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REVIEW

# Oxidant/Antioxidant Disequilibrium in Idiopathic Pulmonary Fibrosis Pathogenesis

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*Abstract*— Idiopathic pulmonary fibrosis is characterised by abnormal reepithelialisation and remodelling consequent to persistent stimuli or injury. The involvement of oxidative stress in alveolar injury, inflammation and fibrosis development has been suggested. Increased concentrations of lipid peroxidation products, oxidised proteins and an altered antioxidant enzyme status with the depletion of glutathione, the most abundant low-molecular-weight antioxidant, have often been reported in epithelial lining fluid of IPF patients. This review describes the sources of free radical generation, ROS-induced signalling pathways and mechanisms of oxidative stress damages in the pathogenesis of idiopathic pulmonary fibrosis.

**KEY WORDS:** idiopathic pulmonary fibrosis; pathogenesis; oxidative stress; oxidant/antioxidant balance.

## INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease (ILD) limited to the lung associated with a severe prognosis (mean survival less than 5 years) and a radiological and histopathological pattern of usual interstitial pneumonia (UIP) [1, 2]. This disease is recognized as an epithelial/fibroblastic disorder that accounts for approximately 25% of all ILDs [3]. ILD development is more common in patients professionally exposed to dust, metals and asbestos fibres as well as cigarette smoke, radiations and chemotherapies inducing reactive oxygen and nitrogen species overproduction [3].

IPF is an ageing-related disorder that generally occurs in over 65 years old subjects with a genetic susceptibility to develop the disease as a consequence of uncontrolled profibrotic stimula determining an overdepositon of extracellular matrix from lung fibroblasts and myofibroblasts [4]. IPF commonly affects smokers or ex-smokers. Cigarette smoking is the most relevant risk factor for IPF development and smoke is considered a negative prognostic factor although its specific pathogenetic role is unclear. It probably facilitates the increase of oxidative stress, the hypoxia-mediated damages and the chronic persistent injuries associated with impaired regenerative responses [5]. No therapy can modify IPF natural history (with the only exception for lung transplantation) and, actually, the objective of the treatment is to stabilise or reduce the disease

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**Abbreviations:** *IPF*, Idiopathic pulmonary fibrosis; *HRCT*, High-resolution computed tomography of the chest; *ILD*, Interstitial lung diseases

progression [6]. Nintedanib and Pirfenidone are the only antifibrotic drugs worldwide approved for the management of the disease and interestingly both can positively interfere with cellular redox status and oxidative stress contributing to IPF progression [7, 8].

The aetiology of IPF is still unknown but several studies demonstrated a pathogenetic role for oxidative/nitrosative stress in IPF [8–10]. The aberrant production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induces cellular damages [11, 12], facilitated in their turn by insufficient antioxidant defences, unable to keep ROS below a toxic threshold with consequent cellular damage at protein, lipid, carbohydrate and nucleic acid levels [13, 14]. Increased oxidative stress might promote disease progression in IPF patients mainly in those who are current and former smokers [15].

In this context, this review will provide an overview of the pathogenetic role of oxidative/nitrosative stress in IPF, considering the oxidant/antioxidant imbalance as one of the major triggers for interstitial and pulmonary vasculature damages. The significant role of oxidant/antioxidant imbalance in IPF represents a hot research topic.

## OXIDANTS IN IDIOPATHIC PULMONARY FIBROSIS

Lung tissue and biological fluids from patients with IPF have been collected in different studies in order to quantify their concentrations of oxidants, demonstrating that both oxidative and nitrosative stress significantly contributes to IPF development and progression [16–18]. A significant increased concentration of carbonyl proteins, isoprostanes, etane, nitrogen oxides and nitrosotyrosines has been demonstrated in bronchoalveolacr lavage (BAL), serum and tissue of IPF patients than controls [19–21]. Our group of research recently assessed elemental metal profiles in BAL from IPF patients and controls: we observed remarkable decreases in average concentrations of Cr, Ni and Zn that are antioxidant trace elements that protect the lungs against oxidative stress. This decrease was the consequence of the enhanced activation of specific immunomodulatory cells and encouraged an overproduction of ROS inducing oxidative stress in IPF [22, 23].

