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Review Article

New frontiers in the treatment of comorbid cardiovascular disease in chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a disease characterised by persistent air-flow limitation that is not fully reversible and is currently the fourth leading cause of death globally. It is now well established that cardiovascular-related comorbidities contribute to morbidity and mortality in COPD, with approximately 50% of deaths in COPD patients attributed to a cardiovascular event (e.g. myocardial infarction). Cardiovascular disease (CVD) and COPD share various risk factors including hypertension, sedentarism, smoking and poor diet but the underlying mechanisms have not been fully established. However, there is emerging and compelling experimental and clinical evidence to show that increased oxidative stress causes pulmonary inflammation and that the spill over of pro-inflammatory mediators from the lungs into the systemic circulation drives a persistent systemic inflammatory response that alters blood vessel structure, through vascular remodelling and arterial stiffness resulting in atherosclerosis. In addition, regulation of endothelial-derived vasoactive substances (e.g. nitric oxide (NO)), which control blood vessel tone are altered by oxidative damage of vascular endothelial cells, thus promoting vascular dysfunction, a key driver of CVD. In this review, the detrimental role of oxidative stress in COPD and comorbid CVD are discussed and we propose that targeting oxidant-dependent mechanisms represents a novel strategy in the treatment of COPD-associated CVD.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is an incurable disease that is characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of lungs to noxious particles and gases [1,2]. COPD is currently the fourth largest cause of mortality globally accounting for over 3 million deaths annually [3]. Moreover, the global financial burden of COPD is tremendous costing € 82 billion annually [2,4–7]. Importantly, much of the disease burden and healthcare utilisation in COPD are associated with the management of its comorbidities, defined as other chronic medical conditions, and infective viral and bacterial acute exacerbations of COPD (AECOPD) [8]. The most common comorbidities of COPD include cardiovascular disease (CVD), skeletal muscle wasting and stroke [4,9–13]. This review outlines the pathophysiology of COPD, the mechanisms underlying the onset and progression of CVD in COPD, the detrimental role of both endogenous and exogenous reactive oxygen species (ROS) and persistent inflammation on comorbid CVD, and current and future COPD treatments and their effectiveness in comorbid CVD. We also propose that targeting oxidant-dependent mechanisms represents a novel strategy in the treatment of COPD-associated CVD.

The persistent respiratory symptoms associated with COPD are caused by abnormalities to the airway and alveoli, which in most cases are caused by repeated exposure to noxious gases and particles.

Cigarette smoke (CS) is the largest cause of COPD, accounting for 95% of all cases in industrialised countries [1,5,14], although environmental pollutants also contribute to the development of this disease. The structural damage to the small airways, large airways and lung parenchyma (emphysema) associated with COPD are caused by chronic pulmonary inflammation, which has adverse effects on the elastic recoil of the lungs during expiration and overall lung function [15,16].

There are three key characteristic symptoms of COPD; these include dyspnea, chronic cough and an increase in sputum production. Progressive dyspnea is the most prominent symptom of COPD, which causes the patients need to increase their effort to breathe, while increasing chest heaviness thus leading to these patients gasping for air [11,13]. The development of chronic cough is generally the first symptom seen in developing COPD, and in some cases, can cause significant limitations to airflow. When a patient has these symptoms in conjunction with increased levels of sputum, COPD is debilitating, and these adverse effects substantially impact the quality of life of these individuals, particularly during periods of exacerbation.

AECOPD

Patients with COPD are susceptible to AECOPD, defined as 'a sustained worsening of patient's condition from the stable state and beyond normal day-to-day variations that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD' [17]. Exacerbations are a common occurrence in COPD patients and contribute mainly to morbidity, mortality and reduced health. Patients with COPD experience on an average between 1 and 3 exacerbations per year, with an inpatient mortality rate of up to 30% [18–20]. Acute exacerbations of COPD are due to a number of aetiological factors, predominantly viral and bacterial infections with 40–60% attributed to viral infections [17].The majority of these infections are due to respiratory syncytial virus (RSV) (22%), influenza A (25%) and rhinovirus (36%) [17]. Even though RSV is the most commonly isolated virus in exacerbations, influenza has the potential to be more problematic due to the likelihood of epidemics and pandemics. It has recently been shown that exacerbations may also be induced or worsened by comorbidities such as other lung diseases (e.g. pneumothorax, pulmonary emboli), CVD or systemic inflammatory responses [21]. Interestingly, changes in airway microbiota may induce exacerbations of COPD rather than single microbes as shown by Charlson et al. [22] who demonstrated that CS changes the upper respiratory tract microbiome causing impaired mucocillary clearance, inducing constant immune activation and targeting of commensal bacteria driving pulmonary inflammation and an altered bacterial composition of the lung in severe patients with COPD. It has recently been shown that the airway microbiota of COPD patients is similar to that found in asthmatic patients, as they showed high levels of Proteobacteria, as well as there being a high prevalence of Firmicutes, particularly *Lactobacillus* spp. [23–25]. It is infection with either *Streptococcus pneumoniae* and Influenza virus that drives AECOPD, leading to the overexuberant pulmonary inflammatory response during exacerbation periods driving reduced expiratory flow and increased dyspnea and associated COPD symptoms.

Mild cases of AECOPD can be reversed with the use of medications such as antibiotics, bronchodilators and corticosteroids, whereas in more severe cases there is a significant increase in the likelihood of mortality and respiratory failure despite treatment with these medications [11]. Bacterial infections are involved in AECOPD, and this is usually due to reduced antibacterial defence mechanisms and impaired lung function [26]. During exacerbations there are increases in both acute phase proteins and inflammatory cell numbers, driving macrophage and neutrophil infiltration, that promotes inflammation within the lung [27]. It has been found that during AECOPD, there can be a 'spill over' of these pro-inflammatory proteins and cytokines from the lungs into the systemic circulation, causing persistent non-resolving low-grade systemic inflammation and an increase in inflammatory biomarkers within the blood [28,29]. The concentration of numerous inflammatory biomarkers has been shown to be useful in both predicting future exacerbations and their severity [2,5,30,31].

Recent studies have shown that elevated serum concentrations of C-reactive protein (CRP), fibrinogen and increased leucocyte cell numbers have been associated with an increase in the likelihood of frequent exacerbations in patients with stable COPD [5,27]. Thomsen et al. [5] showed that an overall increase in the blood serum concentration of at least two of these biomarkers was evident in 95% of the cases prior/during exacerbation periods. The study concluded that there was simultaneous elevation in the concentration of CRP and fibrinogen as well as leucocyte number prior to exacerbations in patients with either mild or moderate COPD [5]. Pro-inflammatory cytokine expression was also analysed and patients that had higher serum concentrations of cytokines such as interleukin (IL)-6 and tumour necrosis factor (TNF)-α were more likely to develop further exacerbations. The nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) pathway is also activated in alveolar macrophages during exacerbations further promoting

a pro-inflammatory environment [4]. Prolonged airway inflammation and increased oxidative stress are associated with enhanced exacerbation severity.

