

## Review Article

# Reliability and Usefulness of Different Biomarkers of Oxidative Stress in Chronic Obstructive Pulmonary Disease

Elisabetta Zinellu,<sup>1</sup> Angelo Zinellu ,<sup>2</sup> Alessandro G. Fois,<sup>1,3</sup> Sara S. Fois,<sup>3</sup> Barbara Piras,<sup>3</sup> Ciriaco Carru ,<sup>2</sup> and Pietro Pirina <sup>1,3</sup>

<sup>1</sup>Respiratory Unit-Azienda Ospedaliero Universitaria, Sassari, Italy

<sup>2</sup>Department of Biomedical Sciences, University of Sassari, Sassari, Italy

<sup>3</sup>Respiratory Unit, Department of Medical, Surgical and Experimental Sciences-University of Sassari, Sassari, Italy

Correspondence should be addressed to Pietro Pirina; [pirina@uniss.it](mailto:pirina@uniss.it)

Received 15 January 2020; Revised 18 April 2020; Accepted 27 April 2020; Published 14 May 2020

Academic Editor: Elena Azzini

Copyright © 2020 Elisabetta Zinellu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Introduction.** Chronic obstructive pulmonary disease (COPD) is a progressive disease characterized by airflow limitation that is not fully reversible after inhaled bronchodilator use associated with an abnormal inflammatory condition. The biggest risk factor for COPD is cigarette smoking. The exposure to noxious chemicals contained within tobacco smoke is known to cause airway epithelial injury through oxidative stress, which in turn has the ability to elicit an inflammatory response. In fact, the disruption of the delicate balance between oxidant and antioxidant defenses leads to an oxidative burden that has long been held responsible to play a pivotal role in the pathogenesis of COPD. There are currently several biomarkers of oxidative stress in COPD that have been evaluated in a variety of biological samples. The aim of this review is to identify the best studied molecules by summarizing the key literature findings, thus shedding some light on the subject. **Methods.** We searched for relevant case-control studies examining oxidative stress biomarkers in stable COPD, taking into account the analytical method of detection as an influence factor. **Results.** Many oxidative stress biomarkers have been evaluated in several biological matrices, mostly in the blood. Some of them consistently differ between the cases and controls even when allowing different analytical methods of detection. **Conclusions.** The present review provides an overview of the oxidative stress biomarkers that have been evaluated in patients with COPD, bringing focus on those molecules whose reliability has been confirmed by the use of different analytical methods.

## 1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide, so much so that it has now become the third leading cause of death by disease [1]. COPD is characterized by a persistent and progressive airflow limitation associated with a chronic inflammatory condition [2]. Cigarette smoking is notably the main cause of COPD, but other factors are being identified at an increasing rate such as occupational exposure, biomass smoke inhalation, and  $\alpha_1$  antitrypsin deficiency [3]. Acute exacerbations and comorbidities contribute to disease severity in individual patients [4]. Tobacco smoke contains  $10^{17}$  oxidant molecules per puff [5]. Exposure to these oxidants causes direct injury

to airway epithelial cells leading to airway inflammation. Inhaled particles and mediators of inflammation activate phagocytic cells such as neutrophils and macrophages, which in turn produce large amounts of reactive oxygen species (ROS) that need to be counterbalanced by antioxidant factors [6]. In addition, ROS may react with nitric oxide (NO) produced by the inducible form of nitric oxide synthase during inflammation, to form various oxidant species such as peroxynitrite [7]. When oxidants are produced in excess of the antioxidant defense mechanisms, oxidative stress occurs resulting in harmful effects, including damage to lipids, proteins, and nucleic acids [8]. It is now well established that oxidative stress plays a key role in the pathophysiology of COPD [9–11]. Several biomarkers of oxidative stress have been

studied, including ROS themselves. Direct measurement of ROS levels is difficult due to their short lifespan and rapid reactivity; indirect determination by examining oxidation target products is therefore a good alternative approach [12]. As regards COPD, various potential biomarkers of oxidative stress have been evaluated in different biological samples, especially in peripheral blood and exhaled breath condensate (EBC), but also in induced sputum, urine, and to a lesser extent, bronchoalveolar lavage fluid (BALF) as well as bronchial or muscle biopsies. In this review, we summarize to the best of our knowledge the main literature findings regarding oxidative stress biomarkers in the abovementioned biological samples in patients with COPD compared to the healthy controls. We have considered and listed our findings based on the method used to detect the biomarkers, because we believe this is likely to be a major influence factor on the results.

## 2. Search Strategy and Eligibility Criteria

We conducted a literature search involving electronic databases such as PubMed, Web of Science, and Scopus from inception to June 2019. The combinations of keywords used were “chronic obstructive pulmonary disease” or “COPD,” “oxidative stress,” and “biomarkers.” All relevant studies were included based on predetermined eligibility criteria. Specifically, we included published case-control studies if (i) they were conducted on human subjects, (ii) there was an assessment of oxidative stress biomarkers, (iii) they were conducted in patients in stable phase of COPD, and (iv) they were written in English.

## 3. Oxidant Biomarkers

**3.1. Lipid Peroxidation Products.** Lipids can be oxidized by various mechanisms that yield different products [13]. These products have received much attention as biomarkers of oxidative stress [14]. The most commonly studied molecules in COPD are malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) as evidenced in Table 1. The constitution of TBARS requires the reaction of MDA with thiobarbituric acid (TBA), resulting in the formation of a byproduct that can be detected with a spectrophotometer or a chromatograph. The levels of MDA molecules are increased in various biological samples in patients with COPD compared to the healthy controls, and increase further with progression of disease (Table 1) [15–45]. A few studies found no differences using this method of detection [46–54]. Some other studies have investigated MDA using other methods of detection, finding an increase of this biomarker in COPD (Table 1) [55–61].

Other biomarkers of lipid peroxidation such as 8-isoprostane, lipid peroxides, conjugated dienes, oxidized low-density lipoproteins, and ethane have been evaluated in COPD, but to a lesser extent than MDA. These molecules were also increased in COPD compared to the controls (Table 1) [17, 55, 62–70].

**3.2. Protein Oxidation Products.** Byproducts of protein carbonylation, a common variety of protein oxidation, have also been assessed in COPD [71]. The most relevant results on these biomarkers are summarized in Table 1 [19, 25, 26, 30, 41, 50, 55, 56, 72–79]. A widely used method for the determination of carbonyl content is based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH), which leads to the formation of a stable product that can be detected spectrophotometrically or immunochemically [80]. The majority of studies have described a significant increase in protein carbonyl groups in patients with COPD in various biological samples compared to the healthy controls and in correlation with disease progression. Another object of research is the advanced oxidation protein products (AOPPs). AOPPs are a family of oxidized compounds formed by the reaction of plasma proteins, mostly albumin, with chlorinated oxidants [81]. They have commonly been evaluated in the peripheral blood of COPD patients by means of a microplate spectrophotometer, finding an increase or no difference in the cases versus controls (Table 1) [22, 30, 47].

**3.3. Reactive Oxygen Species (ROS).** Some authors have explored the level of oxidative stress in COPD patient by looking at the production of ROSs such as superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ). Detection of  $O_2^-$  was based on chemiluminescence assays using a luminometer [82]. Alternatively, enzymatic assays were used to measure the reduction of a substrate by  $O_2^-$ . Superoxide anion blood levels were found increased in COPD patients compared to the healthy controls [22, 26, 29, 47, 48, 74] (Table 2).

Production of  $H_2O_2$  was studied with horseradish peroxidase-containing enzyme assays or by measuring reactive oxygen metabolites (ROMs) with diacron-reactive oxygen metabolites (d-ROM) in EBC. All studies have shown that  $H_2O_2$  is increased in the EBC of patients with COPD compared to the controls as well as in relation to disease progression [43, 64, 83–85] (Table 2).