Some metal ions, especially Fe2+ and Cu2+, are crucial in specific enzymatic pathways participating in redox reactions and in the conversion of active O2− containing compounds [22]. Fe homeostasis is altered in IPF patients. The increase in free radicals is associated with a disordered homeostasis of Fe in IPF [23]. An interesting genetic study demonstrated for the first time the association of high frequency of C282Y, S65C and H63D HFE allelic variants and higher iron-dependent oxygen radical generation in IPF. It was hypothesised that iron dysregulation associated with HFE allelic variants increased the susceptibility to environmental exposures, facilitating lung damage and fibrosis [24]. Moreover, a further study explored alveolar macrophage activation phenotype in IPF patients by RNA sequencing of BAL cells, showing increased iron-dependent ROS generation. Iron accumulation resulted crucial for macrophage activation, inducing inflammatory, tissue remodelling and angiogenic processes in IPF [25]. A very recent paper underlined the pathogenetic role of CD71 expression in alveolar macrophages from IPF patients, related to high levels of transferrin and iron in BAL [26].

A proteomic study compared protein profiles of IPF patients, never-smoker healthy controls and smoker control subjects in BAL. Among proteins potentially related to disease progression and pathogenesis, there were several proteins involved in hypoxia/oxidative stress and iron transport regulation. Several proteins of interest were differently expressed in smokers or ex-smokers being smoke-induced oxidative stress proteins. In particular, Glutathione S transferase P1 was a functional hub downregulated in IPF patients as metabolised in large quantities in IPF patients and healthy smokers because of its antioxidant functions [16].

In another study, the oxidative stress was analysed through determination of BAL protein carbonyl content, looking for an identification of target proteins of oxidation by two-dimensional electrophoretic (2-DE) analysis combined with immuno-blotting with specific antibodies for carbonyl groups. Carbonylated proteins were increased in BAL of patients with IPF in respect to healthy controls, suggesting that the protein carbonylation involved specific carbonylation-sensitive proteins and that in IPF, a greater number of proteins target of oxidation were present [19]. Oxidatively modified protein concentrations were found increased in BAL samples of IPF patients in respect to healthy controls also through different methodologies, and it was observed that proteins that have become dysfunctional by oxidation could play a role in the pathogenesis of diffuse lung diseases (Fig. 1) [20].

Very recently, Fois et al. reported that some oxidative stress reactants may constitute prognostic biomarkers of IPF correlating with progressive worsening of dyspnoea, acute exacerbation incidence and BAL neutrophil count in serum or BAL samples; furthermore, serum levels of ROS negatively correlated with lung function test parameters, predicting disease severity [4].

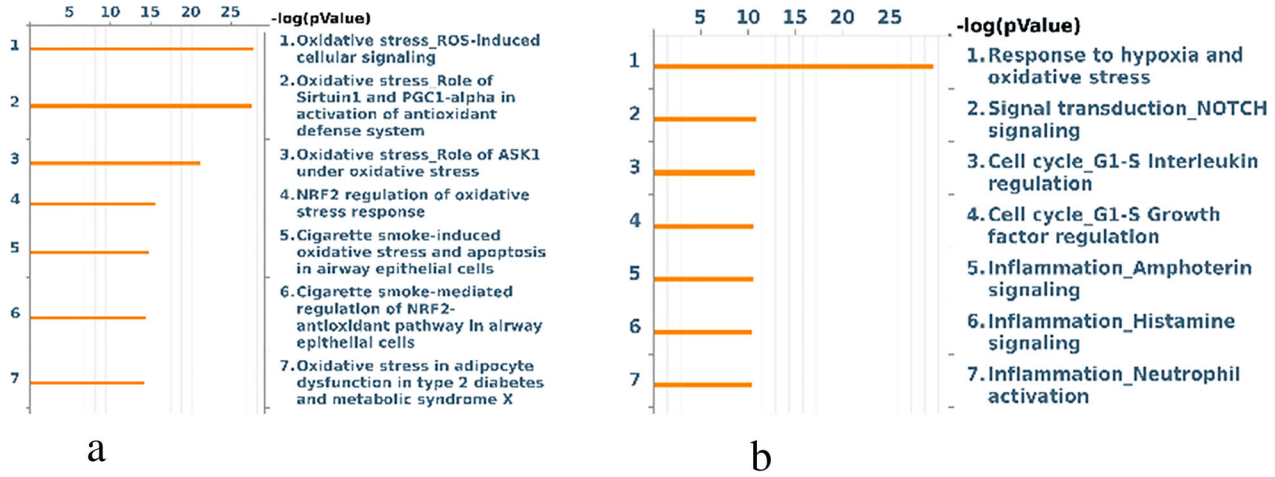


**Fig. 1.** Pathway maps (**a**) and process networks (**b**) analyses comparison by MetaCore software. Orange histograms represent the *p* value of the pathway maps process network of the differential proteins between IPF and healthy controls [27].

