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Chronic Obstructive Pulmonary Disease

Current Concepts and Practice

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE – CURRENT CONCEPTS AND PRACTICE

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Meet the editor

After completing his basic medical education (MBBS) at the National University of Singapore in 1987, Dr Ong was accepted as a member of the Royal College of Physicians (MRCP) in the United Kingdom in 1993 and subsequently completed his specialist training in Respiratory Medicine at the Singapore General Hospital. In 1996 and 1997, Dr Ong was a Fellow in the Department of Pulmonary and Critical Care Medicine, Stanford University Medical Center, USA, and was also a visiting clinical and research Fellow in the Stanford Sleep Disorders Clinic and Research Center, USA. Dr Ong has been in Edinburgh as a Fellow of the Royal College of Physicians (FRCP) since 2002. He is also a Fellow of the American College of Chest Physicians (ACCP). Dr Ong is the President of the Chronic Obstructive Pulmonary Disease Association (Singapore) and is recognized by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as the National Leader in Singapore. He is presently in private specialist practice at the Mount Elizabeth Medical Centre, Singapore.

Contents

Preface

It is indeed heartening to note the ardent interest in Chronic Obstructive Pulmonary Disease (COPD) and the progress that has been achieved in the management of this disorder in recent years. A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years, and the contributions that we have received from numerous distinguished sources as well as the keen anticipation for the publication of this book are testament to this observation.

The 'open-access' format of this book provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. This clearly reflects the wide-ranging academic interest in this disease. Indeed, those of us privileged to have a part in the management of patients with COPD will have known that this disease challenges the whole gamut of Respiratory Medicine – necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of our patients. With that in mind, I sincerely hope that this assemblage of subject reviews and novel insights on COPD will be of benefit for our readers and the patients they are helping.

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Part 1

Basic Science

1

Lung and Systemic Inflammation in COPD

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1. Introduction

Nuclear factor-κB (NF-κB) is a nuclear transcription factor first recognized in 1986 by Sen and Baltimore. Its name derives from the fact that it was first diagnosed in the nuclei of B cells [1- 3] bound to an enhancer element of the immunoglobulin kappa light chain gene [4]. At that time, NF-κB was primarily thought to be a B-cell–specific transcription factor, but it was afterward found to be present in every cell type [5]. NF-κB has been implicated in the regulation of host inflammatory [6-8] and immune responses [9-11], cell adhesion [12], developmental signals [13], cell proliferation, differentiation [14, 15] and in defending cells from apoptosis [16, 17]. In addition, it plays important roles in cellular growth properties by encoding cytokines, chemokines and receptors required for neutrophil adhesion and migration, thus increasing the expression of specific cellular genes [18].

Physical and chemical damage to the lung causes an inflammatory response, thus defending the lung against the causative agents. Inflammation initiates a series of cellular procedures which lead to healing the injury; however, if resolving the inflammatory response is inefficient, the result is a chronic situation. Numerous pathophysiologic conditions and inhaled air pollutants are identified as generating stable stimulation of phagocytic cells, leading to the amplification of proinflammatory cytokines, and mediating chronic inflammation in the lung [19].

Many studies have reported the role of NF-kB in inflammation and proven the association of NF-κB with human inflammatory lung diseases. The point of this short review is to summarize what is known about the molecular biology and activation pathway of NF-κB and to highlight the role of NF-κB in the pathogenesis of inflammatory lung disease, as well as in asthma, COPD, ARDS, and cystic fibrosis.

1.1 Molecular pathway of NF-κB and its activation

In mammals, the NF-κB highly conserved protein family is composed of five members, p50 (precursor protein: p105), p52 (precursor protein: p100) [20, 21], p65 (RelA), c-Rel, and

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RelB [22]; these are encoded by NFKB1, NFKB2, RELA, REL, and RELB, respectively [23], which share the so-called N-terminal Rel homology domain (RHD), responsible for DNA binding and homo- and heterodimerization [24, 25]. Various combinations of dimeric complexes bind to κB sites within the DNA, where they directly regulate transcription of target genes [26]. The major form of NF-κB in cells is a p50/RelA heterodimer [27]. The diverse Rel/NF-κB proteins exhibit different abilities to shape dimers [4], dissimilar preferences for different κB sites [28, 29], and distinct abilities to interact with inhibitory subunits known as IκBs. Because different Rel/NF-κB complexes can be induced in different types of cells and via different signals, they can cooperate in diverse ways with other regulatory proteins and transcription factors to control the expression of particular gene sets [30].

In their unstimulated state, NF-κB dimers can be found in the cytoplasm of a large variety of cells as an inactive complex controlled by their interaction with the κB family of inhibitor proteins (IκB) [31, 32]. They block NF-κB nuclear localization sequences and thus cause its cytoplasmic retention [33, 34]. Numerous IκBs have been identified; there are three typical IκB proteins, IκBα [35], IκBβ [36] and IκBε [37], and two atypical IκB proteins, Bcl-3 [38] and IκBζ, which act in a different way [39]. The precursor proteins p100 (NFKB2) and p105 (NFKB1) also act as inhibitory molecules [40].

Most mediators that activate NF-κB are involved in the phosphorylation-induced degradation of IκB. Phosphorylation of IκB by the multisubunit IκB kinase (IKK) complex in N-terminal regulatory domain at two critical serine residues (S32 and S36) [41] results in the ubiquitination and subsequent degradation of IκB by the 26S proteasome [42-44]. Free NF-κB dimmers translocate into the nucleus, where they bind to specific promoters and affect gene transcription [45, 46].

A variety of upstream extracellular signals, including tumor necrosis factor alpha (TNF-α) [47-50], lipopolysaccharide [51], virus infection (human T-cell leukemia virus, HIV1) [52-54], ionizing radiation [55], interleukins such as IL-1β [48], epidermal growth factor (EGF) [3], mitogens [56], bacteria [52], reactive oxygen species (ROS) [48], environmental hazards such as cigarette smoke [57], and physical and chemical stresses [58], activate the IKK complexes, which are comprised of three subunits: IKKα, IKKβ, and IKKγ/ NEMO. IKKα and IKKβ are catalytic subunits, and IKKγ functions as a regulatory subunit [59-61].

Numerous genes associated with the inflammatory process include proinflammatory cytokines (such as TNF-α), cell adhesion molecules (such as intercellular adhesion molecule 1) [62, 63], or assumed NF-κB binding sites in their promoters that can amplify the inflammatory response and enhance the time of chronic inflammation. NF-κB also induces the expression of enzymes whose proteins have a connection to the pathogenesis of the inflammatory procedure, such as inducible cyclooxygenase (COX-2) [18], which generates prostanoids, and the inducible type of nitric oxide synthase (iNOS), which manufactures nitric oxide (NO) [64, 65]. These facts emphasize the significance of NF-κB as a regulator of inflammatory gene activation and indicate it as a predominant choice for targeted inactivation. In fact, diverse techniques intended to improve or suppress the inflammatory process related to determined pathologies have already been directed at obstructing the biological actions of NF-κB (Figure 1).

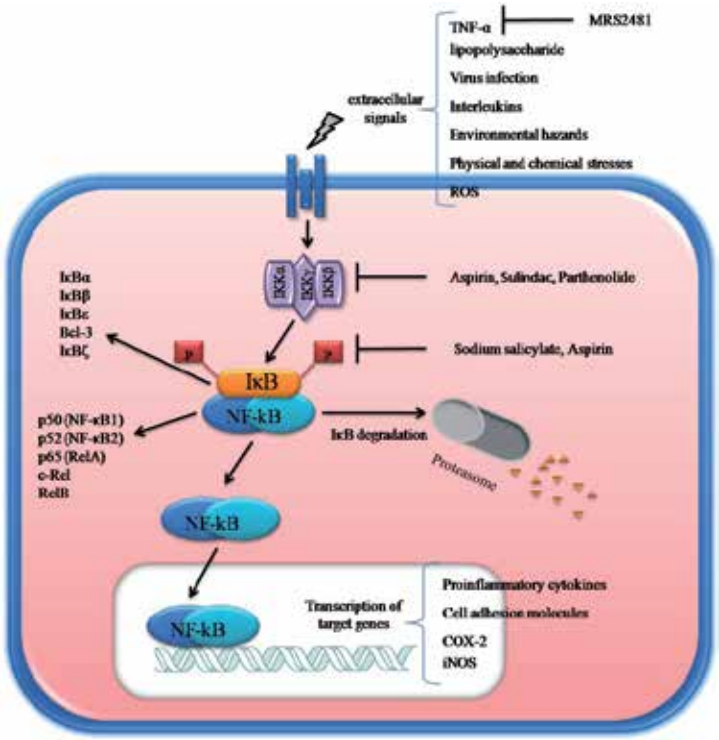

Fig. 1. Schematic representation of NF-κB activation in inflammatory disease. A variety of upstream extracellular signals activate the IKK complexes, which are comprised of 3 subunits: IKKα, IKKβ, and IKKγ. Phosphorylation of IκB by the IKK complex in the N-terminal regulatory domain at two critical serine residues results in the ubiquitination and subsequent degradation of IκB by proteasome. Free NF-κB dimmers translocate into the nucleus, where they bind to specific promoters and affect gene transcription of such molecules as proinflammatory cytokines, cell adhesion molecules, COX-2, and iNOS. Some drugs and agents are able to suppress NF-κB activation via different pathways. Aspirin and sodium salicylate block IkB phosphorylation and degradation. Sulindac, Parthenolide, Aspirin inhibit activation of the NF-kB pathway by suppressing IKK activity and MRS2481 inhibit TNF-α.

1.2 Asthma

Asthma is a chronic inflammatory disease [65, 66] of the airway accompanied by reversible bronchial hyperreactivity. Increased numbers of Th2 lymphocytes [67] and eosinophils in the airway can cause chronic inflammatory response, leading to asthma [68, 69]. In addition to the existence of inflammatory cells in the airway, these patients expose changing levels in structure of airway, termed remodeling [69, 70]. As cited above, NF-κB is one of the most important transcription factors involved in the expression of wide groups of inflammatory proteins, including cytokines, adhesion molecules, and enzymes, which themselves are implicated in the pathogenesis of asthma [71]. Translocation of NF-κB and its binding activity increases in airway specimens from asthmatics, in airway epithelial cells obtained from bronchial mucosal biopsies, and in alveolar macrophages extracted from sputum.

Results show that the agents that are coordinate with deterioration of asthma generally activate NF-κB. Viral infections, allergens [72], and ozone, all of which can cause activation of NF-κB, are related to aggravation of asthma [73].

Viral infections of the upper respiratory airway might intensify asthma by activation of NF-κB. In cell cultures of bronchial epithelial cells, rhinovirus causes induction of oxidative stress and NF-κB activation and increases expression of IL-8, which can in turn participate in neutrophil recruitment into the upper respiratory tract. Respiratory syncytial virus (RSV) has been involved in stimulation of NF-kB and consequent expression of IL-8 and IL-1 in human type II–like alveolar epithelial cells (A549 cells). Thus NF-κB seems to be activated during replication of RSV (Table 1)[73].

In vitro research has revealed that allergens activate NF-κB in bronchial epithelial cells of asthmatic patients. For example, exposure to aerosolized ovalbumin causes profound activation of NF-κB and transcription of inducible nitric oxide synthase in the respiratory tract of sensitized Brown Norway rats [73]. Mice lacking the NF-κB subunits p50 or c-Rel exhibit less airway inflammation in response to an antigen challenge, signifying the fundamental role of NF-κB in allergic respiratory disease [68].