Neutrophilic and eosinophilic inflammation in COPD

Persistent pulmonary inflammation has been shown to involve innate inflammatory mediators such as neutrophils and eosinophils as well as cytokines, chemokines and cellular proteases [16,32,33]. The role of both neutrophilic and eosinophilic inflammation is currently under investigation, with a clinical trial showing that COPD patients with a history of exacerbations showed an overall elevated blood eosinophil count and increased inflammatory burden, with evidence supporting that eosinophilic infiltration is increased in both the blood and lungs of patients with COPD [34]. CS stimulates the production of granulocytes, driving neutrophilic infiltration into the lung in response to neutrophil chemotactic factors such as C–X–C Motif Chemokine Ligand 5. It was also shown that an increase in eosinophilic infiltration and inflammation drives the recruitment of neutrophils and other associated inflammatory cells into the lung, enhancing this further [16]. It is well characterised that the number of activated neutrophils is significantly increased in the bronchoalveolar lavage fluid (BALF) and sputum of patients with established COPD and is directly correlated to disease severity. These neutrophils are activated by sputum supernatant and granule proteins, which are up-regulated in the lungs of COPD patients. Activated neutrophils secrete proteases such as MMP-9, that drive alveolar destruction. During AECOPD neutrophilic inflammation is further enhanced, therefore promoting excessive degradation of the lung parenchyma [16].

A study by Sapey et al. [35] aimed to determine if increased neutrophils in the lungs of COPD patients was due to an increase in inflammatory signalling and cell migration into the lung, or whether this pathological accumulation was due to inaccurate neutrophilic chemotaxis. This study consisted of patients with mild to severe COPD, with age- and sex-matched healthy volunteers as well as matched patients with α-1-antitrypsin deficiency, which is phenotypically like COPD and causes neutrophilic infiltration and airflow obstruction in the lung. The results of this study showed that the neutrophils extracted from COPD patients migrated faster than both control groups, although showed significantly impaired migration accuracy, with no differences in cell surface receptor expression, therefore this response is believed to be due to cell signalling differences, rather than chemoattractant expression alteration.

Neutrophilic inflammation is a key promoter of lung tissue damage through the release of detrimental proteases. A direct correlation has been established between airway neutrophilia and a decline in overall lung function in both smokers and patients with COPD [36,37]. Exposure to pollutants such as CS induces cytoskeletal damage and rearrangement of the lung directly and it is the ineffective clearance and enhanced influx of these neutrophils that promote the lung damage and dysregulated inflammation in COPD patients. We recently showed that glucocorticosteroids were ineffective in the regulation of neutrophil elastase and matrix-metalloproteinase-9 balance in severe COPD [38]. The effectiveness of corticosteroids in modulating lung neutrophilia is currently under debate and these drugs have shown more promising effects in the treatment of pulmonary eosinophilic inflammation.

The role of eosinophilic inflammation in COPD is currently under investigation. Similar to that seen in neutrophilic-derived pulmonary inflammation, eosinophil counts are also significantly elevated in the lungs, airways and BAL fluid of patients with established COPD and furthermore during an AECOPD; conversely other studies have shown that there is no significant increase in eosinophilic infiltration in bronchial biopsies [16,39]. The underlying mechanisms that promote this eosinophil migration in COPD are under investigation although eosinophilic inflammation is believed to be due to increased IL-33 expression, which has been shown in the basal epithelium of the lung in patients with asthma and COPD. This up-regulated IL-33 is believed to promote IL-13 expression, a mediator of allergic inflammation, causing cell injury [16,40]. With eosinophilic inflammation generally being due to an allergic sensitisation and subsequent airway inflammation in asthmatic patients, it is vital that it is understood; eosinophilic inflammation can occur independently of asthma or allergic responses, for example, COPD is associated with T helper 1-mediated immunity, driving a neutrophilic response, although up to 40% of COPD patients experience eosinophilic-derived inflammation [40]. Eosinophilic inflammatory responses should therefore also be used in the assessment of inflammation severity in COPD and asthma due to clinical overlap of these diseases, particularly when administering medications such as corticosteroids, which have been shown to be ineffective in suppressing inflammation in COPD [41]. The exaggerated immune cell infiltration and activation within the lungs of patients with COPD has been associated with inflammation-induced reductions in lung function measured by forced expiratory volume in 1 s (FEV-1), which has been linked to hypoxia and its associated CVD [10,36,42,43].

Although eosinophilic inflammation has been long associated with asthma, it has recently emerged as a diagnostic and therapeutic strategy in COPD. Studies have shown that a subset of COPD patients with eosinophilic airway inflammation exists and that these patients exhibit the greatest response to corticosteroid treatment [44–47]. Whether

blood eosinophils can be used as a surrogate marker for airway eosinophils to direct corticosteroid therapy for the treatment of COPD and its exacerbations has recently been explored. Studies have shown that blood eosinophil counts can predict the effect of inhaled corticosteroids (ICS) in preventing future exacerbations as there was a strong correlation between these treatments having significant effects on patients with high eosinophil counts (>300 cells/μl), although displaying little to no effect in those with lower eosinophil counts (defined as having fewer blood eosinophils than 100 cells/μl) [20,48]. However, various cohort studies have yielded opposing results using blood eosinophil counts as predictors of future exacerbations, with studies showing that enhanced eosinophil numbers are either indicative of AECOPD or not related [49,50]. It is believed that these opposing findings may be a result of differences in previous corticosteroid use and exacerbations. Taken together, the use of blood eosinophil counts as a marker of airway eosinophilia to direct treatment with corticosteroids has been shown to be beneficial in the clinical recovery of COPD, particularly during acute exacerbations, where this eosinophilic inflammation is enhanced further [51]. The mechanism for an increased effect of ICS in COPD patients with higher blood eosinophil counts remains unknown.

The detrimental role of oxidative stress in COPD

Oxidative stress can be defined as an 'imbalance between both oxidant and antioxidant levels in favour of a pro-oxidant environment in cells and tissues' [52,53]. Oxidants are molecules that promote oxidation whereas antioxidants can either inhibit oxidation directly or prevent oxidant formation. Oxidative stress ascends from both a heightened production of ROS along with the failure of antioxidant mechanisms to neutralise these ROS. This resultant oxidative environment causes chronic oxidative damage/modification of DNA, lipids or proteins [54]. Recent studies have shown that oxidative stress plays a key role in the development and progression of COPD because of the increased oxidant exposure in CS with each puff of CS containing more than 10^{14} free radicals that promote the endogenous production of ROS [55]. ROS can perpetuate the inflammatory and immune responses in the lung following exposure to noxious gases or particles, like those found in CS.

NADPH oxidase (NOX), is the primary ROS-generating enzyme that can be found in phagocytic (e.g. macrophages, neutrophils), non-phagocytic (e.g. epithelial cells) and skeletal muscle cells [55–58]. There are seven key NOX isoforms in humans; these are NOX-1, NOX-2, NOX-3, NOX-4 and NOX-5, as well as Dual oxidases (DUOX) DUOX-1 and DUOX-2 [59]. Both macrophages and neutrophils produce ROS in response to CS because of increased NOX enzyme activity (in particular NOX-2). Studies have shown that oxidative stress generated through NOXs, has been associated with obstructive lung disorders such as asthma and emphysema [4,52,60]. Activation of the NOX-2 enzyme by CS leads to the formation of the superoxide radical ($O_2^{\bullet-}$) that can either react with nitric oxide (NO) to create harmful peroxynitrite ($ONOO^-$) or undergo modification through superoxide dismutase (SOD) which converts this $O_2^{\bullet-}$ into harmful hydrogen peroxide (H_2O_2). This hydrogen peroxide in the presence of ferrous iron (Fe^{2+}) can be converted into hydroxyl radicals ($\bullet OH$) through the Fenton reaction which sees the oxidation of Fe^{2+} to Fe^{3+}. Both glutathione peroxidases (Gpx) and catalase (CAT) enzymes are responsible for reducing the oxidative load by converting hydrogen peroxide into oxygen and water, and thus causing a reduction in circulating ROS concentration [14,54,55] (Figure 1).