**3.4. Total Oxidant Status.** A few authors have studied the total oxidative status (TOS) in patients with COPD as a marker of oxidative stress. This can be evaluated by surveying the oxidation of a ferrous ion by the oxidants present in the chosen biological sample or by means of the d-ROM test. Total oxidative status was elevated in the blood of COPD patients in all of the examined studies. [22, 86–91] (Table 2).

**3.5. Oxidatively Damaged DNA.** Products derived from DNA oxidative damage have also been examined in connection with COPD. Comet assay, a single-cell gel electrophoresis, has been used in various studies to detect DNA strand breaks. Another commonly used marker for assessing oxidative damage to nucleic acids is 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). By means of these methods, a significant increase or no differences in the levels of DNA damage has been detected in the blood and urine of COPD patients compared to the controls; however, a significant increase has been found in sputum. [25, 30, 37, 43, 75, 92–97] (Table 2).

**3.6. Peroxynitrites and Nitrotyrosines.** Peroxynitrite is the product of the reaction of nitric oxide and superoxide

TABLE 1: Most relevant findings about lipid and protein oxidation biomarkers.

Specimen	Method of detection	Healthy vs. COPD	In COPD stages
<b>MDA</b>			
Blood	Spectrophotometry following reaction with TBA	↑ [15–35, 38–40]/nd [46–54]	↑ [16, 19, 38, 40–42]/nd [30]
	HPLC following reaction with TBA	↑ [36, 37]	
	Spectrophotometry following reaction with methyl-phenylindole	↑ [55–57]	↑ [55, 56]
	HPLC	↑ [58, 59]	
Urine	HPLC following reaction with TBA	↑ [43]	
EBC	LC-MS following derivatization with DNPH	↑ [60]	
	HPLC following reaction with TBA	↑ [44]/nd [45]	↑ [44]
Sputum	LC-MS following derivatization with DNPH	↑ [60]	
	HPLC following reaction with TBA	↑ [45]	
	Spectrophotometry following reaction with TBA	↑ [31]	
Diaphragm muscle biopsies	HPLC	↓ [61]	
<b>8-Isoprostane</b>			
Blood	Specific enzyme immunoassay	↑ [63]	
Urine	GC/MS assay	↑ [69]	
	Enzyme immunoassay	↑ [70]	
EBC	Enzyme immunoassay	↑ [63–66]	↑ [66]/nd [64]
Sputum	Enzyme immunoassay	↑ [68]	
<b>Lipid peroxides</b>			
Blood	Spectrophotometry using solution containing cholesterol-iodide	↑ [55]	
	Spectrophotometry following reaction with peroxidase	↑ [62]	
<b>Coniugated dienes</b>			
Blood	Spectrophotometry	↑ [17, 55]	
<b>Oxidized LDL</b>			
Blood	Specific enzyme immunoassay	↑ [62]	
<b>Ethane</b>			
EBC	Gas chromatography	↑ [67]	
<b>Protein carbonyls</b>			
Blood	Spectrophotometry following reaction with DNPH	↑ [19, 25, 26, 30, 55, 56, 72]/nd [73]	↑ [55]/nd [19, 26, 30]
	Immunochemistry following reaction with DNPH	↑ [41, 74, 75]/nd [76]	
	Selective radioactive labeling with tritiated borohydride	↑ [50]	↑ [50]
Diaphragm muscle biopsies	Immunochemistry following reaction with DNPH	↑ [77]	
Quadriceps muscle biopsies	Immunochemistry following reaction with DNPH	↑ [76, 78, 79]	
<b>AOPP</b>			
Blood	Spectrophotometer	↑ [22, 30]/nd [47]	nd [30]

↑ indicates increased levels; ↓ indicates decreased levels; nd: no difference.

TABLE 2: Most relevant findings about other oxidative biomarkers.

Specimen	Method of detection	Healthy vs. COPD	In COPD stages
<b>Reactive oxygen species (O<sub>2</sub><sup>-</sup> in blood and H<sub>2</sub>O<sub>2</sub> in EBC)</b>			
Blood	Chemiluminescence	↑ [47, 48, 74]	
	Enzymatic assays	↑ [22, 26, 29]	
EBC	Enzymatic assays	↑ [43, 64, 83]	↑ [43, 64, 83]
	d-ROMs test exhalation kit	↑ [85]	
<b>Total oxidant status</b>			
Blood	Determination of ferrous iron (Fe <sup>2+</sup> ) by colorimetric methods	↑ [22, 86–89]	
	d-ROMs test	↑ [90, 91]	
<b>Oxidatively damaged DNA</b>			
Blood	Comet assay	↑ [25, 30, 92]	
	Quantification of 8-oxodG with ELISA	nd [75, 93]	
	Quantification of 8-oxodG with HPLC	nd [37]	
Urine	Quantification of 8-oxodG with ELISA	↑ [43, 94]/nd [96]	
	Quantification of 8-oxodG with HPLC-MS	↑ [95]	↑ [95]
	Quantification of guanine-derived products with LC-MS/MS	↑ [97]	
Sputum	Quantification of 8-oxodG with ELISA	↑ [93]	
<b>Peroxynitrite</b>			
Blood	Evaluation of nitrophenol formation	nd [30]	
EBC	Oxidation of a fluorescein	↑ [98]	
<b>Tyrosine nitration</b>			
Blood	ELISA	nd [76]	
Sputum	Immunocytostaining with antisera	↑ [99]	
	HPLC	↑ [100]	
BALF	ELISA	↑ [101]	
Bronchial biopsies	ELISA	nd [75]	
Diaphragm muscle biopsies	Immunochemistry	nd [61, 77]	
Quadriceps femoris muscle biopsies	Immunochemistry	↑ [76, 78, 102]	

↑ indicates increased levels; ↓ indicates decreased levels; nd: no difference.

radicals; it is a powerful oxidant that promotes tyrosine nitration with formation of nitrotyrosines. Peroxynitrite levels can be determined using oxidation of a fluorescein or by spectrophotometric detection of nitrophenol, a molecule resulting from the nitrating properties of peroxynitrite itself. Protein tyrosine nitration has also been evaluated by means of ELISA or immunocytostaining in sputum cells. Both peroxynitrites and nitrotyrosines have been studied in patients with COPD compared to the controls in various biological samples and by means of a multitude of assays, finding both an elevation and no difference [30, 61, 75–78, 98–102] (Table 2).

#### 4. Antioxidant Biomarkers

**4.1. Protein and Nonprotein Thiols.** Thiols are organic compounds containing a sulfhydryl group (–SH). In humans, blood proteins harbor the largest amount of thiol groups,

while a smaller proportion is represented by nonprotein thiols such as glutathione (GSH). Thiols can undergo oxidation processes in the presence of oxidants and constitute an important component of the antioxidant defense system. The plasma thiol level is most commonly determined using Ellman's reagent, 5,5'-dithiobis-2-nitrobenzoic acid. This compound is reduced by free thiols in an exchange reaction that releases a product that can be measured with spectrophotometry [103]. In patients with COPD, protein thiols have predominantly been examined in the blood, where a significant reduction compared to the controls has often been discovered; as regards disease progression, both a decrease and no significant difference have been found (Table 3) [19, 20, 26, 29, 30, 36, 38, 51, 52, 54, 104]. Nonprotein thiols, especially reduced GSH, have been investigated in various biological samples of patients with COPD using different assays. Results have often demonstrated a reduction of this marker

TABLE 3: Most relevant findings about nonenzymatic antioxidant biomarkers.

