Thesis

by Khushi Upadhyay

Submission date: 21-May-2026 09:03PM (UTC+0700)
Submission ID: 2966393457
File name: cvd_copd_final.docx (177.46K)
Word count: 11012
Character count: 67316

ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) can co-occur with cardiovascular disease (CVD) in the elderly, raising the possibility of common molecular pathways between the two diseases. Pulmonary inflammation and cardiovascular dysfunction are clinically important and the molecular mechanisms that connect these processes are still not fully understood.

This dissertation provides a thorough systems biology study of the molecular crosstalk between COPD and CVD using integrative computational methods such as transcriptomic analysis, network topology analysis, and pathway enrichment. To identify the common inflammatory mediators of COPD-CVD comorbidity we combined data from public gene expression datasets, publicly available lists of inflammatory genes, and network and pathway-based analyses in a novel systems biology approach. By integrating transcriptomic data from pulmonary and cardiovascular sources, we aimed to reveal conserved inflammatory pathways and molecules that are simultaneously expressed during both diseases. This method provided the ability to uncover common molecular inflammatory signatures in addition to those that may be more specifically related to either COPD or CVD, thereby shedding light on inter-organ inflammation signaling between the lungs and the heart.

The central inflammatory molecule identifying multiple immune and cardiovascular signaling pathways, through network analysis, was found to be interleukin-6 (IL-6) and identified as a crucial bridge between NF-KB, JAK-STAT, ICAM-1 immune and vascular signaling pathways, all of which are essential for the enhancement of inflammation, recrutement of immune cells and endothelial dysfunction and vascular remodeling.

Pathway enrichment analysis showed a simultaneous activation of both pro- and anti-inflammatory pathways (IL-4/IL-13 and IL-10), suggesting immune dysregulation instead of a one-way inflammatory shift. It is suggested that this simultaneous activation of opposing cytokine pathways can be responsible for chronic tissue remodelling, endothelial dysfunction and chronic inflammation that occurs in both COPD and CVD. These results suggest that the inflammatory regulatory axis centred on IL-6 is a potential therapeutic target for multiple diseases. Translational opportunities are discussed, such as strategies like monoclonal antibody-induced IL-6 inhibition or inhibition of IL-6 receptor, or inhibition of JAK/STAT pathway and RNA interference-based IL-6. This dissertation makes a contribution to understanding of the pulmonary-cardiovascular comorbidity in the context of inflammaging and age-related disease, and provides a framework for future experimental validation.

CHAPTER 1: INTRODUCTION

1.1 Background and Context

The global demographic landscape is rapidly changing, with the population of indidviduals aged 60 years and above increasing at an unparalled rate. ARDs like cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), type 2 diabetes, neurodegeneration, and osteoarthritis often do not occur in isolation; rather, they are frequently seen co-occurring in the same individual as complex multimorbidities. Multimorbidities substantially impair the quality of life, lead to increased health care utilization, and are significant contributors to mortality [1]. Current classifications based on single-organ pathology do not adequately capture the systemic nature of ARDs [5]. These disorders are highly recognized as systemic conditions driven by overlapping biological mechanisms turning into the economic and social burden growing exponentially. Of the numerous combinations of ARDs that exist, the combination of CVD and COPD is one of the most prevalent, lethal, and inexplicable [3]. The high co-occurrence of both conditions suggests that they might not simply develop independently as a result of common risk factors but may share underlying common biological mechanisms [8], and exploring the link between them has therefore been the object of substantial biomedical investigation.

Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory disease of the airways that affects nearly 400 million people worldwide [2]. It is currently the third leading cause of death globally creating a long-term condition that involves airflow obstruction and damage to the lungs, with hypersecretion of mucus and a chronic inflammatory condition of the airways. Typically, patients present with shortness of breath, a chronic cough, decreased exercise tolerance and recurrent exacerbations. Similarly, cardiovascular disease (CVD), which includes conditions like coronary artery disease, heart failure, and arrhythmias, is the leading cause of death worldwide [8]. Over the years, growing research has shown a strong link between these two disorders. People with COPD are two to five times more likely to develop cardiovascular problems [3], considering common risk factors such as smoking. The pulmonary and cardiovascular systems are intimately linked and a malfunction in one may directly affect the other in an inflammatory, neuronal or hemodynamic manner [10]. This highlights the close and often overlooked connection between the lungs and the heart.

1.2 Inflammaging and age-related disease

A conceptual framework underlying the biology of age-related diseases is that of inflammaging ‘chronic, low-grade, sterile inflammation characterizing aging’. Unlike acute inflammation that is transient, resolving when the instigating insult is removed, inflammaging is sustained due to cell damage, dysfunctional mitochondria, senescent cells and the constitutive secretion of inflammatory factors known as the senescence-associated secretory phenotype (SASP). The key inflammatory mediators in the SASP include IL-6, IL-1, TNF-, MCP-1 and various matrix metalloproteinases (MMPs), which create a sustained positive feed-forward loop of inflammation [4]. Aging lung tissues, affected by COPD, are particularly susceptible to this loop, and similarly so for aging vascular tissues, affected by atherosclerosis and CVD [4], and these networks likely overlap. These signaling molecules are regulators of major inflammatory signaling pathways, including the NF-B and JAK-STAT pathways. These pathways themselves regulate expression of SASP components and therefore are positively feed-backing loops that

drive inflammation; hence they represent therapeutic targets in an aging biology system, the basis of this dissertation.

1.3 A Systems Biology Approach

Traditionally biomedical research has been reductionist in nature, isolating genes, proteins or pathways to investigate their function. However this approach has severe limitations in polygenic diseases such as COPD and CVD, which result from the interplay of hundreds of genes within many biological networks. An alternative Systems Biology approach is holistic, viewing all biological elements as components within interconnected biological networks that exhibit emergent properties which are more than just the sum of their constituent parts. Within this approach Network medicine applies the principles of graph theory to interaction networks to reveal key 'hub' genes or proteins which are central to network structure and function and also likely critical to disease pathogenesis. Due to their key role within the network hub genes are potentially ideal therapeutic targets, as perturbation should have a broad downstream effect within the network. Many large scale comparative studies have utilized this approach to investigate the shared pathways underlying diseases of disparate types; this is an approach that this dissertation utilizes to identify the shared genetic drivers underlying COPD and CVD. Publicly available, large scale, high-throughput transcriptomic data from public repositories such as the Gene Expression Omnibus (GEO) and curated human-mouse disease-gene interaction databases such as the Comparative Toxicogenomics Database (CTD) make large scale multi-disease analyses tractable, and are the resource pool for this analysis, used with the software package NetworkAnalyst for network construction and Enrichr for functional enrichment analysis.

1.4 Background and Significance

To date most work focused on COPD and CVD has involved the investigation of one or other disease in isolation, with limited emphasis placed on the molecular interface connecting the two and current treatment protocols rarely take the comorbidity into account. The prevalence of COPD/CVD comorbidity has led to increasing recognition that the two diseases can have a detrimental cross talk effect on each other, driving disease progression and increasing mortality. A possible explanation for this cross talk is a sustained mutual inflammatory effect arising from the two different disease locations. Identifying these shared inflammatory hubs provides an explanation and rationale for understanding the poor prognosis of comorbid patients and provides targets for rational drug design that can exploit this interaction. This dissertation utilizes an integrative Systems Biology approach to analyze transcriptomic and gene interaction data from COPD and CVD patient data in order to identify shared genes, pathways and networks that link these two conditions, building on an understanding of their inflamed nature to identify therapeutic targets which could benefit patients with COPD/CVD comorbidity.

1.5 Aim of the study

The study has the following objectives:

i. Obtain and aggregate data relevant to the study, consisting of transcriptomic data of COPD and CVD, retrieved from GEO and disease specific gene lists from CTD.

ii. Intersect the COPD and CVD gene lists to yield a list of genes common to both conditions.

iii. Use the common gene list and NetworkAnalyst software to build context specific co-expression networks and identify potential hub genes based on network topology.

iv. Use functional enrichment analysis to ascertain which biological pathways are represented by the common gene list and therefore presumably involved in the comorbidity.

v. Investigate the use of these hubs as targets for anti-inflammatory intervention strategies in aging biology.

vi. Propose therapeutic interventions based on shared genetic hubs in the context of inflammaging and disease.

1.6 Scope and limitations

This is purely a computational investigation using available databases and tools. The findings therefore, are statistical associations and hypothesis generating in nature, and require experimental validation in vitro and in vivo prior to any clinical application. The focus of the investigation into networks was protein coding genes with direct links to either COPD or CVD from the CTD database; non coding regions of the genome and epigenetic changes were not investigated and thus this dissertation cannot identify non coding DNA sequences, epigenetic modification patterns or protein post translational modification, though these may be involved in the diseases.

1.7 Overview of Dissertation

The dissertation is broken down into six chapters, beginning with an introduction including background information, a rationale and aims in this first chapter, followed by a detailed literature review in chapter two. Chapter three covers the methodology used for obtaining the data and carrying out all the analyses; Chapter four deals with the presentation of the data acquired during the analysis, detailing all common genes identified, hub gene analysis results and functional enrichment results. In chapter five the findings are interpreted in the context of the literature and proposed therapeutics. Chapter six presents a summary of the research findings and identifies directions for future research.

CHAPTER 2: LITERATURE REVIEW

2.1 The Molecular Basis of Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) comprise a group of respiratory illnesses that are chronic and manifested with emphysema and the limitation of airflow accompanied by inflammation. COPD has been accounted for high morbidity and mortality, affecting approximately 3 million individuals globally [2], according to the World Health Organization (WHO). The hallmarks of COPD include bronchiolitis along with fibrotic condition, small airways obstruction, hypersecretion of mucus and emphysema. As compared to other pulmonary diseases, such as asthma, COPD prevails more in the elderly population, affects predominantly the middle-aged and elder individuals. However, the disease is less explored owing to its complexity and population heterogeneity [5].

