



Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation

Ivana Stanojkovic^a, Jelena Kotur-Stevuljevic^a, Branislava Milenkovic^{b,c,*}, Slavica Spasic^a, Tatjana Vujic^c, Aleksandra Stefanovic^a, Aleksandra Ilic^{b,c}, Jasmina Ivanisevic^a

^a Department for Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

^b Faculty of Medicine, University of Belgrade, Belgrade, Serbia

^c Clinic for Pulmonary Diseases, Clinical Centre of Serbia, Belgrade, Serbia

KEYWORDS

COPD;
Inflammation;
Oxidative stress;
Paraoxonase 1;
Prooxidant-antioxidant
balance

Summary

Background: Oxidative stress and inflammation play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD).

Objective: Pulmonary function, oxidative stress parameters and inflammatory markers were measured in 74 patients with severe COPD exacerbation and 41 healthy subjects. In patients all parameters were assessed at two time points: Firstly, one day after admission and secondly, after 7-10 days when they were clinically stable enough to be discharged. Patients were divided in two groups according the presence of ischemic heart disease (IHD): IHD positive (IHD+) patients and IHD negative (IHD-) patients.

Methods and Results: During hospitalisation $O_2^{\cdot-}$, malondialdehyde (MDA), advanced oxidation protein products (AOPP) and total oxidant status (TOS) increased and were higher at discharge compared with admission and the control group. Superoxide dismutase (SOD) activity was significantly lower in COPD patients at both time points compared with the control group. Total antioxidant status (TAS) was significantly lower and the prooxidant-antioxidant balance (PAB) was higher at both time points in COPD patients compared with the control group. High sensitive C-reactive protein (hsCRP) and also the neutrophil count were significantly higher at admission compared with discharge. Paraoxonase 1 (PON1) enzymatic activities in COPD patients did not differ compared with the control group. IHD+ COPD patients had significantly lower PON1 activity but higher PAB levels and hsCRP concentrations, compared with IHD- COPD patients.

Conclusion: The oxidant/antioxidant imbalance was significantly pronounced in patients with COPD exacerbation for at least 24 hours following their admission and when they were clinically stable enough to be discharged. Increased oxidative stress, elevated systemic inflammation and decreased antioxidant defence were common in end-stage disease and particularly COPD patients with ischemic heart disease.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major worldwide health disorder that has increasing prevalence, morbidity and mortality. Neutrophils and macrophages may

infiltrate into lung tissue thereby playing a key role in the production of reactive oxygen species (ROS), in augmenting inducible nitric oxide (NO) synthase expression and the simultaneous production of superoxide ($O_2^{\cdot-}$) anions.^{1,2} Such hyperproduction of ROS leads to oxidative damage of cell proteins, lipids, DNA and carbohydrates. The oxidative stress contributes to irreversible damage of both parenchyma and airway walls, activates molecular mechanisms that initiate lung and systemic inflammation and also to atherosclerosis.^{3,4}

* Corresponding author. Prof. Branislava Milenkovic, MD, PhD.
Clinic for Pulmonary Diseases, Clinical Centre of Serbia,
Koste Todorovica 26, 11000 Belgrade, Serbia.
Tel.: +381 64 111 97 44.
E-mail: branislava.milenkovic@kcs.ac.rs (B. Milenkovic).

Patients with reduced airflow have a significantly higher risk of death from myocardial infarction which is associated with increased systemic oxidative stress, hypoxemia and systemic inflammation observed in COPD patients.⁵⁻⁷

The aim of our study was to evaluate the relationship between respiratory status, oxidative stress status and inflammation in severe COPD exacerbation and during treatment. We also analysed the possible independent correlation between COPD exacerbation and parameters of oxidative status and the relationship between ischemic heart disease (IHD) and oxidative stress/inflammatory markers in COPD exacerbation.

Methods

Study participants

Seventy four patients who presented with severe COPD exacerbation were studied from March 2009 to September 2010. The control group included 41 healthy subjects with no pulmonary, cardiovascular nor oncological disease, inflammation, infection and neurological dysfunction that could influence the oxidative status. COPD was defined according to the Global initiative for Chronic Obstructive Lung Disease (GOLD) criteria.⁸ The severity of exacerbation was categorized in items of clinical presentation and healthcare resource utilization.^{9,10} The patients were separated in two groups according to the presence of IHD.

