07 (0.89,7.39) p = 0.442).

**Correlations of Sesn2 with study parameters**

Table 2 summarizes the major correlation data either for the whole group or/and for the two subgroups (emphysematous lesions in ≥15%, emphysematous lesions in <15%). Sesn2 presented a significant positive correlation to the score of emphysema in HRCT (r_s = 0.87, p<0.001, Fig 2). Similar positive but weaker correlation was observed between Sesn2 and FRC (r_s = 0.27, p = 0.024) and even weaker but negative between Sesn2 and DLCO (r_s = -0.24, p = 0.047). Negative correlations were observed between Sesn2 and either the % of blood eosinophils or/and the blood eosinophils absolute count (r_s = -0.79, p<0.001, and r_s = -0.78, p<0.001 respectively). In those with >15% emphysema a highly significant correlation between Sesn2 and eosinophils either as percentage or as an absolute count was observed (r_s = -0.69, p<0.001, and r_s = -0.75, p<0.001 respectively). However, in the non-emphysema group no correlation between Sesn2 and either the % of blood eosinophils or/and the blood eosinophils absolute count was observed (r_s = -0.26, p = 0.182, and r_s = -0.22, p = 0.249 respectively). Correlations between the levels of Sesn2 and the absolute number of blood eosinophils are shown on Fig 3A–3C. No other significant correlation was observed.
Diagnostic performance of Sesn2 for the presence of significant emphysema in HRCT

Sens 2 levels were a significant predictor of emphysema AUC 0.93, 95% CI (0.85,0.98), p<0.001. Sesn2 levels above 1.87 ng/ml showed a high diagnostic performance for the presence of significant emphysema in HRCT with a 92.5% sensitivity and 96.3% specificity PPV = 100% and NPV = 65.5%. Fig 4.

Discussion

In the present cross-sectional study, we have shown that patients with significant emphysema in HRCT present with significant higher levels of Sesn2 compared to those without emphysema. Additionally, Sesn2 presented a positive correlation to the score of emphysema in HRCT and a negative correlation to DLCO. Finally, Sesn2 was negatively associated to either the % of blood eosinophils or/and the blood eosinophils absolute count.

This is the first study to evaluate Sesn2 levels among COPD patients according to the presence of emphysema. Previous studies [5–9] have shown that Sesn2 inactivation leads to ROS accumulation and oxidative stress, that plays a role in the pathogenesis of COPD. Moreover, ROS are crucial regulators of signal transduction pathways, such as platelet-derived growth factor receptor β (PDGFRβ) and its signaling has been shown to be induced by ROS accumulating [7]. On the other hand, the TGF-β signaling that partly encodes elastin but not the profibrotic genes collagen I and collagen III was selectively up-regulated by the inactivation of Sesn2 in Ltbp4S--/−mice through a ROS independent pathway [7, 9].

Table 1. Demographic, functional and inflammatory characteristics of the study participants (all and according to the presence of emphysema).