COPD is the multilayered effect of genetic, clinical and environmental factors. However, the highest threat for disease development is posed by tobacco smoking. According to the estimates, the percentage risk of mortalities caused by COPD induced by smoking is far more in the high and middle-income countries as compared to the low- income countries. Moreover, genetics also play an inherent role over here. It is unlikely that all the smoking individuals develop COPD. But there are chances that such individuals are highly susceptible to develop COPD due to prolonged smoking, at later stages in life. Other environmental factors contributing to the disease are prolonged exposure to fumes, chemical vapours and dust particles especially metal dust, such as lead, cadmium owing to occupation. Air pollution, exposure to second hand smoking, poor ventilation are also significant causes that worsen the disease development and progression.

Genetic factors contribute significantly, such as the absence of alpha-1 antitrypsin gene encoded by SERPINA1, leads to the protease- antiprotease imbalance inducing the epithelial damage. Several other genes are also accounted for the developing risk for COPD, such as, tumor necrosis factor- alpha (TNF-α), microsomal epoxide hydrolase enzyme and Transforming Growth factor (TGF-β). Lung microbiota dysregulation, due to bacterial or viral infections in the respiratory tract, also comprise an important risk factor. The lung microbiota burden increases under the effect of any infection, thus, there is an increase in release of inflammatory molecules and proteases due to enhanced activation of the immune cells, leading to fibrosis and destruction of the alveolar cells.

Apart from the environmental and genetic factors discussed above, clinical factors also pose a high risk, making individuals more susceptible to COPD. The major factor here is age. Ageing is mediated by the p-16 and sirtuin protein families, impairing the DNA repair induced by oxidative stress at the cellular level. It has been implicated that the depleted levels of SIRT1 gene in elder individuals, placing them at a higher risk for COPD, causing death in extreme conditions [11]. Gender, though not that much significant, cannot be neglected completely. Men possess higher risk towards COPD as compared to women. However, this ratio does not always hold true, especially while considering the socio-economic status or in the scenario of developed countries. Passive smoking and exposure to smoke particles also influence this ratio.

2.1.1 Chronic Obstructive Pulmonary Disease (COPD): Molecular Pathobiology

2.1.1.1 Epidemiology and Clinical Features

Chronic obstructive pulmonary disease is a progressive disease that is largely non-reversible, in which the ability to breathe out is limited. It is a combination of two clinical syndromes: chronic bronchitis (productive cough for at least three months for two years in a row); emphysema, a pathological term referring to permanent enlargement and destruction of alveolar air spaces distal to the terminal bronchioles. COPD is now the third major killer in the world, and is estimated to cause around 3.23 million deaths annually, with estimates indicating it will become even more of a problem in the future due to ageing and continuing high tobacco consumption in developing nations. Clinically COPD is characterised by dyspnoea on exertion, chronic cough and progressive deterioration in lung function revealed by spirometry. Much of the morbidity, hospitalisation and mortality experienced by the disease is associated with exacerbations or acute worsening events, which are caused by respiratory infections or environmental pollutants. COPD is a systemic disease, which is associated with muscle wasting, metabolic syndrome, osteoporosis, anaemia and — most important for the purpose of this dissertation — cardiovascular comorbidities.

2.1.1.2 Pathogenesis and Inflammatory Mechanisms

The disease process of COPD starts when noxious particles and gases, typically tobacco smoke, are inhaled. This activates airway epithelial cells, alveolar macrophages and dendritic cells in the airways and lung parenchyma, which leads to an innate immune response. These cells also secrete a series of chemokines and cytokines (such as CXCL8, CXCL1, IL-1β, TNF-α and IL-6) that cause the entry of neutrophils and monocytes into lung tissue from the circulation [8]. This acute inflammatory response does not resolve, instead becoming a chronic, self-sustaining inflammation, in susceptible individuals. CD8+ cytotoxic T lymphocytes and CD4+ helper T cells, especially Th1 and Th17, are recruited to the lung and trigger persistent inflammation and tissue damage. The protease-antiprotease imbalance (excessive activity of matrix metalloproteinases (MMPs) over the tissue inhibitors of metalloproteinases (TIMPs)) results in the breakdown of extracellular matrix of the alveoli, the characteristic feature of emphysema. Barnes (2016) gave a comprehensive review of the inflammatory mechanisms active in COPD, focusing on alveolar macrophages as coordinators of the innate immune system, and the steroid-resistance of COPD inflammation compared with airway inflammation in asthma. The author identified NF-κB as a "master regulator" of inflammatory gene transcription in COPD which regulates the expression of hundreds of pro-inflammatory genes, including those for TNF-α, IL-1β, IL-6, CXCL8 and ICAM-1 [9].

2.1.1.3 Systemic Effects of COPD

COPD is now known as a systemic disease, affecting much more than the lungs. Systemic inflammation, as evidenced by increased circulating levels of IL-6, CRP, TNF-α and fibrinogen, is a constant component of COPD and linked to poorer systemic outcomes [10]. In a seminal review, Barnes and Celli (2009) reported many systemic effects of COPD, such as skeletal muscle dysfunction, metabolic syndrome, bone density loss, anaemia, and

cardiovascular disease. Importantly, systemic IL-6 levels have been shown to be related to FEV1 decline, hospitalisation rate and mortality in COPD, making IL-6 a potential biomarker and a mechanistic driver in the pathogenesis of COPD.

The systemic inflammatory spillover from the COPD lung is believed to play a role, at least in part, in the increased cardiovascular risk in COPD patients [3]. The effects of chronic low-grade systemic inflammation include endothelial dysfunction, increased atherosclerosis, and decreased autonomic regulation, all of which are essential to the development of CVD. This understanding serves as the conceptual link between the COPD biology literature (above) and the CVD pathobiology literature (below).

2.1.1.4 THE ROLE OF AGEING AND OXIDATIVE STRESS IN COPD

The two major classes of chemically reactive molecules containing free radicals of oxygen and nitrogen are referred to as the reactive oxygen and nitrogen species (ROS and RNS) respectively. Under normal physiological conditions, both ROS and RNS are produced as by-products in redox reactions to maintain redox homeostasis. The production of different ROS is achieved by the reduction step of oxygen (O_2) to water (H_2O) which includes superoxide ($O_2^{\bullet-}$), hydrogen peroxides (H_2O_2), hydroxyl ions (OH^-) and nitric oxide (NO). The major cellular sites to produce ROS include mitochondria, endoplasmic reticulum, peroxisomes, and NADPH oxidase (NOX). Preliminary studies on ROS suggested it to have detrimental effects on normal cellular functions, but later studies revealed its ability to act as redox signal mediators and defense molecules performing immune functions in the body. The major defense mechanisms used by the immune cells (macrophages and neutrophils) against foreign invasion is the production of ROS/RNS that damages target cell lipids, proteins and DNA.

If we think of ROS and RNS as weapons to fight against microbial invasion, we should also be acquainted with the fact that friendly fire is inevitable just like in any other war. This makes us aware of the deleterious effects of ROS/RNS when present in excess than what is required. High levels of ROS results in oxidative stress in the body accompanied by several diseases and disorders including cancer. Oxidative stress can be defined as the imbalance in the production and elimination of oxidants or ROS. The loss of homeostasis due to oxidative stress is associated with oxidative damage such as increased genetic mutations, altered structures of crucial biomolecules such as lipids, proteins, and nucleic acids. This in turn triggers the antioxidant systems operating in the body to counteract stress conditions and re-establish homeostasis.

An Imperative feature of COPD Is oxidative stress. The Inflammatory cells upregulated by the NF-κB transcription factor, such as neutrophils, macrophages, eosinophils, function by producing reactive oxygen species. Superoxide dismutase, encoded by SOD gene, converts the superoxide ions into hydrogen peroxide (H_2O_2), which then can be dismuted by catalase enzyme into water. Also, $O_2^{\bullet-}$ may combine with H_2O_2 and NO to form -OH free radical and peroxynitrite respectively. There is a plethora of antioxidant mechanisms that are able to counteract the oxidative stress generated in the respiratory tract of humans. These include antioxidants like glutathione (GSH), catalase and superoxide dismutase enzymes. An important enzyme is HO-1, haem oxygenase-1 that catalyses the conversion of haem to biliverdin coupled with the release of carbon monoxide (CO), is induced by oxidative stress. It has been observed that CO production is enhanced in COPD patients due to the presence of HO-1 in the airways. The effect of ROS on the human airways may be mediated through both ways- directly or

indirectly. ROS activates the downstream signal transduction and transcription factors primarily by the NF-κB pathway which in turn leads to the activation of the inflammatory genes, thus amplifying the response.
Oxidative stress also induces the expression of AP-1 transcription factor, and is accompanied by the enhanced release of cytokines such as IL-8, CXC chemokines, MMP-8, MMP-9 and TNF-α. An increased oxidative stress in the airways also lead to the destruction of α1-antitrypsin, an anti-protease which results in an accelerated breakdown of elastin in the lung parenchymatous tissue. ROS may also directly induce apoptosis of type -1- pneumocytes. Oxidative stress in turn promotes ageing by causing direct DNA damage and telomere shortening. Cellular senescence is induced by an increased oxidative stress by activation of age-related genes such as cyclin-dependent kinase inhibitor (CDKN1A,), HIF1A, MAX dimerization protein 1(MXD1), and Superoxide dismutase-2 (SOD2). All these four genes have found to be overexpressed in COPD affected individuals [4]. Therefore, there exists a direct link between oxidative stress, ageing and COPD development and progression.