Patients were excluded if they had lung cancer, known psychiatric illness, maintenance treatment with systemic corticosteroids, active tuberculosis or insulin-dependent diabetes mellitus. The diagnosis of IHD was based on the American College of Cardiology guidelines.¹¹

The first blood sample was taken from the patients within 24 hours of admission into the Clinic for Pulmonary Disease, Clinical Centre of Serbia, Belgrade. Prior to admission, the patients had only inhaled bronchodilator therapy in the form of beta-2 agonists and/or ipratropium bromide. Respiratory insufficiency was confirmed if the arterial partial oxygen pressure (P_{O_2}) was less than 8.0 kPa. All participants underwent a clinical and biochemical investigation revealing age, gender and the assessment of risk factors (including familial history of acute coronary disease, arterial hypertension, smoking, dyslipidemia, current medication and other socioeconomic variables). They were categorised as current smokers, ex-smokers or non-smokers. Treatment during the hospitalisation consisted of oxygen (1-2 L/min by nasal prongs), nebulised beta-2 agonists and ipratropium, theophylline, antibiotics and systemic corticosteroids.

The second blood sample was taken and pulmonary tests performed 7-10 days later when the patient's condition was considered clinically stable enough for discharge.

The whole study was planned according to the ethical standards detailed in the Declaration of Helsinki (as revised in 1983). The local Institutional Ethics Committee approved the research proposal and informed written consent was taken from all patients.

Lung function

Spirometry was performed according to the ATS/ERS standardisation guideline using a Master Scope spirometer (Viasys Healthcare GmbH, Germany).¹² Arterial blood gases were measured using a Radiometer analyser ABL5 (Radiometer, Copenhagen, Denmark).

Oxidative status

Oxidative stress parameters

Plasma malondialdehyde (MDA) was measured using thio-barbituric acid reagent; the rate of nitroblue tetrazolium (NBT) reduction was used to measure the rate of superoxide anion ($O_2^{\cdot-}$) generation.¹³ Advanced oxidation protein products (AOPP) were measured according to Witko-Sarsat method and total oxidative status (TOS) according to Erel's method.^{14,15}

Antioxidative defence parameters

Plasma superoxide dismutase (SOD) activity was measured according to a previously published method.¹⁶ The paraoxonase 1 (PON1) status was assessed using a two-substrate activity (paraoxon/diazoxon) method.¹⁶ Total antioxidant capacity (TAS) was determined using an automated method developed by Erel.¹⁵

Oxidant/antioxidant balance

The prooxidant-antioxidant balance (PAB) was measured according to a previously published method.¹⁷

Inflammatory status

The concentration of hsCRP in serum was measured by latex-enhanced immunoturbidimetry (Quantex hsCRP kit, BIODATA, Barcelona, Spain) using an ILAB 600 analyser. Neutrophil number was determined by differential leukocyte counting using an ABX Micros 60 analyser (Horiba Medical, Montpellier, France).

Statistical analysis

All statistical analyses were performed using MedCalc (MedCalc ver. 11.4 Software, Belgium). Data are shown as mean \pm standard deviation for normally distributed variables, geometric mean and 95% confidence interval for log-normal values and as relative or absolute frequencies for categorical variables. For analysis of categorical variables we used the Chi-square test for contingency tables. A comparison of continuous variables was performed by independent *t*-test or ANOVA with Tukey's *post hoc* test for subgroup differences. To determine possible correlation between examined parameters Pearson's correlation analysis was employed. We used binary logistic regression to analyse the possible independent association between COPD exacerbation and parameters of oxidative status. The control group was used as the reference group and was coded 0, while the patients group was coded 1. Adjustment analysis was performed to correct the influence of lipid profile risk factors (t-C, TG, LDL-C and HDL-C) and oxidative stress status parameters (TAS, TOS).

Results

The general characteristics of the study groups are listed in Table 1. They were matched by gender and by smoking habits. COPD patients were significantly older than the control group ($p < 0.001$). To avoid the influence of age all values for control subjects and patients were adjusted for age prior to further statistical analyses.

The diagnosis of IHD was established in 33.8% of patients and respiratory insufficiency in 38.5%.

Table 1
Baseline characteristics by subject category.