| Variable                                | All          | Emphysema | Non-emphysema | p value |
|-----------------------------------------|--------------|-----------|----------------|---------|
| Age (years)                             | N = 67       | N = 40    | N = 27         |         |
|                                         | 68 (61, 71)  | 69(62,73) | 65(54,70)      | 0.071   |
| Sex (male) n(%)                         | 55 (74)      | 32(80)    | 23(85)         | 0.187   |
| BMI kg/m²                                | 26(23,30)    | 25(22,29) | 27(24,30)      | 0.062   |
| Current smokers/ Ex-smokers             | 42/25        | 26/14     | 16/11          | 0.456   |
|                                         | (63/37)      | (65/35)   | (59/41)        |         |
| Pack years                              | 65 (45–88)   | 60(43–80) | 69(48–90)      | 0.104   |
| FEV₁ (%pred.)                           | 51±18        | 49±18     | 54±17          | 0.136   |
| FEV₁ (L)                                | 1.40±0.56    | 1.35±0.58 | 1.49±0.56      |         |
| FEV₁/FVC                                | 53±11        | 51±12     | 56±10          | 0.061   |
| FRC % pred                              | 123 (91–154) | 138±46    | 117±41         | 0.036   |
| DLCO %pred                              | 49±14        | 46±14     | 53±14          | 0.053   |
| ICS use, n (%)                          | 33 (49.2)    | 15(37.5)  | 18(66)         | <0.001  |
| Exacerbations in the previous year (n)  | 1.0 (0.0,1.0)| 1(0.1)    | 0(0.1)         | 0.286   |
| Sesn2 ng/mL                              | 2.45 (0.91,8.9)| 6.7 (2.7,10.9)| 1.09 (0.9,1.9)| <0.001  |
| Emphysema score on HRCT                  | 1.1(0.4,2.7) | 2.25(1,42.3)| 0.40(0,3.5)     | <0.001  |
| Blood Eosinophils %                     | 2(1–3)       | 1(0.2)    | 4(2.4)         | <0.001  |
| Blood eosinophils absolute count (cells/μL)| 110(62–190) | 62(0,110) | 248(180,300)   | <0.001  |
| Metabolic disease n (%)                  | 5(7.5)       | 2(5)      | 3(11)          | 0.350   |

Values are presented as mean (SD) or Median (IQR) for normally and skewed variables respectively, unless otherwise indicated. Abbreviations: BMI: Body mass index, DLCO: Diffusion Lung Capacity, FEV₁: Forced Exhaled Volume in one second, FVC: Forced Exhaled Vital Capacity, FRC: Functional residual capacity, ICS: Inhaled Corticosteroids, Sesn2: Sestrin 2, HRCT: High Resolution Computed Tomography.

https://doi.org/10.1371/journal.pone.0273652.t001
It has been shown that the lack and/or excessive degradation of elastin enhance the emphysema progress. Using a pulmonary emphysema model induced by inactivating mutation of the small splice variant of the Ltbp4 gene (Ltbp4S–/–), Frank et al. demonstrated that the Sesn2 null alleles (Ltbp4S–/–Sesn2–/–) significantly reduced the pulmonary emphysema of Ltbp4S–/–mice, as Ltbp4S–/–Sesn2–/–mice presented less parenchymal lesions and lung compliance. Sesn2 may also inhibit PDGFRβ expression and in another study, it was demonstrated that the

![Fig 1. Serum Sesn2 levels according to the presence of emphysema in HRCT. For data see text. Abbreviations: HRCT: High Resolution Computed Tomography.](https://doi.org/10.1371/journal.pone.0273652.g001)

Table 2. Major correlation data for Sesn2 levels (ng/ml) either for the whole group or/and for the two subgroups (emphysematous lesions in ≥15%, emphysematous lesions in <15%).

| Variable                        | All                   | Emphysema               | Non emphysema          |
|--------------------------------|-----------------------|-------------------------|------------------------|
|                                | N = 67                | N = 40                  | N = 27                 |
| FEV1 (%pred.)                  | r_s = -0.17, p = 0.162| r_s = -0.11, p = 0.496  | r_s = -0.04, p = 0.818 |
| FEV1/FVC                       | r_s = -0.16, p = 0.195| r_s = -0.13, p = 0.413  | r_s = -0.14, p = 0.455 |
| FRC % pred                     | r_s = 0.27, p = 0.024  | r_s = 0.24, p = 0.038   | r_s = -0.01, p = 0.478 |
| DLCO %pred                     | r_s = -0.24, p = 0.047 | r_s = -0.18, p = 0.257  | r_s = 0.04, p = 0.827  |
| Emphysema score on HRCT        | r_s = 0.87, p<0.001   | r_s = 0.90, p<0.001    | r_s = 0.21, p = 0.314  |
| Blood Eosinophils %            | r_s = -0.79, p<0.001  | r_s = -0.69, p<0.001   | r_s = 0.26, p = 0.182  |
| Blood eosinophils absolute count (cells/μL) | r_s = -0.78, p<0.001 | r_s = -0.75, p<0.001   | r_s = 0.22, p = 0.249  |