2.2 Cardiovascular Disease (CVD): Molecular Pathobiology

Atherosclerosis was thought of as a simple slow deposition of lipids, however it is now recognised as a chronic, immune mediated, inflammatory condition of the artery wall which is the pathology of most common cardiovascular event, like myocardial infarction and ischemic strokes. Emerging evidence emphasizes this concept further, proposing atherosclerosis to be an immune-metabolic disorder where impaired lipid metabolism and persistent non-specific inflammation work together to induce damage to the vasculature.

This section synthesizes recent literature to detail the two foundational pillars of atherogenesis: the initiation of endothelial dysfunction and the central, perpetuating role of inflammation in both plaque formation and the critical transition to plaque rupture.

2.2.1 Epidemiology and Classification

Cardiovascular diseases are a broad class of conditions affecting the heart and blood vessels, encompassing coronary artery disease (CAD), myocardial infarction, heart failure, stroke, peripheral artery disease, and hypertension. They are the number one cause of death globally, resulting in about 17.9 million deaths every year, which accounts for 32% of all worldwide deaths. The incidence and burden of CVD is disproportionately large in low and middle income countries due to accelerating urbanization and changing lifestyles which promote epidemics of hypertension, obesity and type 2 diabetes – risk factors for CVD.

Of the many forms of CVD that exist, the vast majority of clinical events including myocardial infarction and ischaemic stroke are as a consequence of the chronic inflammatory disease of the arterial wall known as atherosclerosis. Atherosclerosis begins in childhood with the formation of fatty streaks and develops over many decades via the formation of foam cells, smooth muscle cell migration and proliferation, deposition of extracellular matrix and formation of a fibrous cap which protects a rupture prone atherosclerotic plaque and when ruptures result in a thrombotic event.

2.2.2 Inflammation in Atherosclerosis

The inflammatory component of atherosclerosis was first comprehensively described by Libby in a landmark 2002 review which reframed atherosclerosis not as a storage disease for lipids, but a chronic inflammatory one. Initially there is a trapping of apolipoprotein B-containing lipoproteins in the subendothelial space of the arterial wall, followed by oxidation to oxLDL. This oxLDL is recognised by innate immune receptors in the intima (primarily on endothelial cells and macrophages), triggering a cascade of events resulting in activation of the intima by increased expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin) and chemoattractant factors (MCP-1/CCL2, CXCL1).

Monocytes then transmigrate from the circulation to the intima, where they differentiate to become macrophages and take up oxLDL by phagocytosis to become foam cells. Later, as the atherosclerotic lesion matures, there develops a lipid-rich core of foam cells and crystalline cholesterol, surrounded by a fibrous cap comprised mainly of smooth muscle cells and collagen. Pro-inflammatory cytokines (especially TNF-, IL-1 and IL-6) contribute to destabilizing the plaque by activating matrix degrading enzymes (MMP) which degrade the fibrous cap while inhibiting the synthesis of new collagen, weakening the cap until it ruptures, exposing the thrombotic core and inducing the acute events of myocardial infarction or stroke [6].

2.2.3 Endothelial dysfunction in CVD

Endothelial dysfunction, a state in which the vasculature does not achieve normal vascular tone in response to physiological stimulus, resulting in excess superoxide generation, increased inflammation and endothelial activation - is widely considered to be the earliest and most fundamental lesion in CVD. Under normal physiological circumstances, the endothelium provides protection to the vascular wall by producing endothelial nitric oxide (eNOS/NOS3) from L-arginine which mediates vasodilation, inhibits platelet activation, smooth muscle proliferation and leucocyte adhesion.

Under conditions of increased oxidative stress, excess superoxide radicals neutralize much of the NO and peroxynitrite, a reaction that further depletes bioavailable NO. Furthermore, inflammatory cytokines (particularly IL-6 and TNF-) increase the expression of iNOS/NOS2 in inflammatory cells within the plaque which, together with superoxide, can generate considerable amounts of cytotoxic peroxynitrite. Endothelial dysfunction stimulates leucocyte adhesion to the endothelial wall (through the increase in expression of VCAM-1 and ICAM-1) and increases vascular permeability facilitating increased uptake of LDL.

Both NOS2 and NOS3 (both identified as common genes in this study) have dual roles in the vascular environment. NOS3 promotes a vasoprotective and anti-atherogenic effect while NOS2 produces pro-inflammatory and pro-atherogenic effects [12]. The relative expression of the two isoforms of NOS is a critical factor in the outcome of cardiovascular events, and a switch fromNOS3 to NOS2 driven by inflammation exacerbates pathology.

2.2.4 Vascular remodelling and fibrosis

Apart from the more acute inflammatory and thrombotic aspects, the chronic aspect of cardiovascular disease involves the remodelling of the vasculature including thickening of the intima, hypertrophy of the medial layer and fibrosis of the adventitia. All these structural changes can be related to the activity of various growth factors, cytokines and enzymes that modify the extracellular matrix (ECM). Transforming Growth Factor Beta 1 (TGFB1) (a

common gene in this study) is a cytokine with complex dual roles in vascular biology; it is physiologically important for the maintenance of the vascular wall integrity through induction of differentiation and ECM synthesis in smooth muscle cells, but pathologically drives adverse fibrotic remodelling and loss of endothelial function at higher concentrations.

VEGF-A is primarily an angiogenic factor that promotes vascular proliferation, albeit that angiogenesis can occur at pathological concentrations in the plaque itself and is an indicator of vulnerability. Fibronectin 1 (FN1) (a common gene in this study) is a component of the ECM and wound healing. It increases in atherosclerotic plaques and may promote smooth muscle cell migration and leucocyte adhesion to the intima.

2.3 Common molecular mechanisms in COPD and CVD

Chronic inflammation at a low-grade is central to the underlying molecular mechanisms in both COPD and CVD. The shared molecular pathways in COPD and CVD create a molecular link between them, hence the clinical comorbidities.

2.3.1 Common pro-inflammatory mediators

Arguably the strongest piece of evidence for molecular cross-talk between COPD and CVD comes from the overlap of inflammatory mediator profiles found in these diseases. Indeed, both diseases share elevated systemic concentrations of IL-6, TNF, IL-1, CRP and fibrinogen [6] [8]. Studies have already provided epidemiological data to show that these mediators are not solely a consequence of these respective disease processes but that they are, simultaneously, playing a role in the pathogenesis of both diseases.

A review on IL-6 biology established that this cytokine played a key role in inflammation, immunity and a multitude of diseases across all organ systems. IL-6 works by binding to two separate pathways: either a membrane-bound receptor expressed on endothelial cells, liver and immune cells, or via a soluble receptor (sIL-6R) which, once bound to IL-6, activates nearly all cells that possess the corresponding signal transducer gp130. While it is thought that this trans-signalling pathway primarily activates pro-inflammatory responses such as endothelial activation, recruitment of leukocytes and production of acute phase reactants, classical signalling has been found to promote some pro-regenerative cellular activities.

O'Donovan and colleagues (2010) subsequently went on to demonstrate that elevated resting levels of both IL-6 and TNF were independent predictors of death in elderly people, and that these mediators can thus be considered to act as systemic activators of age-associated diseases across all organs, not specific to a single pathology.

2.3.2 NF-kB signaling as a common regulatory hub

A central mediator of chronic inflammation, the Nuclear Factor-kappa B (NF-κB) signaling pathway is involved in the pathogenesis of chronic obstructive pulmonary disease (COPD) and cardiovascular disease (CVD). NF-κB gets activated by multiple pathways, some of which are not canonical, and leads to the induction of pro-inflammatory mediators that cause tissue damage, immune imbalance, and disease development [9]. The enhanced NF-κB signaling in COPD and CVD patients has been shown, and is known to be a common molecular link between pulmonary and vascular inflammatory disorders. Neutrophils, macrophages, T lymphocytes, endothelial cells, and vascular smooth muscle cells, are only some of the various inflammatory and immune cells regulated by NF-κB that play a role in disease pathogenesis.

NF-κB activation is responsible for the increased production of cytokines, chemokines, MMPs and proteases in COPD, driving chronic airway inflammation and small airway disease. Likewise, NF-κB signaling in CVD also leads to endothelial dysfunction, vascular inflammation, atherosclerotic plaque formation and myocardial remodeling through the production of inflammatory mediators (IL-6, TNF-α, adhesion molecules, and reactive oxygen species (ROS) [9]. Neutrophils are important in pathogenesis of both diseases. In COPD, IL-8, which is a product of NF-κB regulation, causes neutrophilia and neutrophilic infiltration into the small airways. The presence of increased neutrophils in the sputum of COPD patients is associated with disease severity and is a common feature of COPD. Activated neutrophils secrete inflammatory and tissue-destructive mediators, including Cathepsin-G, proteinase-3, MMP-8, MMP-9, and neutrophil elastase, which cause the damage of alveolar tissue and mucous hypersecretion. The recruitment of neutrophils involves adhesion to endothelial cells through E-selectin and migration in response to chemotactic factors such as IL-8, chemokines and leukotrienes. Neutrophils also play a role in endothelial injury and plaque instability in CVD, through the secretion of ROS, proteases and inflammatory cytokines, which cause vascular damage and thrombosis. Another class of NF-κB-regulated inflammatory cells that play a significant role in COPD as well as CVD are macrophages [18]. COPD is characterized by a significant increase in the number of macrophages present in sputum and BAL fluid that is strongly associated with the severity of the disease. Macrophage activation by cigarette smoke induces the release of one or more of IL-8, TNF-α, leukotrienes, ROS and several MMPs, including MMP-9, that is elastolytic and promotes tissue destruction. Likewise, during cardiovascular disease, activated macrophages are found in atherosclerotic lesions and produce inflammatory cytokines, chemokines, ROS and MMPs that lead to plaque development and vascular remodeling. In both diseases, the expression of many of these inflammatory and elastolytic proteins is regulated by transcription mediated by NF-κB. The NF-κB pathway also plays a role in the recruitment and amplification of immune cells and inflammation. Monocytes are recruited and differentiated by chemokines like MCP-1, CXCR2 ligands and GRO-α secreted by macrophages. Activated macrophages also induce the production of chemokines, such as interferon-inducible T-cell α-chemoattractant (I-TAC) and interferon-inducible protein 10 (IP-10) that stimulate cytotoxic CD8+ T-cells. The CD8+ T-cells in COPD damage the alveoli by releasing perforins, granzymes and TNF-α in the small airways. In a similar fashion, activated T-cells invade the vascular lesions in CVD and promote chronic vascular inflammation and plaque instability by producing pro-inflammatory cytokines like IFN-γ and TNF-α. Furthermore, NF-κB signaling is closely linked with oxidative Stress in the two diseases. Cigarette smoke, environmental pollutants, oxidized lipids and inflammatory cytokines activate NF-κB by generating ROS, which in turn leads to a vicious cycle of oxidative stress and inflammation. Chronic activation of NF-κB eventually results in chronic immune dysfunction, tissue remodeling, apoptosis, and progressive loss of function in the lungs and cardiovascular system. Thus, NF-κB is a key common inflammatory pathway involved in the pathogenesis of COPD and CVD, and an attractive therapeutic target for the management of systemic inflammation and disease progression.