Furthermore, activation of NF-κB has also been illustrated in animal models of allergic airway inflammation in airway epithelium. However, inhibition of NF-κB activation in airways did not ameliorate airway hyperresponsiveness, a key characteristic of asthma. These findings reveal that NF-κB activation in airway epithelium is essential to the airways in response to allergen activity via recruitment of inflammatory cells but also exhibits a different segregation between hyperresponsiveness and airway inflammation [68].

Airway irritants such as ozone may also exacerbate asthma symptoms and trigger inflammation through NF-κB activation. Exposure of A549 cells to ozone affects activation of NF-κB and transcription of IL-8. Another study revealed that rats exposed to ozone subsequently show time- and dose-dependent activation of NF-κB and modulate penetration of neutrophils and monocytes into lavageable airspace via expression of CXC and CC chemokines, respectively [73].

Cre/lox molecular techniques have been examined whether inhibiting NF-κB expression only in airway epithelial cells in a mouse model would diminish levels of airway remodeling. In selective airway epithelial cells frominhibitor of κB kinase β (Ikkβ) knockout mice, peribronchial fibrosis had considerably reduced levels of TGF-β in BAL, and numbers of cells had positive peribronchial TGF-β1. Airway epithelial Ikk-β ablation also leads to reduction in levels of mucus and eosinophils in the airway [69].

Reduction in expressions of NF- κB-regulated chemokines such as eotaxin-1 and Th2 cells can diminish airway inflammatory response in the airway as well. These findings support the key role of NF-κB pathway the in bronchial epithelium and its significance in the process of remodeling [69].

As cited above, expression of some cytokines and adhesion molecules as a result of NF-κB activation exacerbates inflammation in airway cells. For example, tumor necrosis factor alpha (TNF-α) is a cytokine produced by macrophages and associated with inflammation. It increases the expression of adhesion molecules for recruitment of immune cells to damaged tissue. TNF-α may also be involved in expression of intercellular adhesion molecule 1

(ICAM-1). It has been illustrated that epithelial upregulation of ICAM-1, which has an important role in cellinteraction, exists in asthmatics. Active bronchial asthma is matched by an amplified level of soluble ICAM-1 in serum and thereby is associated with the pathogenesis of asthma. When rhinoviruses activate NF-κB, it amplifies the gene expression ofICAM-1 in bronchial epithelial cells, because rhinovirus utilizes ICAM-1 as a cellular receptor [73].

1.3 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction which is irreversible. COPD is a complex of two chronic lung diseases: chronic bronchitis and emphysema both caused mainly by a familiar irritant, cigarettes [74]. The inflammatory response in smokers' lungs is not fully understood [75]. One theory is that cigarette smoke disturbs the oxidant/antioxidant balance by induction of oxidative stress, which stimulates activation of redox-sensitive transcription factors such as NF-κB. Transcription factors, including NF-κB (Table 1) and activator protein 1 (AP-1), have a key role through gene transcription of wide range of inflammatory cytokines that cause airway inflammation, including TNF-α interleukin (IL)-8, and interleukin (IL)-6 [41, 76]. As well, NF-κB has been demonstrated to be a mediator of cigarette smoke effects on gene transcription in various cell types. Its activated dimer has been revealed to be induced in bronchial biopsies of smokers [77].

Previous studies have reported that cigarette smoke increases DNA damage in lung fibroblasts and human bronchial epithelial cells; however, this does not lead to necrosis or apoptosis. Lung fibroblasts and human bronchial epithelial cells are capable of repairing DNA damage and forming colonies after sub-culturing in normal medium. Cigarette-smoke-induced DNA damage is involved in modulating cell survival or apoptosis via numerous signaling pathways. It has been elucidated that NF-κB plays a significant role in mediating cell survival [78].

Transcription of genes is not only dependent upon transcription factor bindings; it is also related to the alteration of core histone proteins which adjust the availability of the genome to cofactors and nuclear factors. Octamers are composed of two copies of each histone core protein, H2A, H2B, H3, and H4, and DNA covers them. Post-translational modification of N-terminal side chains of each histone cause conformational changes via phosphorylation, methylation and acetylation[76].

Histone acetyltransferases (HATs) acetylate lysine residues in histones, neutralize their positive charge, and lead to chromatin relaxation, increasing binding of transcription factors and RNA polymerase II, which unwinds DNA and increases gene amplification [76].

The imbalance of acetylation/deacetylation and increase in acetylation might cause transcription of proinflammatory genes mediated by NF-κB and therefore initiate chronic inflammation. Consequently, the imbalance of histone acetylation/deacetylation may have a role in the inflammatory response in "susceptible" smokers who progress toCOPD [76].

When NF-κB translocates into the nucleus and acetylates histone H4, the sequence leads to DNA relaxation and transcriptional accessibility. Research has shown that smoking cessation in patients suffering from COPD causes increased histone H3 acetylation,

illustrating that the stability of the inflammation in the lungs in COPD after smoking cessation may be regulated by H3 acetylation. As cited above, this study shows that cigarette smoking affects chromatin remodeling in the lungs [76]. Smoking has been found to reduce expression of IκB protein dramatically and thus affects regulation of NF-κB. Unexpectedly, in ex-smokers with COPD, a notable depletion of IκBα has been detected. Nevertheless, the NF-κB DNA binding in these patients was similar to that in nonsmokers [76]. Other investigations confirm the enhanced activation of NF-κB in cigarette smoke. Cigarette-smoke-exposed Guinea pigs increase expression of IL-8 in response to NF-κB activation. Furthermore, studies of smokers and number of pack-years reveal a positive correlation with NF-κB activation. Smokers with COPD and currently healthy smokers both increase DNA binding activity of NF-κB [76]. NF-κB expression and its translocation in lung tissue and sputum increase in COPD patients in comparison with non-smoking controls, and this seems to be related to exacerbation [79].

Caramori and coworkers investigated p65 expression in leucocytes extracted from sputum patients with exacerbated COPD and revealed p65 transcription in macrophages but not in neutrophils [80].

Even though an enhanced proinflammatory molecule whose expression is vitally dependent on NF-κB activation has been formerly described in COPD, the role of NF-κB activation has not been determined. We hypothesize that, through COPD exacerbations, initiation factors including viral and bacterial infections could activate NF-κB, generate cytokines and chemokines, and lead to inflammatory cell penetration of the airways. Sputum immunocytochemistry methods have evidenced activation of p65 in alveolar macrophages through COPD exacerbations [80].

As a sign of oxidative stress activation, Di Stefano and colleagues demonstrated increases in activation of NF-κB in segmental and subsegmental bronchial biopsies in COPD subjects and healthy smokers accompanied by enhanced lipid peroxidation products. They reported increased localization of p65 and its immunoreactivity in bronchial epithelium but not in submucosa. Nevertheless, they could not diagnose any difference between healthy smokers and COPD smoking subjects [81]. Similarly, Yagi and coworkers investigated IκBα expression by an immunostaining method to measure NF-κB activation indirectly in airway epithelial cells. They revealed increased levels of phosphorylated IκBα in both ex-smokers with COPD and subjects without COPD. Phosphorylated IκBα underwent degradation and freed NF-κB to bind to enhancers of related genes [76].

Inflammatory molecules in COPD cause increased neutrophils and inflammatory agents in the airways and bronchial tissue of patients [79]. Mishra and colleagues reported that NF-κB can be inhibited independently from IκBα and may be inhibited via a peroxisome proliferator-activated receptor α (PPAR-α). The interaction of PPARα with the p65 and c-Jun subunits of NF-κB and AP-1, respectively, may block their activation, suppressing expression of cytokines such as IL-6 [76].

1.4 Cystic fibrosis

Cystic fibrosis (CF) is a chronic inflammatory airway disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Lung disease in CF expresses a profoundly proinflammatory phenotype related to increased constitutive

viscosity of respiratory secretions and chronic lung infection by *Pseudomonas aeruginosa* and other bacterial species, resulting in considerable morbidity in cystic fibrosis subjects followed by the lack of innate immune responses [73].

Pseudomonas aeruginosa supposedly causes activation of NF-κB and may play an important role in overproduction of mucin caused by the increase in MUC2 mucin transcription (Table 1) [73]. Even though there is not enough data in vivo, enhanced activation of NF-κB and amplification of IL-8 can be observed in bronchial epithelial cells that display CFTR mutations (IB3 cells) in comparison with normal bronchial epithelial cells line (C38 cells). To decrease sputum viscosity in CF patients, inhibition of NF-κB activation might be a useful procedure for decreasing airway inflammation and improve lung function [82]. These findings show that CFTR mutations are related to modification of NF-κB levels and airway inflammation [73]. Another research revealed that, in either wild-type (WT) or mutant (CFTR) isogenic bronchial epithelial cell lines infected by *Pseudomonas aeruginosa*, transcriptional changes occur in cytokine production. For example, NF-κB activates transcription of four -regulated cytokines include ICAM-1, CXCL1, IL-8 and IL-6, but protein expression in both cell lines involves only enhancement of IL-6 and IL-8 expressions. Inhibition of NF-κB prior to countering t *Pseudomonas aeruginosa* revealed different levels of dependence on NF-κB for expression of the cytokines [83].

T. Joseph and colleagues demonstrated that in vitro activation of NF-κB in human airway epithelial cells isolated from CF (DeltaF508/DeltaF508) and non-CF (NCF) patients when infected by *Pseudomonas aeruginosa* elevated nuclear levels of IkBα in CF cells, although this increase was transient. They also showed increased baseline translocation of NF-κB to nuclei in primary CF epithelial cell cultures; following *Pseudomonas aeruginosa* infection, activation of IκBα might suppress that of NF-κB [84].

In a systematic search for drugs for therapeutic treatment that may be utilized for inhibition of IL-8 secretion from these cells, a series of amphiphilic pyridinium salts was examined. The most effective of these salts is a (R)-1- phenylpropionic acid ester known as MRS2481. For optimal activity, it has been demonstrated that the ester ought to be joined to the pyridinium derivative by an eight-carbon chain. MRS2481 seems to be able to suppress signaling of the NF-κB and AP-1 to the IL-8 promoter . Another therapeutic feature is that MRS2481 is an effective inhibitor of TNF-α, which leads to suppression of phosphorylation and proteosomal destruction of IκBα (Figure 1). In this way, IκBα is maintained and keeps the IL-8 promoter silent [85]. Another pharmaceutical strategy against the inflammatory phenotype of the CF lung is Parthenolide, which is sesquiterpene lactone derived from the feverfew plant. Numerous researchers have controversially proposed that this compound suppresses the NF-κB pathway by attenuation of IκBα degradation. As we show in Figure 1, parthenolide inhibits IκB kinase, ensuring the stabilization of IκBα in cytoplasm, hence causing inhibition of NF-κB translocation and reduction of following inflammatory responses, so parthenolide can be an effective treatment for the excessive inflammation in CF [86].

another therapeutic medicine, Azithromycin (AZM), has been shown to modulate airway inflammation in CF subjects. AZM suppressed IL-8 expression in a CF cell line. Because the IL-8 gene is transcripted by NF-κB, it can be concluded that this is the probable pathway by which AZM activates NF-κB in the cell line. Such findings indicate the anti-inflammatory

task of this macrolide. Suppression of NF-κB activity reveals other proinflammatory molecules regulated by this factor as an AZM effect relevant to the treatment of CF [87].

1.5 Acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS) is known for enormous infiltration of neutrophils into the lungs accompanied by leak of serum proteins, especially albumin, into the alveolar space, blood loss in the intra-alveolar space, and interstitial edema, all important and frequent signs in exacerbation of ARDS. In spite of the occurrence of ARDS in all over the world, the precise pathophysiology mechanisms remain to be detailed [88].