Bold letters indicate statistical significance. Abbreviations: DLCO: Diffusion Lung Capacity, FEV1: Forced Exhaled Volume in one second, FVC: Forced Exhaled Vital Capacity, FRC: Functional residual capacity, HRCT: High Resolution Computed Tomography.

https://doi.org/10.1371/journal.pone.0273652.t002
development of cigarette-smoke-induced pulmonary emphysema was prevented by the muta-
tional inactivation of Sesn2 through the up regulation of PDGFRβ expression [7, 9]. These
studies conclusively support a role for Sesn2 in the development of pulmonary emphysema
and are in accordance with our findings showing that emphysematous patients are character-
ized by higher levels of Sesn2. Finally, although emphysema is a radiological diagnosis the
increased levels of Sesn2 in emphysematous patients in combination of the high predictive
value of this biomarker for the presence of significant emphysema, support the hypothesis that
Sesn2 is playing an adverse role in the development of emphysematous lesions [10].

In a previous study [15] has been reported that patients with significant emphysema in
HRCT present lower levels of blood eosinophils while the study of Singh et al. [16], which
included patients from the ECLIPSE cohort, showed that the progression in the

---

Fig 2. Correlation between Sesn2 and emphysema score in HRCT. For data see text. Abbreviations: HRCT: High Resolution Computed Tomography.

https://doi.org/10.1371/journal.pone.0273652.g002
emphysematous lesions was enhanced in subjects with persistent eosinophil counts <2%. These observations are in accordance with our findings showing lower blood eosinophil levels in patients with emphysema, while the presence of negative correlations between Sesn2 and either the % of blood eosinophils or the absolute blood eosinophil count also shows that Sesn2
levels are also related to the presence of emphysematous lesions. Accordingly, one would postulate a role for Sesn2 in the suppression of eosinophilic inflammation in COPD. To our knowledge, currently there are no data connecting Sesn2 with peripheral blood eosinophils. The association between Sesn2 and eosinophils detected in our study might not be causal but simply an indirect one. These correlations between Sesn2 and eosinophils were not observed in the non-emphysema group indicating that this association is indirect, and possibly mainly related to the presence of emphysema. Furthermore, we cannot exclude the possibility that Sesn2 levels were affected by the use of ICS since patients with emphysema used significantly less ICS compared to non-emphysema COPD patients although further studies are needed to evaluate the possible effect of ICS in Sesn2 levels in COPD patients.

Furthermore, the observation that blood eosinophil level was greater in COPD subjects without emphysema, line with increasing evidence that blood eosinophils might have a beneficial, rather than a detrimental, effect in COPD [17, 18]. This finding is of interest considering that therapies geared to reduce eosinophils in COPD had no beneficial effects [19, 20]. Our findings indicate that Sesn2 might serve as a biomarker for emphysema among COPD patients and may also be a candidate biomarker for future treatments that aim at reducing emphysema. Its correlation—though weak—with FRC supports the well-known association of static hyperinflation with COPD and mainly emphysema. We expected a more significant association between Sesn2 and DLCO as the latter is a sensitive marker of emphysema. The small number of patients that comprised our study group as well as the use of a HRCT visual scoring for emphysema might explain the lack of a highly significant association.

Our study has some limitations. First, this is a cross-sectional observational study without the inclusion of a healthy control group while a second group of patients was not used in order to validate our results. Secondly, the quantification of emphysema in HRCT was performed through an observational method, instead of dedicated CT software. However, this method is easy to use in clinical practice and presents excellent correlation with densitometry quantitation. Furthermore, to minimize the potential bias by human rating, both radiologists which scored the emphysematous lesions were blinded to the functional, laboratory and clinical data of the patients. We must also admit that Sesn2 is also elevated in several diseases such as obstructive sleep apnea, atherosclerosis and cardiovascular disease which are common comorbidities in COPD patients [21, 22]. Moreover, the lack of data regarding the time of first COPD diagnosis and duration of COPD may have influenced our findings. Finally, blood eosinophil count may vary from time to time in COPD and a single test is not safe to definitely define whether patients with emphysema had persistently low absolute eosinophil counts.