2.3.3 JAK-STAT signaling in comorbid inflammation

A key mediator of cytokine signaling in both COPD and CVD is the JAK-STAT pathway (Janus Kinase-Signal Transducer and Activator of Transcription). Signaling via IL-6 (mediated by binding to a specific receptor, and subsequent activation of JAK1 and JAK2), results in the

activation of STAT3, which subsequently dimersies, translocates to the nucleus and up-regulates transcription of STAT3 target genes [13]. These target genes encode proteins such as the acute phase reactants fibrinogen and CRP,anti-apoptotic factors (BCL-XL, MCL-1) and cell cycle regulators which all promote inflammation, cell survival and in some cases cellular senescence.

In the lungs, JAK-STAT signaling plays a role in the activation of alveolar macrophages, smooth muscle cells and airway epithelial cells promoting mucin production, airway remodeling and macrophage survival, whereas in the vascular disease setting the activated JAK-STAT3 pathway has been associated withICAM-1 upregulationand leukocyte recruitment by activating the endothelial cells and can have a number of both detrimental and protective effects in cardiomyocytes depending on its timing and duration [13].

The fundamental mechanisms of IL-6-type cytokine signaling, which involves activation of both JAK-STAT and MAPK/ERK signaling through gp130 in order to mediate the transcriptional response to IL-6, IL-11, oncostatin M, Leukemia inhibitory factor, etc. Which contribute to many inflammatory and metabolic processes has been previously described by Heinrich and colleagues (2003). As gp130 is the common mediator of signal transduction initiated by multiple IL-6-like cytokines this explain why so many disease states benefit from IL-6 blockade.

2.3.4 Matrix Metalloproteinases and tissue remodelling

Matrix metalloproteinases are a family of zinc dependent endopeptidases capable of degrading most types of extracellular matrix components. They have key roles in tissue remodelling in both COPD and CVD. MMP-2 (a common gene; MMP2) is involved in the degradation of type IV collagen the main structural component of the basement membrane and also cleaves elastin and fibronectin. In COPD it leads to degradation of the alveolar ECM, destroying the alveolar walls and causing emphysema [14]. In CVD it plays an important role in degrading the fibrous cap of atherosclerotic plaques, inducing smooth muscle cell migration and arterial wall remodelling in atherogenesis.

Thus, the balance of MMP and tissue inhibitor of metalloproteinase (TIMP; a common gene; TIMP1) activity represents a common determinant of structural integrity of the lung and vasculature [14]. Pro-inflammatory cytokines IL-6, TNF and IL-1 are strong promoters of MMP gene expression while inhibitors of TIMP expression, tipping the balance in favour of matrix destruction and tissue degradation. Therefore, the MMP-TIMP axis represents the link between the inflammatory signalling identified here and structural changes characteristic of COPD and CVD.

2.4 IL-6 as a central node

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a crucial role in the immune system and its regulation, in tissue remodeling, in senescence and the progression of chronic diseases, linking all these processes through its role in inflammation.

2.4.1 Sources and Regulation

IL-6 is produced by a variety of immune and non-immune cells such as macrophages, monocytes, dendritic cells, neutrophils, fibroblasts, endothelial cells, epithelial cells, adipocytes, smooth muscle cells and activated T lymphocytes. Recent studies also indicate that

senescent cells or fibroblasts from the stromal parts of tissues and tumor-associated cells are significant pathological contributors of IL-6 in chronic inflammatory states and during ageing.

Multiple inflammatory and stress responsive signaling pathways are involved in the regulation of the expression of IL-6. Pattern recognition receptors (PRRs) are activated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and downstream signaling pathways involve activation of transcription factors such as nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1), CCAAT/enhancer-binding protein beta (C/EBPβ), and signal transducer and activator of transcription 3 (STAT3) which are associated with hypoxia and mitochondrial dysfunction. During inflammatory responses these transcription factors regulate the rapid gene expression of IL-6. Chronic diseases like COPD and CVD cause a sustained activation of NF-κB and STAT3 pathway that leads to continuous production of IL-6 and chronic inflammation. Recent studies have also emphasized the significance of cGAS-STING pathway in the regulation of the expression of IL-6 in sterile inflammation and cellular senescence [15]. Further, the level of IL-6 is also regulated post-transcriptionally via microRNAs and mRNA stabilization. Importantly, IL-6 can also trigger positive feedback loops through STAT3mediated signaling that augment and sustain inflammatory signaling in diseased tissues.

IL-6 has two main pathways that mediate its biological effects, classical signaling and trans-signaling. Classical signaling involves binding of IL-6 to membrane-bound IL-6 receptor (mIL-6R) which is expressed primarily in hepatocytes and certain immune cells, and subsequent association with glycoprotein 130 (gp130). This pathway is typically linked to protective functions (tissue repair, regeneration, host defense). The difference is that trans-signaling is mediated by binding of IL-6 to soluble IL-6 receptor (sIL-6R) which activates gp130 expressing cells in the body. The ability of IL-6 trans-signaling to broaden the spectrum of responsive cells and its link to chronic inflammation, endothelial dysfunction, fibrosis, vascular remodeling, and tissue destruction in COPD and CVD, respectively, indicate the importance of gp130. Therefore, IL-6 is a key molecule that contributes to the progression of chronic inflammatory diseases through persistent activation of the IL-6/JAK/STAT3 axis, resulting in oxidative stress, fibroblast activation, immune dysregulation and cellular senescence.

2.4.2 Role of IL-6 in Chronic Obstructive Pulmonary Disease (COPD)

Inflammation has now been recognized as one of the major underlying mechanisms of cardiovascular disease (CVD) and IL-6 has been characterized as one of the most important inflammatory mediators associated with vascular disease. Elevated circulating levels of IL-6 have been associated with myocardial infarction, atherosclerosis, stroke and heart failure [6].

Endothelial dysfunction, defined as a reduction in the bioavailability of nitric oxide, enhanced oxidative stress and enhanced vascular permeability, is among the earliest events in the development of atherosclerosis. Endothelial dysfunction is one of the earliest consequences of IL-6, in which IL-6 directly induces the expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin on endothelial cells increasing the attachment and migration of leukocytes into the vascular wall.

Recruitment and activation of monocytes and macrophages within atherosclerotic plaques also occurs through IL-6. Activated immune cells will secrete other inflammatory cytokines and reactive oxygen species (ROS) that can enhance inflammation within the vascular wall. In

chronic IL-6 signaling, cell proliferation, migration and ECM remodeling of the vascular smooth muscle cells as well as plaque instability.

A major downstream effect of IL-6 signaling is hepatic induction of acute phase proteins like CRP that itself has downstream effects on endothelial dysfunction and thrombosis. There is a well-established correlation between elevated levels of IL-6 and CRP as well as the association with cardiovascular risk and adverse outcome.

The impact of IL-6 trans-signaling on cardiovascular pathology is starting to be elucidated. Signaling by the soluble IL-6 receptor elicits responsiveness in a wider array of cells, leading to chronic inflammation of the vessel wall and subsequent myocardial remodeling and fibrosis. Furthermore, IL-6/JAK/STAT3 axis is linked to cardiac hypertrophy and the progression of heart failure.

It is also important to note that senescence in aging subjects may promote the production of IL-6 via the senescence-associated secretory phenotype (SASP). Endothelial and vascular smooth muscle cells release IL-6 leading to chronic low-grade inflammation and progression of vascular aging and atherosclerosis.

2.4.3 Role of IL-6 in CVD

Inflammation is recognized as one of the most important components of cardiovascular disease, and IL-6 is one of the most important inflammatory mediators contributing to vascular disease. Elevated serum levels of IL-6 predict myocardial infarction, atherosclerosis, stroke and heart failure.The initial steps in the development of atherosclerosis involve endothelial dysfunction, in which there is reduced bioavailability of nitric oxide, increased oxidative stress and increased permeability of the vascular endothelium. IL-6 is important in mediating this endothelial dysfunction through its promotion of expression of adhesion molecules, ICAM-1, VCAM-1, promoting recruitment and infiltration of leukocytes into the vessel wall.

