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Oxidative stress in chronic obstructive pulmonary disease

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Resumo

A doença pulmonar obstrutiva crónica (DPOC) é caracterizada por uma limitação progressiva, persistente e irreversível do fluxo de ar, causada principalmente pela inflamação e stress oxidativo. O stress oxidativo, ou a produção de espécies reativas de oxigénio (ROS), é principalmente devido a triggers ambientais e tóxicos como $PM_{2.5}$ mas, predominantemente, ao fumo do cigarro. Estes triggers ativam vários mecanismos e fatores de transcrição, amplificando a via pró-inflamatória do fator nuclear kappa B (NF-κB) e diminuindo os níveis e a atividade de histona desacetilase 2 (HDAC2). Acaba por levar a um aumento da inflamação e do dano nuclear e celular, formando um ciclo vicioso onde o stress oxidativo continua a aumentar, deteriorando cada vez mais a função pulmonar e a piorar a DPOC. Adicionalmente, além do aumento do stress oxidativo e dos seus marcadores, como o malondialdeído (MDA), há uma tendência para a redução dos níveis de antioxidantes como superóxido dismutase (SOD), catalase e as proteínas do grupo sulfidrílico. Contudo, pode haver um aumento destes níveis aquando das exacerbações agudas, existindo, portanto, uma tentativa de contrabalançar e combater a inflamação e a oxidação.

Objetivo: fornecer uma revisão sobre o assunto destacando o papel do stress oxidativo na doença pulmonar obstrutiva crónica, suas consequências e os possíveis biomarcadores que derivam dele e suas aplicações.

Métodos: Foi realizada pesquisa bibliográfica no PUBMED utilizando as palavras-chave “DPOC”; "doença de obstrução pulmonar crônica"; “stress oxidativo” e “espécies reativas de oxigénio”. Na sequência desta pesquisa, incluiu-se 69 artigos originais, em inglês, de janeiro de 2015 a dezembro de 2020. Em alguns casos, a bibliografia dos estudos também foi incluída. Foram excluídos todos os artigos que se focavam apenas nas opções de tratamento.

Palavras-chave: DPOC; doença pulmonar obstrutiva crónica; Stress oxidativo e espécies reativas de oxigénio.

Title: Oxidative stress in chronic obstructive pulmonary disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by progressive, persistent, and irreversible airflow limitation caused mainly by inflammation and oxidative stress. Oxidative stress, or (ROS) reactive oxygen species production, is due, principally, by environmental triggers and toxics like $PM_{2.5}$, and predominantly cigarette smoke. They activate various mechanisms and transcription factors, increasing the nuclear factor-κB (NF-κB) pro-inflammatory pathway and diminishing histone deacetylase 2's (HDAC2) levels and activity. In the end, it leads to an escalation in the inflammation and nuclear and cellular damage, forming a vicious cycle where oxidative stress continuous on increasing, worsening lung function, and thus, gradually augmenting the grade and severity of COPD. Additionally, besides the increase in oxidative stress and its markers, like malondialdehyde (MDA), there is a tendency for the reduction of the antioxidant levels such as superoxide dismutase (SOD), catalase and sulfhydryl group proteins. This tends to differ, however, in exacerbations, where, since it is an acute event, there is an attempt to counterbalance and fight the inflammation and oxidation, increasing the antioxidant levels when compared to the stable disease.

Keywords: "COPD"; "chronic obstructive pulmonary disease"; "oxidative stress" and "reactive oxygen species"

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Abbreviations

AChE- Acetylcholinesterase

AECs- Airway epithelial cells

AOPP- Advanced oxidation protein products

CAT- Catalase

CF- Cystic fibrosis

CFTR- Cystic fibrosis transmembrane conductance regulator

CI- chromosomal instability

CRP- C-reactive protein

CSE- Cigarette smoke exposure

FEV_1/FVC- ratio forced expiratory volume in the first second to forced vital capacity