Parameter	COPD patients (n = 74)	Healthy subjects (n = 41)	p*
Sex, % W/M	57/43	61/39	ns
Age, years	63 (15)	42 (12)	<0.01
BMI, kg/m ²	24.4±5.32	26.0±5.11	0.062
Smoking habits, % never/ex/current	11/50/39	68/0/32	<0.01
Smoking pack-years	44.7±44.32	26.9±11.09	<0.01
Therapy, n		NA	
Long-acting bronchodilator, regularly	74		
Inhaled corticosteroids, regularly	32		
Short-acting bronchodilators, as needed	74		
Comorbidities, n		NA	
Arterial hypertension	41		
Diabetes mellitus type II	5		
History of myocardial infarction	11		
History of venous thromboembolism	4		
Osteoporosis	5		
Ischemic heart disease, % No/Yes	66/34	100.0/0.0	<0.001
Resting PaO ₂ below 8.0 kPa, % No/Yes	61.5/38.5	100/0.0	<0.001

Chi-square for categorical variables, Student's *t*-test for continuous variables. ns: non-significant. BMI: body-mass index; NA: not applicable.

Table 2
Values of respiratory status, oxidative stress status and inflammatory markers in COPD patients vs. the control group

Parameter	Control group	Admission	Discharge	P
% predicted FEV ₁	NA	42.2±18.3	50.2±23.0	<0.001
FEV ₁ , L	NA	1.12±0.66	1.32±0.82	<0.001
% predicted FVC	NA	71.9±19.3	82.2±22.6	<0.001
FVC, L	NA	2.23±0.9	2.62±1.10	<0.001
FEV ₁ /FVC ratio	NA	46.3±14.2	48.1±15.6	ns
P _{O2} , kPa	NA	7.49±1.44	8.68±1.97	<0.001
P _{CO2} , kPa	NA	5.81±1.16	5.88±1.24	ns
MDA, μmol/L	1.07±0.36	0.94±0.327	1.60±0.23 ^{**,aa}	<0.001
O ₂ ^{•-} , μmol/L	43.58±19.1	97.2±26.4 ^{***}	100.6±23.4 ^{***}	<0.001
AOPP, μmol/L	22.± 5	55±15 ^{***}	59±21 ^{***}	<0.001
SOD, IU/L	118±46	20±8 ^{***}	19±6 ^{***}	<0.001
POase, IU/L	330±216	417±324	447±374	ns
DZOase, IU/L	9618±3070	11134±3961	11549±4183	0.079
TAS, μmol/L	0.815±0.14	0.74±0.21	0.685±0.14 ^{**}	<0.01
TOS, mmol/L	2.68±1.64	3.02±2.46	5.74±4.60 ^{**,a}	<0.01
PAB, arb. U	72±22	144±43 ^{***}	126±45 ^{***,a}	<0.001
hsCRP, mg/L	0.90 (0.68-1.00)	5.46(3.63-8.20) ^{***}	1.90(1.16-3.14) ^{a,aa}	<0.001
Neutrophiles, %	54.7±5.8	76.8±9.1 ^{***}	65.2±4.6 ^{***aa}	<0.001

Data are presented as mean±standard deviation except for CRP geometric mean (95% CI). P from Student's *t* test for respiratory parameters, for other parameters P from ANOVA test with Tukey's post hoc test:

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control group; ^ap < 0.05, ^{aa}p < 0.01 vs. admission. ns: non-significant.

FEV₁: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity, MDA: malondialdehyde; NA: not applicable; O₂^{•-}: superoxide anion; AOPP: advanced oxidation protein products; SOD-superoxide dismutase; POase: paraoxonase PON1 activity; DZOase: diazoxonase PON1 activity; TAS: total antioxidant defence; TOS: total oxidant status; PAB: prooxidative-antioxidative balance; hsCRP: high sensitivity C-reactive protein.

Pulmonary function, oxidative stress parameters and inflammatory markers at admission and discharge are shown in Table 2.

Oxidative stress status markers indicated free radical generation during the course of COPD exacerbation. The level of O₂^{•-} was significantly higher in patients

Table 3
Logistic regression analysis for the association of PAB with severe COPD exacerbation on admission and discharge.

Parameter	OR (95% CI)	
	Admission	Discharge
PAB unadjusted ^a	1.073 (1.038-1.109)***	1.042 (1.021-1.063)***
PAB adjusted:		
Model 1	1.076 (1.035-1.120)***	1.066 (1.029-1.104)***
Model 2	1.055 (1.017-1.095)*	1.030 (1.027-1.107) ^{p=0.062}
Model 3	1.072 (1.002-1.149)*	0.000 (0.000-0.001) ^{ns}

^a OR (95% CI): odds ratio (95% confidence interval); *P < 0.05, ***P < 0.001, ns: non-significant.