Varying expression levels of proinflammatory cytokines are associated with the progression of ARDS. overexperssion of proinflammatory cytokines such as TNF-α, IL-6 and IL-8 in the lung has been demontrated in bronchoalveolar lavage (BAL) of ARDS patients and is correlated with poor outcome [88].

Patients with proved ARDS revealed increased activation of NF-κB in alveolar macrophages, in comparison with control subjects without acute lung injury [73]. Because there were no notable increases in the levels of transcription factors, including CREB, AP-I , or SP- I activation, in alveolar macrophages from patients with ARDS, NF-κB is suggested to be a significant upstream regulator for cytokine gene expression in ARDS patients, because of its existence on the enhancer of proinflammatory cytokines (Table 1). The level of subunits p50, p65, and c- Rel decreased in cytoplasm of alveolar macrophages in ARDS subjects, proving the existence of an ongoing stimulus for NF-κB activation. Increased levels of oxygen radicals, proinflarnmatory cytokines, and endotoxin in ARDS might be associated with NF-κB activation. TNF-a and IL-8 are increased in BAL of ARDS subjects [88].

NF-κB activation can alsobe caused by oxygen radicals. Our in vivo data from a hemorrhage-induced murine model of ARDS indicates an outstanding role for xanthine oxidase, a kind of oxygen radical, in stimulation of NF-κB in lung cells [88]. Cytoplasmic and nuclear levels of IκBa are not notibly dissimilar in alveolar macrophages from ARDS subjects and controls, so these findings are rather unexpected, because signals that cause activation of NF-κB would be expected to generate phosphorylation. Alveolar macrophages have a significant protective role in mediating NF-κB activation in the lung and in initiation of neutrophilic inflammation [73, 88].

2. Inhalation of some agents cause activation of the NF-κB inflammatory pathway in the lung

Asbestos

Asbestos belongs to a group of physically occurring, hydrated mineral silicate fibers that are causally related to the progression of pulmonary diseases [88]. Iron, which exists in asbestos fibers, cause cellular redox changes by generation of intracellular reactive oxygen species, leading to activation of NF-κB. It has been shown that, after inhalation of crocidolite and chrysotile asbestos, nuclear translocation of RelA increases in rat airway epithelial cells (Table 1). The main reason is that macrophages phagocytize asbestos but cannot ''digest'' these fibers. Because the asbestos harms them, these macrophages secret TNF-α, and this cytokine mediates activation of NF-κB [73, 89-92].

inflammatory lung disease	The role of NF-κB
Asthma	Respiratory syncytial virus (RSV) and Rhinovirus cause induction of NF-κB activation
COPD	Cigarette smoke stimulates activation of redox-sensitive transcription factors such as NF-κB
CF	Activation of NF-κB may overproduct mucin during the increase of MUC2 mucin transcription
ARDS	NF-κB suggested to be a significant upstream regulator for cytokine gene expression
Inhalation of proinflammatory agents	Translocation of RelA increases in rat airway epithelial cells and activates the p38 and JNK MAPK pathways and cause the activation of NF-κB

Table 1. The implication of NF-κB in inflamatory lung disease.

2.1 Sulphur mustard Inhalation

Sulphur mustard (SM) is a chemical weapon used during the Iraq war against Iran of the late 1980s [93, 94]. It can produce damage in skin, eyes, and, , most importantly, in lung. 2-Chloroethyl ethyl sulphide (CEES) is a sulphur vesicating agent and an analogue of SM. Both of these agents are alkylating agents that affect cellular thiols and are highly toxic. CEES appears to decrease iNOS expression by associating with the LPS-induced stimulation of transcription factor NF-κB. CEES also alkylates the NF-κB consensus sequence, thus suppressing the binding of the NF-kB to the iNOS promoter. Even though the activation of NF-κB due to SM or CEES countering has been elucidated in different cell lines, the exact mechanism of this pathway is still poorly understood, and the question of whether activated NF-κB induces an inflammatory pathway remains to be elucidated [95].

2.2 Diesel exhaust

Diesel exhaust (DE) is a major pollutant;exposure increases a prominent inflammatory response in the airways, with induction of cytokines such as IL-8, IL-13 and activation of redox sensitive nuclear factors (NF-κB, AP-1) in the bronchial epithelium, including upregulation in the transcription of ICAM-1 and vascular endothelial adhesion molecules (VCAM-1). It has been established that DE activates the p38 and JNK MAPK pathways and causes the activation of NF-κB and AP-1 [96].

3. Strategies to block NF-κB activation

Several strategies have been proposed to block the activation of NF-κB. An extensive diversity of molecules (both natural and synthetic) has been highlighted as having an effect on activation of NF-κB and being able suppress it. These compounds suppress NF-κB

activation through various pathways by blocking NF-κB activation. Subsequent information has provided strategies for suppressing NF-κB activation in response to different type of stimuli. Both steroids and nonsteroidal anti-inflammatory agents are helpful (Table 2). Hence, it is important to get a better understanding of the activation of NF-κB and release of prostaglandins [64]. Glucocorticoids, including dexamethasone and prednisone, are commonly prescribed for their anti-inflammatory and immunosuppressive effects [97-99]. These components interact with the steroid receptor and cause reduction of the expression of particular genes that control the inflammatory procedure. NF-κB can be inhibited via glucocorticoids in different ways. Dexamethasone induces the expression of IκBα, which causes retention of NF-κB in the cytoplasm, especially of p65. Synthesis of IκBα by dexamethasone is likely to be dependent on p65 in pre-existing NF-κB complexes. These findings show that quick degradation of IκBα may be blocked by consequent expression of IκBα following dexamethasone treatment. Another pathway implicated in glucocorticoid-mediated repression of the NF-κB is that dexamethasone may inhibit the expression and p65-dependent transactivation in endothelial fibroblasts in murine models, but it does not have any effect on the IκB level. In the same way, dexamethasone alters NF-κB–mediated transcriptional activity in endothelial cells, but it does not alter IκB levels either [64].

type	Name
Steroids	Dexamethasone
	Prednisone
Nonsteroids (NSAIDs)	Aspirin
	Sodium salicylate
	Tepoxaline
	Defereoxamine
	Ibuprofen
	Mesalamine
	Sulindac
	MRS2481
	Parthenolide
	Azithromycin (AZM)

Table 2. Therapeutic agents and drugs which block NF-κB activation.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively applied to improve the therapeutic status of chronic inflammatory states. The most widely hypothesis for the inhibitory property of these compounds on the inflammatory response supposes that NSAIDs inhibit COX activity to suppress prostaglandin synthesis [64].

NSAIDs such as Aspirin and sodium salicylate correlate with NF-κB inhibition. At concentrations measured in the serum of patients treated with these drugs for chronic inflammatory situations, both aspirin and salicylate suppress NF-κB activation, and aspirin has been demonstrated to inhibit the activation of the IκB kinase complex [97, 100]. In particular, Aspirin and sodium salicylate prevent NF-κB nuclear translocation by blocking IkBα phosphorylation and degradation (Figure 1) [3, 100]. These drugs also inhibit TNF-α-induced mRNA transcription of adhesion molecules such as ICAM-1 in endothelial cells. Penetration of neutrophils from endothelial cells can be prevented following NF-κB inhibition in these cells. Recently, Yin *et al.* have reported that Aspirin can bind to and prevent the kinase activity of IKKβ by decreasing its capacity to bind ATP. Other NSADs, such as tepoxaline, defereoxamine, and ibuprofen, are also capable of suppressing NF-κB activity [100].

An aminosalicylate derivative with anti-inflammatory aspects, mesalamine, prevents IL-1–mediated activation of p65 phosphorylation without suppressing IκBα degradation [64]. Indomethacin, is another NSAID, is able to inhibit inflammatory responses via suppressing COX activity, but it does not prevent activation of the NF-κB pathway [64]. Sulindac is illustrated in Figure 1 as a NSAID that is structurally correlated with indomethacin and can inhibit activation of the NF-kB pathway by suppressing IKK activity [64, 97].

These findings suggest that inhibition of the NF-κB pathway might be implicated in the anti-inflammatory pathways as well as participation of NSAIDs in growth inhibitory properties.

4. Conclusion

NF-κB is one of the most important transcription factors and has an important role in inflammatory special lung disease [6]. The exact pathophysiological mechanism of NF-κB that leads to inflammation continues to be better understood. Pharmacologic therapy used for blocking this molecule can be useful for treatment of lung disease. The major recommendation for further research is to define the exact molecular mechanisms of each inflammatory lung disease that involves NF-κB. This is critical because the glucocorticoids which benefit patients with asthma do not work for COPD. Future research will to elucidate new methods of treatment for those patients [101].

5. Acknowledgement

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6. References

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2

Homocysteine is Elevated in COPD

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1. Introduction

Homocysteine was first described by Butz and du Vigneaud in 1932 (Butz 1932). An association between elevated homocysteine levels and human disease was first suggested in 1962 by Carson and Neil (Carson 1962). They had found high homocysteine concentrations in the urine of some children with mental retardation.

In 2000, Yi and Melnyk found that plasma total homocysteine is positively associated with parallel increases in plasma S-adenosylhomocysteine and concentrations and lymphocyte DNA hypomethylation. This lead Medina and Urdiales (2001) to speculate on an indirect mechanism for homocysteine pathogenicity secondary to inhibition of DNA methytransferase and that is the disruption of DNA methylation patterns leading to alterations in gene expression which may be of significance in chronic diseases many of which are associated with elevation in homocysteine. Elevated plasma homocysteine has been associated with neural tube defects, cognitive impairment in the elderly, psoriasis and some tumours (Refsum 1998). Hyperhomocysteinaemia has also been associated with cardiovascular disease, atherosclerosis, venous thrombosis, diabetes mellitus and renal failure (Okuyan et al, 2010; Refsum et al, 1998; Givvimani et al, 2011; Kim et al, 2011; Hankey & Eikelboom, 1999; Dominguez et al, 2010; Wile et al, 2010; Austen et al, 2003). Plasma HCY has also been related to clinical outcome in acute respiratory diseases (Tsangaris et al, 2009). This widespread involvement of homocysteine in disease explains the current interest of both basic and clinical biomedical scientists in this amino acid and thus the explosion of articles containing homocysteine as keyword.

There has hitherto not been much interest in homocysteine disorders in respiratory disease. Sanguinetti was one of the first researchers to postulate that there was an imbalance between redox reactions in COPD (Sanguinetti 1993). In an elegant series of experiments, Rahman et al showed that reduced glutathione was depleted by exposure to cigarette smoke in alveolar epithelial cells (Rahman et al 1995). Further work by this group revealed that there is loss of antioxidant capacity in COPD relative to healthy non-smokers (Rahman et al

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2000). These results were supported by Andersson who showed that high plasma homocysteine levels were associated with low reduced glutathione levels in 2000 in the plasma of COPD patients (Andersson 2000). Thus establishing an almost inverse relation between the levels of homocysteine and reduced glutathione and giving rise to the hypothesis that homocysteine should be elevated in COPD because of impaired oxidative stress. Taken together this series of studies demonstrate that COPD, the most common chronic respiratory disorder, is linked to hyperhomocysteinaemia.

Chronic obstructive pulmonary disease is a disease mainly of the middle-aged and elderly. It results from an abnormal pro-inflammatory response of the lung to inhaled noxious stimuli that leads to an unrelenting accelerated decline in forced expiratory volume in the first second of exhalation (FEV1) and is characterised by a ratio of FEV1 to forced vital capacity (FVC) of less than 70%. The disease is currently estimated as the fourth leading cause of death world-wide and it is expected to become the third leading cause within the next ten years (GOLD 2010).