Recent studies have shown that there is a decrease in circulating antioxidant levels in smokers and COPD patients, which may account for the increased circulating levels of ROS. It was also discovered that CS exposure promotes the conjugation of glutathione within the epithelium of the lung, reducing resident antioxidant levels and thus allowing for an increase in pro-inflammatory responses [61–63]. The antioxidant enzyme Gpx-1 has a key regulatory role in the inflammatory response within the lungs. The key function of Gpx-1 is to convert harmful H_2O_2 into harmless water and oxygen, maintaining oxidative balance within the lung. In smokers there is an increase in both localised pulmonary Gpx-1 concentration and its associated gene expression; conversely in patients with COPD there is an impairment in these protective mechanisms and depletion of Gpx-1, hence the overstated inflammatory response and oxidative burden in COPD patients [64,65].

Gpx-1 is expressed ubiquitously in the body and can be found in alveolar macrophages, alveolar epithelial lining fluid and the lung epithelium [65,66]. Recent studies have shown that drugs which mimic the actions of Gpx-1 (e.g. ebselen) in CS-induced lung inflammation may be of therapeutic potential in COPD patients [64,65]. Exhaled breath condensate studies have found increased levels of H_2O_2 in COPD patients which are further increased during an exacerbation [67,68], indicating that a reduction in Gpx-1 activity is evident during these periods leading to a subsequent increase in the inflammatory response and oxidative burden. Mouse models have shown that Gpx-1 knockout (KO) mice exposed to CS have a significant increase in the number of BALF macrophages and neutrophils driven by increased expression of macrophage and neutrophil chemotactic factors (e.g. IL-17A and MIP-1a) indicating that Gpx-1 protects against CS-induced lung inflammation [64].
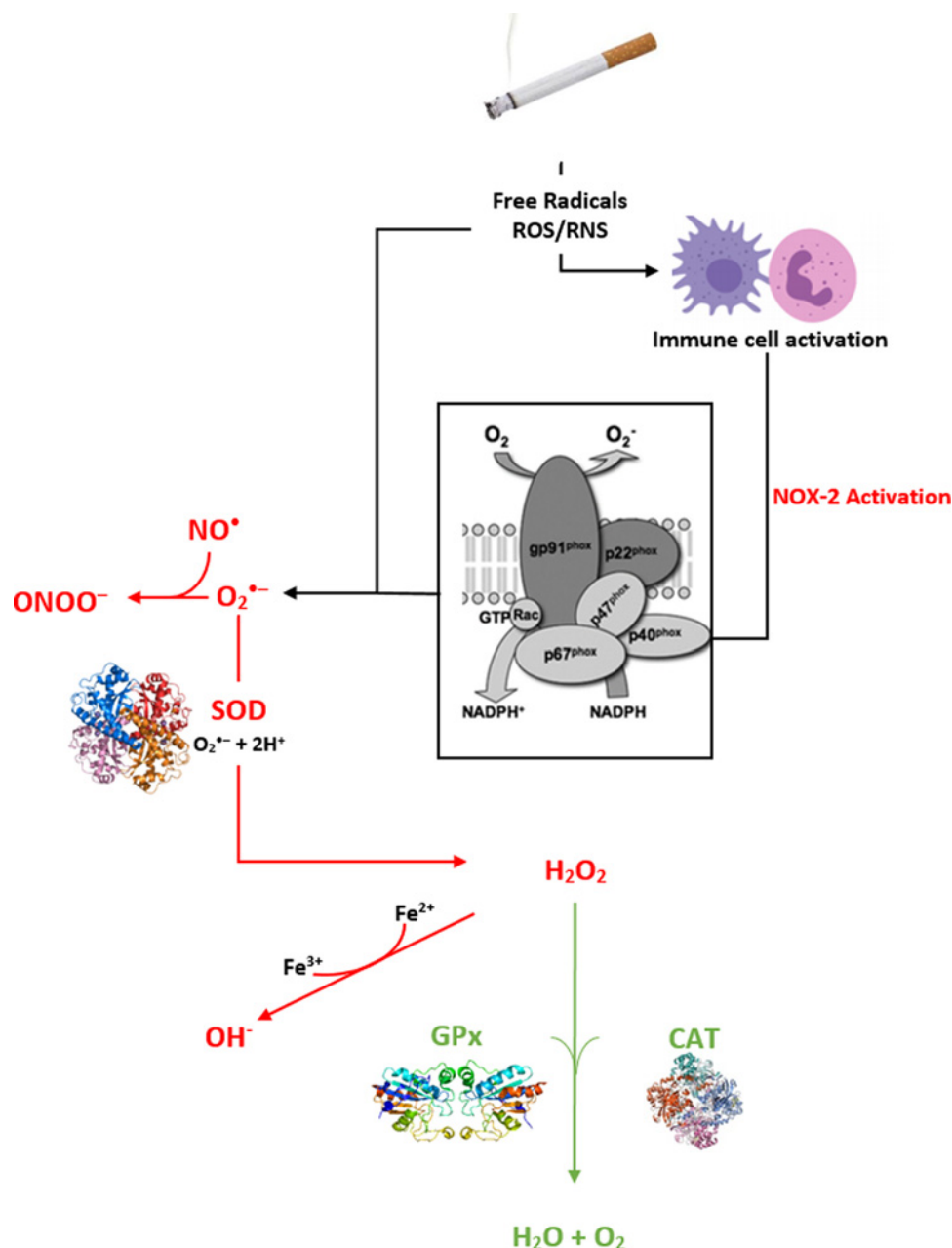
Figure 1. Formation of ROS and RNS in response to CS

CS exposure activates immune cells, which generate superoxide radical ($O_2^{\bullet-}$), through activation of NOX-2, which either reacts with NO to form harmful peroxynitrite ($ONOO^-$) or be converted into harmful hydrogen peroxide (H_2O_2) under the influence of SOD, which in the presence of ferrous iron (Fe^{2+}) is converted into hydroxyl radicals ($\bullet OH$) via the Fenton reaction. Both GPx and CAT convert H_2O_2 into H_2O and O_2, reducing circulating ROS. Abbreviation: RNS, reactive nitrogen species.