FRC- Functional residual capacity

GPx- Glutathione peroxidase

GSK3β- Glycogen synthase-3β

GST- Glutathione S-transferase

HDAC2- Histone deacetylase 2

MAO- Monoamine oxidase

MAPK- p38 mitogen activated protein kinase

MDA- Malondialdehyde

miRNAs- Micro-RNAs

MMPs- Matrix metalloproteinases

mtDNA- Mitochondrial DNA

NF-κB- Nuclear factor-κB

Nrf-2- Nuclear factor erythroid-2-related factor 2

LDH- Lactate dehydrogenase

LDL- Low-density lipoprotein

$PM_{2.5}$- Particulate matter <2.5 μm in diameter

PI3K- phosphatidylinositol 3-kinase

PON1- Paraoxonase 1

PTEN- Phosphatase and tensin homolog

RNS- Reactive nitrogen species

ROS- Reactive oxygen species

RV- Residual volume

SIRT- Sirtuins

SOD- Superoxide dismutase

TGF-β- Transforming growth factor beta

TAS- total antioxidant status

TOS- total oxidant status

TEAC- Trolox equivalent antioxidant capacity

Introduction

Chronic obstructive pulmonary disease is a preventable pathology with very high morbidity and mortality around the world[1]. It is characterized by progressive, persistent, and irreversible airflow limitation causing dyspnoea, chronic bronchitis and wheezing.[2]

The airflow limitation is usually triggered and accentuated by an increase in the lungs and airways inflammatory response due to harmful particles and gases.[2] The most common and reported is the tobacco smoke[3]. This inflammation leads to obstruction of the small airways and sometimes destructions of the lung parenchyma (emphysema)[4] with collapse of the small airways (due to loss of alveolar support and decreased pulmonary elastance)[5].

Therefore, COPD results from complex interactions between individual's genetic susceptibility[6], for example the α1-antitrypsin deficiency[7], and harmful exposure to environmental triggers/ particles and gases.[1]

Particularly the cigarette smoke itself contains about 10^{15}-10^{17} oxidizing molecules per puff[8] which causes direct damage to airway epithelium initiating an inflammatory response[3] with proteolytic enzymes and reactive oxygen species (ROS) release by lymphocytes, neutrophils, and macrophages. This release, if not properly counterbalanced by anti-proteases and antioxidants, will cause oxidative stress and, thus, cellular damage[9].

Oxidative stress consists on a significant increase in the reductive potential leading to harmful production of reactive oxygen species such as free radicals and peroxides in aerobic cells. In patients with COPD, the increase in oxidative stress results from an increase in inhaled oxidants and the amount of reactive oxygen species (ROS) and free radicals that are produced by inflammatory aerobic, immune and epithelial cells in the airways[10]. It is thus caused by the reduction of the antioxidant potential in combination with an increase in oxidative load. Some reactive oxygen species are superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), Ozone (O_3), and singlet oxygen ($1\ O_2$).[11]

A quick note about the treatment that usually is given to these patients, firstly, it is highly advised to cease smoking and avoid other environmental toxics and pollutants[2]. In addition to this, recommended therapy consists of bronchodilators (beta-agonists and anticholinergic) and anti-inflammatories (inhaled or systemic corticosteroids, phosphodiesterase inhibitors).[2]

The aim of this review and thesis paper is to provide a revision on this matter highlighting the role of oxidative stress in chronic obstructive pulmonary disease, its consequences and the possible biomarkers that derive from it and their applications. Along with the additional purpose of integrating the project that characterizes the chromosomal instability (CI) (result of damage by oxidative stress at a DNA level) in diseases of the airway such as asthma, COPD and bronchiectasis in development at Cytogenetics Laboratory of the Institute of Biomedical Sciences Abel Salazar.

Methods

A bibliography research was conducted on PUBMED using the key words "COPD"; "chronic obstructive pulmonary disease"; "oxidative stress" and "reactive oxygen species". Following this research, it included 69 original research articles in English from January 2015 till December 2020. In addition, in some cases, the bibliography of the studies was also included. It was excluded all articles that focused just about treatment options.