In this chapter we will examine the evidence for the association of hyperhomocysteinaemia and COPD and discuss its implications.

2. Homocysteine metabolism

Homocysteine is a 4-carbon amino acid attached to a sulphydryl group. Homocysteine is involved in the transfer of methyl groups when it is synthesized from S-adenosylmethionine methylase and adenosyl-homocysteinase (please see Figure 1). Homocysteine may also be transformed back to methionine or catabolised to cystathionine. In the latter pathway, homocysteine combines with serine via cystathione beta-synthase to yield cystathionine which, via a gamma-lyase enzyme, is cleaved to yield free cysteine and a ketobutyrate. Cysteine is then metabolized via gamma-glutamyl synthase/glutathione synthase to reduced glutathione (GSH) which is important for electron storage with oxidized glutathione (GSSG), as shown in Figure 1. Homocysteine is therefore linked to two important pathways in the body one involving methylation processes and the other a transsulphuration pathway that may be of importance in redox reactions in the maintenance of homeostasis (Medina et al, 2001; Giusti et al, 2008). Figure 1 shows how closely intertwined these two pathways are.

A further role for homocysteine may arise out of its capacity to bind to transfer ribonucleic acid (tRNA) which in certain circumstances is thought to produce a highly reactive derivative, homocysteine thiolactone (Jakubowski & Goldman, 1993; Jakubowski 2000). Homocysteine is usually immediately methylated to methionine-tRNA but when this process is impaired or inadequate, the reactive species, homocysteine thiolactone, is formed (Antonia et al 1997). This form of HCY can rapidly homcysteinylate any of several enzymes causing alteration in enzyme activity thus leading to disordered homeostasis and redox imbalance (Booth et al, 1997).

In spite of the above rather interesting theory it was not known how plasma HCY enters cells to affect such change. The transporter for HCY into the endothelial cell has recently been found and shown to be sodium and lysozyme dependent (Jiang et al 2007) and this explains how HCY can enter endothelial cells and become incorporated into proteins (Jakubowski et al, 2000). It is not known whether such mechanisms exist for non-endothelial cells, in particular for alveolar epithelial cells.

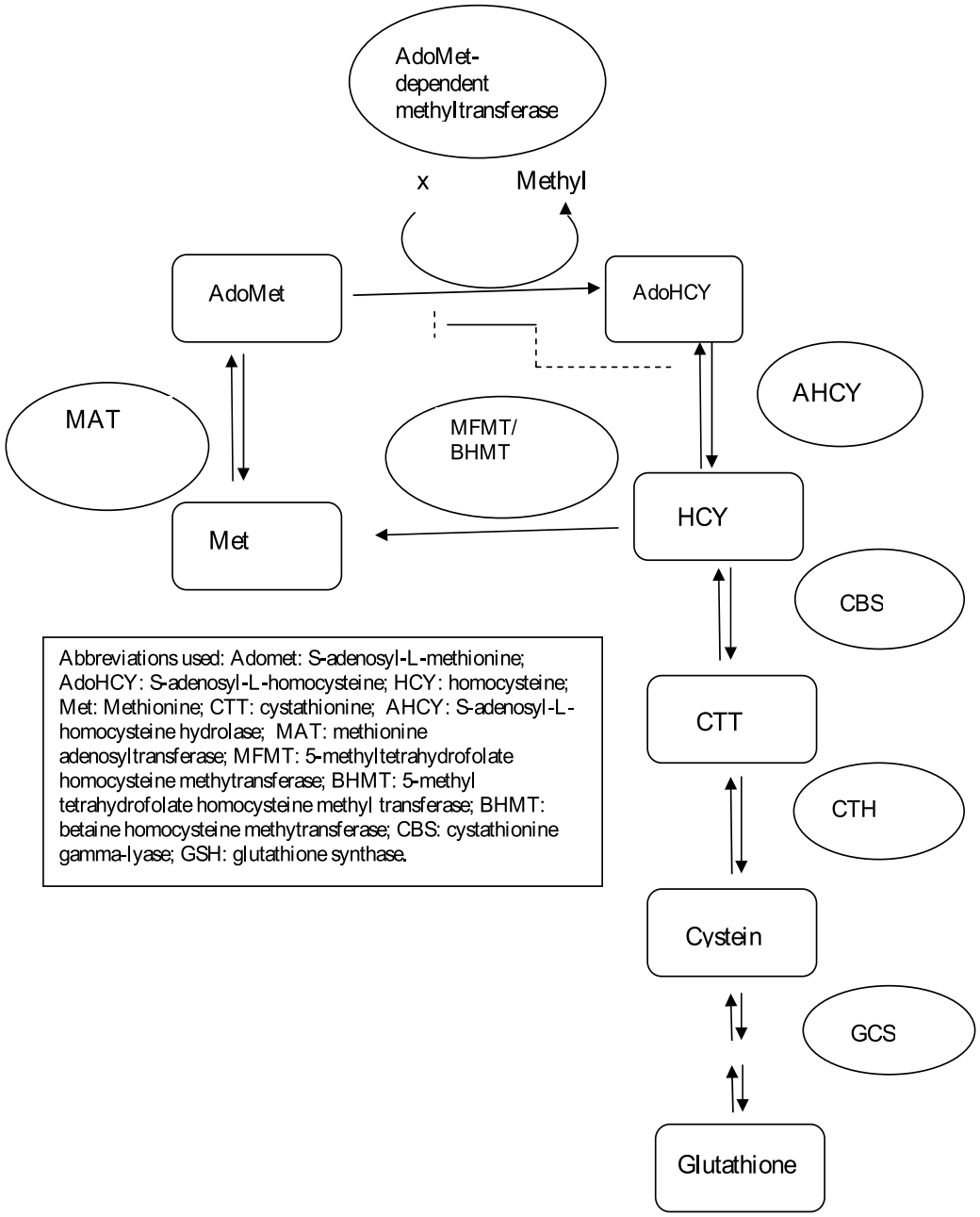

Fig. 1. Modified from Tehlivets (2011). The Figure shows the linkage between HCY and glutathione.

3. Measurements of homocysteine

Human plasma contains both free homocysteine (HCY) and its oxidised form, homocystine (HCY-HCY), where two molecules are bound via a disulphide bond. About 99% of

homocysteine exists in the oxidised form in plasma. About 75% of total homocysteine is protein bound. Plasma HCY concentrations may be altered by several physiological factors: age, gender and body mass.

Kai et al and Fimognari et al measured HCY by high performance liquid chromatography with fluorescence detection and Seemungal et al used a polarization immunoassay technique (Kai et al 2006, Figmonari 2011). Though the techniques were different their results were similar and are compared below.

4. Why study homocysteine in the COPD patient?

Smoking is by consensus the most important risk factor in the development of COPD (GOLD 2010). Cigarette smoking causes elevation of plasma HCY (Bazzano et al 2003, Kai et al 2006) though the effect may be variable (Nygard 1998). Smoking is also a risk factor for the development of vascular disease and cessation of smoking contributes to cardiac risk reduction (Ford 2007). Several studies have linked and continue to link homocysteine with cardiovascular risk (Homocysteine Studies 2002, Givvimani 2011, and Tehlivets 2011). Since both cardiovascular disease and COPD share a common cause (Izquierdo et al, 2010) which itself causes hyperhomocysteinaemia, it is reasonable to expect that COPD should be associated with elevated HCY.

5. Homocysteine and COPD and oxidative stress

The first study to find a difference in homocysteine in COPD patients was reported by Andersson and colleagues (Andersson 2001). They examined the plasma from 19 patients with COPD and 29 healthy subjects. They found that total plasma homocysteine levels were higher in COPD than controls. But also that there was a decreased concentration of reduced glutathione and decreased reduced to total glutathione ration nine COPD. They speculated on a relationship between HCY as a surrogate marker of extracellular pro-oxidant activity and plasma homocysteine.

6. In vivo studies of homocysteine in COPD patients

Table 1 summarises the subject characteristics on patients in the three studies of lung function, COPD and homocysteine. All of the studies are relatively small but all involved a control arm of asymptomatic subjects. All are cross-sectional studies of COPD outpatients. The first study to link HCY and lung function in COPD was a Japanese study of Kai et al who measured lung function twice within a 1-year interval. In all studies post-bronchodilator FEV1 was measured though it is not clear whether this was done for the controls in the Fimognari et al study. Reversibility was measured only in the Kai et al study. Table 1 shows that the Seemungal et al study enrolled slightly younger patients than both other studies with the Kai et al study enrolling only males. The CRP in both Seemungal et al and Fimognari et al studies was measured using immunometric assays.

The BMI in the Kai et al study was low at 20 kgm^{-2}. Some of the controls, though asymptomatic, may have had abnormal lung function in the Kai et al and Seemungal et al studies as the Mean FEV1 was 76 to 83% but this is unlikely in the Fimognari et al study as the Mean FEV1was 104%. The Kai et al study had COPD patients with the more severe

Variable		Kai 2006	Seemungal 2007	Fimognari 2009
Number	Controls	23	25	29
	COPD	24	29	42
Age	Controls	66.4	64.8	70.6
	COPD	70.7	69.1	71.3
% Males	Controls	100	64	72.0
	COPD	100	79	85
BMI (kgm^{-2})	Controls	24.2	27.4	28.1
	COPD	20.0	24.0	26.5
HCY* (micro Mol/L)	Controls	9.8	8.2	11.5*
	COPD	12.0	12.0	13.9*
FEV1 (L)	Controls	2.58	2.25	N/A
	COPD	1.12	1.43	N/A
FEV1%	Controls	83.3	76.1	104.5
	COPD	38.5	49.1	52.8
FEV1/FVC%	Controls	88.4	78.1	78.0
	COPD	42.7	53.1	53.0
CRP (mg/L)	Controls	N/A	0.89	2.3
	COPD	N/A	2.05	5.5

*Median Values only shown in paper.

Table 1. Comparison of Patient Characteristics in three Lung Function Studies in COPD (Data are expressed as means except where otherwise stated.)

COPD (Mean FEV1 38%) compared to Fimognari et al 53%. In the controls, the HCY levels appeared much lower in the Seemungal et al study than the others. The HCY levels in the COPD patients were identical in the Kai et al and Seemungal et al studies but higher in the Fimognari et al study though in the latter only medians are shown. Also, CRP levels were significantly lower in the Seemungal et al study than in the Fimognari et al Study.

The BMI in the Kai et al study was low at 20 kgm^{-2}. Some of the controls, though asymptomatic, may have had abnormal lung function in the Kai et al and Seemungal et al studies as the FEV1 was 76 to 83% but this is unlikely in the Fimognari et al study as the FEV1 was 104%. The Kai et al study had COPD patients with the more severe COPD (FEV1 38%) compared to Fimognari et al 53%. In the controls, the HCY levels appeared much lower in the Seemungal et al study than the others. The HCY levels in the COPD patients were identical in the Kai et al and Seemungal et al studies but higher in the Fimognari et al study though in the latter only medians are shown. Also, CRP levels were significantly lower in the Seemungal et al study than in the Fimognari et al Study.

In conclusion there are differences in the patients between the three studies that make it difficult to actually compare *all* of the findings.

7. Homocysteine, lung function and lung function decline

The major manifestation of airflow obstruction in COPD is reduced maximum expiratory flow and slow forced emptying of the lungs (FEV1) and these features do not change

markedly over months (GOLD 2010). Most of the lung function impairment is progressive and thus rate of decline in FEV1 is an important outcome measure in COPD. COPD may be accompanied by airway hyperactivity and partial reversibility which when present increases the variance in FEV1 and FVC measurements. To eliminate this all three studies used post-bronchodilator lung function measurements (Kai et al 2006; Seemungal et al, 2007; Fimognari et al,2009).