Model 1: adjusted for t-C, TG, LDL-C and HDL-C.

Model 2: adjusted for TAS, TOS.

Model 3: adjusted for t-C, TG, LDL-C, HDL-C, TAS and TOS.

Table 4
Influence of ischemic heart disease (IHD) presence on values of oxidative status markers.

Parameter	IHD- COPD patients		IHD+ COPD patients		p
	Admission	Discharge	Admission	Discharge	
FEV ₁ , %	42.1±3.1	51.7±3.6	42.2±4.2	47.9±4.5	<0.01
P _{O2} , kPa	7.51±0.3	8.85±0.3	7.46±0.3	8.42±0.4	ns
POase, IU/L	459±68	548±72	198±143	312±134	<0.05
PAB, HKU	139±7	125±8	152±13	121±12	ns
hsCRP, mg/L	5.40 (3.3-8.9)	1.89 (1.0-3.6)	5.55 (2.6-11.8)	1.94 (0.8-4.5)	ns
Neutrophils %	73.5±2.1	64.7±2.6	75.7±3.0	66.3±3.9	0.082

Data are presented as mean ± standard deviation except for CRP median (25th-75th percentiles). p: Two-way ANOVA; ns: non-significant. FEV₁: Forced Expiratory Volume in the first second; POase: paraoxonase PON1 activity; PAB: prooxidative-antioxidative balance, hsCRP: high sensitivity C-reactive protein.

compared with the control group. At discharge the patients had significantly higher levels of the oxidative stress marker, MDA, compared to admission ($p < 0.001$). In addition, serum AOPP concentrations paralleled those of MDA concentrations. No significant difference in POase and DZOase activities between patients and controls was noted ($p = 0.119$ and $p = 0.079$, respectively). The patients' PON1 activities were higher at discharge compared with admission, although the difference was not statistically significant.

The patients had significantly higher TOS concentrations ($p < 0.001$) and significantly lower TAS concentrations ($p < 0.001$) compared with the controls. PAB levels were higher in patients compared to controls, both at the point of admission and at discharge. The difference in PAB values at the point of admission and at discharge was not statistically significant, although slightly lower at discharge.

Logistic regression models were constructed in order to test the potential independent association of the analysed oxidative stress status parameters and COPD status in patients on admission and discharge. The best uni-variant disease predictor was PAB concentration. Subsequent multi-variant logistic regression analysis was performed on this parameter (Table 3). The models incorporated adjustments for lipid profile parameters (Model 1), adjustments for the general oxidative stress status parameters (Model 2) and for all parameters included in analysis (Model 3). The association between the PAB concentrations and COPD status remained strong regardless of the confounding

variable at admission. It proved itself to be independently associated with acute COPD exacerbation before the treatment (Table 3). In contrast, the observed association was lost after taking into account other investigated parameters (Model 2 and Model 3) in patients at discharge (Table 4).

Inflammatory markers (hsCRP and the percentage of neutrophils) were significantly higher in COPD than controls. The hsCRP concentrations ($p < 0.001$) and neutrophil count decreased on discharge ($p < 0.01$). Significant positive correlation was found between PAB and hsCRP suggesting a possible relationship between oxidative stress and inflammation (Pearson's correlation $r = 0.388$, $p < 0.05$, Fig. 1).

In order to determine any additional influence of previously diagnosed IHD on COPD outcome we sub-divided our patients according to IHD+ and IHD-. The patients' respiratory status parameters showed no differences between IHD+ and IHD- at admission, but FEV₁ was higher in IHD+ patients on discharge (Table 4). The presence of hypoxemia was noticed in all patients at admission but oxygen saturation significantly improved only in IHD- patients after one-week of treatment.

Patients with IHD had significantly lower POase activity compared with IHD- patients, both at the time of admission and at discharge (Table 4). IHD+ patients had higher PAB values at the time of admission. Furthermore, IHD+ patients had a tendency to develop more inflammation that was documented by slightly higher hsCRP and the percentage of neutrophils.