Molecular Mechanisms

- NF-κB

Excess of oxidative stress provokes a redox system imbalance. In order to try to maintain this homeostasis, there is an increase in antioxidant genes transcription. This is mediated by ROS, NADPH oxidase and NF-κB (nuclear transcription factor) in the NADPH oxidase-ROS-NF-κB transduction pathway, occurring a rise in their levels to combat the higher oxidative stress present in COPD[1]. Monoamine oxidase (MAO)-B can also initiate this pathway causing induction of various antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT) and even glutathione peroxidase (GPx).[12] MAO-B's activity and expression is elevated due to cigarette smoke exposure (CSE) of airway epithelial cells (AECs), being one of the mechanisms that helps fight oxidative stress rise caused by CSE.[12]

On the other hand, however, through the NF-κB/MAPK (p38 mitogen activated protein kinase) signalling pathway, there is an inflammation boost, as well as oxidative stress causing lung injury/apoptosis and pulmonary function deterioration.[13-15] This pathway is mediated by CSE[16] but on a molecular level can be activated by IL-33 which is released when inflammation or necrosis arises, being increased in COPD's lungs.[13] It can also be stimulated by chloride channel cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction or decrease. Since CFTR

is dysfunctional in COPD, it initiates the NF-κB/MAPK signalling pathway increasing oxidative stress, which in turn causes lower CFTR levels, creating a vicious cycle.[14, 17] In similitude with CFTR, the kinase glycogen synthase-3β (GSK3β), is diminished in oxidative stress and immune cells in COPD[18]. This decrease ultimately causes glucocorticoid insensitivity in the airways via the NF-kB/MAPK signalling pathway among other routes as the phosphatidylinositol 3-kinase (PI3K)/Akt signalling system.[18] Likewise, PI3K/Akt specific pathway can be continuously and persistently activated as a result of PTEN (Phosphatase and tensin homolog) protein levels inhibition, leading to inflammation.[19] This inhibition was initially done by oxidative stress derived from cigarette smoke, and it enlightens the continuation of disease progression even after smoking cessation.[19]

Oxidative or nitrosative stress excess, besides reducing antioxidant system levels, can also induce STAT1 phosphorylation that stimulates transcriptional responses to pro-inflammatory cytokines which, in turn, lead to MAPK phosphorylation.[16] Therefore, as a result of the redox imbalance, MAPKs/STAT1 pathway causes lung parenchyma injury, leading to accelerated lung aging in COPD.[16]

A variation of the MAPK, MAP3K19, is over-expressed in oxidative stress and, consequently, in COPD, leading, through NF-κB pro-inflammatory pathway, to chemokines CXCL-8, CCL-7 and CCL-20 secretion and expression, as well as neutrophils recruiting.[20] An alternative pathway this kinase could activate is the TGF-β related mechanism, that sequentially causes tissue remodelling and airspace expansion.[20]

- Antioxidants

In order to combat oxidative stress, our organism has natural endogenous antioxidants to regulate redox balance and to prevent damage that may consequently come from excess of ROS/RNS. However, in COPD, it appears to be a decrease in antioxidant levels as vitamin C[21], reactive persulfides and polysulfides[22] and paraoxonase 1 (PON_1)[23]. That may be due to an increase in these molecules consumption by oxidants and/or a downregulation of the mechanisms responsible for their production. For instance, the activity of PON_1, an enzyme in charge of preventing low-density lipoprotein (LDL) oxidation, decreases in conformation with disease progression, being negatively associated with FEV_1/FVC.[23] An example of this production downregulation is H_2S. H_2S is produced by various enzymes, one of the most important is cystathionine-γ-lyase. This enzyme is related with smoking status, being decreased in COPD along with smokers[24] causing a decrease in H_2S and therefore in its antioxidant potential. This loss leads

to reduction of other antioxidants (GSH and SOD), to IL-8 and TNF-α secretion and finally to inflammation, more oxidative stress, and glucocorticoid insensitivity.[24]

- Environmental triggers

The major environmental causes of COPD are cigarette smoke and exposure to other toxic particles present in the outside environment like airborne fine particulate matter <2.5μm in diameter ($PM_{2.5}$). This particulate matter, although being very small, can easily disturb the pulmonary barrier and cause apoptosis by reducing tight junction proteins and breaking the cell membrane, all while still increasing ROS levels.[25] This increase occurs through the aryl hydrocarbon receptor pathway that, sequentially, augments the risk of COPD by depleting the zona occludens and α1-antitrypsin.[25]

When it comes to cigarette smoke, one of the most abundant reactive compounds present is acrolein. This specific compound is increased in COPD, being directly linked to oxidative stress, increased FRC and RV and, of course, smoking history.[26]

- Erythrocyte changes and mechanisms

Oxidative stress affects blood cells as well. It can cause various effects, from a decrease in antioxidants activity like SOD and GPx[27], to repercussions in erythrocyte membrane. Usually, it triggers thiol groups oxidation, decreases ATPases activity and most importantly, increases acetylcholinesterase (AChE) activity[27], a biomarker of membrane integrity that can neutralize acetylcholine and subsequently diminish the inflammatory response [28].