IL-6 further promotes the recruitment and activation of monocytes and macrophages within atherosclerotic lesions. Activated monocytes and macrophages produce more inflammatory mediators and reactive oxygen species, exacerbating the ongoing inflammation in the vessel wall. Chronic activation of the IL-6 signaling pathway further promotes endothelial cell proliferation, migration and matrix deposition and also stimulates proliferation and migration of vascular smooth muscle cells.

One of the major downstream actions of IL-6 is stimulation of hepatic acute-phase proteins, most notably C-reactive protein, which in turn also mediates endothelial dysfunction and thrombosis. Increased serum levels of both IL-6 and CRP are predictive of adverse cardiovascular outcomes.More recently it has become clear that the signaling through IL-6 trans-signaling has an important role in cardiovascular disease pathogenesis. The use of soluble IL-6 receptor mediates a broader pattern of cells that respond to IL-6 which contributes to chronic vascular inflammation, fibrosis and myocardial remodeling. Continued stimulation of the IL-6/JAK/STAT3 axis has been described in heart failure and cardiac hypertrophy.

There is also evidence for age related increased production of IL-6 via the senescence associated secretory phenotype (SASP). Senescent cells in the vasculature, such as endothelial cells and vascular smooth muscle cells secrete IL-6 thus perpetuating the low-grade

inflammation characteristic of vascular disease and accelerating vascular aging and atherosclerosis [16].

2.4.4 IL-6 and the Senescence-Associated Secretory Phenotype (SASP)

IL-6 is a central and non-redundant element of the senescent cell associated secretory phenotype (SASP)- a repertoire of inflammatory cytokines, chemokines, and proteases secreted from irreversibly arrested senescent cells. Causes of senescence such as telomere shortening, oxidative damage, DNA damage and cigarette smoking are all relevant to both COPD and CVD. Once cells are senescent, transcription factors like NF-B, C/EBP and mTORC1 drive the constant expression of IL-6. Crucially, secreted IL-6 exerts both autocrine and paracrine autocrine regulation of senescence by virtue of JAK/STAT3 mediated upregulation of cell cycle inhibitors p21 and p16 [4], this positive feedback loop of senescence induces IL-6 and that IL-6 propagates senescence. This is the reason that even a small population of senescent cells in an organ can drive inflammatory pathology across that organ. In COPD, cigarette smoke acts to drive senescence in alveolar epithelium, airway epithelium, fibroblasts and pulmonary vasculature: IL-6 is expressed by senescent alveolar epithelium and drives metalloproteinase production, alveolar collapse (emphysema) and inflammation of the airways [11]. In CVD, senescence drives proliferation of endothelial cells and vascular smooth muscle in atherosclerotic plaques: senescent endothelium upregulates endothelial activation factors like ICAM-1 in an IL-6 mediated fashion [16], and senescent macrophages are found to fail to phagocytose immune debris yet remain IL-6 secreting, the overall process leads to continued plaque inflammation and unstable plaques. The most compelling reason for our analysis, and which allows us to explain co-progression of COPD-CVD comorbidity, is the fact that IL-6 mediated reciprocally drives organ-level Senescence-SASP driven systemic inflammation- lung Senescence-SASP drives the production of circulating IL-6, which acts on vasculature-driven organ level Senescence-SASP in the endothelium and vice versa. In terms of therapeutic targets, senolytics (drugs which kill senescent cells, like dasatinib/quercetin) and senomorphics (drugs which target secretion of the SASP, without killing senescent cells, like metformin) are available which may prevent IL-6 secretion and have pre-clinical lung and vascular efficacy [17].

The central connectivity, its dual role as a SASP effector and a senogenic factor, and the fact that IL-6 is 'targetable' with biologicals like tocilizumab and its mechanism in bridging pulmonary and cardiac systemic immune dysfunction with COPD-CVD comorbidity suggests that interventions targeting this pathway may be the most potent approach to resolving the comorbidity and slowing age-related progression.

CHAPTER 3: METHODOLOGY

3.1 Computational Pipeline overview

A step-wise computational pipeline was adopted for exploring the molecular crosstalk of COPD and CVD. It consists of 5 stages: (i)dataset acquisition, (ii)gene selection and curation, (iii)identification of common genes, (iv)network construction and topology analysis, and (v)pathway enrichment and therapeutic target assessment. The details of each step are introduced below. The software packages and databases used were standard in the field of bioinformatics with reproducible and transparent methodologies.

3.2 Dataset Acquisition

Microarray expression datasets from both Chronic Obstructive Pulmonary Disease (COPD) and cardiovascular disease (CVD) were systematically retrieved from the Gene Expression Omnibus (GEO) database, which is a public repository of high throughput genomics data. The datasets were selected based on the basis of criterias like human tissue samples, case-control study designs, and availability of raw or normalized expression matrices etc. for each disease. The COPD datasets collected primarily contained lung tissue of COPD patients and control healthy subjects, while the CVD datasets contained either cardiac or vascular tissue samples from patients diagnosed with atherosclerotic cardiovascular disease and a comparison control group.

3.3 Gene Selection

A comprehensive resource integrating chemical-gene-disease interactions, such as the Comparative Toxicogenomics Database (CTD), was utilised for systemic gene retrieval. For each disease under study (COPD and CVD), the first 100 disease-related genes were retrieved with official names or the respective GEO ID. The disease-relatedness of genes was ranked using the pre-compiled gene-disease association score of the CTD (which is an aggregated measure of direct and inferred interactions reported in published literature). To assure biological relevance and limit false positives, only genes with a high confidence association score were taken.

3.4 Common Genes Identification

The identification of overlapping genetic signatures between COPD and CVD was performed computationally using Python programming language. Specifically, the panda's library—a highly efficient data processing and analysis toolkit—was employed for data manipulation and intersection operations. The top 100 gene lists for COPD and CVD were loaded as separate pandas Series objects, and the common gene set was derived using set intersection methods. The resulting list of shared genes represented the molecular intersection between the two diseases, providing a focused gene set for subsequent network and pathway analyses. All operations were validated by manual cross-referencing to ensure accuracy.

3.5 Network and Pathway Analysis

The set of common genes was subjected to an integrative analysis in a web-based comprehensive gene expression analysis and visualization network tool called Network Analyst. A tissue-specific co-expression network was generated by plotting the set of common

genes onto precomputed co-expression data from two related human tissues, lung tissue (representing the etiology of COPD) and atrium tissue (representing cardiovascular pathogenesis). Lung and atrium tissues were selected based on their roles in organ systems related to the pathophysiology of COPD and CVD, respectively. Using topological scoring algorithms of Network Analyst, the highest connected nodes (degree centrality) with highest betweenness centrality of the bipartite co-expression network were designated as the hub genes.

Next, functional enrichment analysis was performed in the highly utilized gene set enrichment analysis tool, Enrichr. Significant biological pathways were identified from the Reactome Pathways 2024 database, which has a collection of curated, peer-reviewed human biological pathways and reactions. Statistical significance of each pathway was determined using adjusted p-values (Benjamini-Hochberg correction for multiple tests), with $p < 0.05$ representing significance. The pathways with the highest enrichment were highlighted in regards to inflammation, immune response, and vasculature dysfunction.

3.6 Evaluation of Therapeutic Target

The hub genes from tissue-specific co-expression network were examined as potential therapeutic target using a two- prong approach. Firstly, structural and functional interconnectedness among hub genes in common disease network was identified, where we search for the nodes that act as link between two disease subnetworks (COPD and CVD subnetworks). Second, the hub genes were correlated with aging related pathological progression and in particularly focused on inflammaging, a concept defining chronic low-grade inflammation in aging system and cellular senescence. The hub genes, which not only display significant interconnectedness among in disease network but also have established connection with inflammaging or cellular senescence related pathways were chosen as ideal therapeutic targets. The two-prong evaluation helped us identifying the targets that can link two diseases by bridging them in mechanistic way and also can be addressed as targeting the age-related immune dysregulation.

CHAPTER 4: RESULTS

4.1 Overview of Results

We integrated publicly available transcriptomic and disease gene association data for both COPD and CVD. This integration revealed a congruent set of molecular observations that describe the common inflammatory basis of these two diseases. We show the findings, in the following order: (i) Identification of genes that are common; (ii) Network topology and hub gene analysis; (iii) Pathway enrichment; (iv) Cytokine profile and immune dysregulation.

4.2 Identification of Common Genes Between COPD and CVD

Computation intersection analysis on top 100 COPD related genes and top 100 CVD related genes obtained from CTD database yielded 19 shared genes between COPD and CVD. The shared genes list shown in Table 1 and covered with a wide range of functional genes involved in, such as cytokine and mediator of inflammation (IL6, IL10, IL1B, TNF, CRP, TGFB1), adhesion molecule (ICAM1, FN1), transcription factor and co-activator (RELA, FOS, TP53), growth factor (VEGFA), vasoactive mediator (EDN1), antioxidant enzymes (CAT, SOD2), nitric oxide synthase (NOS2, NOS3), matrix remodeling enzymes and inhibitors (MMP2, TIMP1).

The varied list of functional genes identified reflected the multi-dimensional features of common pathology. This list contains not only those with broad range transcriptional control genes (RELA, TP53, FOS) at the upstream level but also those with direct product-mediated tissue damage and endothelial dysfunction genes (ICAM1, MMP2, VEGFA) at the effector level, suggesting the involvement of multi-layer shared regulatory mechanisms at both transcriptional and effector levels in COPD and CVD.