All three studies agree that HCY is higher in COPD patients than in controls (Kai et al, 2006; Seemungal et al, 2007; Fimognari et al, 2009). But only one study found that HCY was higher in the more severe COPD (please see Figure 2) (Seemungal et al, 2007). Kai et al found that HCY was higher in patients with a higher FEV1 – an opposite finding to the Seemungal et al group.

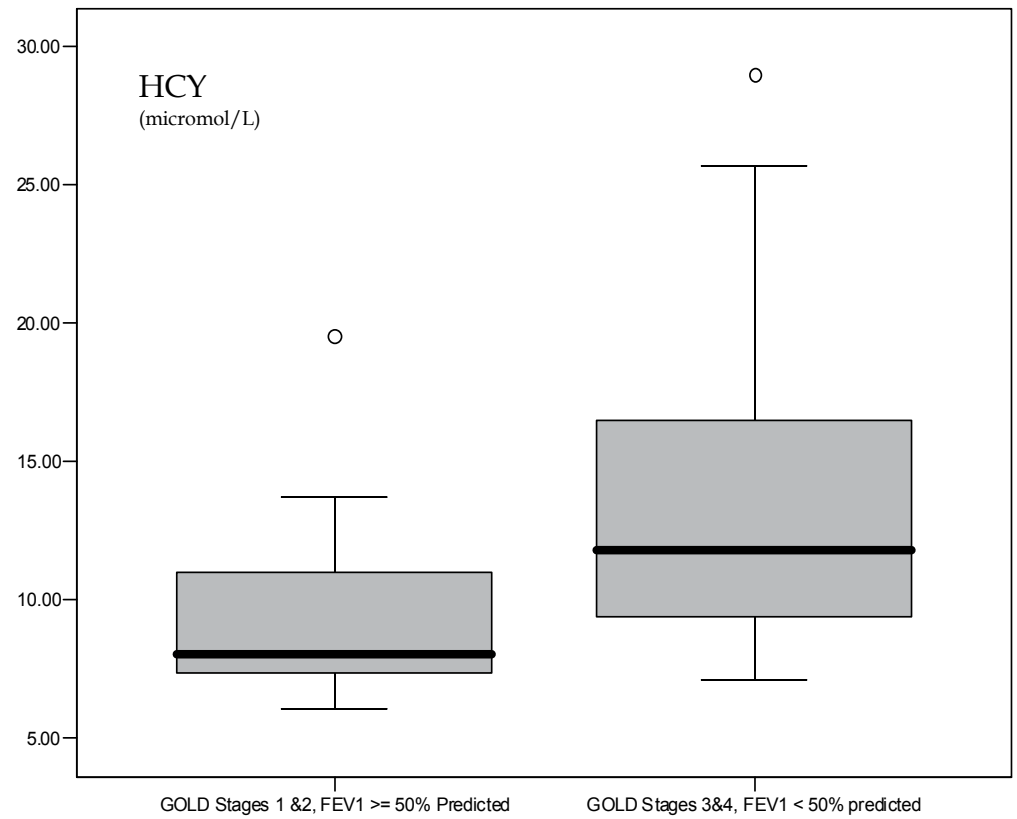

Fig. 2. Homocysteine and COPD severity based on Seemungal et al, 2006.

Kai et al are the only group so far to look at annual decline in FEV1 and HCY. In this study FEV1 decline varied between 0 ml/year and 275 ml /year. However the correlation was positive (r=0.40 and p-value = 0.02), that is, a high HCY was related to a more rapid decline in lung function. The authors have not explained the apparent contradiction between their findings in the cross-sectional analysis (of low HCY related to low lung function) and the paired analysis where FEV1 decline was faster in those with high plasma homocysteine (Kai

et al, 2006). Rather, the relationship with annual decline in FEV1 would appear to support the conclusion of Seemungal et al that COPD severity is related to a higher HCY (Seemungal et al, 2007).

In a subset analysis of the COPD-only group, Kai et al found that those with FEV1 less than 30% (N = 7) had a lower arterial oxygen tension and lower HCY than those with FEV1 greater than 60% (N = 8) – a very small sample in an already small study. However in the entire COPD sample there was no significant correlation between arterial oxygen tension and HCY. Kai et al used this finding to hypothesize that (a) hypoxia could easily occur on exertion in the patients with severe COPD and that (b) there is a possibility that hypoxia played a role in the reduction of the plasma HCY concentration via down regulation of methionine adenosyltransferase gene transcription. The difficulty with this hypothesis is that it is based on a very small subset difference in an already small study (Kai et al, 2006).

8. Homocysteine and CRP: Evidence for immune activation?

Serum C-reactive protein (CRP), is a ubiquitous marker of systemic inflammation, mortality and hospitalisation in COPD (Dahl et al, 2007; Man et al, 2004), cardiac disease in COPD (Sin et al, 2003) and of cardiac disease in the elderly (Zakai et al, 2007). High CRP levels have also been shown to correlate with low 6-min walk test scores (de Torres et al, 2007).

As shown in Table 1, both Seemungal et al and Fimognari et al measured serum CRP in their normal and COPD subjects and though their samples showed significantly different values for mean CRP, they both agreed that CRP was elevated in the COPD subjects compared to asymptomatic controls. The CRP levels in the normal controls in the Seemungal et al study was similar to that in previously published American and Dutch controls (Broekhuisen et al, 2005; Sin & Man 2003) and the greater value of CRP in COPD in the Seemungal et al study is the same as that attributed to COPD by Gan et al in their metaanalysis (Gan et al, 2004).

The Seemungal et al study found a correlation between CRP and HCY which was not found (rho= 0.377, p = 0.005) in the Fimognari et al study. The clinical implication of this finding from the Seemungal et al study is unclear at present more so because it was not supported by the Fimognari et al study. However, a similar correlation between HCY and CRP (as observed by Seemungal et al) has been reported in psoriatic arthritis (Sattar et al, 2007), in cancer (Schroecksnadel 2007) and in elderly patients with cardiovascular disease and dementia (Ravaglia et al, 2004). Taken together these results suggest that HCY may play a role in immune activation in some chronic diseases (Schroecksnadel et al, 2007) and its relationship to HCY in COPD may be a further indicator of the role of HCY in oxidative stress in COPD (Folchini et al, 2011).

9. Homocysteine and quality of life

The St. Georges Respiratory Questionnaire (SGRQ) (Jones et al, 1992) assesses quality of life in three domains: symptoms, activities and impacts. Scores in three domains are combined to give weighted average called the total score (Jones et al, 1992). The SGRQ has been shown to be sensitive to different levels of health (Jones 1997). As a standardised questionnaire the SGRQ has the advantage of allowing direct comparison between different patient

populations and treatment groups and has been shown to be responsive when used for these comparisons (Jones et al, 1991; Jones & Lasserson, 1994). The Symptoms score assesses the degree of distress due to frequency and severity of respiratory symptoms, whilst the impacts component addresses psychosocial effects (Jones & Booth 1997).

Of the three studies, only the Seemungal et al study assessed quality of life via the St. Georges Respiratory Questionnaire (SGRQ) in the COPD subjects. All of the quality of life indices (total, symptoms, impacts and activities) were related to HCY levels with a minimum correlation of: symptoms score 0.295, impacts score 0.330 and total score 0.289. The activities score was the only component not related to HCY. The HCY scores were higher in patients with worse quality of life scores – consistent with the relationships found between FEV1 and HCY (Seemungal et al 2007). The SGRQ scores have been shown to be an important outcome measures in COPD and predict frequent exacerbations and hospitalisation (Seemungal et al, 1998; Wilkinson et al, 2004). Though few serum parameters have been shown to predict exacerbations apart from CRP (Dahl et al 2007), the relationship between HCY and SGRQ does raise intriguing possibilities. This is the only result so far available for HCY and life style in COPD, HCY has been related to life style determinants in cardiac disease (Nygard et al, 1998). Further the relationship of elevated CRP to ten year mortality in COPD (Dhal et al 2007) and of HCY to mortality in coronary artery disease (Nygard et al, 1997; Ford et al, 2007) raises the issue of whether HCY is also related to mortality in COPD which would only be revealed by long term studies of COPD.

10. Effects of diet, renal disease on homocysteine – Other diseases

Kai et al did not assess dietary indices. Prior studies have all found that low vitamin B12 and or folic acid are related to hyperhomocysteinaemia (D'Angelo et al, 1997; Clarke et al, 2003; Kluijtmans et al, 2003). Seemungal et al estimated dietary intake of vitamins using the food frequency questionnaire and found no relation to plasma HCY values but Fimognari et al estimated serum vitamin B12and folic acid levels directly. The Fimognari et al study also attempted to determine if there was a role for co-morbidities in the elevation of HCY in COPD. Thus they attempted to control for those factors known to be associated with hyperhomocysteinaemia such as vascular disease, renal disease and diabetes (Dominguez et al, 2010; Austen et al 2003). When they controlled for these factors in a multivariate analysis in the COPD patients only, they found that the best predictors of high HCY were low serum folic acid, vitamin B12 and triglycerides. This has been supported by further work from the Andersson et al group (Andersson et al, 2007)

Fimognari et al did not measure vitamin B6 levels. Further these multivariate analysis, did not include the normal subjects therefore did not include COPD as a factor even though a prior analysis of all subjects in the Fimognari et al study had shown a relationship between both presence of COPD as well as FEV1% and HCY. It is therefore not clear whether a repeat analysis using all subjects in the study with COPD and FEV1% as independent variables would have yielded significant relationships with three B vitamins.

11. Homocysteine elevation in COPD: Pathogenesis or epiphenomenon?

Three studies shave shown that HCY is elevated in COPD relative to asymptomatic controls. The Kai et al study showed that COPD patients with a high HCY were likely to have faster

decline in FEV1. Seemungal et al showed that HCY was related to COPD severity. Taken together these results suggest that HCY is involved in COPD pathogenesis. In 2001 Andersson et al showed that HCY was elevated in COPD and that patients with high HCY were more likely to have a low reduced GSH and low GSH:GSSG ratio (Andersson et al, 2001; Sibrian-Vazquez et al, 2010). Further there is evidence from a laboratory study that low levels of reduced glutathione are associated with emphysema in the rat (Hamlet et al, 2007). These studies suggest that HCY is involved in redox pathways in COPD and that a high HCY reflects an imbalance in the redox state favouring oxidative stress. However only cohort studies will allow us to determine which comes first the oxidative stress or the elevation in HCY.

12. Implications for management

The implications for management of COPD are not yet known. However, for now, COPD patients with an elevated HCY should be screened for cardiac disease and more closely monitored for evidence of a faster decline in lung function. Investigations into the role of antioxidants that may effectively lower HCY are ongoing (Zinellu et al 2008).

13. Concluding comments

Homocysteine is a ubiquitous amino acid, elevation of which is associated with several diseases as diverse as thrombotic disorders and psoriasis. There is a strong link between cardiac disease and homocysteine levels. The cause and effects of HCY elevation in COPD are unknown but preliminary studies suggest that HCY is related to COPD pathogenesis and is likely to be associated with disorders in the redox pathway leading to oxidative stress in COPD. It is unknown whether HCY infiltrates the epithelium of the airway but HCY may well affect the endothelium of the lung.