Specimen	Method of detection	Healthy vs. COPD	In COPD stages
<b>Protein SH groups</b>			
Blood	Ellman's assay	↓ [19, 20, 26, 38, 54, 104]/nd [29]	↓ [38]/nd [19, 26]
	Quantification of albumin via chemistry automated analyzer	↓ [36]	
	Subtraction of GSH from total thiols	↓ [51]/nd [30, 52]	
<b>Reduced GSH</b>			
Blood	Ellman's assay in plasma	↓ [18, 24, 30, 31, 33, 35, 51, 52]/nd [33]	nd [30]
	Ellman's assay in whole blood	↓ [40, 96]	nd [40]
	Ellman's assay in erythrocytes	↓ [16, 27]	↓ [16]
	Colorimetric determination in erythrocytes	nd [46]	
Sputum	Subtraction of oxidized glutathione from total GSH	nd [105]	
	Colorimetric determination	↓ [31]	
BALF	Reaction with Ellman's reagent in plate reader	↓ [106]	
<b>Total GSH</b>			
Blood	Reaction with Ellman's reagent and glutathione reductase in plasma		nd [42]
	Reaction with Ellman's reagent and glutathione reductase in whole blood	↑ [19, 26]	↑ [19]
Sputum	Spectrophotometry following reaction with Ellman's reagent and glutathione reductase	↑ [105, 107]	
<b>Total thiols</b>			
Blood	Reaction with Ellman's reagent in plasma	↓ [49]/↑ [72]	↓ [30]
<b>Total antioxidant capacity</b>			
Blood	FRAP assay	↓ [19, 26]	↓ [19, 26, 111]
	TEAC assay	↓ [30, 39, 53, 104, 110–112]/nd [47, 89]	nd [30]
	Assay based on preventing oxidation of orthodiansidine molecules by hydroxyl radicals	↓ [21, 22, 87]	
BALF	Spectrophotometric monitoring of inhibition of ABTS radical formation by antioxidants	nd [113]	

↑ indicates increased levels; ↓ indicates decreased levels; nd: no difference.

compared to the controls and in a few cases, an elevation or no difference at all (Table 3) [16, 18, 19, 24, 26, 27, 30, 31, 33, 35, 40, 42, 46, 49, 51, 52, 72, 96, 105–107].

**4.2. Total Antioxidant Capacity.** Many researchers have investigated total antioxidant capacity in the blood and bronchoalveolar lavage fluid of patients with COPD using different assays, particularly the FRAP (ferric-reducing ability of plasma) [108] and the TEAC (Trolox equivalent antioxidant capacity) assays [109]. The largest proportion of these studies has highlighted a decrease of this biomarker in COPD that positively correlates with disease progression (Table 3) [19, 21, 22, 26, 30, 39, 47, 53, 87, 89, 104, 110–113].

**4.3. Antioxidant Enzymes.** Antioxidant enzymes are an important component of the antioxidant defense system. Some of them have been evaluated in patients with COPD to assess oxidative stress. A few studies have focused on these biomarkers analyzing the sputum, BALF, bronchial biopsies, diaphragm or quadricep muscle biopsies, and above all,

peripheral blood samples. Most studies concentrated on specific antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx), and to a lesser extent, glutathione-S-transferase (GST) and paraoxonase 1 (PON1).

SOD catalyzes the dismutation of superoxide anion  $O_2^-$  to hydrogen peroxide  $H_2O_2$ , which is subsequently detoxified to oxygen and water by other enzymes. The majority of research work on SOD has so far utilized the assay method developed by Mc Cord and Fridovich [114]. This is an enzymic function where the superoxide anion generated by xanthine and xanthine oxidase reacts with a tetrazolium salt to form red formazan dye, which is then used as a flag detector. SOD inhibits the formation of the formazan dye, and the activity is measured as percent inhibition. Other assays can measure SOD activity using its ability to inhibit other reactions such as the autooxidation of epinephrine to adrenochrome, the autooxidation of pyrogallol, and the formation of nitrite from the reaction of  $O_2^-$  with hydroxylamine hydrochloride [115–117]. In other instances, SOD protein

TABLE 4: Most relevant findings about enzymatic antioxidant biomarkers.

Specimen	Method of detection	Healthy vs. COPD	In COPD stages
<b>SOD activity/levels*</b>			
Blood (erythrocytes)	McCord and Fridovich assay	↑ [26, 72]/↓ [40, 111, 112]/nd [23]	↓ [40]/nd [41]
	Inhibition of epinephrine autooxidation	↓ [16]/↑ [17]	
	Inhibition of pyrogallol autooxidation	↓ [19]/nd [30]	
	Inhibition of nitrite formation by superoxide radical	↓ [20]	
Blood (plasma)	McCord and Fridovich assay	↑ [57]/↓ [31, 120]/nd [74, 75]	nd [42]
	*ELISA	↓ [118]/nd [119]	
Sputum	McCord and Fridovich assay	↓ [31]	
	*ELISA	↑ [119]	nd [119]
BALF	McCord and Fridovich assay	nd [113]	
Bronchial Biopsies	McCord and Fridovich assay	nd [75]	
Diaphragm muscle biopsies	*Immunochemistry	nd [77]	
Quadriceps muscle biopsies	McCord and Fridovich assay	↑ [76, 78]	
	*Immunochemistry	↑ [102]/nd [76]	
<b>Catalase activity/levels*</b>			
Blood (erythrocytes)	Measurement of H <sub>2</sub> O <sub>2</sub> decomposition rate	↓ [16, 19, 40, 111, 112]/nd [17, 23, 26, 30]	↓ [16, 40]/nd [30, 41]
Blood (plasma)	Measurement of H <sub>2</sub> O <sub>2</sub> decomposition rate	↓ [24, 33, 120]/nd [42]	
	Peroxidatic function of catalase assay	nd [75]	
	*ELISA	nd [118]	
Bronchial biopsies	Peroxidatic function of catalase assay	nd [75]	
Diaphragm muscle biopsies	Measurement of H <sub>2</sub> O <sub>2</sub> decomposition rate	↑ [61]	↑ [61]
	*Immunochemistry	nd [77]	
Quadriceps muscle biopsies	Peroxidatic function of catalase assay	nd [76]	
	*Immunochemistry	nd [76, 102]	
<b>GSHPx activity/levels</b>			
Blood (erythrocytes)	Quantification of oxidation of NADPH	↓ [16, 17, 19, 23, 26, 40, 111]	↓ [40, 41, 121]
Blood (plasma)	Quantification of oxidation of NADPH	↓ [24, 31, 33]/↑ [26, 118]/nd [57]	
	Quantification of reduced glutathione	↑ [30]	
Whole blood	Quantification of oxidation of NADPH	↓ [72]	
Sputum	Quantification of oxidation of NADPH	↓ [31]	
BALF	Spectrophotometry following reaction with Ellman's reagent	nd [113]	
<b>GST activity/levels*</b>			
Plasma	Reaction with 1-chloro-2,4-dinitrobenzene	↓ [111]/nd [57]	
Sputum	*Western analysis	↑ [122]	
<b>PON1 activity/levels</b>			
Plasma	Paraoxon/diazoxon	nd [22, 86]	
	Paraoxon alone	↓ [34]/nd [38, 54]	↓ [55]

↑ indicates increased levels; ↓ indicates decreased levels; nd: no difference.

levels have been determined using an ELISA kit. Superoxide dismutase has been by far the most studied among antioxidant enzymes biomarkers in COPD and also the one that

presented with the most varied results, having been found diminished, increased, or similar to the controls in different studies (Table 4) [16, 17, 19, 20, 25, 26, 30, 31, 40–42, 57,