- Other molecular stress-induced mechanisms

Usually, Butyrylcholinesterase (BChE) is a good marker of oxidative stress, being inhibited by it[29]. However, its value can also be increased according to other studies[28]. Regardless of this, its activity does not seem to change with different smoking habits, implying that cigarette smoking has no effect on this enzyme.[28]

One of the consequences of oxidative stress increase is the irreversible formation and accumulation of the Advanced glycation end products (AGEs) in tissue, especially the skin.[30] If they bind to their receptor, they can even cause further damage. Typically, in COPD, their accumulation on the skin is high, being associated with lower sRAGE (soluble form) and lower lung function. The soluble form prevents AGEs accumulation, acting as a decoy, thus being inversely associated.[30]

Oxidative stress can also be produced endogenously. It's frequently generated to help with host defence against foreign microorganisms in phagocytes. In COPD, the myeloid leukaemia cell differentiation protein (Mcl-1) is overexpressed, causing, unfortunately, a deficiency in the production of ROS.[31] The end result is a defect in bacterial killing's delayed phase and a decrease in lung pathogens removal.[31]

Cell organelles are not immune to oxidative stress; they are just as vulnerable to it, bearing in mind that the longer the exposure, the higher the probability of permanent changes to these organelles. More specifically in COPD, Endoplasmic reticulum (ER), becomes less reticulated, and there are more clusters and dotty structures. Golgi complex, as it is very vulnerable to stress, shrinks in size. Lastly, lysosomes are smaller and/or not as abundant.[32] Overall, this all hints to the fact that oxidative stress causes a defect with protein trafficking through the cell.

Mitochondria mechanisms

Mitochondria, like other organelles, are widely affected by oxidative stress causing some morphological changes like mitophagy or mitochondrial fission/fusion.[33] They are as well a major source of ROS with the help of the mitochondrial ROS modulator 1 (Romo1), a membrane protein, that is involved in the production of ROS. Its level is increased in COPD, correlating with lung function (negative association with FEV_1), inflammation and oxidative stress.[34]

In spite of being one of the major sources of ROS, mitochondria are also highly susceptible to it, especially in peripheral blood leukocytes. In these leukocytes, there is a decrease in mitochondrial DNA (mtDNA) number of copies, protection and biosynthesis, originated by a dysfunctional mitochondria that releases ROS, and is continuously being impaired by it[35], forming a vicious cycle.

Mitochondria dysfunction is also seen in airway smooth muscle cells (ASM) in COPD.[36] In these cells, excessive mitochondrial ROS causes a drop in mitochondrial membrane potential, in the ATP content, as well as in complex protein production and function.[36] This can result in lung inflammation and airway hyperresponsiveness (AHR).[36]

Mitochondrial dysfunction is, as a result, a very important factor that contributes to COPD onset.[35]

Muscle mechanisms

Myotubes and myoblasts are vulnerable to ROS and oxidative stress. It can impair skeletal muscle performance by reducing sodium/potassium pump activity, sarcoplasmic reticulum function, myosin ATPase, and cause dysfunction.[10] The augment of ROS instigates higher formation of autophagosomes, expression of autophagy markers and enhanced autophagic flux and signalling[37] leading to a decrease in myotube diameter and consequently to muscle atrophy [37, 38]. ROS can also induce atrophy through the ubiquitin/proteasome system: the FoxO1/MuRF1/atrogin-1 signalling pathway - causing, additionally, cellular damage.[39]

Regarding muscle mitochondria in COPD, mtDNA replication is defective resulting in a chronically low mtDNA copy number, which, in turn, makes myocytes extra sensitive to oxidative damage, exacerbating muscle oxidative deficit and contributing to COPD disease pathophysiology. This proves that the decrease in the fatigue resistance is part of the disease instead of it being just a consequence of the sedentary behaviour common in COPD patients.[40]