Table 1: Common genes identified between COPD and CVD

S.No.	Gene Symbol	Full Name	Primary Function
1	TP53	Tumor Protein p53	Apoptosis, cell cycle arrest, senescence regulation
2	TIMP1	TISS Inhibitor of Metalloproteinase 1	ECM remodeling inhibition, anti-apoptotic
3	RELA	NF-kB p65 subunit	Transcriptional activator of inflammatory genes
4	ICAM1	Intercellular Adhesion Molecule 1	Leukocyte-endothelial adhesion, vascular inflammation
5	CAT	Catalase	Antioxidant enzyme, H2O2 decomposition
6	CRP	C-Reactive Protein	Systemic inflammation marker, complement activation

S.No.	Gene Symbol	Full Name	Primary Function
7	VEGFA	Vascular Endothelial Growth Factor A	Angiogenesis, vascular permeability
8	IL6	Interleukin-6	Pleiotropic cytokine, acute phase response hub
9	TGFB1	Transforming Growth Factor Beta 1	Fibrosis, immune suppression, TGF-β signaling
10	IL10	Interleukin-10	Anti-inflammatory cytokine, immune resolution
11	IL1B	Interleukin-1 Beta	Pro-inflammatory cytokine, inflammasome product
12	EDN1	Endothelin-1	Vasoconstriction, vascular tone regulation
13	TNF	Tumor Necrosis Factor	Pro-inflammatory cytokine, NF-kB activator
14	FOS	FBJ Murine Osteosarcoma Viral Oncogene	AP-1 transcription factor, stress response
15	MMP2	Matrix Metalloproteinase 2	ECM degradation, tissue remodeling
16	SOD2	Superoxide Dismutase 2	Mitochondrial antioxidant defense
17	NOS2	Nitric Oxide Synthase 2 (iNOS)	Inflammatory NO production
18	NOS3	Nitric Oxide Synthase 3 (eNOS)	Vascular NO synthesis, endothelial function
19	FN1	Fibronectin 1	ECM glycoprotein, cell adhesion and migration

4.3 Tissue-Specific Co-expression Network Construction

The 19 common genes were subjected to NetworkAnalyst in order to establish tissue-specific co-expression networks from lung and cardiac (atrial) expression data. The constructed network was composed of the 19 input nodes and several other interacting partners selected from tissue-specific co-expression networks. The visualised network presented one large central cluster with several Highly interconnected nodes and some of the genes showed clearly higher degree centrality than others (Figure 1).

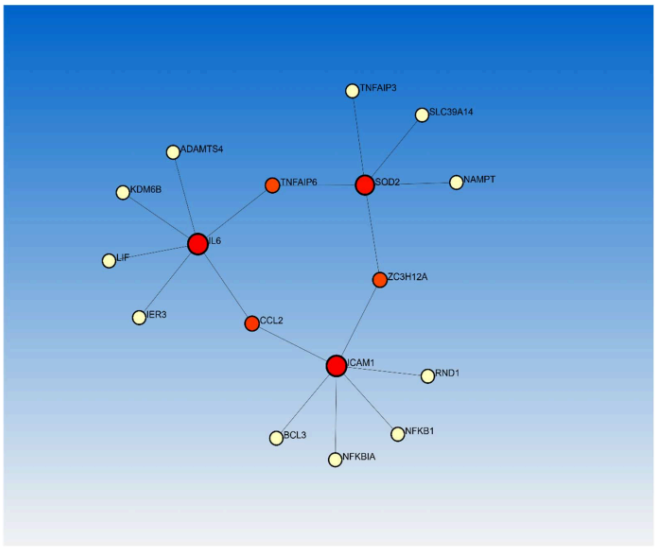


Fig. 1: The tissue specific co-expression network indicating the central hub genes (red)

4.3.1 Hub Gene Identification

Network topology analysis indicated IL-6 to be the primary hub gene with the largest values for degree centrality, betweenness centrality and closeness centrality across all network nodes. IL-6 showed connections to, but was not limited to, ICAM1, NFKBIA, TNFAIP3, SOD2 and CCL2. IL-6 thus shows regulatory links with numerous elements throughout the network, with its most key functional connections being with cytokine signaling, NF-kB regulation and endothelial activation, key pathway in both COPD and CVD.

Other hub genes were TNF, RELA, and IL1B with high connectivity within the network, as these are known to have regulatory relationship with IL-6 in terms of being upstream and downstream elements, respectively. The significant hub status of RELA (p65 NF-B) is noteworthy, reflecting the central role that NF-kB signaling appears to play in integrating inputs from numerous pathways of inflammation in both diseases.

4.3.2 The IL-6 sub-network architecture

Further scrutiny of the IL-6 sub-network revealed an ordered and nested interaction set. NFKBIA (IB), an immediate inhibitor of NF-kB was an immediate IL-6 network interactor, indicating the co-operative control between IL-6/JAK-STAT signaling and NF-kB activation. TNFAIP3 (A20), an NF-kB pathway-inhibiting deubiquitinase, was another IL-6 immediate

network interactor, suggesting the activation state induced by IL-6 was regulated by negative feedback control of A20 in this network setting. The linkage between IL-6 and ICAM1 is functionally interesting in this context. ICAM-1 is an expressed gene on activated endothelial cells and its expression is responsible for the firm adhesion of leukocytes (neutrophils, monocytes, lymphocytes) to the endothelial cell and thus critical for leukocyte trans endothelial migration into tissues during inflammatory responses. This upregulation of ICAM-1 by IL-6 represents a potential functional link between IL-6 induced inflammatory response in the system and inflammatory infiltration and tissue destruction in the lungs (COPD) and atherosclerotic plaques.

4.3 Pathway Enrichment Analysis

In total 10 significantly enriched pathways were found (adjusted $p < 0.05$) upon conducting a functional enrichment analysis using Enrichr on Reactome Pathways 2024. The 10 most significant pathways are shown in Table 2.

Table 2: Significantly enriched Reactome pathways for the common gene set

Pathway	p-value	Genes Involved	Disease Relevance
IL-4 and IL-13 Signaling	< 0.001	IL6, IL1B, STAT6, TGFB1	Th2-driven airway inflammation (COPD)
IL-10 Signaling	< 0.01	IL10, STAT3, IL6	Anti-inflammatory resolution pathway
Cytokine Signaling in Immune System	< 0.001	TNF, IL6, IL1B, RELA	Cross-disease inflammatory hub
NF-kB Signaling	< 0.001	RELA, TNF, IL1B, ICAM1	Endothelial activation in CVD & COPD
JAK-STAT Signaling	< 0.01	IL6, STAT3, TGFB1	Systemic cytokine amplification
Signal Transduction	< 0.05	VEGFA, TGFB1, EDN1	Vascular remodeling
Hemostasis	< 0.05	FN1, MMP2, VEGFA	Vascular integrity and coagulation
Innate Immune System	< 0.001	CRP, TNF, IL1B, NOS2	Pattern recognition and first response
Cellular Responses to Stress	< 0.01	TP53, SOD2, CAT	Oxidative stress and senescence
Cellular Responses to Stimuli	< 0.05	FOS, TGFB1, VEGFA	Adaptive cellular programming

Fig. 2 : Reactome Pathway representing shared inflammatory pathways

4.4.1 Interpretation of pro-inflammatory pathway enrichment

The enrichment of IL-4/IL-13 and general interleukin signaling pathways demonstrates the marked enrichment of cytokine genes (IL6, IL1B, IL10, TGFB1, TNF) in the common gene set. IL-4 and IL-13 are classically viewed as Th2 cytokines associated with allergic inflammation and asthma but their signaling pathways are intertwined with the global inflammatory network active in COPD and CVD. Activation of JAK1/TYK2 by IL-4 and STAT6 by IL-13 leads to IgE synthesis, macrophage polarization, and increased airway smooth muscle responsiveness. Their enrichment in the common gene set shows that elements of Th2 immunobiology are present in the inflammatory milieu shared by COPD and CVD. This may reflect variability within COPD etiologies in terms of their tendency to induce airway inflammation driven by Th2 activity.

4.4.2 Concomitant activation of opposing signaling pathways

Most importantly, enrichment of IL-4/IL-13 signaling pathways (pro-inflammatory with regard to tissue eosinophilia and remodeling) along with IL-10 signaling (anti-inflammatory, immunosuppressive) suggests that the common gene set contains both amplifiers and resolvers of inflammation. The concomitant activation of opposing cytokine signaling pathways does not fit the paradigm of a singular, pro-inflammatory state, rather, it is descriptive of an immunoregulatory imbalance where the "amplifiers" and the "resolvers" of immune activation act simultaneously, without complete resolution of homeostatic mechanisms.

This paradox of concurrently active pro- and anti- inflammatory signaling has been characterized in chronic inflammatory diseases and inflammaging as "immune exhaustion" or "regulatory stress." Continuous induction of NF-kB driven pro-inflammatory gene transcription while negative feedback signals via IL-10 and TNFAIP3 are also activated does not result in resolution of inflammation but rather an unbalanced, sustained, inflammatory state. The unchecked, persistent, inflammatory stimuli in the lung and vasculature drive chronic tissue damage in COPD and CVD.

CHAPTER 5: DISCUSSION

5.1 IL-6 as the Central Molecular Hub

IL-6 emerged as a dominant molecular hub between COPD and CVD. This finding agrees with previous studies showing a high level of comorbidity between these two diseases. IL-6 plays a crucial role in mediating inflammatory networks by triggering itself as well as NF-B activation, initiating a positive feed forward loop amplifying the inflammatory response. Localized NF-B activation happens in immune and structural cells of the lungs and vasculature, and also systemically in the liver and endothelial cells throughout the body; thus, IL-6 has the potential to link COPD and CVD. The self-amplifying inflammatory loop is even more problematic in comorbidity. Clinical studies corroborate our network architecture: blood IL-6 is significantly increased in comorbidity as compared to individual diseases, and correlates with severity of disease. In fact, cardiovascular event rates were attenuated in the CANTOS trial by IL-1 inhibition, which increases IL-6, demonstrating the causal involvement of IL-6 pathway in disease processes. Our network shows NFKBIA (IB) and TNFAIP3 (A20) co-expressed with IL-6; these genes usually function to limit NF-B mediated inflammation but, since they were upregulated along with IL-6, show failed termination of the inflammatory loop, consistent with the hyperactivation of NF-B which both conditions share. In fact, while IL-6 does function differentially (damaging in lung and vessels, and stimulating acute phase response in liver), the major role in a common hub gene for both diseases stands out. Treatment targeted to IL-6 pathway may prove beneficial in comorbidity.