As mentioned, oxidative stress has numerous adverse effects, such as directly causing damage to the lung tissue through modification of DNA, lipids or proteins as well as initiating cellular responses that can drive the inflammatory response within the lung, leading to lung tissue degradation (emphysema). Activation of pro-inflammatory signalling pathways such as the NFκB pathway by ROS, perpetuates the lung inflammatory response by inducing pro-inflammatory mediator gene expression, driving direct lung tissue damage and worsening the effects of COPD patients [60,69–71]. The production of pro-inflammatory chemotactic factors also causes T cells, neutrophils and
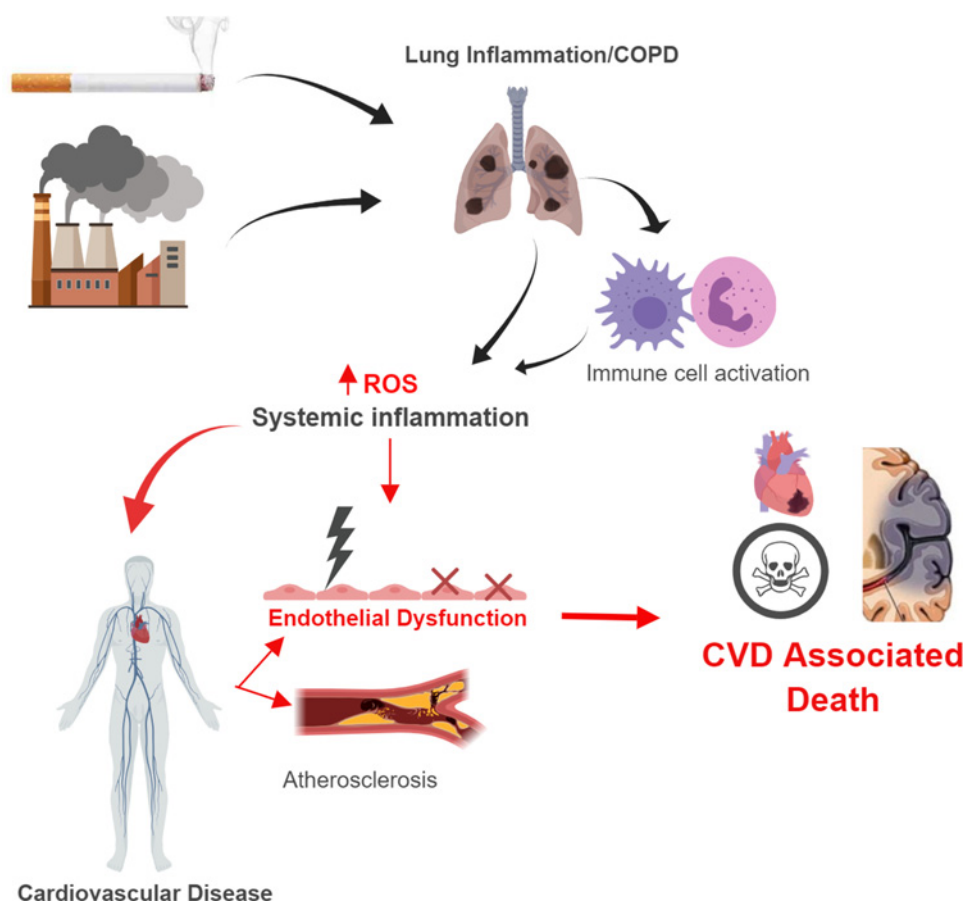
Figure 2. Schematic of lung inflammation and pathogenesis of COPD and comorbid CVD
Exposure to CS and air pollution activates immune cells (e.g. macrophages, neutrophils) which drives ROS production and systemic inflammation which promote CVD onset and progression ultimately leading to CVD-associated death.

macrophages to be recruited to the lung where they further contribute to an increase in oxidative stress, inflammation and ROS production, as well as causing emphysema, fibrosis of small airways and remodelling of airway walls, all of which impair lung function [52,60,62].

CVD: onset and progression in COPD

CVD is the leading cause of morbidity and mortality in patients with existing COPD, as it has been linked to various comorbidities such as myocardial infarction, angina, arrhythmias and stroke, [72,73]. Approximately 30–50% of deaths in COPD patients are attributed to CVD [74–76] especially congestive heart failure, arrhythmia and acute myocardial infarction [10]. CVD also accounts for 42% of first hospitalisations and 44% of second hospitalisations in COPD [77]. A study of patients with COPD admitted to hospital with acute respiratory failure found that arrhythmias were associated with 70% in-hospital mortality and no survival at 2.4 years [78]. In another study, hospital mortality was 31% in patients with severe COPD and arrhythmia, compared with 8% in patients without arrhythmias [79]. It has also been shown that there is increased arterial stiffness seen in these patients, indicating that there may be a link between the reduced FEV-1 and increased mortality due to COPD-induced cardiovascular complications [80]. CVD and COPD share various risk factors including hypertension, sedentarism, smoking and poor diet [29,54,55]. These risk factors contribute to the development and progression of comorbid CVD and recent studies have concluded that oxidative stress triggered by persistent low-grade lung and systemic inflammation has been associated with comorbid CVD seen in COPD patients [80,81] (Figure 2).

It is understood that the increased arterial stiffness associated with CVD is due to the dysfunction of endothelial cells and abnormal arterial walls, which could be a consequence of prolonged low-grade systemic inflammation [82].

The combined effects of systemic inflammation and immune cell activation contributes to the thickening of arterial walls as well as increasing the likelihood of atherosclerotic plaque or lesion formation, both of which can lead to myocardial infarction or stroke. It has been well characterised that under states of prolonged stress and inflammation, atherosclerotic plaques grow and rupture, causing sudden luminal thrombosis and tissue injury increasing the likelihood of clot formation within the vasculature or vessel occlusion, inducing an ischaemic stroke [83–87]. CS exposure and COPD, particularly during an AECOPD enhance this pathway by inducing thrombosis, through an overexuberant secretion of procoagulant factors and systemic inflammation [88,89]. A study by Lahousse et al. [90] used high-resolution MRI to analyse human atherosclerotic plaque structure in detail, providing valuable information into size, plaque core lipid content, signs of plaque haemorrhage and fibrous cap thickness. The findings of this study showed that patients with severe COPD were at a heightened risk of having a vulnerable atherosclerotic plaque, defined as a plaque with a high lipid content, than those without COPD. This study then went on to analyse mild to moderate COPD, with similar results, indicating that plaque instability occurs early in the pathogenesis of COPD [90]. The underlying mechanism has not yet been completely established, although animal models of COPD have been used to show that pulmonary inflammation in COPD promotes inflammation and causes inflammatory cell recruitment into atherosclerotic plaques [91]. This then drives lipid infiltration and plaque cell turnover, therefore resulting in larger, less stable atherosclerotic plaques that are prone to rupture [91]. The development of atherosclerosis because of COPD will be discussed later in this review.

Inflammatory status has also been directly correlated with endothelial dysfunction, a major causative factor of CVD. A recent study showed that if inflammatory mediators (e.g. IL-6) are removed, vascular endothelium integrity can be restored, ultimately reducing the effects of CVD [30,92]. Increased oxidative stress and inflammation as seen in COPD, have adverse effects on stroke outcomes as these factors cause the alteration and structural remodelling of cerebral vessels and promote blood–brain barrier disruption [86,93,94]. This pro-inflammatory state further increases ROS production via NOX-NADPH oxidase activity, causing inflammation of the vessel wall through nuclear factor κ-light-chain-enhancer of activated B cell (NFκB) signalling [94]. Therefore, increased oxidative burden and inflammation are key causative factors of comorbid CVD in COPD patients, therefore modulation of these crucial oxidative stress and inflammatory pathways may be a potential therapeutic target.

Airway remodelling and pulmonary hypertension: promoters of CVD

The persistent chronic pulmonary inflammation observed in COPD has been shown to alter the healing process of damaged lung tissue, and thus can lead to airway remodeling. The extracellular matrix (ECM) becomes degraded upon injury to the lung tissue. Abnormal deposition of ECM proteins has been observed in the lung healing processes of patients with COPD, therefore contributing to structural alterations to the airway including increased airway stiffness and obstruction [15]. Airway remodeling can be attributed to the imbalance between cellular proteases and their associated inhibitors [15]. Pulmonary inflammation in COPD has been related to increased production of the enzyme elastase that subsequently promotes the degradation of elastic fibres and enhances fibroblast migration within the ECM.

Emphysema is a key pathological feature of COPD where the lung tissue involved in gas exchange is destroyed. Under normal circumstances bronchioles dilate allowing air to leave the lungs during expiration, although when damaged, the bronchioles collapse and prevent the complete expiration of air from the alveoli [95]. Emphysema causes the lung to lose its elastic recoil, impacting both expiration and the effectiveness of gas exchange. Reduced expiration of air from the alveoli causes an over-inflation of alveoli, which results in a reduction in breathing rate and shortness of breath due to there being little gas exchange at the alveolar level, resulting in persistent hypoxia, a key driver of pulmonary hypertension.