Inflammation

Cigarette smoke (CS) is an important cause of COPD that leads to lung inflammation which in turn accelerates COPD development. In small airways, increased oxidative stress, or ROS activation, derived from tabaco smoke and other endogenous sources, leads to a protease/anti-protease imbalance, that results in anti-proteases inactivation, upregulation of pro-inflammatory mediators like GM-CSF, TNF-α, and IL-1, autophagy-impairment, aggresome formation, apoptosis, and alveolar airspace expansion.[38, 41, 42] It starts by activating pattern recognition receptors (PRRs) like the Toll-like receptors (TLRs) that cause epithelial and immune cells to release chemokines and proteases (like MMP-9), which attract more inflammatory cells to the area and cause, ultimately, emphysema and elastin and tissue degradation, respectively.[38] In this cycle, usually induced by a toxic trigger, there is also IL-17A (interleukin) release, which contributes to type II alveolar cell apoptosis and neutrophil recruitment as well as IL-22 upregulation capable of tissue regeneration and protection. Both, however, increase MMP levels, such as MMP-12, which are responsible for tissue degradation[38] and worsening lung function[21].

Also involved in neutrophil activation and recruitment is sialic acid-binding immunoglobulin-type lectin-9 (Siglec-9). Its value rises in COPD, acting as a negative feedback keeping neutrophil activation to a minimum.[43] However, the extracellular region of Siglec-9, or soluble Siglec-9 (sSiglec-9), can act as a competitive ligand, enhancing neutrophil's oxidative burden and

chemotaxis to IL-8.[43] Its levels are also increased in COPD.[43] As a result, anti-inflammatory effects of Siglec-9 are disrupted or even reversed.

In the other hand, CS causes transcription factor EB (TFEB) accumulation in perinuclear aggresome-bodies, preventing it from performing its normal function as a crucial autophagy activator resulting in accumulation of misfolded or impaired proteins in aggresome-bodies.[42] This accumulation is worse with emphysema severity and COPD patient smoking history.[42]

Besides neutrophils, that can be recruited and even boost IL-17A development by IL-21 stimulation via STAT3 phosphorylation causing degranulation and ROS production, in COPD there are also prevalent macrophages and CD8 T lymphocytes. In contrast to healthy patients, macrophages in COPD divide in two phenotypes: M1 phenotype (classic pro-inflammatory, its polarization is triggered and contributes to the inflammation itself) and the M2 phenotype (alternative phenotype, linked to tissue remodelling and repair, polarization increases with disease incidence).[38] Less prevalent are CD4 cells that secrete IL-9, activating STAT3 leading to IL-8 release and enhancing inflammatory and oxidative stress and exacerbating lung injury and corticoid insensitivity.[44, 45]

COPD's systemic inflammation and oxidative stress are also exacerbated by C reactive protein (CRP) and ROS, having elevated serum levels of CRP circulating being linked to a decrease in FEV_1. [34, 46, 47] In sputum, there is, as well, positive associations between nitrosative stress markers and inflammatory mediators.[41] Other inflammatory mediators are also mentioned. For example, serotransferrin, a negative acute phase protein involved in antioxidant defence mechanisms, is found to be increased in COPD. [48] In comparison, however, vitamin D binding protein (VDBP), apolipoprotein H (ApoH) and prothrombin are increased in response to amplified inflammation and oxidative stress burden, but it is only a temporary phenomenon that quickly becomes overwhelmed by the chronic exposure of airway and lungs to oxidative stress and inflammation.[48]

Finally, although many studies have been conducted about this topic, it is still not entirely clear whether systemic inflammation is the cause or the consequence of the COPD pathology.

Genetics

- HDAC2

Histone deacetylases are responsible for manipulate gene expression by deleting acetyl groups on histones. One of the most important functions is to suppress the expression of various inflammatory genes associating negatively with viral load and nitrite levels besides airway inflammation.[41] HDAC2's levels and activity are decreased in smoker's lungs, COPD[24], and emphysema patients [49]. This drop is substantially more marked in severe COPD.[41] HDAC2 levels and activity reduction, caused mainly by cigarette smoke, is responsible for increasing DNA damage, cellular senescence and proinflammatory responses like the organization of lymphoid follicles[49]. However, that does not seem to improve host defence, increasing the severity of clinical disease after virus infections[41]. Overall, it leads to a deterioration in lung function and to airspace expansion causing in the end, emphysema, and COPD.[49] HDAC2 can also be inactivated by nitrosylation via peroxynitrite as a result of oxidative and nitrosative stress.[41]

