Role of Statin Therapy as Anti-inflammatory in Chronic Obstructive Pulmonary Disease (COPD) Patients

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Abstract: Introduction*: Statins* have a role in reducing the chronic progressive inflammatory status of COPD. One of the inflammatory markers which are evaluated in COPD patients is BAL neutrophils count. The aim of this study is to evaluate evidence the role of statin in treatment of COPD. Patients & Method: 23 adult male patients aged >40 years, with COPD, FEV1 30-70% predicted FEV1/FVC< 70% (with no exacerbation attacks in the last two months prior to the study, Smoker or ex-smoker with smoking index > 400 were studied by Spirometry, smoking index, fiber optic bronchoscopy and broncho-alveolar lavage. Total cell count & neutrophils levels in broncho-alveolar lavage were measured in these patients pre- and post- statin therapy using 40 mg simvastatin once daily for 8 weeks. Results: Statins reduced the inflammatory state by decreasing Broncho-alveolar lavage (BAL), total cell count (TCC) & neutrophils levels in all stages of COPD. As regards total cell count (TCC), there was statistically significant difference between pre- and post- statin therapy in moderate, severe and very severe COPD. As regards BAL absolute neutrophils count, there was statistically significant difference between pre- and post- statin therapy in moderate and severe COPD while no statistically significant difference in very severe COPD. As regards BAL relative neutrophils count, there was statistically significant difference between pre- and post- statin therapy in severe COPD while no statistically significant difference in moderate and very severe COPD. Conclusion: Treatment with statins for 8 weeks in COPD patients decreases inflammatory mediators (broncho-alveolar lavage (BAL), total cell count (TCC) & neutrophils levels in all stages of COPD).

1. Introduction:

In 1995 The American Thoracic Society (ATS) defined COPD as a disease state characterized by the presence of airflow obstruction due to chronic bronchitis or emphysema; and obstruction is progressive and may be partially reversible[1]. The Global initiative of chronic Obstructive Lung Disease **(GOLD, 2008)** defined Chronic Obstructive Pulmonary Disease (COPD) as a preventable and treatable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patients [2]. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.

COPD is a leading cause of morbidity and mortality worldwide and results in economic and social burden. COPD prevalence, morbidity, and mortality vary across countries and across different groups with direct relation to the prevalence of tobacco (GOLD, 2007) [3]. (GOLD) has estimated that this disease is probable to be the third cause of death in the world by the year 2020 [4].

One of the inflammatory markers which is increasingly evaluated in COPD patients is BAL neutrophils count [5].It has been shown to be increased in COPD in stable condition [6] and during exacerbations [7]. Also, it is known as a predictive factor for the course of COPD [8].

Stages of COPD (GOLD, 2008)

- Stage I: Mild COPD: - Mild airflow limitation (FEV1/FVC < 70%, FEV1 ≥80% predicted).

- Stage II: Moderate COPD: - Worsening airflow limitation (FEV1/FVC < 70%, 50% ≤ FEV1 < 80% predicted).

- Stage III: Severe COPD:- Further worsening of airflow limitation (FEV1/FVC < 70%, 30% ≤ FEV1 < 50% predicted).

- Stage IV: Very Severe COPD:- Severe airflow limitation

(FEV1/FVC < 70%; FEV1 < 30% predicted) or FEV1 < 50% predicted plus chronic respiratory failure.

Statins, the most potent cholesterol-lowering agents available, Statins reduce cholesterol levels by inhibiting 3‐hydroxy‐3-methylglutaryl coenzyme A (HMG-CoA) reductase and have an established role in