5.2 Immune Dysregulation as a driver of comorbidity

The simultaneous enrichment of pro-inflammatory (IL-4/IL-13) and anti-inflammatory (IL-10) signalling cascades in the common gene set suggests novel mechanisms that could contribute to the chronicity of the COPD-CVD comorbidity. One traditional concept for chronicity in inflammatory disease is an amplification of the pro-inflammatory signalling cascade and persistent tissue damage. The pathway enrichment results presented in this study challenges that concept by showing evidence for both amplification of inflammatory response and resolution programmes in the shared network, but that a homeostatic equilibrium is not established.

These data are compatible with the immunological concept of regulatory stress: a state of inflammation characterized by activation of simultaneously offensive (pro-inflammatory) and defensive (anti-inflammatory) immune pathways without resolution of underlying conflict. Operationally, the observation that both inflammation and resolution signalling pathways are activated in the shared molecular network, suggests that only suppressing the pro-inflammatory signalling pathway (e.g. With a monotherapy targeting anti-TNF or anti-IL-1) may not be effective to restore homeostatic immune functioning, as the resolution pathway is simultaneously deficient (e.g. deficiency in IL-10 signaling). These data thus provide a rationale for targeting both the pro-inflammatory and the resolution pathways of immune signaling in combination for therapeutic approaches.

5.3 Therapeutic implications

The results of this study have several important therapeutic implications. 1. The fact that IL-6 acts as a central nodal point within the COPD-CVD inflammatory network is strongly indicative of the use of anti-IL-6 therapies for patient management where comorbid COPD and

CVD coexist. Currently, only two IL-6 receptor antagonists, approved for the management of rheumatoid arthritis (tocilizumab and sarilumab), have been explored, and to our knowledge have never been investigated in this particular condition of combined comorbidity. An interventional study with spirometric decline, exacerbation rate, and cardiovascular events as its co-primary end points will definitively establish or refute the therapeutic benefit of such treatment. 2. Enrichment for the JAK-STAT pathway, downstream of IL-6, provides substantial grounds for a therapeutic approach utilizing JAK inhibitors (e.g., baricitinib, upadacitinib) to ameliorate the inflammatory environment within multiple comorbid diseases. As this patient population poses an increased risk of adverse cardiovascular events, comprehensive evaluation of cardiovascular function while utilizing these agents will be imperative. 3. The presence of senescent pathways and the ubiquity of the TP53 gene further suggest a need for investigation into the use of senolytic (e.g., dasatinib plus quercetin) and senomorphic (e.g., rapamycin based strategies) therapies as potential therapeutic avenues for aging comorbidities, especially when employed in animal models of emphysema and atherosclerosis. 4. ICAM-1 serves not only as a highly accurate marker of anti-IL-6 treatment efficacy when its serum levels are analyzed, but also as a direct target for drug development that is downstream of IL-6 (mediated by cell adhesion). Collectively, numerous treatments can be derived from our observations that will serve to revert the dysregulation of the aged immune system in comorbid conditions.

5.4 Study Limitations

There are several key limitations of this computational study that should be noted. Firstly, the method used for gene selection was the top 100 CTD ranked genes for each disease. However, this may not represent the complete set of genes involved in the disease state and may have missed genes with lower inference scores but higher biological relevance. Future analyses including all CTD associated genes, or using multiple databases for gene-disease association (DisGeNET, OMIM) would result in a broader common gene set. Secondly, the gene co-expression network shown above represents correlational relationships between gene expression profiles and is not a representation of causal regulatory interactions. Two genes co-expressed does not necessarily mean they interact at protein level and such interactions require empirical confirmation. In the future, the integration of protein-protein interaction data into co-expression networks would lead to more biologically relevant networks. Thirdly, the pathway enrichment analysis performed used a list of 19 genes and so could have been limited by statistical power and incomplete pathway coverage. The results are dependent upon the definition of background genes and pathway database; here the latest version (2024) of the Reactome Pathways database was used to give the best possible, current evidence of pathway interactions. Other pathways such as KEGG and GO biological process could be searched in future studies to provide more comprehensive information. Fourthly, sex differences, aging and ancestry have been excluded as covariates or factors to consider for this study, as all three are known to influence the expression of inflammatory genes and risk for both COPD and CVD. These factors would need to be examined in future studies in stratification and comparative analyses.

5.5 Future directions

From the current results several lines of research can be initiated. On the experimental side, the therapeutic hypotheses generated by the network analysis should be investigated in primary

human bronchial epithelial and alveolar macrophage as well as human endothelial cells cultured in cell culture system. This experiment, treatment of the cells with IL-6 followed by investigation of ICAM-1, NFKBIA and TNFAIP3 expression will serve as the proof of in silico networks. This in vitro proof of principle for therapy targeting IL-6R pathway would be obtained when endothelial cells, alveolar macrophages and bronchial epithelial cells are treated with anti-IL-6R antibodies and NF-B activation, ICAM-1 cell surface expression and MMP secretion are investigated.

This proposed network analysis and subsequent therapeutic hypotheses also require confirmation by experimental approaches in animal models where both emphysema and atherosclerosis are present such as cigarette smoke-exposed ApoE/ mice to demonstrate that either IL-6 blockade or JAK inhibition attenuates lung inflammation and atherosclerotic burden at the same time. The results obtained could support further translation into human clinical trials in COPD-CVD comorbidities.

On the computational level, by combining scRNA-seq from both lungs and atherosclerotic arteries of COPD and atherosclerotic subjects, cell-type specific resolution of the network will be possible that may suggest individual cell types (i.e., inflammatory macrophages and senescent endothelial cells) to be important hubs in disease crosstalk that cannot be revealed by the bulk transcriptome analysis. This single-cell resolution marks the future of research on molecular comorbidity.

CHAPTER 6: CONCLUSION AND FUTURE PERSPECTIVES

6.1 Summary of main conclusions

This dissertation has conducted a systemic, biology-based study of the molecular cross-talk between Chronic Obstructive Pulmonary Disease (COPD) and Cardiovascular Disease(CVD), the overall aims of which were to elucidate shared, underlying molecular drivers for the comorbidity and to propose the molecularly rational therapeutic approach.

The main conclusions from the investigation are:

i. A common set of 19 genes was derived, the overlap between the COPD and CVD gene sets. These genes fall within the broader functional classes of cytokines, adhesion molecules, transcription factors, antioxidant enzymes, nitric oxide synthases and matrix remodelling proteins, and these constitute the network of genes involved in common inflammation and structural pathological processes which underly both disease entities.

Ii. In tissue-specific co-expression network analyses, IL-6 was found to be the most connected gene in the common network, having the highest degree, betweenness and closeness centrality. It forms a hub structure which directly connects genes involved in cytokine signalling, negative feedback via NFKB, endothelial activation and oxidative stress (i.e. ICAM1, NFKBIA, TNFAIP3, SOD2) within both pulmonary and cardiac contexts.

Iii. Pathway enrichment analysis demonstrated simultaneous enrichment of both pro-inflammatory pathways (IL-4/IL-13, innate immune signalling) and anti-inflammatory pathways (IL-10). Thus, immune dysregulation- the so-called 'regulatory stress' - characterises the common molecular context of COPD and CVD where the amplificatory and resolution arms of immune response are simultaneously activated but homeostasis is not restored.

Iv. The IL-6 signalling axis (IL-6, the JAK-STAT pathway, NF-B, the downsteam target ICAM-1) emerges as the most promising target cluster. Monoclonal antibodies targeting IL-6/IL-6R, JAK inhibitors, RNA interference of IL-6 are suggested for experimental validation in the comorbid COPD/CVD patient population.