Additionally, HDAC2 is related to the suppression of the inflammation by glucocorticoids via NF-kB. It binds to the promoter and mediates deacetylation of glucocorticoid receptor allowing it also to bind to NF-kB complex, inhibiting the pro-inflammatory pathway.[49]

- Sirtuins

Sirtuins (SIRT) are histone deacetylase enzymes that catalyse deacetylation and ribosylation of certain proteins.[50] Specifically, SIRT1 and SIRT6 are decreased via oxidative stress in COPD[46, 50, 51] through up-regulation of miR-34a expression by the PI3K pathway [50].

SIRT1 has a direct association with FEV_1/FVC and FEV_1, and total antioxidant capacity correlating, naturally, indirectly to airway obstruction and its severity.[51] One of the main mechanisms by which cigarette smoke seems to downregulate SIRT1 in COPD is via IL-6 expression.[46]

- Nrf-2

Nuclear factor erythroid-2-related factor 2 (Nrf-2) is a transcription factor that controls antioxidant-response elements (ARE) production[52] like heme oxygenase-1 (HO-1). It is generally upregulated in COPD[47, 53] by oxidative stress and inflammation usually caused by active smoking[54], and it is directly correlated with lung function (inversely associated with FEV_1)[47] This is an important determinator of Nrf-2 response: according to disease severity[47, 53].

- Oxidative nuclear damage and other transcriptional factors

Additional transcription factors can be affected in COPD. While Forkhead box C1 (Foxc1) is stimulated by cigarette smoke, has an antioxidant, anti-inflammatory, and anti-apoptotic role

and correlates with COPD progression[55], Peroxisome proliferator-activated receptor γ (PPARγ), for example, is downregulated by CS, causing a decrease in antioxidants activity (like GPx3) provoking further inflammation and oxidative stress[15].

Oxidative stress can also directly affect the DNA. With $PM_{2.5}$ exposure causing, reflexively, ROS formation, it leads quickly to DNA strand breaks[25]. Higher oxidative stress, usually seen in worse stages of COPD, is, as well, involved in DNA methylation reducing [56].

- Genotypes, functional variants, and polymorphisms

Some genotypes have a protective role when it comes to oxidative burden hence COPD. For example, the I/I genotype of the angiotensin-converting enzyme (ACE) gene is known to produce a lesser amount of ROS than the other genotypes of the enzyme[57]. In a similar manner the -21TT genotype of SOD1, causes an increase of catalase activity, helping shield against oxidative load. [58]

In the other hand, genotypes like AA genotype of NFE2L2 (rs35652124)[52] and MMP1 GG/GG genotype[59] are associated with COPD incidence, whereas the MMP12 A>G variant is linked with early disease onset.[59] The AA genotype of NFE2L2 is also associated with a decrease in FEV_1.[52] Another risk factor for COPD is the PTEN polymorphism[19].

Concerning functional enzyme variations, there are a few that are worth mentioning. Beginning with GSTM1 and GSTT1 (members of the Glutathione S-transferase GST family), the null variants, provoke a total lack of enzymes activity, causing, in turn, an increased risk for COPD development[59, 60]. The microsomal epoxide hydrolase (mEH), however, depending on the degree of activity it may correspond to a higher risk for lung function deterioration: low activity does not signify a risk [60], but intermediate and high activity do. Lastly, in COPD, the functional variation of SOD1 +35A/C is linked to disease severity.[58]

Pathophysiology

Oxidative stress, along with protease-antiprotease imbalance are the main exacerbating elements in COPD pathogenesis[61]. Yet, oxidative stress levels, seems to be correlated with cigarette smoking rather than COPD itself[62].

In addition, oxidation products like AOPPs (advanced oxidation protein products) are also very important, correlating directly with Metabolic syndrome prevalence in COPD, with COPD severity and with worse pulmonary function[63]. Metabolic syndrome refers to a set of risk factors for

cardiovascular disease, and other health problems like diabetes and obesity (insulin resistance, dyslipidaemia, central obesity, hypertension, and impaired glucose tolerance).[63]

Lastly, acute exacerbations are found to be more common in environments with higher ozone oxidant capacity and $PM_{2.5}$[64], each aggravation leading to loss of lung function[41] in COPD.