72, 74–78, 102, 111–113, 118–120]. Catalase is involved in the detoxification of  $H_2O_2$  to molecular oxygen and water. Its activity has been measured in COPD by monitoring of the decomposition rate of  $H_2O_2$  at the spectrophotometer, and its levels have been estimated with ELISA kits. Results have shown a reduction as well as no difference in different biological samples of patients with COPD (Table 4) [16, 17, 19, 23, 24, 26, 30, 33, 40–42, 61, 75–77, 102, 111, 112, 118, 120]. GSHPx converts reduced GSH to oxidized glutathione (GSSG) while reducing organic peroxides or  $H_2O_2$ . Spectrophotometry has been used to determine GSHPx activity by direct evaluation of the content of reduced GSH or by metering the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), a coenzyme that reduces the GSSG formed in the abovementioned reaction. As opposed to superoxide dismutase, studies on GSHPx activity have produced much less conflicting results and have consistently described a reduction of this biomarker in COPD and also in correlation with disease severity (Table 4) [16, 17, 19, 23, 24, 26, 30, 31, 33, 40, 41, 57, 72, 111, 113, 118, 121]. Glutathione-S-transferase catalyzes the formation of glutathione-S conjugates between GSH and certain electrophilic substrates. It has been studied using 1-chloro-2,4-dinitrobenzene as an artificial substrate or investigating the content of isoenzymes by western blot analysis. PON1, an esterase associated with high-density lipoproteins (HDL), protects against the toxicity of some organophosphates and contributes to the antioxidant protection conferred by HDL on low-density lipoprotein oxidation. Its activity has been evaluated in COPD using a two-substrate (paraoxon/diazoxon) activity method or by the hydrolysis of paraoxon alone. GST and PON1 activities have been studied to a minor extent, and a reduction or no difference was described in COPD compared to the controls (Table 4) [22, 34, 38, 54, 55, 57, 86, 111, 122].

**4.4. Antioxidant Elements.** Various antioxidants elements have been measured in COPD by means of spectrophotometric or chromatographic methods: vitamin A, C, and E and  $\alpha$ - and  $\beta$ -carotenes that contribute to the antioxidant defense system; essential trace elements that play a role in oxidant/antioxidant pathways; and uric acid, a powerful antioxidant protecting lipoproteins from oxidation and acting as a scavenger of oxygen radicals. These biomarkers have especially been studied in the blood of patients with COPD, where a reduction or no difference was found compared to the healthy controls [15, 17, 18, 20, 30, 34, 46, 47, 62, 72, 92, 94, 96, 110, 123].

## 5. Conclusions

This review summarizes the main findings on biomarkers of oxidative stress in patients with stable COPD compared to healthy individuals and in relation to disease progression. The most studied biological sample in this context is the peripheral blood, probably because it is an easily accessible source of information and it allows repeated measurements. Urine test and exhaled breath condensate analysis are other noninvasive monitoring tools, even if EBC analysis is indeed difficult to standardize [124]. Sputum induction, on the other

hand, presents some degree of invasiveness, and sampling repetition at regular intervals has been reported to produce conflicting results [125]. As regards BALF and bronchial or muscles biopsies, their invasiveness makes repeated measurements very limited.

Numerous biomarkers of oxidative stress have been investigated in the blood, particularly lipid peroxidation products and protein carbonyls. The majority of these studies have reported an increase of these biomarkers in patients with COPD compared to the healthy controls and sometimes in relation to disease progression. Superoxide anion and total oxidative statuses have always been found to be increased in the blood in the examined case-control studies, even if different assays have been used to investigate them. Other oxidant biomarkers such as oxidatively damaged DNA, peroxynitrites, and nitrotyrosines were increased in some studies, but with no difference between the cases and controls in other research works. As regards antioxidant markers, protein and nonprotein SH groups have provided different results. Nevertheless, most studies have reported a reduction in plasma protein SH groups and reduced GSH. As for total antioxidant capacity, most of the studies examined have consistently reported a significant decrease of this parameter in COPD using various methods of analysis. Conversely, the analysis of blood antioxidant nutrient levels such as vitamins A, C, and E and the analysis of enzymatic antioxidant activities in blood have given conflicting results, being found sometimes decreased, and then again sometimes similar to the controls. Fewer markers have been studied in exhaled breath condensate compared to the blood, particularly oxidant markers such as  $H_2O_2$ , 8-isoprostane, malondialdehyde, ethane, and peroxynitrites. All of them have been reported to be increased in COPD compared to the controls, except MDA for which not all studies have shown an elevation. In urine, isoprostanes, MDA, and 8-oxodG have always been found increased in COPD, so is the case of the oxidant markers MDA, 8-isoprostane, nitrotyrosines, and 8-oxodG in induced sputum. In BAL, elevated nitrotyrosine levels and reduced GSH levels have been described, while no differences have been observed for antioxidant markers. A few studies have considered bronchial biopsies and muscle biopsies, finding an increase in some oxidant markers such as protein carbonylation, lipid peroxidation, and 3-nitrotyrosine. Conversely, conflicting results have been found in some enzymatic activities.

The underlying reason for the conflicting findings of some studies could be attributed to certain variables that must be taken into account during the sample preparation. These variables that are difficult to control can influence the measurements and therefore the results. We must also consider the human biological variation that inevitably affects any clinical study that is conducted in different populations. Lifestyle variables such as nutrition, smoking, and physical activity can also influence the imbalance between oxidants and antioxidants. Nonetheless, despite these complexities, the different methods used, and the variety of biological samples, the present review highlights that various researchers actually share a few concordant results on this topic. Indeed, it is clear that COPD patients do present oxidative stress,

showing higher levels of oxidants, especially lipid and protein oxidation products, and diminished antioxidant defenses, particularly protein SH groups, reduced GSH, and total antioxidant capacity, compared to healthy individuals. Suitable biomarkers to accurately diagnose COPD and to monitor its progression and its response to therapy have not yet been identified. In this context, this review provides a full picture of the oxidative stress biomarkers that have so far been evaluated in patients with COPD, highlighting those whose reliability is confirmed in different biological samples applying various analytical methods. There is no doubt that further research is needed for better validation of these markers in well-characterized populations.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## References