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3

Chronic Obstructive Pulmonary Disease: Emphysema Revisited

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1. Introduction

The chronic obstructive pulmonary disease (COPD) encompasses two phenotypically related diseases, chronic bronchitis and emphysema [1-2]. Although the hallmark of COPD is inflammation and inability to maintain efficient gas exchange, emphysema is often characterized by atypical over-distension of the alveoli and permanent destruction of the surrounding supporting structures leading to irreversible damage to gaseous exchange. Statistically, it is rapidly approaching a leading cause of mortality in the United States [3], with a morbidity of 4.9 million [4] and mortality rate at 4.2 per 100,000 [5]. Even with a higher prevalence of COPD related incidences in chronic bronchitis, mortality from emphysema (12,790) had exceeded that of chronic bronchitis (667) in pulmonary-related deaths [5] making early diagnosis and treatment of emphysema an alarming and continued cause for concern.

2. Pathogenesis of emphysema

The pathogenesis of emphysema is embodied by enlargement of alveolar space, progressive destruction of connective tissue and loss of elasticity leading to eventual collapse of the small airways and destruction of pulmonary-alveolar units, all of which combine to limit gas exchange and airflow out of the lungs. These physical manifestations are usually initiated by chronic inflammation and induction of persistent oxidative stress mediated primarily by the neutrophil, a key innate immune cell residing in the conducting airways [6]. The persistent thought for the past four decades was that the tenuous balance between proteases and anti-proteases determined the severity of airway damage and alveolar enlargement, with the main protease-inhibitor, alpha1 anti-trypsin (AAT1) [6] severely lacking to combat the secretagogue elastase, from the chronically activated resident neutrophils [7-8]. Activated neutrophils also promote the secretion of mucus and inhibit the clearance ability of mucocilliary cilia thus adding to the severity of disease.

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The role of another innate cell, the alveolar macrophage which resides in the alveolar region [9-10] has been demonstrated to be markedly influenced by the pro-inflammatory milieu and also promote the secretion of elastase [11] primarily matrix-metalloproteases (MMP), and key cytokines/chemokines responsible for sustaining inflammation and inhibition of the healing or resolution process [11]. Of all the MMP implicated, MMP-9 was demonstrated to be markedly elevated in patients with emphysema [12-13]. Even though MMP-9 is capable of breaking down extra cellular matrices and cause destruction of the septal and alveolar walls, recent evidence has questioned its role [14-15] suggesting that some of the MMP may not be directly associated with the progression of disease as inhibiting the expression does not affect the severity in smoke-induced emphysema in rodents.

A majority of recent documented emphysema cases is primarily attributed to inhalation of damaging stimuli such as cigarette smoke (CS) [16]. The emergent contribution of oxidants is integral to the development of emphysema. CS either in the form of chronic smoking or in the laboratory to induce experimental-emphysema in rodents, consists of both reactive oxygen and reactive nitrogen species, both of which are robust inducers of inflammation [17]. Additionally, other components of CS such as particulate matter, in itself a potent activator of macrophages [18-19], is able to induce the generation of macrophage-derived oxidants, thus adding to the persistence of a pro-inflammatory milieu. These CS- and macrophage-derived oxidants can contribute to the inactivation of AAT1, promote the activation of MMP and impair the pulmonary-alveolar units (PAU) *in situ,* therefore exaggerating the already compromised situation [20]. Another index of emphysema which has recently emerged is the increased pulmonary capillary endothelial cell turnover as a direct result of the destruction of the PAU [21], resulting in the elaboration of endothelial microparticle (EMP). Using stringent criteria to determine the source of PAU, the authors confirmed that emphysematous patients have increased circulating plasma levels of pulmonary capillary endothelium-derived EMP as a result of increased endothelial turnover. They also added that the detection of EMP in the plasma superseded any spirometry evidence of emphysema, thus adding an early novel albeit invasive biomarker of emphysema.

There are three major causative factors for the development of emphysema; environmental, social and genetic predisposition. Coal miners and those working with silica often inhale airborne toxicants that contribute to initial oxidative stress [22]. It is the inability to remove these toxicants that lead to progressive inflammation and destruction of respiratory tissue. In addition to the anti-protease role of AAT1, recent evidence documenting the role of AAT1 as an anti-inflammatory protein [reviewed in 23] only highlights the importance of those who inherited this deficiency. The influence of smoking or 'inhalation of toxicants by choice' remains a controversial and somewhat contentious causative factor of emphysema. However, the combination of AAT1 deficiency and chronic smoking or coal miners who are heavy smokers almost always result in the progressive development of emphysema.

3. Presentation of COPD: Emphysema

Chronic obstructive pulmonary disease (COPD) has significant impact on not only patients inflicted with the disease but also on the healthcare system under which they are taken care of.

Patients with severe COPD have significant physical impairment with reduction in quality of life and can result in death. The healthcare system requires that patients have multiple office visits and frequent hospitalizations on top of chronic lifelong therapy that includes medications and long-term oxygen treatments. Early diagnosis of COPD can help early management of symptoms and make lifestyle changes such as the cessation of smoking, as ultimately these practices are the only options to slowing the progression of disease. However, this disease is still underdiagnosed with only 15% of smokers diagnosed with COPD [24].

Patients with emphysema can have varying degrees of symptoms ranging from few to chronic respiratory complaints with acute exacerbations. Some symptoms include dyspnea, cough, wheezing, and acute chest illnesses. Any patient with a chronic cough, dyspnea or a history of any exposure to risk factors such as CS, occupational dusts, chemicals and smoke from home or heating fuels should have the diagnosis of emphysema suspected for their symptoms [25]. Some patients may unknowingly restrict their own activities since emphysema is slowly progressive and persistent. Physical examination findings are usually only present in patients with severe disease which includes, over distention of the lungs in the stable state with the chest held near full inspiratory position at the end of normal expiration, and a low diaphragmatic position resulting in retraction of the lower intercostal spaces (Hoover's sign) [26]. Additionally, there is decreased intensity of breath and heart sounds accompanied by a prolonged expiratory phase [27]. Wheezes on auscultation on slow or forced breathing with a prolongation of forced expiratory time may be evidence of airflow obstruction. Some of the frequently classically described characteristics include purse-lip breathing, accessory respiratory muscle usage of the neck and shoulder girdle muscles. Other findings may be unusual positions to relieve dyspnea such as leaning forward with arms outstretched (tripod position) [28], digital clubbing, dependent edema in the absence of right heart failure, neck vein distention and an enlarged liver due to right heart failure.

Patients with suspected emphysema may present at various stages of the disease process. Chest radiography is usual the initial study performed or ordered but will not be diagnostic except in severe cases, however, is still important to exclude other lung diseases. Signs of hyperinflation on the chest radiograph include; rapidly tapering vascular shadows with increased radiolucency of the lung, a flat diaphragm and a long narrow heart shadow on posterior-anterior films and flat diaphragmatic contour and increased retrosternal airspace on a lateral radiograph (*Figure 1a-b*). Bullae may also be identified on a chest radiograph. Computed tomography (CT) is better able to characterize the involvement pattern as either centriacinar or panacinar. Centriacinar usually involves the upper lobes in the center of secondary pulmonary lobules, in contrast to panacinar with involves the lung bases and the entire secondary pulmonary nodule with generalized paucity of the vascular structures. CT has become the mainstay in evaluating patients for lung volume reduction surgery [29-30].

Pulmonary function tests (PFTs) have become the cornerstone of the diagnostic evaluation of patients because these patients may be symptomatic but have no physical exam findings [31]. PFTs can determine the severity of the airflow obstruction and can also be used to follow the disease progression, with spirometry being the essential confirmatory test that not only stages COPD but also distinguishes the phenotype of COPD [32]. The forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) is needed to

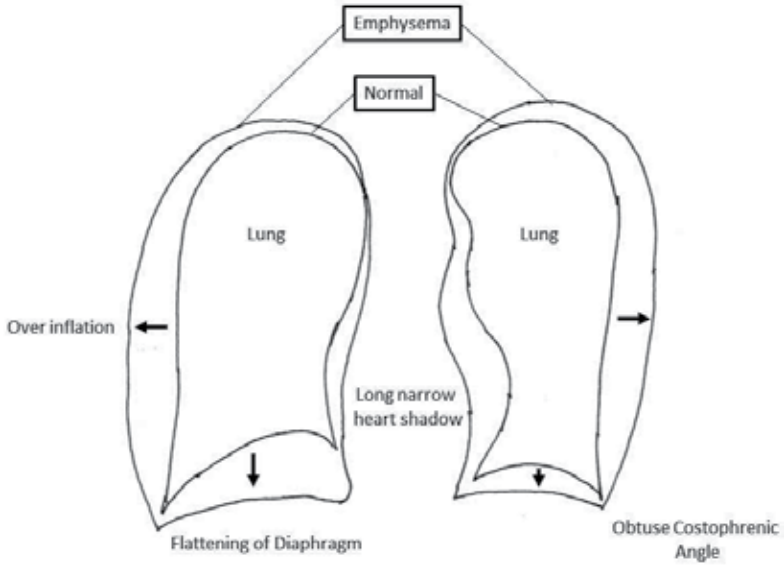

Fig. 1a. Schematic of typical anteroposterior

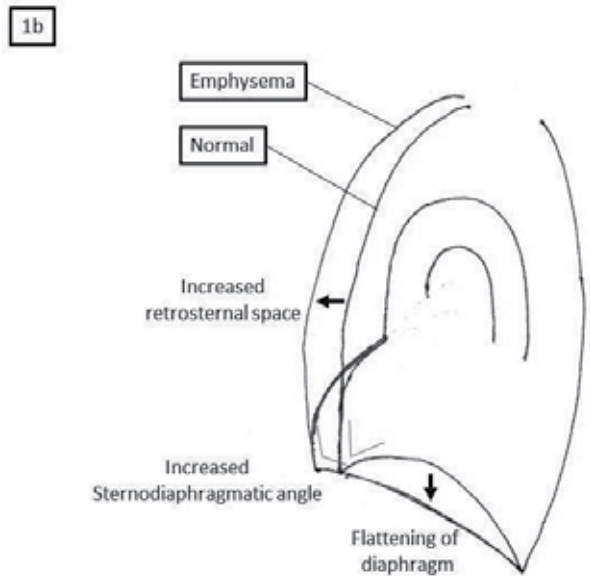

Fig. 1b. Lateral chest radiograph findings of emphysema.

establish the presence of obstruction, with a ratio of less than 0.70 being significant for obstruction [33]. The inspiratory capacity is usually decreased with tachypnea due to dynamic hyperinflation and increased total lung capacity, functional residual capacity and residual volume. The measurement of carbon monoxide diffusing capacity can help to establish the presence of emphysema but is not used in the routine diagnosis of COPD. Arterial blood gases (ABG) is used to correlate symptoms with blood oxygenation levels but is not needed in mild to moderate airflow obstruction. ABG is optional in moderately severe

airflow obstruction however, for severe disease, ABG then becomes the major monitoring tool once hypoxemia with hypercapnia develops.

4. Treatment of emphysema

The treatment of pulmonary emphysema has not only puzzled surgeons but has also attracted their interest throughout history. Many operations have been proposed however, it was not until further understanding of the physiological impairment of the disease was understood that appropriate surgical treatment evolved. Surgical treatment for patients on maximal medical therapy but remain symptomatic carries both morbidity and mortality; however, the operations also carry with them the hope of relief from dyspnea. Three typical operations performed for emphysema are bullectomy, volume reduction, and lung transplantation.