In IPF, ROS and RNS amplify the transforming growth factor β (TGF-β)-mediated pathway through oxidation of redox-sensitive proteins, such as thioredoxin, favouring fibrogenesis [28]. TGF-β induces mitochondrial oxidant radical formation in lung fibroblasts by enhancing NADP oxidase, inhibiting sirtuin 3 expression and inactivating Nrf2. ROS support the profibrotic TGF-β downstream signalling at different levels facilitating IPF development and progression [29, 30]. The pathogenetic role of NADPH oxidase-4 (NOX4) has been confirmed by several studies [31, 32]. NOX4 is highly expressed in fibroblast foci of IPF increasing the expression of α-smooth muscle actin, fibronectin and procollagen. It also induces apoptotic resistance in fibroblasts/myofibroblast and epithelial-to-mesenchymal transition in alveolar epithelial cells [33, 34]. There is evidence that oxidative mechanisms can lead to remodelling and fibrosis-inducing epithelial-to-mesenchymal transition degradation in IPF lung [29]. By immunohistochemistry, it has been demonstrated that an increased expression of matrix metalloproteinases and other extracellular matrix-degrading enzymes can be found inside the fibrotic lung areas [35]. Moreover, low-molecular fragments of extracellular matrix components have been described in IPF and have been suggested to promote fibrosis and inflammation [32]. A recent study focuses on tenascin C (TNC) synthesis and expression in lungs from idiopathic pulmonary fibrosis patients and controls. This profibrotic factor, induced by TGF beta, was almost absent in normal lungs while it was significantly upregulated in fibrotic foci areas of IPF patients together with versican and fibronectin glycoproteins. It was demonstrated that fibroblasts from IPF patients constitutively synthesised higher levels of TNC than normal fibroblasts supporting that the increase of this protein in IPF may cause the aberrant wound healing occurring in this disease [36]. Sonic hedgehog represents another protein of interest interacting with TGF-β and ROS in IPF patients [37]. The oxidative stress stimulates the release of sonic hedgehog and the transcription of tenascin-C in IPF facilitating oxidative mediated lung damage [36, 38].

The role of nitrosative stress in IPF has been recently evaluated; in the animal models of lung fibrosis, NO seems to promote TGF-β and ECM-degrading enzymes in fibroblasts [37, 39]. In lungs, NO is synthesised by NO synthase (NOS) family, which includes endothelial (eNOS), neuronal (nNOS) and inducible isoforms (iNOS) [40]. RNS are mostly produced by iNOS, which can be expressed by a large variety of cells within the respiratory system, including fibroblasts, in response to an aberrant TGF-β stimulation [41]. In IPF, there is an excess of iNOS expression and nitrotyrosine production by epithelial cells, macrophages and fibroblasts, leading to an abnormal nitrosative stress *in loco* that facilitates fibrogenesis and disease progression [42]. At the same time, Hsu et al. demonstrated that an increase of NO pathway can enhance TGF-β expression in lung fibroblasts, triggering a vicious circle that perpetuates nitrosative stress in fibrotic lungs [43]. Furthermore, NO production depends also on the availability of L-arginine and to the concentrations of asymmetric and symmetric dimethylarginine (ADMA, L-NMMA and SDMA),

regulated by dimethylarginine dimethylaminohydrolases (DDAH). In IPF, DDAH is overexpressed in pneumocytes type 2, breaking down ADMA levels and inducing an aberrant production of NO [44] that can be detected also in exhaled breath [45–47]. DDAH inhibition demonstrated to reduce collagen production and epithelial proliferation in a murine model of bleomycin-induced pulmonary fibrosis, corroborating the substantial contribution of NO-ADMA-DDAH pathway to the progression of lung fibrosis [44].