- [1] R. Lozano, M. Naghavi, K. Foreman et al., "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010," *The Lancet*, vol. 380, no. 9859, pp. 2095–2128, 2012.
- [2] B. R. Celli and W. MacNee, "standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper," *The European Respiratory Journal*, vol. 23, no. 6, pp. 932–946, 2004.
- [3] D. M. Mannino and A. S. Buist, "Global burden of COPD: risk factors, prevalence, and future trends," *The Lancet*, vol. 370, no. 9589, pp. 765–773, 2007.
- [4] M. Decramer, W. Janssens, and M. Miravittles, "Chronic obstructive pulmonary disease," *The Lancet*, vol. 379, no. 9823, pp. 1341–1351, 2012.
- [5] W. A. Pryor and K. Stone, "Oxidants in cigarette Smoke radicals, hydrogen Peroxide, peroxyxynitrate, and peroxyxynitrite," *Annals of the New York Academy of Sciences*, vol. 686, pp. 12–27, 1993.
- [6] W. MacNee, "Oxidative stress and lung inflammation in airways disease," *European Journal of Pharmacology*, vol. 429, no. 1–3, pp. 195–207, 2001.
- [7] J. S. Beckman and W. H. Koppenol, "Nitric oxide, superoxide, and peroxyxynitrite: the good, the bad, and ugly," *The American Journal of Physiology*, vol. 271, no. 5, pp. C1424–C1437, 1996.
- [8] I. Rahman, S. K. Biswas, and A. Kode, "Oxidant and antioxidant balance in the airways and airway diseases," *European Journal of Pharmacology*, vol. 533, no. 1–3, pp. 222–239, 2006.
- [9] P. A. Kirkham and P. J. Barnes, "Oxidative stress in COPD," *Chest*, vol. 144, no. 1, pp. 266–273, 2013.
- [10] B. M. Fischer, J. A. Voynow, and A. J. Ghio, "COPD: balancing oxidants and antioxidants," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 10, pp. 261–276, 2015.
- [11] E. Zinellu, A. Zinellu, A. G. Fois, C. Carru, and P. Pirina, "Circulating biomarkers of oxidative stress in chronic obstructive pulmonary disease: a systematic review," *Respiratory Research*, vol. 17, no. 1, p. 150, 2016.
- [12] I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini, and A. Milzani, "Biomarkers of oxidative damage in human disease," *Clinical Chemistry*, vol. 52, no. 4, pp. 601–623, 2006.
- [13] E. Niki, Y. Yoshida, Y. Saito, and N. Noguchi, "Lipid peroxidation: mechanisms, inhibition, and biological effects," *Biochemical and Biophysical Research Communications*, vol. 338, no. 1, pp. 668–676, 2005.
- [14] E. Niki, "Lipid peroxidation products as oxidative stress biomarkers," *BioFactors*, vol. 34, no. 2, pp. 171–180, 2008.
- [15] N. Dhakal, M. Lamsal, N. Baral et al., "Oxidative stress and nutritional status in chronic obstructive pulmonary disease," *Journal of Clinical and Diagnostic Research*, vol. 9, no. 2, pp. BC01–BC04, 2015.
- [16] C. Arja, K. M. Surapaneni, P. Raya, C. Adimoolam, B. Balisetty, and K. R. Kanala, "Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease," *Respirology*, vol. 18, no. 7, pp. 1069–1075, 2013.
- [17] A. Woźniak, D. Górecki, M. Szpinda, C. Mila-Kierzenkowska, and B. Woźniak, "Oxidant-antioxidant balance in the blood of patients with chronic obstructive pulmonary disease after smoking cessation," *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 897075, 9 pages, 2013.
- [18] C. Cristóvão, L. Cristóvão, F. Nogueira, and M. Bicho, "Avaliação do equilíbrio entre oxidantes e antioxidantes na patogénese da doença pulmonar obstrutiva crónica," *Revista Portuguesa de Pneumologia*, vol. 19, no. 2, pp. 70–75, 2013.
- [19] A. Ahmad, M. Shameem, and Q. Husain, "Altered oxidant-antioxidant levels in the disease prognosis of chronic obstructive pulmonary disease," *The International Journal of Tuberculosis and Lung Disease*, vol. 17, no. 8, pp. 1104–1109, 2013.
- [20] A. M. Raut, A. N. Suryakar, and D. Mhaisekar, "A study of oxidative stress, thiol proteins and role of vitamin E supplementation in chronic obstructive pulmonary disease (COPD)," *Al Ameen Journal of Medical Sciences*, vol. 6, pp. 134–137, 2013.
- [21] M. Gencer, N. Aksoy, E. C. Dagli et al., "Prolidase activity dysregulation and its correlation with oxidative-antioxidative status in chronic obstructive pulmonary disease," *Journal of Clinical Laboratory Analysis*, vol. 25, no. 1, pp. 8–13, 2011.
- [22] I. Stanojkovic, J. Kotur-Stevuljevic, B. Milenkovic et al., "Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation," *Respiratory Medicine*, vol. 105, pp. S31–S37, 2011.
- [23] P. Joppa, D. Petrášová, B. Stančák, Z. Dorková, and R. Tkáčová, "Oxidative stress in patients with COPD and pulmonary hypertension," *Wiener Klinische Wochenschrift*, vol. 119, no. 13–14, pp. 428–434, 2007.
- [24] A. Vibhuti, E. Arif, D. Deepak, B. Singh, and M. A. Qadar Pasha, "Correlation of oxidative status with BMI and lung function in COPD," *Clinical Biochemistry*, vol. 40, no. 13–14, pp. 958–963, 2007.
- [25] E. Ceylan, A. Kocyigit, M. Gencer, N. Aksoy, and S. Selek, "Increased DNA damage in patients with chronic obstructive pulmonary disease who had once smoked or been exposed to biomass," *Respiratory Medicine*, vol. 100, no. 7, pp. 1270–1276, 2006.
- [26] A. Nadeem, H. G. Raj, and S. K. Chhabra, "Increased oxidative stress and altered levels of antioxidants in chronic obstructive pulmonary disease," *Inflammation*, vol. 29, no. 1, pp. 23–32, 2005.
- [27] M. Calikoglu, A. Unlü, L. Tamer, B. Ercan, R. Buğdayci, and U. Atik, "The levels of serum vitamin C, malonyldialdehyde