Biomarkers

- Malondialdehyde

Malondialdehyde (MDA) is a lipid peroxidation product that serves as an indicator of free radical activity in the body. [65] It reacts with free amino groups (-NH_2) in proteins, phospholipids, and nucleic acids, causing structural changes in the immune system as well as changes in the inflammatory cascade.[66] MDA blood levels are significantly higher in COPD patients [21, 28, 29, 55, 65-69] (especially in males) [65] correlating negatively with disease severity (implying a more severe redox imbalance) [67], lung function (FEV_1 and FVC) [47, 65] and with antioxidant levels like catalase, SOD and copper [65]. It appears to correlate positively with number of pack years and increasing age[65]. In acute exacerbations (AECOPD), sputum MDA levels appear to be increased, diminishing upon treatment of exacerbation.[70] These findings show that increased levels of lipid peroxidation, and consequently oxidative stress, play a role in COPD aetiology making MDA a good biomarker to measure oxidative stress in these patients..

- Superoxide dismutase and Catalase

SODs are metalloenzymes that catalyse superoxide anion dismutation to molecule oxygen and hydrogen peroxide, whereas catalase is implicated in H_2O_2 conversion to molecule oxygen and water [70] making part of the antioxidant enzymes. SOD and catalase blood levels are diminished in COPD patients[29, 55, 65, 69], more so in females[65] depleting drastically with the progression of the disease.[65, 69] Catalase levels also correlated negatively with iron and positively with SOD and copper.[65] Curiously, during exacerbations there was no alteration in SOD concentration and serum catalase, however, there was a boost in both enzymes sputum concentration [70]. That may be due to the fact that the response during AECOPD is to combat the damaging effects of oxidants exerting a defensive mechanism. It also shows that, to monitor acute exacerbations, blood may not be the best sample for measuring antioxidant activity that is existing locally in the airways, sputum is.[70] However in erythrocytes, SOD is increased[71].

- 8-Hydroxy-2'-deoxyguanosine

8-Hydroxy-2'-deoxyguanosine (8-OHdG), continuously produced in living cells, is an end-product of oxidative DNA damage [72] being therefore a biomarker of DNA oxidation. 8-OHdG levels were higher among COPD patients[41, 73]. Particulate matter radioactivity (gamma) was associated positively to 8-OHdG with higher effects for indoor exposures rather than ambient [74], this proposes that ambient exposures are infiltrating the homes of COPD patients and that even though most COPD patients spend most of their time indoors, staying indoors is, apparently not protective of ambient PM gamma activities effects.[74] When it comes to acute exacerbations, plasma levels of 8-OHdG are elevated in AECOPD patients (compared with stable disease) and even more so in patients with smoking history [72]. 8-OHdG levels are positively associated with symptoms severity and lung function in AECOPD patients[72], implying that oxidative stress hastened lung function deterioration. Overall, using 8-OHdG to diagnose, monitor and guide treatment in acute exacerbations is an asset.

Besides 8-OHdG, there are other products of oxidation of nucleic acids, for example, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo), the primary oxidation product of guanosine (Guo) and a recognized marker of RNA oxidation [75] being another product of oxidation of guanosine, 8-oxo-7,8-dihydroguanosine (8-oxoGuo). They can be both excreted through urine. In critical patients, who need mechanical ventilation (MV), the oxidative burden rises, increasing urine concentrations of these products,[60, 75] being 8-oxoGuo alone the best and most sensitive biomarker of oxidative stress capable of discriminating from patients and healthy subjects.[75]

- Sulfhydryl groups of proteins

In peripheral circulation, albumin is the primary source of protein sulfhydryl/thiol groups.[67] The -SH (sulfhydryl group) radical is oxidized in the presence of OS being also another antioxidant biomarker [63]. SH values are independently associated with mild COPD being an early indication of oxidative stress by reduction in their concentration.[67, 69, 76, 77] They are also a protective factor for COPD severity, reducing the prevalence of COPD advanced stages.[63]

GSH, an important antioxidant against free radicals, is decreased in COPD patients[15, 28, 33, 53, 68, 69, 71] as well as GPx and total antioxidant status [15, 62, 69, 71, 78]. They are also inversely proportional to the COPD severity.[47, 53, 69]