COPD is the largest causative factor of pulmonary hypertension, a major contributing factor of myocardial infarction and heart failure in patients with this disease [96,97]. COPD drives pulmonary hypertension by inducing hypoxic vasoconstriction, systemic inflammation, endothelial dysfunction and polycythaemia as well as the persistent lung inflammation and impaired lung function, all of which promote remodelling of the pulmonary arterioles, causing them to narrow, promoting subsequent increases in pulmonary blood pressure (BP), as well as worsening hypoxia [98]. Increased pulmonary BP often leads to enlargement of the right side of the heart, otherwise known as cor pulmonale, causing ventricular failure due to excess strain put on the heart muscle. This persistent pulmonary inflammatory response, is driven by the increase in ROS and reactive nitrogen species (RNS) in COPD patients, thus pulmonary artery stiffness/cor pulmonale and other associated cardiovascular manifestations such as pulmonary hypertension being often associated with respiratory diseases such as COPD [99–101].

Patients with COPD who are subject to an acute myocardial infarction have ∼20% lower survival rate than normal healthy patients. This is due to these patients not being able to tolerate cardiac injury as well as others, although this is multifactorial, studies have shown that it is largely due to impaired lung function and inflammation-induced arterial remodelling [55,102,103]. Structural remodelling of vasculature has been observed in patients with severe COPD; a recent study by Santos et al. [104] showed that pulmonary hypertension has been commonly associated with COPD. Post-mortem studies have shown severe changes in the vasculature of the lung. It was shown that the ECM proteins elastin and collagen as well as smooth muscle cell proliferation, caused the thickening of pulmonary artery intimas in both smokers and patients with mild to moderate COPD, suggesting that these changes to pulmonary vasculature occur at the early stages of CS-induced respiratory damage/disease [104–106]. This further reinforces that pulmonary vascular remodeling may be a causative factor of CVD onset in patients suffering from severe lung diseases, due to the constant hypoxia and spill over of these inflammatory cytokines from the lung into the systemic circulation.

It has been shown in several studies that a reduction in lung function, particularly in COPD patients, significantly increases the likelihood of the development of both non-fatal ischaemic heart disease and CVD such as heart failure and hypertension [107–109]. Further studies have also shown a relationship between this reduced lung function and both fatal and non-fatal stroke [54,110]. These findings suggest that the increased incidence of stroke outcomes are due to patients having increased blood viscosity and infection susceptibility particularly during an exacerbation [111]. A recent study by Liao et al. [112] showed that reduced pulmonary function was linked to an increased risk of cerebral infarction and lesions to white matter, indicating that these abnormalities may be early indicators of stroke outcomes within these patients. Furthermore, chronic lung diseases such as COPD induce severe localised inflammation of the lungs, and it is the 'spill over' of these inflammatory mediators that contribute to the persistent low-grade systemic inflammatory responses within these patients, ultimately heightening the risk of cardiovascular complications [54]. A reduction in oxidative stress, through either CS cessation or antioxidant treatment may reduce pulmonary remodelling, thereby preventing further lung degradation and improving lung function in patients with chronic lung diseases.

COPD-induced hypoxia drives CVD pathogenesis

It has been well documented that hypoxia, a reduction in the level of oxygen reaching the tissues, plays a detrimental role in CVD progression [113,114]. Hypoxia has been shown to alter cardiac output and BP as well as impact vascular function. Circulating oxygen levels play a pivotal role in maintaining the secretion of endothelial-derived vasoactive substances, such as NO and various prostaglandins (e.g. PGE1 and PGE2), all of which control vascular tone. Hypoxia is seen in patients with COPD and other chronic lung diseases, as the overall integrity of the lung is reduced. Being in a state of hypoxia increases oxidative stress and ROS production, which in the vasculature alters the regulation of ion channels and cell signal pathways that maintain normal vascular tone [115,116]. This increased ROS production leads to oxidative damage through the activity of various cellular processes, such as: mitochondrial ROS production and up-regulated NOX activity, all of which promote the oxidation of various biomolecules such as DNA and proteins. Under hypoxic conditions, patients have blunted vasodilatory responses to acetylcholine, indicating that the increased oxidative burden due to these hypoxic conditions is limiting endothelial-dependent vasodilatory responses [117]. A study by Kato et al. [117] showed that vascular smooth muscle response to the NO donor, sodium nitroprusside was not different between non-hypoxic and hypoxic cohorts. This finding indicates that hypoxia only alters endothelial-dependent vasodilation while having no effect on vascular smooth muscle [117].

Normal vascular function and tone relies on the production and modulation of NO. The enzyme responsible for NO production within the endothelium, endothelial NO synthase (eNOS), may undergo post-translational modification due to increased oxidative stress and hypoxic conditions, like those seen in patients with COPD and other chronic lung diseases [118]. Post-translational modifications of eNOS alter enzyme kinetics, and acetylation of this enzyme negatively impacts overall NO synthesis, promoting vascular dysfunction, a key driver of CVD onset and progression. A study by Arunachalam et al. [119] has shown that CS induces the acetylation of eNOS in endothelial cells. eNOS acetylation down-regulates enzyme activity, resulting in reduced NO mediated signalling and subsequent endothelial dysfunction. Modulation of SIRT1 (silent mating type information regulation 2 homologue), an enzyme that deacetylates proteins in response to stress such as CS exposure was shown to be down-regulated under hypoxic conditions. Oxidative stress-induced reduction in SIRT1 has been linked to eNOS acetylation and inactivation, thus implicating SIRT1 in hypoxia-induced endothelial dysfunction [119].

Transcription factors Hypoxia Inducible Factor (HIF)-1α (HIF-1α) and HIF-2α regulate cellular responses to hypoxic conditions. It has recently been established that HIF-α proteins promote inflammation, leading to vascular remodelling and atherosclerosis [115]. Elevated blood serum levels of CRP and other pro-inflammatory mediators

in response to hypoxia have been seen in patients with heart failure and hypertension as a result of increased HIF-α signalling. Various studies have shown that there is a direct relationship between heart failure severity and the concentration of these pro-inflammatory mediators [116,120,121]. This localised inflammation in the vessel walls is a crucial factor in underlying vessel dysfunction and endothelial damage. HIF-1α activation does not occur under normoxic conditions, although during hypoxia there is constant HIF signalling activation, causing severe vascular remodelling. Suppression of HIF signalling may be beneficial in the treatment of vascular dysfunction, conversely in stroke or ischaemia HIF-α up-regulation plays a beneficial role in angiogenesis and vascular endothelial growth factor (VEGF) secretion, restoring blood flow to areas of ischaemic damage [122,123]. Alterations to HIF signalling have been observed in vascular diseases and thus may be a potential pharmacological target when targeting CVD [124].

Atherosclerosis and impaired blood vessel function in COPD

CVD, is a broad term used to describe a disease that impairs the heart and/or blood vessel function. Both cardiac and vascular complications due to this disease are highly complex, as genetics, environmental and lifestyle factors all play a role in its onset [125,126]. Prevention strategies such as cessation of cigarette smoking, and improved treatment options have assisted in decreasing cardiovascular-related mortality [127]. In patients with COPD, asymptomatic CVD progression significantly heightens the risk of comorbid CVD-associated mortality, and this is largely due to these patients being at heightened risk of atherosclerosis, due to the persistent systemic inflammation associated with this disease. Atherosclerosis is defined as a condition that causes the innermost layer of a blood vessel (endothelial layer), to become narrowed and hardened due to a build-up of fatty tissue and subsequent plaque formation, possibly leading to cardiovascular complications such as vascular occlusions and ischaemic stroke [85]. Key risk factors of atherosclerosis include cigarette smoking, elevated BP and high cholesterol associated with poor lifestyle (e.g. sedentarism) and diet (e.g. sodium-rich fatty foods), all of which contribute to endothelial dysfunction [86,128–130]. A damaged endothelium signals the recruitment of immune cells, in particular monocytes and low-density lipoprotein (LDL) cholesterol to the area, further promoting the local inflammatory response and altering blood vessel function [87]. The presence of atherosclerotic plaques causes the narrowing of arteries, impairing blood flow and oxygen delivery to tissue [84,131]. Complete occlusion of a vessel is also quite common, and this leads to damage to the myocardium and subsequent infarction [87].