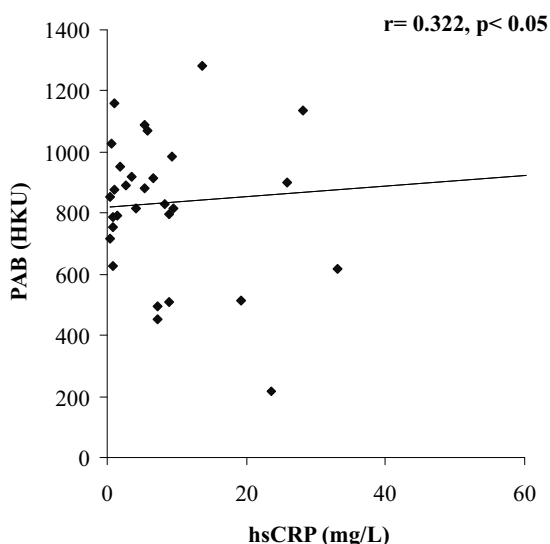


Fig. 1. Pearson's correlation graph between hsCRP and PAB.

Discussion

In this study we assessed changes in respiratory function, oxidative stress and inflammatory status in patients with severe COPD exacerbation from their hospital admission to discharge.

All respiratory status markers were significantly improved at discharge compared with those at admission, but were still lower than reference values (Table 1).

Oxidative stress was evident as revealed by $O_2^{\cdot-}$, MDA, AOPP and TOS. Oxidative stress plays a key role in the pathogenesis and progression of COPD.¹⁸ ROS may contribute to the pathophysiology of COPD in several ways. It is well-known that increased ROS concentrations also activate specific "stress sensitive" signalling pathways leading to activation of the transcription factor NF- κ B and as a result the transcription of many inflammatory genes increases.⁴ The levels of hydrogen peroxide in patients with COPD, particularly in a state of exacerbation, are higher than those in healthy subjects.¹⁹ Such a huge increase in hydrogen peroxide levels could be explained by a consequence of large scale release of $O_2^{\cdot-}$ by alveolar neutrophils. Moreover, we predict that SOD dismutated $O_2^{\cdot-}$ which probably leads to hydrogen peroxide generation. The level of $O_2^{\cdot-}$ rose during the whole period of patients' monitoring in our current study, which confirmed previously published data.^{20,21} Increased release of $O_2^{\cdot-}$ continued during the next 7-10 days of hospitalisation, leading to increased lipid peroxidation, assessed through the level of MDA, an end product of lipid peroxide degradation (Table 2). COPD patients show increased levels of lipid peroxidation products such as F2-isoprostanes, 4-hydroxynonal and MDA, especially during exacerbation.^{22,23} Herein, MDA serum concentrations were initially similar to those in healthy subjects, but then rose significantly until discharge. TOS values increased constantly during hospitalisation and were proportional to other oxidative stress markers, which could imply general oxidative stress fluctuations of other measured markers. Plasma SOD activity in patients with COPD was significantly lower compared to healthy subjects. This fact can be explained by sustained increased levels of $O_2^{\cdot-}$ in

COPD patients which were also noted by Rahman et al.²⁰ As a result the antioxidant defence system is exhausted. A decrease in antioxidant defence was confirmed by lower TAS levels in our COPD patients. Rahman et al. measured changes in TAS values in COPD patients from the point of exacerbation to discharge as in our study, but instead they found elevated TAS values (opposite to our results).²² This could be explained by different therapeutic approaches. The above-mentioned study included patients treated with corticosteroids for a week before admission to hospital; we examined only previously untreated patients. Specifically, the TAS values in our patients decreased during the period between the two measurements. Furthermore, we measured the PAB level as a marker of total oxidation status. According to our knowledge, this marker has never been used before to reveal the oxidative status of COPD patients. The PAB levels were significantly higher in patients compared to controls, suggesting pro-oxidant prevalence.

The most important matter that we addressed here was the potential application of parameters of oxidative stress status determination for COPD patients' assessment. Our aim was to examine if any of these parameters could be independently associated with acute non-treated COPD exacerbation. To gain insight into this feature we calculated odds ratios (ORs) for parameters of oxidative stress status. We found that the level of PAB was potentially the strongest indicator of COPD exacerbation existence (Table 3). Moreover, the association between PAB and COPD exacerbation remained significant even after adjustment for lipid profile and for other oxidative stress status parameters (Table 3). This suggests possible involvement of oxidative stress in COPD pathogenesis. In addition, it is interesting, that the association of PAB and COPD was lost at the time of patient discharge (Table 4). There was a significant decrease in the PAB level at discharge compared with admission. This resulted in the loss of PAB's predictive potential for COPD patient separation from control subjects (Table 4). These results directly show the benefit of implemented therapy on oxidative stress status in COPD patients.