the treatment of atherosclerotic disease. Recent research has identified anti-inflammatory properties of statins. [9]. This review considers the evidence for the anti-inflammatory properties of statins in the lung, and how these effects are being applied to research into the role of statins as a novel treatment of respiratory diseases. Statins have several possible mechanisms of action that may be interrelated which result in the reduction of inflammation. These include (1) modulating the cholesterol content and thus reducing the stability of lipid raft formation and subsequent effects on the activation and regulation of immune cells, and (2) preventing the prenylation of signaling molecules and subsequent down regulation of gene expression, both resulting in reduced expression of cytokines, chemokines, and adhesion molecules with effects on cell apoptosis or proliferation (fig. 1).
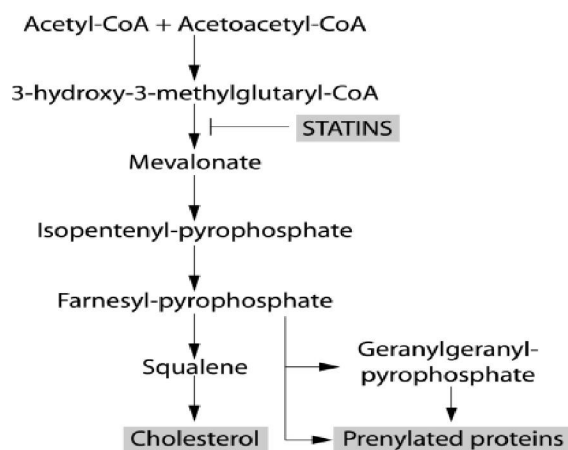
Figure 1 Cholesterol biosynthesis pathway showing potential effects of inhibition of 3‑hydroxy‑3‑methylglutaryl coenzyme A (HMG‑CoA) reductase by statins, causing a decrease in prenylation of signaling molecules as well as derivatives from mevalonate and cholesterol.

2. Patients and methods:

This is a prospective cohort study conducted on 23 adult male patients with COPD attending to the chest outpatient clinic of Bani-Suef University hospital in the period between April 2009 and March 2011. After approval from the Local Institutional Review Committee, Inclusion Criteria: Male, aged > 40 years, physician labelled diagnosis of chronic obstructive pulmonary disease. Patients were COPD patients (by spirometry: improvement of FEV1< 12% or 200 ml after bronchodilators(, with no exacerbation attacks in the last two months prior to the study, smoker or ex-smoker with a pack year smoking history of greater than 20 pack years with smoking index >400, FEV1 30-70% predicted, FEV1/FVC< 70%.Exclusion Criteria: Cardiac or pulmonary disease

other than COPD, respiratory infection, severe or uncontrolled co-morbid disease, patients receiving a statin prior to entry into the study and hypersensitivity to simvastatin or to any of the excipients. All patients were also excluded of having tuberculosis, bronchiectasis, and malignancy or connective tissue disorders. By spirometery: improvement of FEV1> 12% or 200 ml after bronchodilators.

All patients were submitted to the following: Full history taking and examination, chest X ray to exclude other chest lesions, spirometry, pre and post bronchodilators, fiber optic bronchoscopy and broncho-alveolar lavage, calculation of smoking index and Staging according to GOLD.

Spirometry: (Master screen- Jaeger- 781040 Hochberg, Germany)

The nose was clipped by the nose clip and the patient is connected to the mouth piece. The patient was instructed to breath tidally for several times then to inhale slowly till TLC is reached, then to exhale forcibly as much as he can, till RV is reached, then the patient was instructed to inhale forcibly till TLC. This procedure is repeated three times and the best result was selected according to FVC and FEV1. Every patient performed 3 successive trials pre-bronchodilator; the one with the best performance was chosen. Also every patient performed the test 3 successive times 15 minutes post-bronchodilator to determine reversibility of airways obstruction. Inhaled bronchodilator given by metered dose inhaler (MDI). B2-adrenergic aerosol (Salbutamol 400 µg) was used because it has rapid onset of action, usually within 5 minutes.

Fiber optic bronchoscopy: (Olympus CLK-4. Japan).

Patients were submitted to fibro-optic bronchoscope using (Olympus CLK-4. Japan). Premedication was given as intramuscular atropine 1 mg. Spray a 2% lidocaine (lignocaine) solution into both nostrils using an atomizer, with the advice to the patient to 'sniff it back' and a warning about its foul taste. Anaesthetize the cords under direct vision from above while advancing the bronchoscope, additional 2.5-mL aliquots of 2% lidocaine (50mg) may be instilled down the suction channel of the bronchoscope if needed, using boluses from 10-mL syringes made up to volume with air in order to allow for the instrument's dead-space and to rapidly empty all the local anesthetics from its channel. All through the procedure supplemental O_2 was used. All bronchoscopies were performed with the subject in the supine position.