In conclusion, we have shown that patients with significant emphysema present higher levels of Sesn2 and these levels correlate to the score of emphysema in HRCT. This finding may imply that Sesn2 has an enhancing effect on the development of pulmonary emphysema and could serve as a potential biomarker of COPD and mainly emphysema. Accordingly, patients with emphysema might benefit from treatment with antagonists of Sesn2.

Supporting information

S1 Data.
(XLSX)

Author Contributions

Conceptualization: Andriana I. Papaioannou, Spyridon Papiris, Nikoalos Koulouris, Stelios Loukidis, Petros Bakakos.
Data curation: Leonidas Angelakis, Evgenia Papathanasiou, Argiro Mazioti, Maria Kallieri, George Papatheodorou, George Patentalakis, Georgios Hillas.

Formal analysis: Evgenia Papathanasiou, Maria Kallieri, George Papatheodorou, George Patentalakis, Georgios Hillas.

Investigation: Leonidas Angelakis, Andriana I. Papaioannou, Maria Kallieri, George Patentalakis, Georgios Hillas.

Methodology: Leonidas Angelakis, Andriana I. Papaioannou, Evgenia Papathanasiou, Argiro Mazioti, George Papatheodorou, Georgios Hillas, Spyridon Papiris, Nikolaos Koulouris, Stelios Loukides, Petros Bakakos.

Project administration: Leonidas Angelakis.

Software: Argiro Mazioti, Maria Kallieri, George Papatheodorou.

Supervision: Andriana I. Papaioannou, Spyridon Papiris, Nikolaos Koulouris, Stelios Loukides, Petros Bakakos.

Validation: Stelios Loukides, Petros Bakakos.

Visualization: Argiro Mazioti, Spyridon Papiris, Nikolaos Koulouris, Stelios Loukides, Petros Bakakos.

Writing – original draft: Leonidas Angelakis, Andriana I. Papaioannou, Evgenia Papathanasiou, Argiro Mazioti, Maria Kallieri, George Papatheodorou, George Patentalakis, Georgios Hillas, Stelios Loukides, Petros Bakakos.

Writing – review & editing: Andriana I. Papaioannou, Spyridon Papiris, Nikolaos Koulouris, Stelios Loukides, Petros Bakakos.

References

1. Global Strategy for the Diagnosis, Management and Prevention of COPD. Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016. Available from: http://www.goldcopd.org. 2016.

2. Rennard SI. Looking at the patient—approaching the problem of COPD. N Engl J Med 2004; 350: 965–6. https://doi.org/10.1056/NEJMoa048022 PMID: 14999106

3. Celli BR. Roger s. Mitchell lecture. Chronic obstructive pulmonary disease phenotypes and their clinical relevance. Proc Am Thorac Soc 2006; 3: 461–5. https://doi.org/10.1513/pats.200603-029MS PMID: 16921104

4. Wouters EF. Approaches to improving health status in chronic obstructive pulmonary disease: one or several? Proc Am Thorac Soc 2006; 3: 282–9. https://doi.org/10.1513/pats.200511-118SF PMID: 16636096

5. Wang M, Xu Y, Liu J, Ye J, Yuan W, Jiang H, et al. Recent Insights into the Biological Functions of Sestrins in Health and Disease. Cell Physiol Biochem 2017; 43: 1731–41. https://doi.org/10.1159/000484060 PMID: 29050006

6. Budanov AV, Lee JH, Karin M. Stressin’ Sestrins take an aging fight. EMBO Mol Med 2010; 2: 388–400. https://doi.org/10.1002/emmm.201000097 PMID: 20879813

7. Heidler J, Fysikopoulos A, Wempe F, Seimetz M, Bangsow T, Tomasovic A, et al. Sestrin-2, a repressor of PDGFRβ signalling, promotes cigarette-smoke-induced pulmonary emphysema in mice and is upregulated in individuals with COPD. Dis Model Mech 2013; 6: 1378–87. https://doi.org/10.1242/dmm.013482 PMID: 24046361