4.1 Bullectomy

Bullae are defined as emphysematous spaces larger than 1 cm in diameter in the inflated lung, usually demarcated from surrounding lung tissue and pathologically consists of enlarged airspaces covered by visceral pleural. Bullae have been characterized into three different types with Type I and II associated with diffuse emphysema, with type III representing complete loss of parenchymal architecture throughout lung fields [34]. Bullae in emphysematous disease is believed to arise *via* a ball-valve mechanism where air is allowed to enter the airspace but not allowed to escape with progressive enlargement of the airspace over time [35-36]. The enlarged space-occupying lesion leads to compression of the surrounding emphysematous lung tissue with preferential filling of the bullae, when exposed to the same negative intrapleural pressure results in continued enlargement [37-39]. The indication for a bullectomy is to permit expansion of the previously collapsed surrounding lung tissue to regain function as well as restore physiologic respiratory function [40]. Compression of surrounding lung tissue by bullae impairs overall gas exchange due to low ventilation to perfusion ratios in the compressed lung region. Furthermore, bullae can result in increased intra-thoracic pressures eventually resulting in hemodynamic dysfunction from compression of the pulmonary arterial system, decrease in systemic venous return, and increased expiratory airway resistance [40]. Large bullae my also restrict the function of the diaphragm and the thoracic chest wall muscles. Bullectomy would remove the space-occupying lesion, reduce expiratory airway resistance, and reduce dead-space ventilation that may result in overall improvement in respiratory function.

The benefit of a bullectomy must be determined based on careful selection of individuals based on the size of the bullae, the degree of compression and whether underlying condition of compressed lung parenchyma exists [42-45]. The highest predictor for benefit from a bullectomy is that young individuals with large localized unilateral bullae that are nonventilated, nonperfused, with significant compression of surrounding lung tissue that has good perfusion and early emphysema [43]. Although posteroanterior and lateral radiographs can identify bullae, CT has become the mainstay imaging technique in delineating the anatomy of bullae [46]. The operative approach for bullectomy is variable and is dependent on the anatomic details of the bullae and specific techniques deployed by the surgeon in question. Single large bullae with a small pedicle may be excised using either a muscle-sparing thoracotomy or a video-assisted thorascopic surgery (VATS), and resected

with a stapler (*Figure 2)*. In some instances, large bullae may have completely destroyed an entire lobe requiring lobectomy.

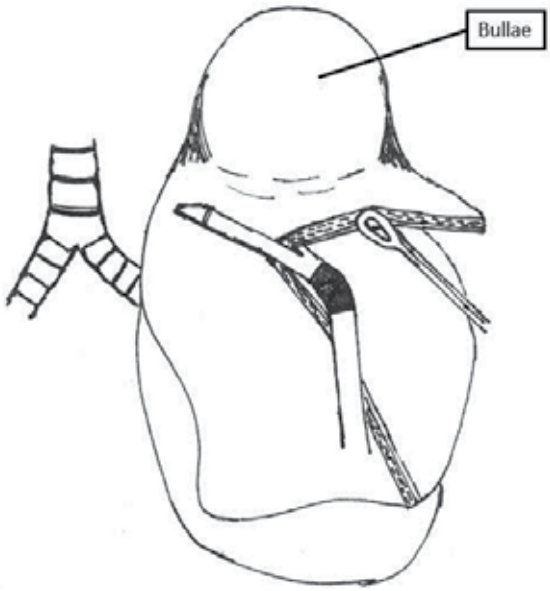

Fig. 2. VATS stapled bullectomy.

Mortality rates of bullectomy should range from 1 to 5%. The mortality rate of approximately 2.3% in well-selected patients was established more than 30 years ago by Fitzgerald *et al* with similar results in modern studies [42, 47]. Similar rates have been seen in patients undergoing VATS compared to thoracotomy approaches. Delayed expansion of the remaining lung tissue, parenchymal air leaks and pulmonary infections are some known post-operative complications, with air leaks being the most frequently occurring. Persistent air-leaks can be managed with the use of a Heimlich valve [48]. Results from bullectomies are difficult to analyze given that there are no prospective randomized clinical trials comparing medical therapy with surgery, as all previous studies were retrospective case series. The most recent series by Schipper *et al* looking at intermediate- to long-term results showed improvement in functional status up to 3 years after resection of giant bullae [47].

4.2 Lung volume reduction (LVR)

Lung volume reduction (LVR) is similar to bullectomy, the difference being that LVR is an extension performed for diseases that affect the entire lung. LVR was pioneered by Otto Brantigan in the 1950s but was not adopted due to a high mortality rate of 18% [49]. Brantigan's initial hypothesis was that the diseased portions of the lung resulted in loss of elasticity and that removing the most diseased portions permitted and maintained patency of the remaining bronchioles to improve airflow [49]. It was not until several decades later that Delarue *et al* [50] and Dahan [51] *et al* re-introduced the concept in patients with end-stage emphysema. However, the role of LVR would not become popular until 1994 when Cooper [52] adapted Brantigan's initial concept. The underlying pathology of end-stage emphysema is characterized by distended airspaces that are inadequately ventilated but

with continued perfusion (ventilation-perfusion mismatch) nevertheless, resection of the diseased portions would result in improved ventilation to other functional regions. LVR also serves to re-establish normal chest wall dynamics and may result in improvement in hemodynamic function from the lowering of intra-thoracic pressure throughout the respiratory cycle.

Selection of candidates for LVR is dependent on the anatomic characteristics of the diseased portion of the lung with ideal candidates having heterogeneous upper-lobe involvement [53]. There is less dramatic improvement in candidates undergoing LVR with lower lobe involvement [54]. Patients being considered for LVR usually undergo scintigrams to identify potential targets for resection. The most important factor for success of LVR has been the meticulous selection of patients based on the National Emphysema Treatment Trial or NETT criteria [55]. Again CT has become the mainstay for characterization of possible resection margins. The mortality rate ranges from 0 to 7.5% with varying surgical approaches [52, 56-58]. Multiple approaches have been used including median sternotomy, bilateral thoracotomies or VATS (*Figure 3)*. All have similar results with functional improvement disappearing over a period of 3 to 5 years, but LVR patients continue to have a clinical advantage over medical treatment for those 3-5 years with substantial gains in exercise tolerance, freedom with oxygen therapy, and overall improvement in quality of life. The most significant trial, NETT, reported the results which included 1218 patients randomized between LVRS and medical therapy between January 1998 and July 2002 [59]. This trial reported a 90-day surgical mortality of 7.9% without a significant difference in surgical approach. Patients with upper-lobe predominant emphysema had a greater survival benefit from surgery while those with lower-lobe predominant emphysema demonstrated survival benefits from medical therapy. Based on these promising results, LVR can be performed for patients who are not candidates for lung transplant.

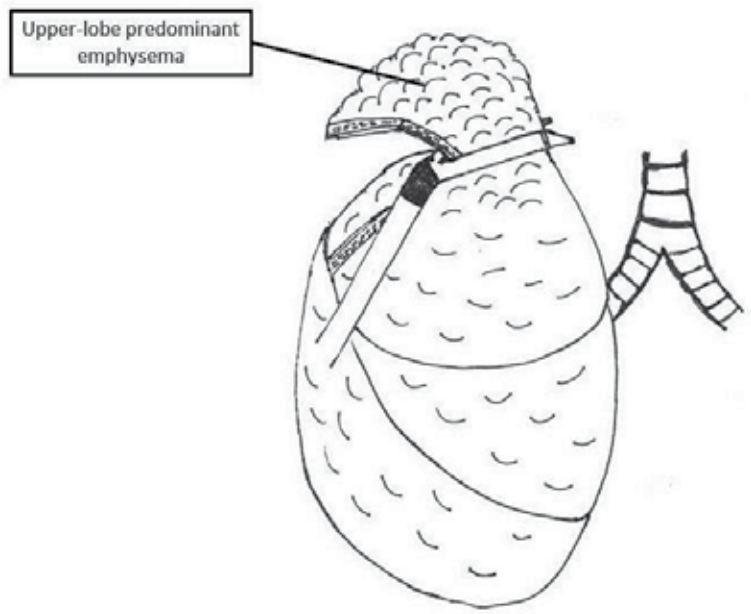

Fig. 3. VATS lung volume reduction surgery for upper-lobe predominant emphysema.

A new technique of lung volume reduction with less morbidity and mortality compared to surgical LVR is bronchoscopic lung volume reduction (LVR). Bronchoscopic LVR aims to achieve the same goals as LVRS: improve physiologic mechanics of the chest wall and diaphragm, restore ventilation-perfusion matching, and improve expiratory airflow. The method by which this is achieved is through the use of a bronchoscope to deploy one-way valves, administer sealants, or apply thermal ablation to exclude diseased portions of the lung [60-62]. It is important to note that these techniques have not been approved by the Food and Drug Administration (FDA) for the treatment of severe emphysema and are currently utilized on experimental basis only. The most developed and well-studied of the bronchoscopic techniques is the one-way valve. One-way valves allow air and mucus to escape from the excluded portion of lung yet concomitantly excluding that portion of the lung from normal physiological function. [61] Multiple valve designs have been tested, the largest on which was the Endobronchial Valve for Emphysema Palliation Trial (*Figure 4)* (VENT) [63-64] There was significant improvement in dyspnea, exercise capacity, and quality of life but not as significant as that which is seen in LVRS. [64]. Possible explanations for the minor improvement seen compare to LVRS have been attributed to collateral ventilation through incomplete lobar fissures. There were more complications of pneumonia, hemoptysis, and pneumothorax in the treatment group, all of which would be expected from an invasive procedure. [64]. The administration of sealants is far less developed and studied when compared to that of one-way valves, and includes the use of fibrin-thrombin mixtures to create a scaffold for collagen deposition by fibrblasts [65]. Preliminary studies show minor improvements in pulmonary function tests but no significant clinical benefits [66-67].

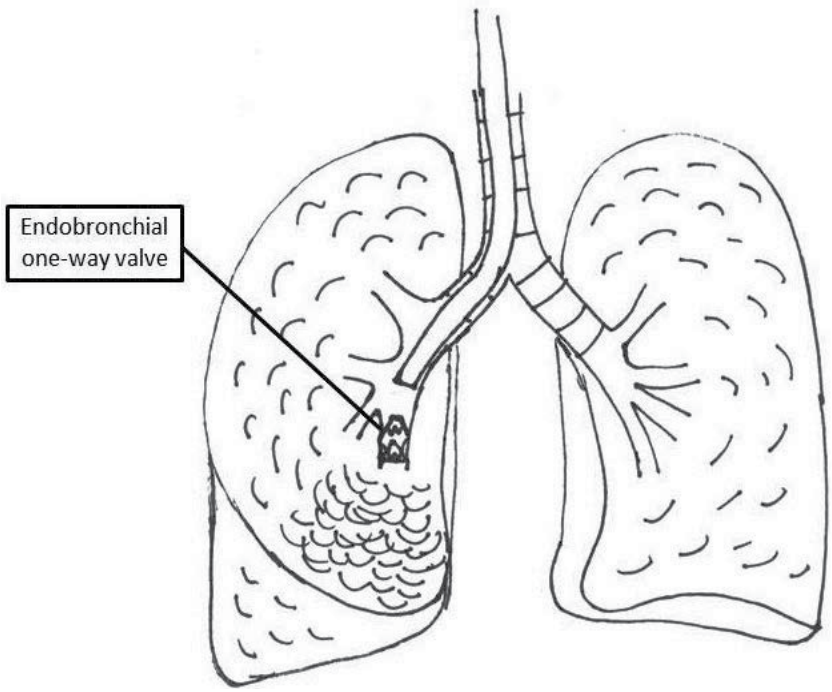

Fig. 4. Endobronchial lung volume reduction with one-valve for lower-lobe predominant emphysema.