Broncho-alveolar lavage: With the bronchoscope wedged in medial segment of right lower lobe BAL was obtained by instilling sterile, non-bacteriostatic, normal saline, either at room temperature or warmed to 37°C. Aliquots of 20 mL are injected and

immediately re-aspirated using low-pressure suction with gentle suction a proportion of the volume used may be recovered after each instillation, the fluid being ideally aspirated into a container, centrifuged and stored at -20°C in 0.5 cc vials. All patients received 40 mg simvastatin once daily for 8 weeks.

Pre-therapy: BAL sample taken from patient to perform: Total cell count (TCC), absolute & relative count (neutrophils). Post-therapy: BAL sample and perform the same tests as pre-therapy. The following variables, which are known to predict outcome in COPD, were evaluated: degree of airflow obstruction, via FEV1 exercise capacity, smoking index

Statistical analysis: Statistical presentation and analysis of the present study was conducted, using the mean with standard error. Comparison between groups was made using paired & independent student T test by SPSS V.22.

3. Results:

In this study we classified COPD patients according to GOLD (2007). where 8 patients out of 23 patients with a percentage 34.8 % were classified as stage II (FEV1/FVC<70%, 50%≤ FEV1<80% predicted), 11 patients with a percentage 47.8% as stage III (FEV1/FVC<70%, 30%≤ FEV1<50% predicted), and 4 patients with a percentage 17.4% as stage IV (FEV1/FVC<70%, FEV1<30% predicted, or FEV1<50% plus chronic respiratory failure), this may be due to the selection criteria where the patient should be a stable COPD with no exacerbations in the last 2 months. (fig. 2).
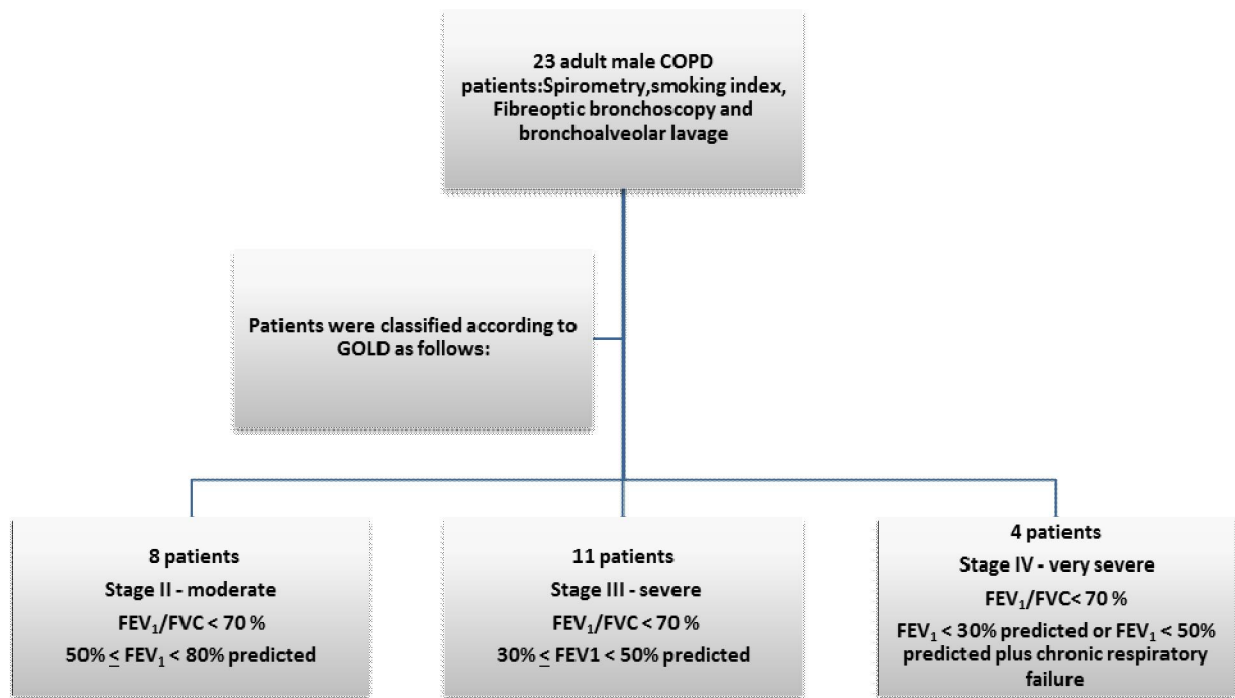