8. Tuder RM, Petrecha I. Pathogenesis of chronic obstructive pulmonary disease. J Clin Invest 2012; 122: 2749–55. https://doi.org/10.1172/JCI60324 PMID: 22850885

9. Wempe F, De-Zolt S, Koli K, Bangsow T, Parajuli N, Dumitrascu R, et al. Inactivation of sestrin2 induces TGF-beta signaling and partially rescues pulmonary emphysema in a mouse model of COPD. Dis Model Mech 2010; 3: 246–53. https://doi.org/10.1242/dmm.004234 PMID: 20106877

10. Haidurov A, Budanov AV. Sestrin family—the stem controlling healthy ageing. Mech Ageing Dev 2020; 192: 111379. https://doi.org/10.1016/j.mad.2020.111379 PMID: 33022334
11. Macintyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CP, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005; 26: 720–35. https://doi.org/10.1183/09031936.05.00034905 PMID: 16204605

12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–38. https://doi.org/10.1183/09031936.05.00034805 PMID: 16055882

13. Park KJ, Bergin CJ, Clausen JL. Quantitation of emphysema with three-dimensional CT densitometry: comparison with two-dimensional analysis, visual emphysema scores, and pulmonary function test results. *Radiology* 1999; 211: 541–7. https://doi.org/10.1148/radiology.211.2.r99ma52541 PMID: 10228540

14. Boschetto P, Quintavalle S, Zeni E, Leprotti S, Potena A, Ballerin L, et al. Association between markers of emphysema and more severe chronic obstructive pulmonary disease. *Thorax* 2006; 61: 1037–42. https://doi.org/10.1136/thx.2006.053821 PMID: 16769715

15. Papaioannou AI, Kostikas K, Papaportyriou A, Angelakis L, Papathanasiou E, Hillas G, et al. Emphysematous Phenotype is Characterized by Low Blood Eosinophils: A Cross-Sectional Study. *COPD* 2017; 14: 635–40. https://doi.org/10.1080/15412555.2017.1386644 PMID: 29099646

16. Singh D, Kolsum U, Brightling CE, Locantore N, Agusti A, Tal-Singer R. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. *Eur Respir J* 2014; 44: 1697–700. https://doi.org/10.1183/09031936.00162414 PMID: 25323230

17. DiSantostefano RL, Hinds D, Le HV, Barnes NC. Relationship between blood eosinophils and clinical characteristics in a cross-sectional study of a US population-based COPD cohort. *Respir Med* 2016; 112: 88–96. https://doi.org/10.1016/j.rmed.2016.01.013 PMID: 26872700

18. Turato G, Semenzato U, Bazzan E, Biondini D, Tinè M, Torrecilla N, et al. Blood Eosinophilia Neither Reflects Tissue Eosinophils nor Worsens Clinical Outcomes in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med* 2018; 197: 1216–9. https://doi.org/10.1164/rccm.201708-1684LE PMID: 29305097

19. Criner GJ, Celli BR, Brightling CE, Agusti A, Papi A, Singh D, et al. Benralizumab for the Prevention of COPD Exacerbations. *N Engl J Med* 2019; 381: 1023–34. https://doi.org/10.1056/NEJMoa1905248 PMID: 31112385

20. Tinè M, Biondini D, Semenzato U, Bazzan E, Cosio MG, Saetta M, et al. Reassessing the Role of Eosinophils as a Biomarker in Chronic Obstructive Pulmonary Disease. *J Clin Med* 2019; 8. https://doi.org/10.3390/jcm8070962 PMID: 31269773

21. Chen Y, Huang T, Yu Z, Yu Q, Wang Y, Hu J, et al. The functions and roles of sestrins in regulating human diseases. *Cell Mol Biol Lett* 2022; 27: 2. https://doi.org/10.1186/s11658-021-00302-8 PMID: 34979914

22. Liu Y, Li M, Du X, Huang Z, Quan N. Sestrin 2, a potential star of antioxidant stress in cardiovascular diseases. *Free Radic Biol Med* 2021; 163: 56–68. https://doi.org/10.1016/j.freeradbiomed.2020.11.015 PMID: 33310138