The endothelial layer of a blood vessel plays a pivotal role in the regulation of vascular tone, as well as controlling blood fluidity and flow. Endothelial function is crucial in maintaining homoeostasis as it controls the fine balance between both vasoconstrictive and vasodilatory stimuli, particularly in the presence of NO [132]. Endothelial dysfunction, defined as an imbalance between these two elements, can be caused by various factors, such as an increase in localised ROS production and subsequent inflammation, both of which can be a direct result of CS exposure [133,134]. ROS have also been shown to directly alter blood vessel function; through vascular remodelling. Emerging evidence indicates that increased oxidative stress induces both DNA and mitochondrial DNA (mtDNA) damage, which are additional key contributing factors of CVD. Although ROS serve an important role in maintaining antimicrobial defence mechanisms and cellular signalling, there is a direct relation between an increase in ROS levels and CVD onset [135]. Harrison et al. [136] showed vascular cells subject to an oxidative environment had increased mtDNA damage. Subsequently, this led to a reduction in smooth muscle and endothelial function due to the alteration in mitochondrial metabolic activity. Shenouda et al. [137] showed that alterations in mitochondrial homoeostasis were in fact associated with an increase in mitochondrial ROS production. A subsequent impairment in the release of both cGMP and agonist-stimulated NO production through up-regulation of eNOS activity was observed [137]. This increase in ROS as well as the down-regulation in eNOS production led to reduced availability of NO, therefore preventing vasodilation, as cGMP and NO both promote endothelial and smooth muscle vasodilatory responses, that are crucial in maintaining normal vascular function.

Numerous studies have also shown that exposure to CS causes a marked decrease in the bioavailability of L-arginine within the vasculature, therefore reducing the release of NO and down-regulating the activity of NOS due to increased oxidative stress [138,139]. Zhang et al. [140] conducted a series of experiments investigating the role of arginine metabolism in a highly oxidative environment. Throughout the study human endothelial cells exposed to 10% CS extract (CSE) showed a significant impairment in arginine metabolism, with a 57 and 23% reduction in both N-hydroxy-L-arginine and arginine, respectively. It was also discovered that endothelial cells cultured with the CSE growth medium showed notable reductions in overall eNOS activity and NO production, further reinstating that exposure to CS directly impairs the endothelium and therefore down-regulates the L-arginine NO synthase pathway (Figure 3).
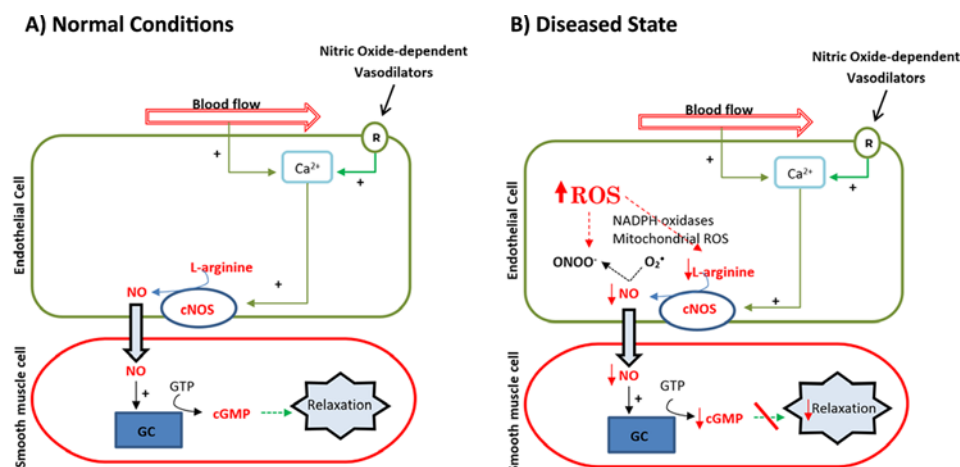
Figure 3. Blood vessel homoeostasis

The role of NO synthase (NOS) and L-arginine metabolism in endothelial-dependent blood vessel dilation (**A**) under normal conditions and (**B**) in an oxidative environment such as that seen in COPD. NO-dependent vasodilators or an increase in localised blood flow stimulate the release of intracellular calcium through receptor (R) binding or an increase in shear flow, respectively. This in turn up-regulates the activity of constitutive NO synthase (cNOS), causing the catalytic conversion of the amino acid L-arginine into NO. NO then diffuses from the superficial endothelial cell layer to the underlying smooth muscle. In smooth muscle cells, guanylyl cyclase (GC) activity is induced by an increase in NO, causing this enzyme to convert guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP) and thus resulting in a vasodilatory response. In an oxidative environment (i.e. smokers with COPD) there is an overall reduction in the bioavailability of L-arginine because of overexuberant ROS production and therefore reduced NO expression and impaired vasodilation.

It has been well established that a hallmark feature of CVD is a dysfunctional endothelium as well as a down-regulation of the L-arginine and NO pathways, both of which significantly contribute to the pathogenesis of CVD. It has been well characterised that there is pulmonary endothelial dysfunction in COPD, however little is known about COPD-induced vascular alteration in peripheral blood vessels [141]. This is of clinical importance as comorbid CVD may induce endothelial dysfunction, which may contribute to the heightened risk of cardiovascular-related mortality within these patients.

The role of platelets in COPD, thrombosis and stroke

Excessive low-grade systemic inflammation as seen in patients with COPD may place these patients at an increased risk of CVD and coronary thrombosis. These pro-inflammatory pathways are further up-regulated during acute exacerbations, which induces inflammatory cells and cytokines to further perpetuate the inflammatory response, contributing to the formation of atheromatous plaques [88,130]. This response in conjunction with the vascular injury associated with persistent inflammation activates circulating platelets which up-regulate P-selectin, von Willibrand factor (vWF) and CD40 expression, causing the adhesion of activated platelets to the arterial wall and collagen fibres [142]. Once bound to the lumen, activated platelets secrete thromboxane A_2 and adenosine diphosphate, which causes the recruitment of other platelets to the site which undergo a conformational change allowing for the binding of fibrinogen and various coagulation factors that ultimately form thrombin that leads to healing of the wound [143]. Conversely, activated platelets recruit inflammatory cells and secrete pro-inflammatory chemokines, which are beneficial to the healing process under normal pathological conditions although when this complex pathway becomes deregulated, prolonged inflammation (as seen in patients with COPD) and platelet hyperactivation causes platelet–monocyte aggregate formation, which further contributes to atherothrombosis [88,144] (Figure 4). Atherothrombosis is characterised by atherosclerotic lesion disruption with superimposed thrombus formation and is the major cause of acute coronary syndrome (ACS) and cardiovascular death [145]. Patients with COPD have increased H_2O_2 and ROS levels, which promotes a pro-thrombotic environment within these patients [67].