## ANTIOXIDANTS IN IDIOPATHIC PULMONARY FIBROSIS

The interest on antioxidant defensive role in IPF pathogenesis started more than a decade ago. An altered antioxidant enzyme status with the depletion of glutathione, the most abundant low-molecular-weight antioxidant, has often been described in IPF patients. At that time, a research demonstrated that antioxidant defences are regulated by the redox-sensitive transcription nuclear factor, erythroid 2. It counteracts the enhanced oxidative burden occurring in IPF promoting antioxidant enzyme functions. It was also hypothesised that polymorphisms in transcription nuclear factor erythroid 2 may contribute to IPF susceptibility [48]. More recently, it was demonstrated that antifibrotic Pirfenidone exerts its antioxidant effect in IPF patients through interaction with nuclear factor-erythroid-related factor 2 (Nrf-2) [49].

Different antioxidant enzymes have been evaluated in tissue and biological fluids from IPF patients. Haem oxygenase (HO)-1 is an antioxidant protein involved in the pathogenesis of IPF and its expression was evaluated in BAL alveolar macrophages, reporting a significant reduction responsible of the disequilibrium between oxidants and antioxidants [27]. Moreover, in fibroblastic foci from IPF patients, other antioxidant enzymes, including extracellular superoxide dismutase, and pathways, such as Nrf-2, have been demonstrated to be downregulated [50]. In a proteomic study, familial IPF proteome was compared with sporadic IPF: in both groups, upregulated proteins were mainly related to oxidative stress responses (*i.e.* isocitrate dehydrogenase, peroxiredoxin 1, antithrombin III, complement factor B) [51]. Isocitrate dehydrogenase cytoplasmic-1 emerged as an antioxidant enzyme overexpressed in BAL of sporadic IPF patients that protects the lungs against premature senescence induced by ROS and RNS. Another crucial protein differently expressed in the lung and BAL of IPF patients is antioxidant peroxiredoxin 1 that at the intracellular level catalyses, the reduction and elimination of several ROS and act as protein chaperone and signalling regulator [51]. This protein, downregulated in IPF, appears to anticipate the senescence contributing to the altered fibrotic microenvironment of IPF lung [16, 52]. Analogously, the upregulation of selenium-binding protein 1, a protein crucial for intracellular selenium transport and regulation of extracellular glutathione, was demonstrated in BAL of IPF patients [51].

Concentrations of transition metals in BAL from IPF patients can likely play a role, complementary to traditional biological approaches in oxidant/antioxidant balance. The increase in free radicals and oxidative stress are likely responsible for the significant decrease of Zn, Mn and Cr concentrations in BAL samples. Chromium is not a true antioxidant; however, Cr (III) competes with Fe for a binding site in transferrin. Zinc is involved in numerous aspects of cellular metabolism, acts as an antioxidant and is involved in the structural remodelling of lung tissue through metallo-proteases. Manganese also promotes the production of antioxidants such as the mitochondrially expressed Mn-SOD, which is induced by acute inflammatory stages of the lung parenchyma, although its antioxidant defence is probably impaired during the progression of fibrogenesis [22].

## CONCLUSION AND FUTURE PERSPECTIVES

The oxidant/antioxidant disequilibrium and the accumulation of ROS and RNS can damage DNA, lipids, proteins and carbohydrates participating to IPF pathogenesis. Increased lipid peroxidation products, DNA oxidation or protein carbonyl formation have been demonstrated in lung tissue and biological fluids from IPF patients [1–4] while several antioxidant scavengers have been found drastically reduced in this disease [4].

N-acetyl-cysteine (NAC) is an antioxidant (acting as scavenger and restoring glutathione), mucolytic and anti-inflammatory drug widely tested in lung diseases yielding contrasting results [30, 53]. Although there is evidence of an improvement of 6-min walking test distance, NAC therapy is not recommended (even when combined with antifibrotic drugs) for IPF therapy due to the lack of beneficial effects in pulmonary functional tests parameters such as DLCO and VC, and in the mortality rate [54].

Interestingly, pharmacogenomics seems to affect the NAC therapy in IPF, as reported by the differential response of a patient with different *TOLLIP* genotypes [55]. For this reason, recent insights in personalised medicine are oriented towards implementing the efficiency of

antioxidant therapy in selected stratified patients. No data is currently available concerning the potential efficacy of specific antinitrosative treatment, although experimental studies in murine model showed promising results in reducing collagen production and slowing disease progression [44]. This could represent an intriguing research area to develop new drugs for IPF, especially since neither pirfenidone nor nintedanib has shown any significant effect on nitrosative stress in these patients.

The monitoring of oxidative/nitrosative stress markers as indicators of treatment response could be helpful in the optimization of individual treatment.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of interest.** The authors declare that they have no conflict of interest.

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