6.2 Contribution to the field

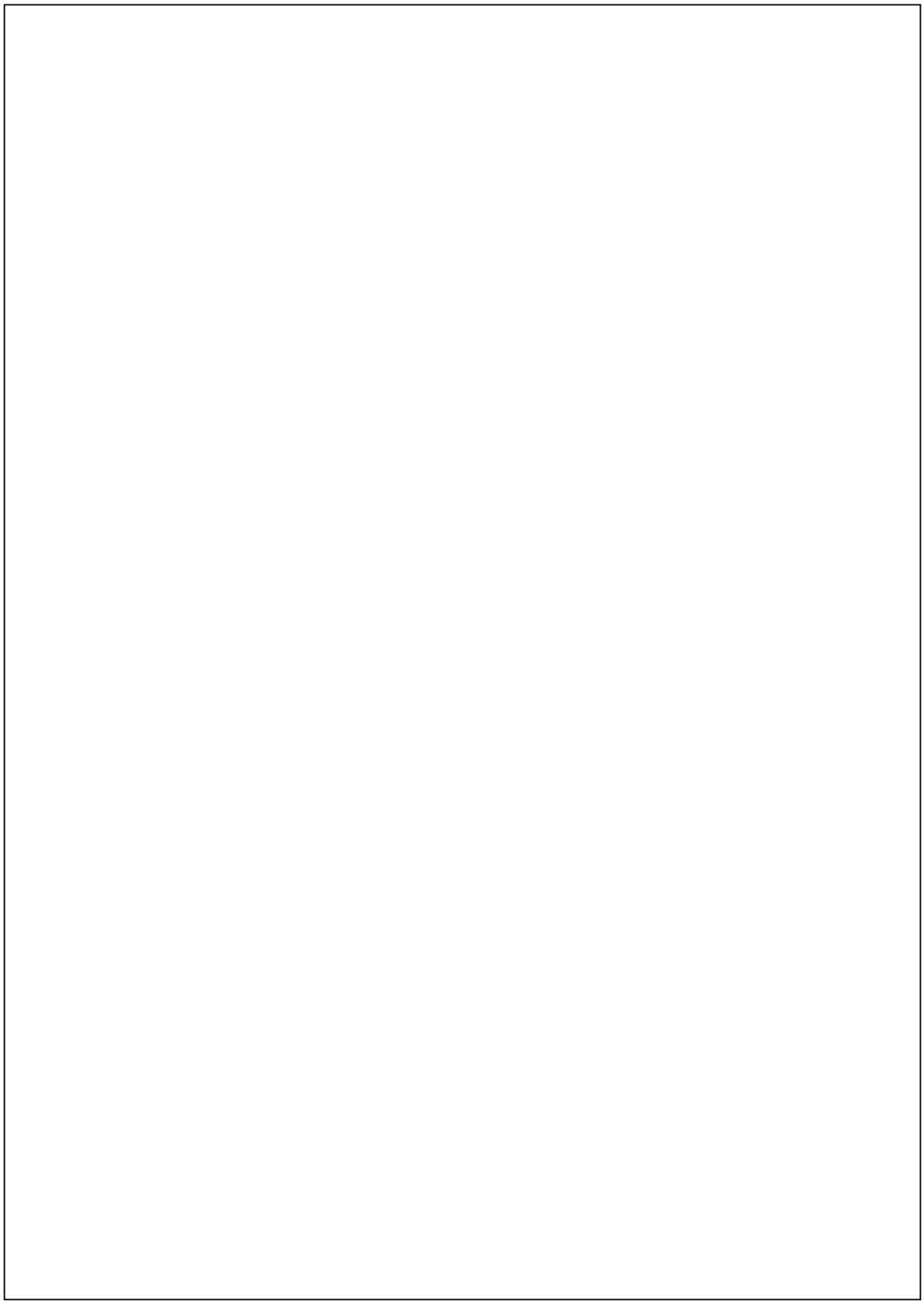
This dissertation offers the following contributions to the burgeoning field of comorbidity biology and network medicine. Unlike standard protein interaction networks, it provides biologically supported, tissue relevant, discovery of shared molecular hubs by applying tissue-specific co-expression network analysis. By placing these findings in the context of inflammaging and cellular senescence, the dissertation integrates network analysis with age related disease biology and provides a mechanistic basis to the COPD-CVD comorbidity being more prevalent in the older cohort. By integrating a large review of literature with novel computational results, the dissertation provides a useful resource for all those involved in pulmonary-cardiovascular interaction, researchers and clinicians alike.

6.3 Clinical Relevance

The clinical relevance of the research detailed in this thesis stems from the identification of druggable targets for a currently undertreated population. Patients co-existing with COPD and CVD present a worse prognosis than the patients who possess either individual condition, and the current treatments for each of these individually designed diseases do not fully tackle the common molecular drivers of the comorbidity. The therapeutic approach framed around IL-6, proposed in this dissertation, supports the potential to reposition existing anti-IL-6 biologics and JAK inhibitors - already proven to be safe and effective in other inflammatory conditions - to target COPD-CVD comorbidity. Clinical trials using this paradigm with correctly defined comorbid populations are therefore the crucial next step to translating this research to patients.

6.4 Future Perspectives

Several future technological and conceptual advancements are anticipated to significantly advance research into pulmonary-cardiovascular comorbidity. Single-cell multi-omics, which aims to integrate single-cell transcriptomics, epigenomics, and proteomics, will facilitate an improved cell-type resolution map of shared inflammatory pathways. Spatial transcriptomics will identify precisely where this shared gene expression programme is occurring within the disease lungs and vasculature, pinpointing where the molecular crosstalk is strongest, such as the perivascular space or the alveolar-capillary interface. AI and machine learning will also play a role in dissecting multi-disease genomic data and to help predict which COPD patients are at highest cardiovascular risk based on their molecular signature [19]. Patients stratified by molecular sub-type will allow a more precise form of medicine, targeting them to particular therapy according to their own molecular disease profile. Furthermore, new drug delivery mechanisms for biological and RNA-based therapies, such as inhaled or intravascular systems, will allow increased organ-specific targeting of the pulmonary-cardiovascular molecular interface [20].



Thesis

ORIGINALITY REPORT

9%	6%	7%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.mdpi.com Internet Source	1%
2	www.frontiersin.org Internet Source	<1%
3	Wadhwa, Ridhima. "Understanding and Targeting Molecular Mechanisms of Respiratory Diseases.", University of Technology Sydney (Australia) Publication	<1%
4	pmc.ncbi.nlm.nih.gov Internet Source	<1%
5	www.science.gov Internet Source	<1%
6	P.J. Barnes, S.D. Shapiro, R.A. Pauwels. "Chronic obstructive pulmonary disease: molecular and cellularmechanisms", European Respiratory Journal, 2003 Publication	<1%
7	biomateng.com Internet Source	<1%
8	Proteases and Their Receptors in Inflammation, 2011. Publication	<1%
9	Submitted to Wageningen University Student Paper	<1%
10	www.scinapse.io Internet Source	<1%

11	Aristidis Tsatsakis. "Telomeres - Biomarkers of a Healthy Life and Successful Aging", Jenny Stanford Publishing, 2025 Publication	<1 %
12	Massimo Volpe. "Cardiovascular Disease Prevention", CRC Press, 2026 Publication	<1 %
13	mdpi-res.com Internet Source	<1 %
14	www.spandidos-publications.com Internet Source	<1 %
15	Andrzej Mackiewicz, Irving Kushner, Heinz Baumann. "Acute Phase Proteins - Molecular Biology, Biochemistry, and Clinical Applications", CRC Press, 2020 Publication	<1 %
16	gut.bmj.com Internet Source	<1 %
17	www.ncbi.nlm.nih.gov Internet Source	<1 %
18	Submitted to Roehampton University Student Paper	<1 %
19	www.precisionbusinessinsights.com Internet Source	<1 %
20	Debasis Bagchi, Kiran Bhupathiraju, Samudra Prosad Banik. "Health Benefits of (Shatavari) - A Comprehensive Guide to the Medicinal and Nutritional Potential of Shatavari", CRC Press, 2026 Publication	<1 %
21	Kemunto, Brenda. "Differential Susceptibility of APOE4 and APOE3 Mice to LPS-Induced	<1 %

	Neuroinflammation: Effect of SIRT3 Activator Honokiol.", North Carolina Central University Publication	
22	scholarship.rice.edu Internet Source	<1%
23	Dilip Ghosh, Prasad Thakurdesai. "Fenugreek - Traditional and Modern Medicinal Uses", CRC Press, 2022 Publication	<1%
24	Submitted to Eastern Oregon University Student Paper	<1%
25	Tianming Feng, Yixiao Wei, Zihan Wang, Jia Sun, Ruigong Zhu. "Diet, the protective bridge connecting nutrition and cardiovascular health: A review", Food Chemistry: X, 2026 Publication	<1%
26	Dilyara G. Yanbaeva, Mieke A. Dentener, Eva C. Creutzberg, Emiel F. M. Wouters. "Systemic Inflammation in COPD: Is Genetic Susceptibility a Key Factor?", COPD: Journal of Chronic Obstructive Pulmonary Disease, 2009 Publication	<1%
27	Peter Gloviczki, Michael C. Dalsing, Bo Eklöf, Fedor Lurie, Thomas W. Wakefield, Monika L. Gloviczki. "Handbook of Venous and Lymphatic Disorders - Guidelines of the American Venous Forum", CRC Press, 2017 Publication	<1%
28	Shamim I. Ahmad. " Aging: Exploring a Complex Phenomenon", CRC Press, 2017 Publication	<1%
29	Submitted to University of Central Florida Student Paper	<1%

30	Razia Khanam, Divya Vohora. "Senotherapeutics - From Cellular Senescence to Disease Management", CRC Press, 2026 Publication	<1 %
31	mycanadianpharmacystore.com Internet Source	<1 %
32	www.healthvermont.gov Internet Source	<1 %
33	Submitted to UMass Amherst Canvas Student Paper	<1 %
34	Submitted to University of Wales Swansea Student Paper	<1 %
35	openscience.ink Internet Source	<1 %
36	zfin.org Internet Source	<1 %
37	Giuseppe Mancia, Guido Grassi, Konstantinos P. Tsioufis, Anna F. Dominiczak, Enrico Agabiti Rosei. "Manual of Hypertension of the European Society of Hypertension", CRC Press, 2019 Publication	<1 %
38	file-thesis.pide.org.pk Internet Source	<1 %
39	vdoc.pub Internet Source	<1 %
40	www2.mdpi.com Internet Source	<1 %
41	www.grin.com Internet Source	<1 %
42	daayitwa.org Internet Source	<1 %

43	hdl.handle.net Internet Source	<1%
44	jcdr.net Internet Source	<1%
45	jneuroinflammation.biomedcentral.com Internet Source	<1%
46	journals.lww.com Internet Source	<1%
47	Jing Hu, Lei Miao, Jingxian Liao, Huaining Hu. "Integrating blood lactate levels and nutritional status in early risk stratification for delirium and mortality in elderly septic patients: A retrospective cohort study", Medicine, 2025 Publication	<1%
48	academic.oup.com Internet Source	<1%
49	brightideas.houstontx.gov Internet Source	<1%
50	respiratory-research.biomedcentral.com Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		