Regarding atherosclerotic plaque instability, it has been found that inflammation is a major pathophysiological factor within the vessel walls that ultimately causes plaque destabilisation and rupture, putting patients at increased risk of myocardial infarction and stroke. There are various cell types responsible for the increased inflammatory response,
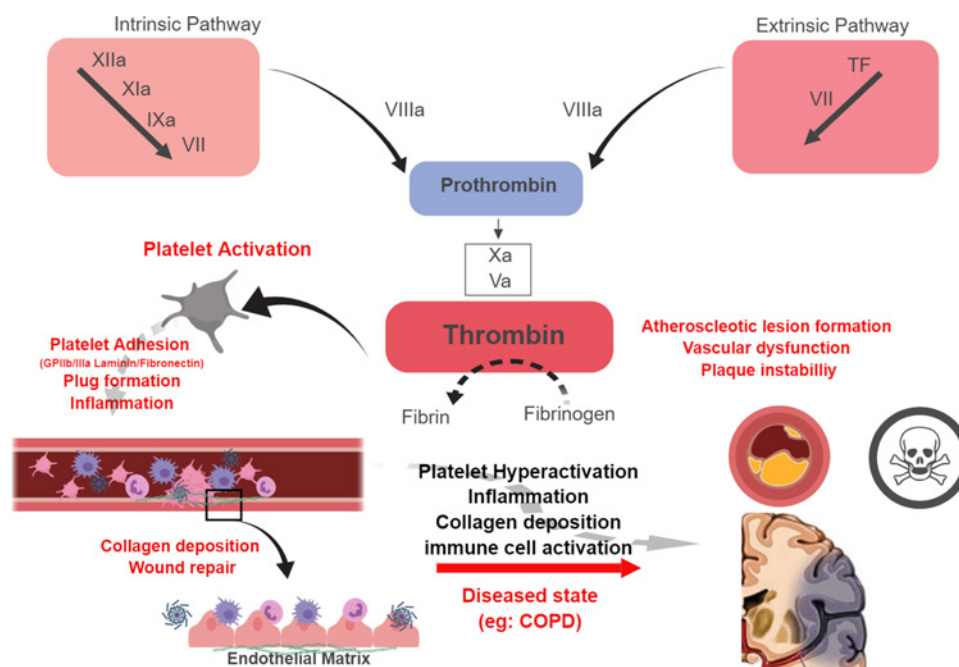
Figure 4. Intrinsic and extrinsic coagulation pathways, platelet activation, adhesion and aggregation, in both healthy and diseased states

Activation of both the intrinsic and extrinsic coagulation pathways drives prothrombin activation, in turn up-regulating thrombin-dependent conversion of fibrinogen into fibrin, promoting platelet activation. This platelet activation causes platelet plug formation and a localised inflammatory response under normal pathological conditions, which cause collagen deposition and wound repair. In a diseased state like that seen in COPD, this process becomes dysregulated, driving platelet hyperactivation, exaggerated inflammatory responses, collagen deposition and immune cell activation, all of which are contributing factors of atherosclerotic lesion formation, vascular dysfunction and plaque instability that can ultimately lead to myocardial infarction, cerebral artery occlusion (stroke) and death.

particularly in patients with COPD, these include: T lymphocytes and monocyte-derived macrophages, which can secrete chemokines, disintegrins, enzymes, growth factors, ROS and pro-inflammatory cytokines. The secretion of these molecules further promotes smooth muscle cell proliferation, endothelial cell activation and progression of the lesion [146]. Overall these cellular processes cause the degradation of the matrix surrounding the plaque and furthermore degrade the fibrous cap [146] leading to instability and rupture of the plaque which can enter circulation and cause the blockage of various arteries, some of which provide blood to the brain. Occlusion of these arteries causes ischaemic stroke and in some cases death. A recent study by Maclay et al. [88] showed that patients with stable COPD have significantly increased circulating levels of platelet–monocyte aggregates, and this is further increased during an exacerbation, although the levels of the cell surface receptors P-selectin and CD40 were not stimulated. The findings from this study show that patients with COPD are at an increased risk of stroke and developing CVD. Thus, preventing the formation of platelet–monocyte aggregates may be a useful strategy to reduce the development of CVD in patients with COPD [88].

Platelet hyper-activation has been shown to be a significant factor in athero-thrombotic disease and has been observed in patients following ischaemic stroke, in particular atherosclerotic ischaemic stroke [147]. Findings from a study conducted by Cha et al. [148] suggested that platelet hyper-activation during ischaemic stroke is directly related to the ongoing atherogenesis of the carotid and vertebra-basillar arteries, rather than acute vascular events. Platelet expression of CD63 and P-selectin as well as platelet aggregation to collagen and ADP remain up-regulated following atherosclerotic ischaemic stroke [148]. Although platelet aggregation was reduced significantly 72 h post stroke, the expression of CD63 and P-selectin remained elevated for 90 days post stroke injury, suggesting that platelet hyper-activation may be sustained for a prolonged period of time following stroke, which may lead to further ischaemic brain injury or infarction [148].

Mean platelet volume (MPV) has been used to evaluate platelet function as well as being linked to inflammatory status, myocardial infarction and stroke in chronic diseases such as COPD [149,150]. MPV is regularly used to determine the rate of platelet production, activation and stimulation. Larger platelets (those with higher volumes) are more reactive and adherent. It has been shown that MPV is of clinical relevance, particularly in COPD patients. Under hypoxic conditions there is an increase in MPV, which stimulates platelet production within the bone marrow [151]. Agapakis et al. [152] investigated the role of platelets in relation to inflammation, with the findings suggesting that an elevated MPV can be used as an inflammatory marker as well as a predictor of future exacerbations. It has also been shown that platelet volume is significantly increased during acute exacerbations in severe cases of COPD [152]. During AECOPD it has been reported that there is both increased platelet aggregation and an increase in the likelihood of venous and arterial thrombosis, due to the increased inflammatory state seen in COPD patients [153]. Studies have shown that increased CRP levels correlate with increased coagulation following stroke, indicating there may be a relationship between inflammatory responses and coagulation [67].

Anti-platelet therapy may be useful in the protection of patients with COPD from the increased thrombotic risk associated with this disease, causing a reduction in likelihood of both myocardial infarction and stroke [154–156]. Anti-platelet drugs such as aspirin have been shown to significantly reduce the risk of mortality in COPD patients, by inhibiting platelet hyper-activation and aggregation, thus preventing thrombosis [154]. A recent study by Harrison et al. [156] showed that patients treated with anti-platelet medications (such as aspirin, warfarin, Clopidogrel) showed a significant reduction in mortality within 1 year following exacerbation. The results from this study suggest that platelets play a role in inflammation and possible hypoxemia, thus anti-platelet treatment during AECOPD could be used therapeutically to prevent platelet aggregation and thrombosis, to reduce mortality in this cohort.

The immune response and ROS following neurovascular stroke injury

New evidence is emerging linking NOX activity to post-ischaemic stroke vascular injury. A study conducted by De Silva et al. [157] showed that there is excessive superoxide and nitrosative production as well as a reduction in NO function in mouse cerebral arteries after ischaemia–reperfusion. The deficit in NO-dependent vasodilation reduces the reperfusion rate of the ischaemic area, further promoting inflammation and neuronal damage. NOX-2 is widely expressed in the endothelium of cerebral arteries and believed to be a major source of superoxide production within the brain. The study concluded that vascular NOX-2 caused a significant impairment in NO endothelial-dependent vasodilation, through direct peroxynitrite inactivation of NO. Modulation of ROS may potentially be beneficial in reducing the overall neuroinflammatory response following ischaemic stroke, thus preventing excess neuronal death. Exuberant oxidative stress and systemic inflammatory responses, like those seen in COPD may promote cerebral vascular dysfunction, drive immune cell activation and further promote neurovascular stroke injury.