- and erythrocyte reduced glutathione in chronic obstructive pulmonary disease and in healthy smokers," *Clinical Chemistry and Laboratory Medicine*, vol. 40, no. 10, pp. 1028–1031, 2002.
- [28] I. Hanta, A. Kocabas, N. Canacankatan, S. Kuleci, and G. Seydaoglu, "Oxidant-antioxidant balance in patients with COPD," *Lung*, vol. 184, no. 2, pp. 51–55, 2006.
- [29] I. Rahman, D. Morrison, K. Donaldson, and W. MacNee, "Systemic oxidative stress in asthma, COPD, and smokers," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 4, pp. 1055–1060, 1996.
- [30] A. ben Anes, H. Fetoui, S. Bchir et al., "Increased oxidative stress and altered levels of nitric oxide and peroxynitrite in Tunisian patients with chronic obstructive pulmonary disease: correlation with disease severity and airflow obstruction," *Biological Trace Element Research*, vol. 161, no. 1, pp. 20–31, 2014.
- [31] M. Zeng, Y. Li, Y. Jiang, G. Lu, X. Huang, and K. Guan, "Local and systemic oxidative stress status in chronic obstructive pulmonary disease patients," *Canadian Respiratory Journal*, vol. 20, no. 1, pp. 35–41, 2013.
- [32] H. Tsukagoshi, Y. Shimizu, S. Iwamae et al., "Evidence of oxidative stress in asthma and COPD: potential inhibitory effect of theophylline," *Respiratory Medicine*, vol. 94, no. 6, pp. 584–588, 2000.
- [33] A. Vibhuti, E. Arif, A. Mishra et al., "CYPIA1, CYPIA2 and CYBA gene polymorphisms associated with oxidative stress in COPD," *Clinica Chimica Acta*, vol. 411, no. 7–8, pp. 474–480, 2010.
- [34] B. Isik, R. S. Isik, A. Ceylan, and O. Calik, "Trace elements and oxidative stress in chronic obstructive pulmonary disease," *Saudi Medical Journal*, vol. 26, no. 12, pp. 1882–1885, 2005.
- [35] R. Premanand, S. Kumar, and A. Mohan, "Study of thiobarbituric reactive substances and total reduced glutathione as indices of oxidative stress in chronic smokers with and without chronic obstructive pulmonary disease," *The Indian Journal of Chest Diseases & Allied Sciences*, vol. 49, no. 1, pp. 9–12, 2007.
- [36] L. Milevoj Kopčinović, A. M. Domijan, K. Posavac, I. Čepelak, T. Žanić Grubišić, and L. Rumora, "Systemic redox imbalance in stable chronic obstructive pulmonary disease," *Biomarkers*, vol. 21, no. 8, pp. 692–698, 2016.
- [37] A. Sunnetcioglu, H. H. Alp, B. Sertogullarından, R. Balaharoglu, and H. Gunbatar, "Evaluation of oxidative damage and antioxidant mechanisms in COPD, lung cancer, and obstructive sleep apnea syndrome," *Respiratory Care*, vol. 61, no. 2, pp. 205–211, 2016.
- [38] A. Zinellu, A. G. Fois, S. Sotgia et al., "Arginines plasma concentration and oxidative stress in mild to moderate COPD," *PLoS One*, vol. 11, no. 8, article e0160237, 2016.
- [39] T. Aggarwal, R. Wadhwa, V. Rohil, and P. K. Maurya, "Biomarkers of oxidative stress and protein-protein interaction in chronic obstructive pulmonary disease," *Archives of Physiology and Biochemistry*, vol. 124, no. 3, pp. 226–231, 2017.
- [40] S. Singh, S. K. Verma, S. Kumar et al., "Evaluation of oxidative stress and antioxidant status in chronic obstructive pulmonary disease," *Scandinavian Journal of Immunology*, vol. 85, no. 2, pp. 130–137, 2017.
- [41] Z. Kluchová, D. Petrášová, P. Joppa, Z. Dorková, and R. Tkáčová, "The association between oxidative stress and obstructive lung impairment in patients with COPD," *Physiological Research*, vol. 56, no. 1, pp. 51–56, 2007.
- [42] F. Folchini, N. L. Nonato, E. Feofiloff, V. D'Almeida, O. Nascimento, and J. R. Jardim, "Association of oxidative stress markers and C-reactive protein with multidimensional indexes in COPD," *Chronic Respiratory Disease*, vol. 8, no. 2, pp. 101–108, 2011.
- [43] E. M. Mercken, G. J. Hageman, A. M. Schols, M. A. Akkermans, A. Bast, and E. F. Wouters, "Rehabilitation decreases exercise-induced oxidative stress in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 8, pp. 994–1001, 2005.
- [44] M. L. Bartoli, F. Novelli, F. Costa et al., "Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases," *Mediators of Inflammation*, vol. 2011, Article ID 891752, 7 pages, 2011.
- [45] B. Antus, G. Harnasi, O. Drozdovszky, and I. Barta, "Monitoring oxidative stress during chronic obstructive pulmonary disease exacerbations using malondialdehyde," *Respirology*, vol. 19, no. 1, pp. 74–79, 2014.
- [46] Y. Jammes, J. G. Steinberg, A. Ba, S. Delliaux, and F. Brégeon, "Enhanced exercise-induced plasma cytokine response and oxidative stress in COPD patients depend on blood oxygenation," *Clinical Physiology and Functional Imaging*, vol. 28, no. 3, pp. 182–188, 2008.
- [47] C. Koechlin, A. Couillard, J. P. Cristol et al., "Does systemic inflammation trigger local exercise-induced oxidative stress in COPD?," *The European Respiratory Journal*, vol. 23, no. 4, pp. 538–544, 2004.
- [48] A. Couillard, C. Koechlin, J. P. Cristol, A. Varray, and C. Prefaut, "Evidence of local exercise-induced systemic oxidative stress in chronic obstructive pulmonary disease patients," *The European Respiratory Journal*, vol. 20, no. 5, pp. 1123–1129, 2002.
- [49] E. S. Erden, S. Motor, I. Ustun et al., "Investigation of Bisphenol A as an endocrine disruptor, total thiol, malondialdehyde, and C-reactive protein levels in chronic obstructive pulmonary disease," *European Review for Medical and Pharmacological Sciences*, vol. 18, no. 22, pp. 3477–3483, 2014.
- [50] Y. D. Torres-Ramos, M. L. García-Guillen, I. M. Olivares-Corichi, and J. J. Hicks, "Correlation of plasma protein carbonyls and C-reactive protein with GOLD stage progression in COPD patients," *Open Respiratory Medicine Journal*, vol. 3, pp. 61–66, 2009.
- [51] S. B. Moussa, I. Sfaxi, Z. Tabka, H. B. Saad, and S. Rouatbi, "Oxidative stress and lung function profiles of male smokers free from COPD compared to those with COPD: a case-control study," *Libyan Journal of Medicine*, vol. 9, no. 1, article 23873, 2014.
- [52] S. Ben Moussa, S. Rouatbi, and S. H. Ben, "Incapacity, handicap, and oxidative stress markers of male smokers with and without COPD," *Respiratory Care*, vol. 61, no. 5, pp. 668–679, 2016.
- [53] I. Rahman, E. Skwarska, and W. MacNee, "Attenuation of oxidant/antioxidant imbalance during treatment of exacerbations of chronic obstructive pulmonary disease," *Thorax*, vol. 52, no. 6, pp. 565–568, 1997.
- [54] A. Zinellu, A. G. Fois, S. Sotgia et al., "Plasma protein thiols: an early marker of oxidative stress in asthma and chronic obstructive pulmonary disease," *European Journal of Clinical Investigation*, vol. 46, no. 2, pp. 181–188, 2016.