- Others

There are other substances that have been researched that could be used as biomarkers in COPD. For example, Iron (a marker of oxidative stress) is expectantly higher in COPD patients

(especially males) contrary to copper who acts like an antioxidant enzyme correlating inversely with MDA and iron levels[65]. Iron levels, on the other hand, are correlated positively with COPD severity.[65] Selenium levels in plasma, per se, are also lower in COPD patients [71]. Others like Serum Romo1 are significantly increased in COPD patients and associated with lung function, inflammation, and oxidative stress. Serum Romo1 can be used as a biomarker of disease progression and cardiac comorbidity in COPD patients[34]. Further oxidative stress parameters such as oxidized low-density lipoprotein (ox-LDL), total oxidant status, systemic oxidative stress index (TOS/TEAC ratio)[51] and ischemia-modified albumin (IMA), an indicator for hypoxia and oxidative stress, are higher in COPD.[71]

Other molecules that interfere in different pulmonary functions and immunity were also investigated. One of them is oxidized alpha-1-antitrypsin (OxyA1AT). Its main difference compared to the non-oxidized A1AT, is the inability to defend lungs against neutrophil elastase[79]. Its level is increased in COPD, mainly due to cigarette smoke, relating to a poor prognosis for COPD.[79] Regarding inflammation and immune responses, neopterin (NP), a risk factor for acute respiratory infections and a measure of oxidative stress, is raised in COPD, with its level rising quickly according to immunological stimulus[68]. Similarly, ceruloplasmin, a globulin that catalyses redox responses, is increased in COPD directly linking to systemic inflammation[48, 67].

As regards to the best indicators to predict COPD and distinguish COPD patients from healthy individuals, a few names come up. Continuing with ceruloplasmin, if it is integrated with albumin and thiol levels (which are usually decreased in COPD) in a multivariate model, it can diagnose correctly around 94% of COPD patients[67]. Serotonin (5-HT), however, can also help in discriminating patients from healthy subjects. Since it is a vital neurotransmitter that affects several pulmonary functions and its levels is augmented in COPD, it is no surprise that it is inversely correlated with FEV_1 and FVC[80]. Although it may not have better discriminating capacity than the multivariate model, it can still be a predictive marker of the onset of COPD[80]. It may also be possible to integrate serotonin in the referred model, causing it possibly to improve the diagnostic accuracy.

Lastly, it is important to refer the ultrafine particles (UFP) and its relation to COPD. These particles are even smaller than $PM_{2.5}$, penetrating deeper and therefore, being even more toxic.[73] Since they can seep into the blood stream easier, their concentration in the exhaled breath condensate (EBC) in COPD is much smaller compared to healthy subjects, increasing in blood samples[73]. Their concentration in EBC is also inversely related with number of pack years, peripheral eosinophil counts and with markers of systemic inflammation (PCR and LDH)[73]. The

decrease in the value of UFP EBC in conjunction with CRP levels are predictors of exacerbations: If UFP value is ≤0.18 E8/mL (cut-off value) and CRP ≥5 mg/L, the COPD patient has more exacerbations.[73]

Conclusion

Chronic obstructive pulmonary disease is associated with an increase in oxidative stress. This is due, mainly, by environmental triggers and toxics like cigarette smoke and $PM_{2.5}$, among others. They activate various mechanisms and transcription factors increasing the NF-κB pro-inflammatory pathway and diminishing HDAC2's levels and activity. In the end, it leads to an escalation in inflammation and nuclear and cellular damage, forming a vicious cycle where oxidative stress continuous on increasing, worsening lung function and thus, gradually augmenting the grade and severity of COPD.

Additionally, besides the increase in oxidative stress and its markers, like MDA, there is a tendency for the reduction of the antioxidant levels such as SOD, catalase and -SH group proteins. This tends to differ, however, in exacerbations, where, since it is an acute event, there is an attempt to counterbalance and fight the inflammation and oxidation, increasing the antioxidant levels when compared to the stable disease.

One final note just to consider that the pathogenesis of COPD may differ slightly according to sex. The increase of oxidant levels is more marked in males, while in females the decrease in the antioxidant levels was the main cause for oxidative stress.

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