**Figure (2): Classification of COPD patients according to GOLD (2007).**

Table (1) shows statistically significant decrease in BAL total cell count (422.78± 16.67) before simvastatin therapy compared with (339.60± 10.92) after simvastatin therapy among whole COPD patients in all stages which indicates that simvastatin significantly decrease BAL total cell count (TCC) (fig. 3). Also shows statistically significant decrease in BAL neutrophils absolute count (5.781±.401) before simvastatin therapy compared with (3.896±.226) after simvastatin therapy among whole

COPD patients in all stages which indicates that simvastatin significantly decrease BAL neutrophils absolute count (fig. 4). And shows statistically significant decrease in BAL neutrophils relative count (1.372 % ±.072) before simvastatin therapy compared with (1.155 % ±.058) after simvastatin therapy among whole COPD patients in all stages which indicates that simvastatin significantly decrease BAL neutrophils relative count (fig.5).
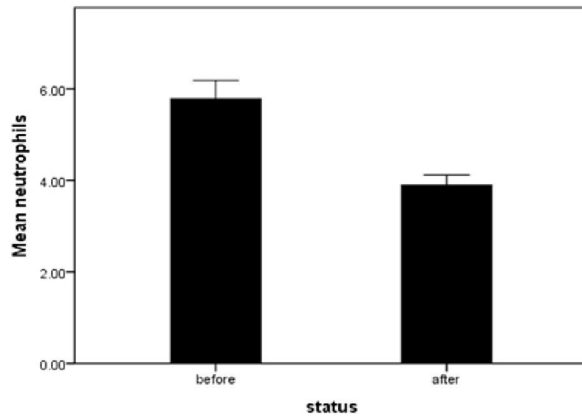
Table (1): Comparison between BAL neutrophils absolute count before & after treatment in all stages

|	BAL total cell count (TCC) No. of cells $*10^3 \backslash$ ml	BAL Neutrophils absolute count No. of cells $*10^3 \backslash$ ml	BAL neutrophils relative count No. of neutrophils per ml /total No. of cells per ml %	NO.
Before TTT	422.78± 16.67	5.781±.401	1.372±.072	23
After TTT	339.60± 10.92	3.896±.226	1.155±.058	23
P value	0.000	0.000	0.003	

Values are mean ± SD. Significance value is regarded as *p* value <0.05
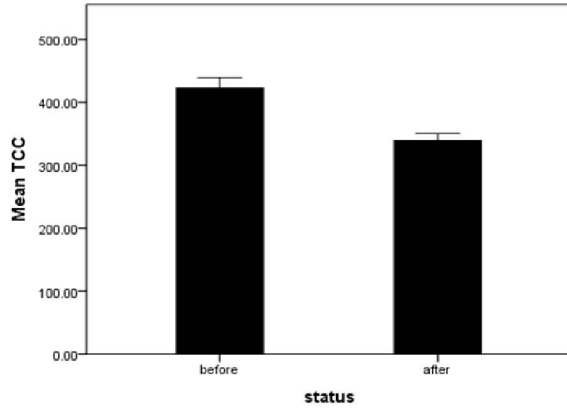
Figure (3): Comparison between Mean TCC before & after treatment in all stages

Figure (4): Comparison between Mean BAL neutrophils absolute count before & after treatment in all stages
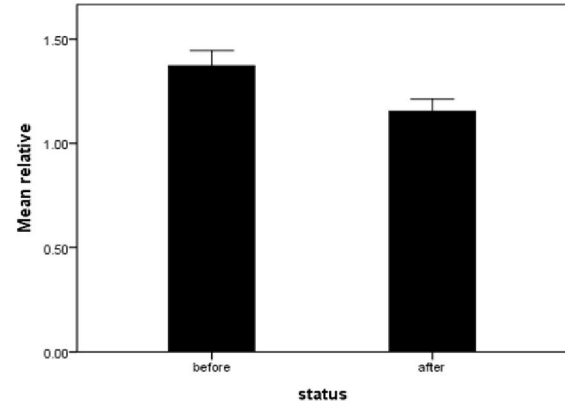
Figure (5): Comparison between Mean BAL neutrophils relative count before & after treatment in all stages

Table 2, shows a statistical comparison between pre- and post-statin therapy parameters in moderate COPD group. There was a statistically significant decrease in mean BAL total cell count & absolute neutrophils count after use of simvastatin. There was no statistically significant change in mean BAL relative neutrophils count after use of simvastatin.