It is understood that following ischaemic stroke, immune system activation occurs in response to the neurovascular injury. Initially, there is a rapid immune response via the innate immune system which causes inflammation, cytokine production and inflammatory cell infiltration into the ischaemic brain tissue. Following the innate response, adaptive immune response activation occurs which should allow for tissue repair and regeneration to the vasculature. However, recent studies have shown that following acute stroke (particularly ischaemic stroke) there is immunosuppression occurring preventing these endogenous repair mechanisms from healing the damage induced by the stroke [158].

As a result of ischaemia and cellular stress, 'danger signals' known as alarmins are secreted from the dying or injured cells of the neurovascular unit. These danger-associated molecular patterns (DAMPs) react with pattern-associated molecular patterns (PAMPs) and interact with toll-like receptors (TLRs), that signal to both antigen-presenting cells and chemotaxins [159,160]. DAMP signalling therefore links the innate immune response to the adaptive (humoral) immune response following ischaemic brain injury. Up-regulation of these alarmins, in particular high mobility group box 1 (HMGB1), which is a ubiquitous protein released from the nuclei of damaged cells, and various heat shock proteins have been shown to be increased following ischaemic stroke and can be used as a biomarker in the prediction of functional outcomes of ischaemic stroke [161]. Conversely, DAMPs can also stimulate further activation of the inflammasome and the innate immune system, therefore further enhancing the already up-regulated inflammatory response in patients with COPD and the likelihood of injury.

Inflammatory mediators such as cytokines and chemokines play an important role in cerebral ischaemic stroke [162]. These mediators have been shown to be expressed both centrally (at the ischaemic core) and peripherally following stroke injury [163,164]. Levels of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α are increased

in the serum following stroke and are directly related to both stroke severity and infarct size [163,164]. Both experimental and human models have shown that blocking IL-1 production or neutralising its actions provides a degree of protection towards stroke injury by reducing the effects of acute phase inflammatory responses [165]. The pro-inflammatory mediators IL-6 and CRP have been shown to be directly correlated to infarct volume and stroke severity, although whether IL-6 is a detrimental contributing factor in the pathogenesis of stroke injury or beneficial due to its anti-inflammatory neurotrophic effects remain unknown [166]. These findings indicate that modulation of the inflammatory response at certain stages of stroke injury, may provide a novel method of reducing infarct volume and aid in neurotrophic recovery.

TNF-α has also been shown to be both neuroprotective and neurotoxic following ischaemic stroke [167–169]. Low-level exposure to this pro-inflammatory cytokine has been used to precondition brain tissue towards ischaemia experimentally prior to stroke, although when expression levels are drastically enhanced, the presence of this cytokine becomes neurotoxic causing cerebral tissue to become more susceptible to ischaemic injury [167–169]. TNF-α over-expressing transgenic rats, showed worsened ischaemic injury following middle cerebral artery occlusion, due to enhanced neuroinflammation and apoptosis, when compared with their wild-type counterparts, which exhibited significantly lower expression levels of TNF-α in brain homogenates [169]. Further studies are being conducted to observe the effects of acute inhibition of these inflammatory cytokines in regard to cerebral ischaemic stroke injury, with the results showing neuroprotection towards ischaemic injury in animal models [169].

Overall the role of these cytokines as well as the immune response differs depending on the phase of the injury response (innate or adaptive), thus further research into the role of feedback mechanisms that regulate these inflammatory pathways is needed. Recently it has been found that following ischaemic stroke, peroxiredoxin (an alarmin) causes the up-regulation of the innate inflammatory response and can possibly be used as a therapeutic target in the future [170].

Current and future COPD treatments and their effectiveness in comorbid CVD

Modulation of the inflammatory response has been tested extensively to reduce the detrimental lung degradation in COPD. Rahman and Adcock [62] have shown that oxidative stress regulates key histone modifications and cell signal pathway activation, driving the overexuberant pulmonary inflammatory response in COPD. Based on the spill over hypothesis, through modulation of lung inflammation and restoration of oxidative balance, subsequent reductions in systemic inflammation should become evident, reducing the structural remodelling and pulmonary vascular dysfunction in COPD, as well as CVD onset and severity. Inflammatory mediators such as TNF-α have been shown to deplete antioxidant glutathione in the airway epithelium and endothelium, promoting further inflammation and emphysema [70]. In patients with COPD ICS are often used to reduce the pulmonary inflammatory burden, although non-responsiveness to these drugs is quite well documented. Novel therapeutics have been developed to help reduce the oxidative burden that drives these inflammatory responses due to overwhelming evidence relating oxidative stress to COPD pathogenesis. There is an urgent need for the development of ROS scavenging therapeutics as typical treatments such as dietary supplementation and vitamin E treatment, showed minimal antioxidant effects [171].

ROS scavengers and modulation of ROS generating enzymes have shown beneficial effects, with NOX inhibitors such as apocynin, preventing the assembly of NOX subunits resulting in reduced NOX-dependent ROS formation and a direct reduction in pulmonary inflammation in mice [172]. SOD mimetics such as AEOL 10150 have also been shown to significantly reduce lung inflammatory responses in rats following CS exposure, when compared with a matched vehicle treated group [173]. With ROS being a hallmark feature of both COPD and CVD, NOX activity is up-regulated in both disease states, therefore further enhancing this oxidative burden and subsequently promoting inflammation in the pulmonary vasculature. The blood flow from the lungs to the systemic circulation, is believed to carry pro-inflammatory mediators, driving CVD in smokers and furthermore in COPD patients. The impact of ROS modulation in the systemic vasculature is largely unknown, therefore further investigation is warranted.

It has been documented that current treatments available for CVD such as ACE inhibitors and β-receptor blockers may not be as beneficial in the context of comorbid CVD in COPD patients. With a study finding that pulmonary symptoms associated with COPD were worsened upon treatment with these drugs, conversely both angiotensin receptor blockers and ACE inhibitors have shown to reduce hypertension and have antioxidant effects [55]. Combinational therapies such as antioxidant treatments/ROS modulators in conjunction with the current CVD treatments available, may present novel and effective treatment of COPD and its associated cardiovascular comorbidities.

Conclusion

Lung and systemic inflammation, ROS production and platelet hyper-activity observed in COPD all contribute to the worsened CVD outcomes in these patients. It is also clear that increased oxidative stress observed in COPD is a major driver of not only the onset and development of COPD, but in also orchestrating and perpetuating the CVD comorbidities associated with COPD. Given the lack of effective treatments for COPD, and an even greater lack of research regarding interventions that treat both COPD and its comorbidities, targeting oxidant-dependent mechanisms represents an exciting and unique therapeutic opportunity to treat COPD and its related CVD comorbidities.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AECOPD, acute exacerbation of chronic obstructive pulmonary disease; BALF, bronchoalveolar lavage fluid; BP, blood pressure; CAT, catalase; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CS, cigarette smoke; CSE, CS extract; CVD, cardiovascular disease; DAMP, danger-associated molecular pattern; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; FEV-1, forced expiratory volume in 1 s; Gpx, glutathione peroxidase; HIF, hypoxia inducible factor; ICS, inhaled corticosteroid; IL, interleukin; MPV, mean platelet volume; mtDNA, mitochondrial DNA; NFκB, nuclear factor κ-light-chain-enhancer of activated B cell; NO, nitric oxide; NOX, NADPH oxidase; ROS, reactive oxygen species; RSV, respiratory syncytial virus; SIRT1, silent mating type information regulation 2 homologue; SOD, superoxide dismutase; TNF-α, tumour necrosis factor α.

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