- [55] Y. D. Torres-Ramos, A. M. Guzman-Grenfell, and A. Montoya-Estrada, "RBC membrane damage and decreased band 3 phospho-tyrosine phosphatase activity are markers of COPD progression," *Frontiers in Bioscience*, vol. E2, no. 4, pp. 1385–1393, 2010.
- [56] A. Guzmán-Grenfell, N. Nieto-Velázquez, Y. Torres-Ramos et al., "Increased platelet and erythrocyte arginase activity in chronic obstructive pulmonary disease associated with tobacco or wood smoke exposure," *Journal of Investigative Medicine*, vol. 59, no. 3, pp. 587–592, 2011.
- [57] M. Montaña, J. Cisneros, A. Ramírez-Venegas et al., "Malondialdehyde and superoxide dismutase correlate with FEV<sub>1</sub> in patients with COPD associated with wood smoke exposure and tobacco smoking," *Inhalation Toxicology*, vol. 22, no. 10, pp. 868–874, 2010.
- [58] E. Avci and G. A. Avci, "Important biomarkers that play a role in the chronic obstructive pulmonary disease process," *Journal of Medical Biochemistry*, vol. 37, no. 1, pp. 46–53, 2018.
- [59] T. Tug, F. Karatas, and S. M. Terzi, "Antioxidant vitamins (A, C and E) and malondialdehyde levels in acute exacerbation and stable periods of patients with chronic obstructive pulmonary disease," *Clinical and Investigative Medicine*, vol. 27, no. 3, pp. 123–128, 2004.
- [60] M. Corradi, P. Pignatti, P. Manini et al., "Comparison between exhaled and sputum oxidative stress biomarkers in chronic airway inflammation," *The European Respiratory Journal*, vol. 24, no. 6, pp. 1011–1017, 2004.
- [61] H. J. Wijnhoven, L. M. Heunks, M. C. Geraedts, T. Hafmans, J. R. Viña, and P. N. Dekhuijzen, "Oxidative and nitrosative stress in the diaphragm of patients with COPD," *International Journal of Chronic Obstructive Pulmonary Disease*, vol. 1, no. 2, pp. 173–179, 2006.
- [62] J. Maury, F. Gouzi, P. De Rigal et al., "Heterogeneity of systemic oxidative stress profiles in COPD: a potential role of gender," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 201843, 11 pages, 2015.
- [63] M. Kaźmierczak, M. Ciebada, A. Pękala-Wojciechowska, M. Pawłowski, A. Nielepkowicz-Goździńska, and A. Antczak, "Evaluation of markers of inflammation and oxidative stress in COPD patients with or without cardiovascular comorbidities," *Heart, Lung & Circulation*, vol. 24, no. 8, pp. 817–823, 2015.
- [64] K. Kostikas, G. Papatheodorou, K. Psathakis, P. Panagou, and S. Loukides, "Oxidative stress in expired breath condensate of patients with COPD," *Chest*, vol. 124, no. 4, pp. 1373–1380, 2003.
- [65] P. Montuschi, J. V. Collins, G. Ciabattini et al., "Exhaled 8-isoprostane as an In Vivo Biomarker of lung oxidative stress in patients with COPD and healthy smokers," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 3, pp. 1175–1177, 2000.
- [66] F. W. S. Ko, C. Y. K. Lau, T. F. Leung, G. W. K. Wong, C. W. K. Lam, and D. S. C. Hui, "Exhaled breath condensate levels of 8-isoprostane, growth related oncogene  $\alpha$  and monocyte chemoattractant protein-1 in patients with chronic obstructive pulmonary disease," *Respiratory Medicine*, vol. 100, no. 4, pp. 630–638, 2006.
- [67] P. Paredi, S. A. Kharitonov, D. Leak, S. Ward, D. Cramer, and P. J. Barnes, "Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 2, pp. 369–373, 2000.
- [68] V. L. Kinnula, H. Ilumets, M. Myllärniemi, A. Sovijärvi, and P. Ryttilä, "8-Isoprostane as a marker of oxidative stress in nonsymptomatic cigarette smokers and COPD," *The European Respiratory Journal*, vol. 29, no. 1, pp. 51–55, 2007.
- [69] D. Praticò, S. Basili, M. Vieri, C. Cordova, F. Violi, and G. A. Fitzgerald, "Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F<sub>2</sub>alpha-III, an index of oxidant stress," *American Journal of Respiratory and Critical Care Medicine*, vol. 158, no. 6, pp. 1709–1714, 1998.
- [70] P. Santus, A. Sola, P. Carlucci et al., "Lipid peroxidation and 5-lipoxygenase activity in chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 8, pp. 838–843, 2005.
- [71] I. Dalle-Donne, R. Rossi, and D. Giustarini, "Protein carbonyl groups as biomarkers of oxidative stress," *Clinica Chimica Acta*, vol. 329, no. 1-2, pp. 23–38, 2003.
- [72] M. C. Santos, A. L. Oliveira, A. M. Viegas-Crespo et al., "Systemic markers of the redox balance in chronic obstructive pulmonary disease," *Biomarkers*, vol. 9, pp. 461–469, 2004.
- [73] P. Gopal, N. L. Reynaert, J. L. Scheijen et al., "Plasma advanced glycation end-products and skin autofluorescence are increased in COPD," *The European Respiratory Journal*, vol. 43, no. 2, pp. 430–438, 2014.
- [74] E. Puig-Vilanova, D. A. Rodriguez, J. Lloreta et al., "Oxidative stress, redox signaling pathways, and autophagy in cachectic muscles of male patients with advanced COPD and lung cancer," *Free Radical Biology & Medicine*, vol. 79, pp. 91–108, 2015.
- [75] E. Barreiro, C. Femoselle, M. Mateu-Jimenez et al., "Oxidative stress and inflammation in the normal airways and blood of patients with lung cancer and COPD," *Free Radical Biology & Medicine*, vol. 65, pp. 859–871, 2013.
- [76] D. A. Rodriguez, S. Kalko, E. Puig-Vilanova et al., "Muscle and blood redox status after exercise training in severe COPD patients," *Free Radical Biology and Medicine*, vol. 52, no. 1, pp. 88–94, 2012.
- [77] E. Barreiro, B. de la Puente, J. Minguella et al., "Oxidative stress and respiratory muscle dysfunction in severe chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 10, pp. 1116–1124, 2005.
- [78] E. Barreiro, R. Rabinovich, J. Marin-Corral, J. A. Barberà, J. Gea, and J. Roca, "Chronic endurance exercise induces quadriceps nitrosative stress in patients with severe COPD," *Thorax*, vol. 64, no. 1, pp. 13–19, 2009.
- [79] E. Barreiro, A. M. Schols, M. I. Polkey et al., "Cytokine profile in quadriceps muscles of patients with severe COPD," *Thorax*, vol. 63, no. 2, pp. 100–107, 2008.
- [80] R. L. Levine, "Carbonyl modified proteins in cellular regulation, aging, and disease," *Free Radical Biology & Medicine*, vol. 32, no. 9, pp. 790–796, 2002.
- [81] W. Cao, F. F. Hou, and J. Nie, "AOPPs and the progression of kidney disease," *Kidney International Supplements*, vol. 4, pp. 102–106, 2014.
- [82] T. Münzel, I. B. Afanas'ev, A. L. Kleschyov, and D. G. Harrison, "Detection of superoxide in vascular tissue," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 11, pp. 1761–1768, 2002.