Table 3, shows a statistical comparison between pre- and post-statin therapy parameters in severe COPD group. There was a statistically significant decrease in mean BAL total cell count, absolute & relative neutrophils count after use of simvastatin.

In table 4, there is a statistical comparison between pre- and post- statin therapy parameters in very severe COPD group. There was a statistically significant decrease in mean BAL total cell count after use of simvastatin. There was no statistically significant change in mean BAL absolute & relative neutrophils count after use of simvastatin.

Table (2): Comparison between (TCC) before & after treatment in stage 2

	BAL total cell count TCC No. of cells $*10^3 \backslash$ ml	BAL neutrophils absolute count No. of cells $*10^3 \backslash$ ml	BAL neutrophils relative count No. of neutrophils per ml /total No. of cells per ml %	NO
Before TTT	363.375± 18.839	4.613±.503	1.261 ±.11	8
After TTT	300.375± 15.947	3.225±.363	1.065 ±.09	8
P value	0.009	0.005	0.105	

Values are mean ± SD. Significance value is regarded as p value <0.05

Table (3): comparison between TCC before & after treatment in stage 3

	BAL total cell count (TCC) No. of cells $*10^3 \backslash$ ml	BAL Neutrophils absolute count No. of cells $*10^3 \backslash$ ml	neutrophils relative count No. of neutrophils per ml /total No. of cells per ml %	NO
Before TTT	440.82± 23.85	6.282 ±.556	1.418±.103	11
After TTT	359.09± 14.22	4.127 ±.182	1.164±.063	11
P value	0.001	0.001	0.038	

Values are mean ± SD. Significance value is regarded as *p* value <0.05

**Table (4): Comparison between TCC before & after treatment in stage 4**

|	BAL total cell count (TCC) No. of cells $*10^3 \setminus$ ml	BAL neutrophils absolute count No. of cells $*10^3 \setminus$ ml	BAL Neutrophils relative count No. of neutrophils per ml \total No. of cells per ml %	NO
Before TTT	492.00± 24.40	9.55 ± 2.6	1.89 ±.42	4
After TTT	364.50± 20.30	6.00 ± 1.36	1.65 ±.35	4
P value	0.009	0.076	0.104	

Values are mean ± SD. Significance value is regarded as *p* value <0.05
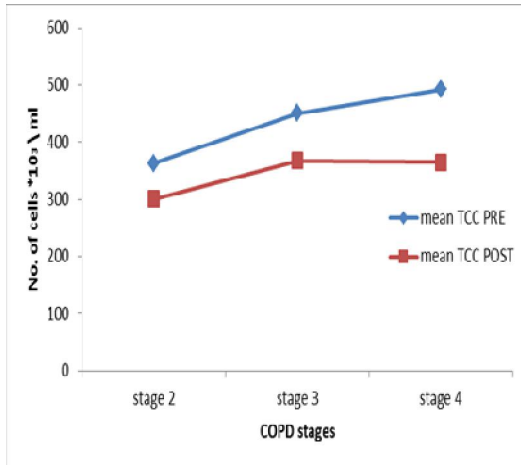
Figure (6): Total cell count (TCC) difference between pre- and post- statin therapy in COPD stages.
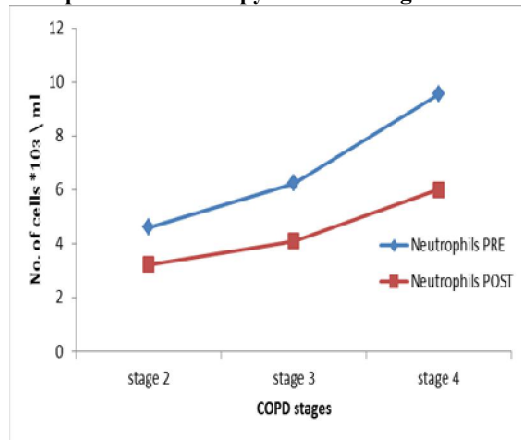
Figure (7): BAL absolute neutrophils count difference between pre- and post- statin therapy in COPD stages.
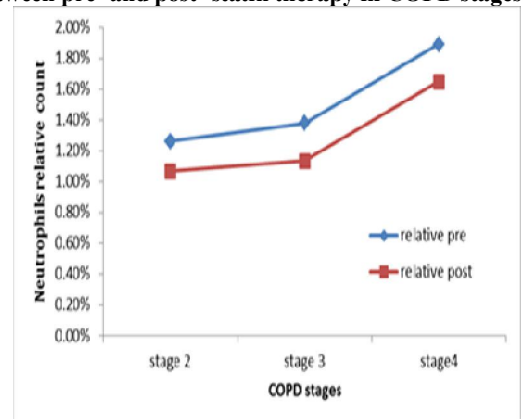
Figure (8): BAL relative neutrophils count difference between pre- and post- statin therapy in COPD stages.