Oxidative stress may also be a mechanism to enhance lung inflammation that is a characteristic feature of COPD. Lipid peroxidation products can act as signalling molecules and cause the release of inflammatory mediators from lung cells. The release of these inflammatory mediators is associated with increased expression of genes encoding other inflammatory mediators. Therefore, inflammation which itself drives higher oxidative stress production, creates a vicious circle of enhanced inflammation resulting from increased oxidative stress.¹⁸ Increased systemic inflammation in our patients was evident through increased levels of hsCRP. CRP is a biomarker of systemic inflammation but also a marker of increased cardiovascular risk.²¹ Links between oxidative stress and inflammation were documented by positive correlation between PAB levels and hsCRP concentrations in COPD patients on admission in acute exacerbation (Fig. 1). Positive correlation between neutrophil count and hsCRP suggested a connection between the main source of ROS and inflammation.

The presence of cardiovascular disturbances in patients with COPD is no coincidence.²¹ There are data that suggest that factors such as systemic inflammation, oxidative stress, hypoxemia, cachexia, endothelial dysfunction and even aging could also be involved.^{21,24-26} Approximately 25% of

patients with COPD die from cardiovascular disease.²⁴ Our results indicated increased systemic inflammation through elevated hsCRP in both groups (IHD+ and IHD-), slightly higher in IHD+ COPD patients. The oxidative stress status in COPD patients with IHD indicated an oxidant/antioxidant imbalance. Namely, PAB levels in those patients were more elevated than in IHD- patients. Alamdari et al. already considered PAB as a new cardiovascular risk factor.¹⁷ Higher PAB, as a measure of oxidative imbalance, indicates that IHD+ patients have a tendency to develop greater oxidative stress compared with IHD- patients. Previous studies showed a decrease in PON1 activity in patients with asthma during exacerbation and a decrease in PON1 gene expression during development of COPD symptoms.^{27,28} PON1 activities in our COPD patients were slightly higher than in control subjects. However, POase PON1 activity in IHD+ COPD patients was significantly lower comparing to IHD- patients, especially at the point of admission after exacerbation (Table 4). Our previous study suggested a role for decreased PON1 activity in coronary artery disease development.¹⁶ PON1 activity in IHD+ patients at the time of admission was similar to that of PON1 activity in three end-stage COPD disease patients who later died (results are not shown because of the small number of deceased patients). According to this result we could speculate that lower PON1 activities could be connected with increased risk of death in COPD. COPD and chronic heart failure are both chronic diseases which activate similar responses and also share similarities with chronic inflammation.^{25,26} This could explain gradual ischemic heart disease development during the course of COPD.

Limitations of our current study: Our conclusions are based upon results from a cross-sectional study and some of the aims could have been better addressed in a prospective study with more studied subjects. Our study included a relatively small number of participants because patients with severe COPD exacerbation are usually treated with antibiotics and systemic steroids before admission to hospital, which therefore prevents them from being part of this study. We wanted to exclude the influence of such drugs on oxidative status. We plan to include new COPD patients and follow them whilst in a stable state one month after the acute exacerbation event.

Conclusion

Our data add further support to the concept of oxidative-stress-induced increase in COPD exacerbations. The oxidant/antioxidant imbalance was significantly pronounced in patients with COPD exacerbation, most markedly at admission. Oxidative stress damage was greater at discharge than the admission, most likely due to poor and/or limited antioxidant defence. A persistently decreased antioxidant capacity was seen in the plasma of patients with an acute exacerbation of COPD for at least 24 hours following their admission, with a rise at the end of the exacerbation when they were considered to be clinically stable enough for discharge. Also, it is important to notice that for the first time PAB was demonstrated as a marker of oxidative stress which is associated with COPD exacerbation and could be modulated by medical treatment. Simultaneous systemic inflammation was also evident in

COPD exacerbation. Increased oxidative stress, elevated systemic inflammation and decreased antioxidant defence were common in end-stage disease and COPD patients with ischemic heart disease.

Future studies should consider development of novel therapeutic interventions with antioxidant therapy as adjunct therapy for COPD exacerbation.

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Conflict of interest statement

The authors declare that they have no competing interest.

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