- [83] P. N. Dekhuijzen, K. K. Aben, I. Dekker et al., "Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 3, pp. 813–816, 1996.
- [84] A. Antczak, M. Ciebiada, T. Pietras, W. J. Piotrowski, Z. Kurmanowska, and P. Górski, "Exhaled eicosanoids and biomarkers of oxidative stress in exacerbation of chronic obstructive pulmonary disease," *Archives of Medical Science*, vol. 8, no. 2, pp. 277–285, 2012.
- [85] K. Murata, K. Fujimoto, Y. Kitaguchi, T. Horiuchi, K. Kubo, and T. Honda, "Hydrogen peroxide content and pH of expired breath condensate from patients with asthma and COPD," *COPD: Journal of Chronic Obstructive Pulmonary Disease*, vol. 11, no. 1, pp. 81–87, 2014.
- [86] I. Stanojkovic, J. Kotur-Stevuljevic, S. Spasic et al., "Relationship between bone resorption, oxidative stress and inflammation in severe COPD exacerbation," *Clinical Biochemistry*, vol. 46, no. 16–17, pp. 1678–1682, 2013.
- [87] E. Ceylan, M. Gencer, E. Uzer, and H. Celik, "Measurement of the total antioxidant potential in chronic obstructive pulmonary diseases with a novel automated method," *Saudi Medical Journal*, vol. 28, no. 9, pp. 1339–1343, 2007.
- [88] S. Ekin, A. Arsoy, H. Gunbatar et al., "The relationships among the levels of oxidative and antioxidative parameters, FEV1 and prolidase activity in COPD," *Redox Report*, vol. 22, no. 2, pp. 74–77, 2016.
- [89] U. Can, F. H. Yerlikaya, and S. Yosunkaya, "Role of oxidative stress and serum lipid levels in stable chronic obstructive pulmonary disease," *Journal of the Chinese Medical Association*, vol. 78, no. 12, pp. 702–708, 2015.
- [90] M. P. Foschino Barbaro, G. E. Carpagnano, A. Spanevello, M. G. Cagnazzo, and P. J. Barnes, "Inflammation, oxidative stress and systemic effects in mild chronic obstructive pulmonary disease," *International Journal of Immunopathology and Pharmacology*, vol. 20, no. 4, pp. 753–763, 2007.
- [91] N. Markoulis, K. I. Gourgoulis, A. Moulas, E. Gerogianni, and A. P. Molyvdas, "Reactive oxygen metabolites as an index of chronic obstructive pulmonary disease severity," *Panminerva Medica*, vol. 48, no. 4, pp. 209–213, 2006.
- [92] Y. C. Lin, T. C. Wu, P. Y. Chen, L. Y. Hsieh, and S. L. Yeh, "Comparison of plasma and intake levels of antioxidant nutrients in patients with chronic obstructive pulmonary disease and healthy people in Taiwan: a case-control study," *Asia Pacific Journal of Clinical Nutrition*, vol. 19, no. 3, pp. 393–401, 2010.
- [93] E. G. Tzortzaki, K. Dimakou, E. Neofytou et al., "Oxidative DNA damage and somatic mutations: a link to the molecular pathogenesis of chronic inflammatory airway diseases," *Chest*, vol. 141, no. 5, pp. 1243–1250, 2012.
- [94] T. Igishi, Y. Hitsuda, K. Kato et al., "Elevated urinary 8-hydroxydeoxyguanosine, a biomarker of oxidative stress, and lack of association with antioxidant vitamins in chronic obstructive pulmonary disease," *Respirology*, vol. 8, no. 4, pp. 455–460, 2003.
- [95] Z. Malic, A. Topic, D. Francuski et al., "Oxidative stress and genetic variants of xenobiotic-metabolising enzymes associated with COPD development and severity in Serbian adults," *COPD*, vol. 14, no. 1, pp. 95–104, 2017.
- [96] Y. Kodama, Y. Kishimoto, Y. Muramatsu et al., "Antioxidant nutrients in plasma of Japanese patients with chronic obstructive pulmonary disease, asthma-COPD overlap syndrome and bronchial asthma," *The Clinical Respiratory Journal*, vol. 11, no. 6, pp. 915–924, 2017.
- [97] Y. M. Shih, M. S. Cooke, C. H. Pan, M. R. Chao, and C. W. Hu, "Clinical relevance of guanine-derived urinary biomarkers of oxidative stress, determined by LC-MS/MS," *Redox Biology*, vol. 20, pp. 556–565, 2019.
- [98] G. O. Osoata, T. Hanazawa, C. Brindicci et al., "Peroxynitrite elevation in exhaled breath condensate of COPD and its inhibition by fudosteine," *Chest*, vol. 135, no. 6, pp. 1513–1520, 2009.
- [99] M. Ichinose, H. Sugiura, S. Yamagata, A. Koarai, and K. Shirato, "Increase in reactive nitrogen species production in chronic obstructive pulmonary disease airways," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 2, pp. 701–706, 2000.
- [100] H. Sugiura, M. Ichinose, M. Tomaki et al., "Quantitative assessment of protein-bound tyrosine nitration in airway secretions from patients with inflammatory airway disease," *Free Radical Research*, vol. 38, no. 1, pp. 49–57, 2004.
- [101] F. L. Ricciardolo, G. Caramori, K. Ito et al., "Nitrosative stress in the bronchial mucosa of severe chronic obstructive pulmonary disease," *The Journal of Allergy and Clinical Immunology*, vol. 116, no. 5, pp. 1028–1035, 2005.
- [102] E. Barreiro, J. Gea, J. M. Corominas, and S. N. Hussain, "Nitric oxide synthases and protein oxidation in the quadriceps femoris of patients with chronic obstructive pulmonary disease," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 6, pp. 771–778, 2003.
- [103] G. L. Ellman, "Tissue sulfhydryl groups," *Archives of Biochemistry and Biophysics*, vol. 82, no. 1, pp. 70–77, 1959.
- [104] I. Rahman, E. Swarska, M. Henry, J. Stolk, and W. MacNee, "Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic obstructive pulmonary disease?," *Thorax*, vol. 55, no. 3, pp. 189–193, 2000.
- [105] K. M. Beeh, J. Beier, N. Koppenhoefer, and R. Buhl, "Increased glutathione disulfide and nitrosothiols in sputum supernatant of patients with stable COPD," *Chest*, vol. 126, no. 4, pp. 1116–1122, 2004.
- [106] E. M. Drost, K. M. Skwarski, J. Saulea et al., "Oxidative stress and airway inflammation in severe exacerbations of COPD," *Thorax*, vol. 60, no. 4, pp. 293–300, 2005.
- [107] T. Turgut, N. Ilhan, F. Deveci, N. Akpolat, E. Ş. Erden, and M. H. Muz, "Glutathione and nitrite levels in induced sputum at COPD patients and healthy smokers," *Journal of Thoracic Disease*, vol. 6, no. 6, pp. 765–771, 2014.
- [108] I. F. Benzie and J. J. Strain, "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.
- [109] N. J. Miller, C. A. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner, "A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates," *Clinical Science*, vol. 84, no. 4, pp. 407–412, 1993.
- [110] G. J. Hageman, I. Larik, H. J. Pennings, G. R. Haenen, E. F. Wouters, and A. Bast, "Systemic poly(ADP-ribose) polymerase-1 activation, chronic inflammation, and

- oxidative stress in COPD patients," *Free Radical Biology & Medicine*, vol. 35, no. 2, pp. 140–148, 2003.
- [111] R. Lakhdar, S. Denden, M. H. Mouhamed et al., "Correlation of EPHX1, GSTP1, GSTM1, and GSTT1 genetic polymorphisms with antioxidative stress markers in chronic obstructive pulmonary disease," *Experimental Lung Research*, vol. 37, no. 4, pp. 195–204, 2011.
- [112] H. Tavilani, E. Nadi, J. Karimi, and M. T. Goodarzi, "Oxidative stress in COPD patients, smokers, and non-smokers," *Respiratory Care*, vol. 57, no. 12, pp. 2090–2094, 2012.
- [113] M. Yigla, Y. Berkovich, and R. M. Nagler, "Oxidative stress indices in COPD—Broncho-alveolar lavage and salivary analysis," *Archives of Oral Biology*, vol. 52, no. 1, pp. 36–43, 2007.
- [114] J. M. McCord and I. Fridovich, "Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein)," *The Journal of Biological Chemistry*, vol. 244, no. 22, pp. 6049–6055, 1969.
- [115] H. P. Misra and I. Fridovich, "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase," *The Journal of Biological Chemistry*, vol. 247, no. 10, pp. 3170–3175, 1972.
- [116] S. Marklund and G. Marklund, "Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase," *European Journal of Biochemistry*, vol. 47, no. 3, pp. 469–474, 1974.
- [117] K. Das, "A modified spectrophotometric assay of superoxide dismutase using nitrate formation by superoxide radical," *Indian Journal of Biochemistry & Biophysics*, vol. 37, pp. 201–204, 2000.
- [118] V. N. Ambade, A. N. Sontakke, M. S. Barthwal, R. Tyagi, and D. R. Basannar, "Diagnostic utility of biomarkers in COPD," *Respiratory Care*, vol. 60, no. 12, pp. 1729–1742, 2015.
- [119] E. A. Regan, W. Mazur, E. Meoni et al., "Smoking and COPD increase sputum levels of extracellular superoxide dismutase," *Free Radical Biology and Medicine*, vol. 51, no. 3, pp. 726–732, 2011.
- [120] J. Bajpai, V. Prakash, S. Kant et al., "Study of oxidative stress biomarkers in chronic obstructive pulmonary disease and their correlation with disease severity in north Indian population cohort," *Lung India*, vol. 34, no. 4, pp. 324–329, 2017.
- [121] R. Tkacova, Z. Kluchova, P. Joppa, D. Petrasova, and A. Molcanyiova, "Systemic inflammation and systemic oxidative stress in patients with acute exacerbations of COPD," *Respiratory Medicine*, vol. 101, no. 8, pp. 1670–1676, 2007.
- [122] T. Harju, W. Mazur, H. Merikallio, Y. Soini, and V. L. Kinnula, "Glutathione-S-transferases in lung and sputum specimens, effects of smoking and COPD severity," *Respiratory Research*, vol. 9, no. 1, p. 80, 2008.
- [123] E. Pirabbasi, M. Najafiyani, M. Cheraghi et al., "What are the antioxidant status predictors' factors among male chronic obstructive pulmonary disease (COPD) patients?," *Global Journal of Health Science*, vol. 5, no. 1, pp. 70–78, 2012.
- [124] P. Kubán and F. Foret, "Exhaled breath condensate: determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review," *Analytica Chimica Acta*, vol. 805, pp. 1–18, 2013.
- [125] O. Holz, K. Richter, R. A. Jorres, P. Speckin, M. Mucke, and H. Magnussen, "Changes in sputum composition between two inductions performed on consecutive days," *Thorax*, vol. 53, no. 2, pp. 83–86, 1998.