4. Discussion:

We assessed in this study current evidence that statins are powerful enough to produce significant changes in the selected inflammatory BAL fluid and to determine the value of total cell count, absolute & relative neutrophils count as a biomarker of the inflammatory process in COPD patients and to measure how smoking affect BAL neutrophils levels in COPD patients, independently from the degree of airflow obstruction. In our study simvastatin 40 mg for 8 weeks reduced the inflammatory state by decreasing BAL TCC & neutrophils levels in all stages of COPD.

Statins are widely used clinically as lipid lowering drugs; however they have also been shown to exhibit anti-inflammatory and anti-oxidant properties [9]. Recently published large retrospective cohort studies, in patients with COPD, suggest that statins reduce mortality and COPD related admissions [10]. Possible mechanisms of action include effects on cell adhesion molecules, changes in inflammatory mediator release, antioxidant effect and increased clearance of apoptotic cells.

Simvastatin has been shown to reduce the development of smoking induced emphysema in rats with reductions in MMP-9 activity and simvastatin withdrawal leads to increased MMP levels in hypercholesterolaemic patients. Serum concentrations of TNFa and high sensitive C Reactive protein [11] (hs-CRP) are reduced with simvastatin therapy in patients with hypercholesterolaemia and risk of cardiovascular disease respectively. No clinical trial has directly evaluated the clinical effects of statins in patients with COPD in terms of induced sputum MMP profile, alveolar nitric oxide or pulmonary physiology.

Current evidence is insufficient to determine whether smoking status influences the beneficial effects of statin therapy. Yet interestingly, present data, including the study by Alexeeff *et al.* [12], suggest that statins exhibit beneficial effects in current smokers as well as those who are not currently smoking.

The relation of duration and dose of statin therapy to clinical outcomes in COPD or anti-inflammatory effects is unclear. A study of 107 hypercholesterolemic patients treated with simvastatin for 6 weeks showed a significant decline in cytokine levels; however, greater reductions were observed after 6 months [13]. Keddissi et al. described a trend towards an association between the change in FEV1

and FEV and the duration of treatment with statins, but this did not reach statistical significance [14].

Different statins could possess different modes of action, with resulting variations in outcomes. The lack of information on the effects of specific statins in most of the reviewed observational studies precludes further detailed analysis. Kiener *et al.* showed that the differential actions of statins are, in part, related to their lipophilicity [15]. Lipophilic statins such as simvastatin and atorvastatin have the greatest anti-inflammatory potential. No difference in FVC or FEV1 decline was seen between the different statins in the study by Keddissi *et al.* where 80% of patients received simvastatin, and the other patients received lovastatin, atorvastatin or fluvastatin [14]. However, all those statins are lipophilic, whereas pravastatin, which was used in the RCT by Lee *et al.* [16], is hydrophilic.

Conclusion:

We conclude that treatment with simvastatin 40 mg for 8 weeks reduced the inflammatory state by decreasing BAL (TCC) & neutrophils levels in all stages of COPD. As regards total cell count (TCC), there was statistically significant difference between pre- and post- statin therapy in moderate, severe and very severe COPD while BAL absolute neutrophils count was statistically significant difference between pre- and post- statin therapy in moderate and severe COPD while no statistically significant difference in very severe COPD but BAL relative neutrophils count was statistically significant difference between pre- and post- statin therapy in severe COPD while no statistically significant difference in moderate and very severe COPD.

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