Thermal ablation is the least developed and studied of the bronchoscopic LVR techniques. The use of heated vapor to induce an inflammatory response resulting in occlusion of a diseased portion of lung has only been tested in feasibility studies with further exploratory efforts required due to small sample sizes [68]. Although bronchoscpoic LVR is an emerging technique, further analyses as well as long-term follow-up studies are needed.

4.3 Lung transplantation

Lung transplantation was originally thought to not be a feasible treatment for emphysema. It wasn't until after the seminal transplantation of single lungs demonstrating significant improvement in symptoms, that lung transplantation became a mainstay for the end-stage emphysema [69-71]. Currently, the most common indication for lung transplantation is idiopathic diffuse emphysema and AAT1 deficiency, two criteria that account for the majority of lung transplants [70]. Lung transplant patients are usually so critically ill that the risk of death from their lung disease enables the actual lung transplant operation to appear quite equitable. The advantages of a lung transplant result in complete replacement of the diseased lung with significant improvements in symptoms [72]. There are however, significant disadvantages to lung transplantation including higher mortality (5 to 15%), lifelong immunosuppression resulting in risks of serious infection and rejection with a cumulative survival rate of around 50% [73-74].

5. Summary

Emphysema can be a preventable and equally treatable pulmonary disease. With the advent of new diagnostic criteria such as the emergence of a key biomarker, circulating levels of EMP may lead to efficient diagnosis and preventative care. Patients with emphysema can present a varying array of symptoms and physical examination findings. While the majority of patients can be managed with medical therapy, those who continue to progress may require surgical intervention based on their diagnostic studies. The ideal surgical treatment of emphysema is dictated by a rigorous selection criteria for each of the possible interventions described and can dramatically improve the quality of life of individuals inflicted with this disease. New and innovative methods for treating crippling emphysemic patients who are not candidates for surgical treatments include bronchoscopic placement of one-way valves into diseased segments of lung tissue or airway bypass by means of inserting stents between bronchi and adjacent lung tissue [75-78], however, these emergent techniques necessitate further exploratory and long term studies.

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4

Diverse Activities for Proteinases in the Pathogenesis of Chronic Obstructive Pulmonary Disease

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1. Introduction

COPD remains a major public health problem. It is the fourth leading cause of chronic morbidity and mortality in the United States, and is projected to rank fifth in 2020 in burden of disease caused worldwide, according to a study published by the World Bank/World Health Organization. COPD is a preventable and treatable disease, with some significant extra-pulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. Smoking remains the major risk factor for this disease, but inhalation of other pollutants and genetic factors also play a role.

Inhalation of cigarette smoke and other pollutants leads to a chronic inflammatory process in the small airways and the lung parenchyma, which includes an influx of macrophages, polymorphonuclear neutrophils (PMN), T lymphocytes (with CD8+ T cells exceeding the numbers of CD4+ T cells), and B lymphocytes (1-4). This inflammatory process over a prolonged period, leads to destruction of the alveolar walls leading to airspace enlargement, loss of lung elasticity, closure of small airways, and irreversible airflow obstruction. Pathological changes also include mucous metaplasia and mucus hyper-secretion. The small airways narrow due to the combined effect of mucus plugging, inflammation in the airways walls and lumen, and subepithelial fibrosis and can become obstructed (1). COPD is a complex disorder with many processes at play but there is strong evidence that proteinases make critical contributions to all the pathologic processes detected in the lungs of COPD patients.

2. Classification of proteinases

Proteinases are named for their action, i.e. to cleave the internal peptide bonds of polypeptides. In human biology they are classified into 4 groups based on the chemical

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nature of their active site: serine, metallo-, cysteine, and aspartic proteinases. Serine proteinases and MMPs are the major players in extracellular proteolysis and are optimally active at neutral pH. Cysteine and aspartic proteinases work mainly in the cell in the breakdown of proteins in lysosomes. These are optimally active at acidic pH. These acid proteinases can potentially breakdown extracellular proteins if they can keep catalytic activity at neutral pH or are released into an environment having an acidic pH, such as the pericellular environment of activated macrophages (5,6). Proteinase inhibitors are generally specific to individual classes of proteinases. Proteinases of the serine, metallo- and cysteine proteinase classes have been shown to have activities that contribute to COPD pathogenesis.

2.1 Serine proteinases

Members of this group that are implicated in COPD include PMN-derived serine proteinases, urokinase-type plasminogen activator, granzymes, and plasmin.

PMN-derived serine proteinases

Neutrophil elastase (NE), proteinase 3 (PR3), and cathepsin G (CG) make up this group. The proteinases are stored in an inactive form within granules in PMN (Figure 1) and pro-inflammatory monocytes {8}. When the cells are stimulated by pro-inflammatory mediators they degranulate releasing the enzymes (7,8). These serine proteinases have a broad action against extracellular matrix (ECM) proteins (especially elastin) and non-ECM proteins (7). Figure 2 illustrates how the catalytic triad at the active site of NE (His^{41}-Asp^{99}-$Ser1^{73}$) cleaves the internal peptide bonds of proteins.

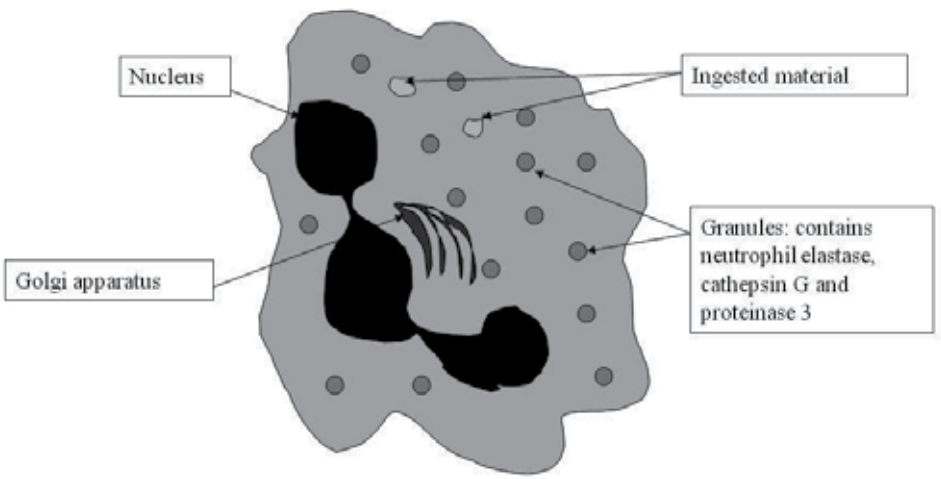

Fig. 1. Structure of neutrophil: Proteinases are stored in an activated form in the azurophilic granules within the neutrophil

Urokinase type plasminogen activator (uPA)

This enzyme is expressed by PMN, monocytes, and macrophages. This enzyme is also stored in and released from the specific granules of PMN. The expression of uPA is regulated at the transcriptional level in mononuclear phagocytes by pro-inflammatory

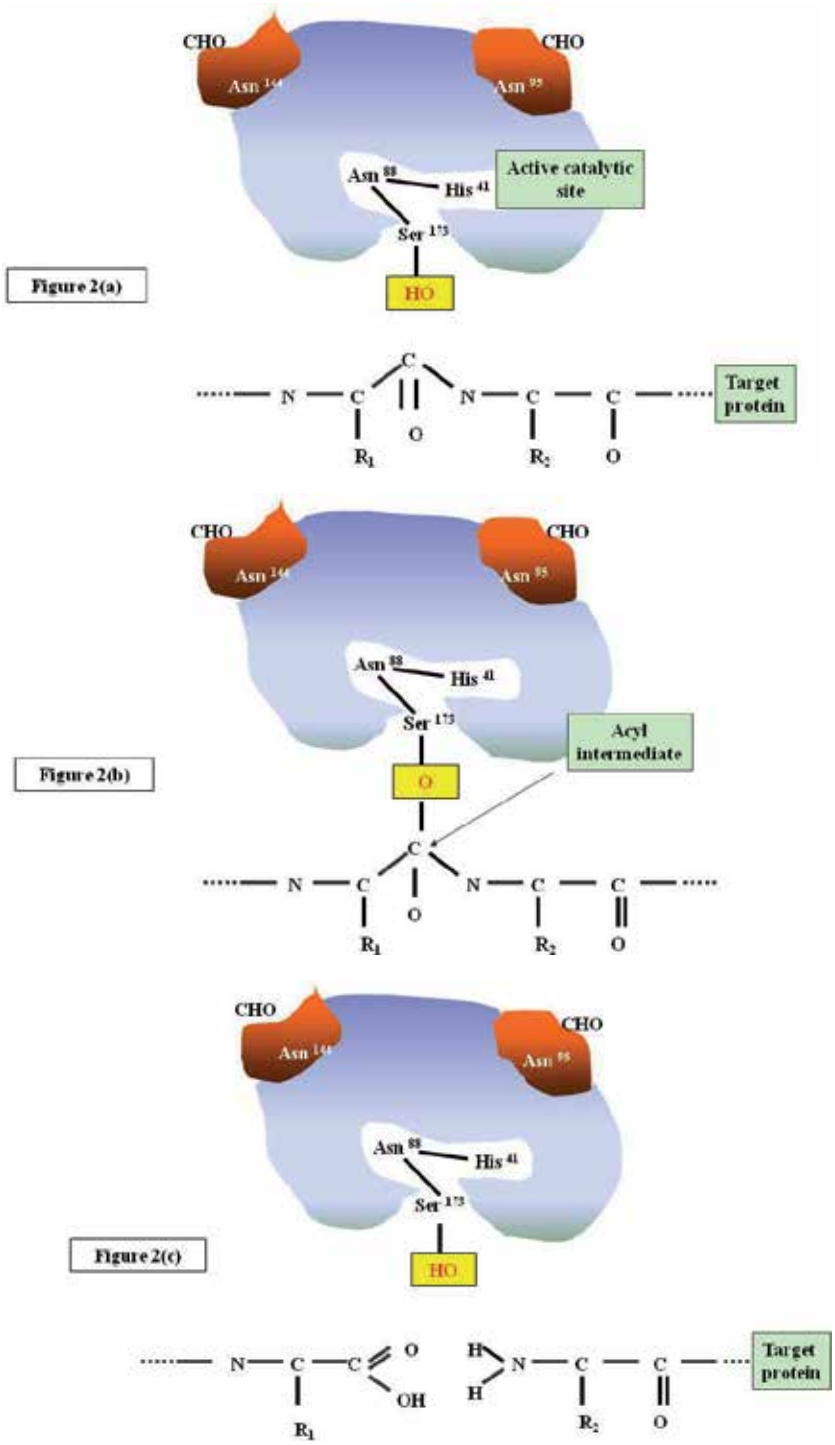

Fig. 2. (a) Mechanism by which NE cleaves a target protein. The NE molecule has two complex carbohydrate side chains attached to Asn[95] and Asn[144]. The catalytic site of the NE

molecule is an indentation of the molecule and is composed of the molecule and the triad His41-Asp88-Ser173, in which the γ-oxygen of serine becomes a powerful nucleophile able to attack a suitably located carbonyl group on the target substrate. The bond to be cleaved must fit into the active site pocket of the NE held there by charge interactions mediated by the residues forming the pocket. The peptide bond under attack is between two amino acid residues recognized by their side chains R_1 and R_2. (b) An acyl-enzyme intermediate molecule is formed between serine and the carbonyl group on the target protein. (c) The acyl-enzyme complex is hydrolysed with subsequent regeneration of active NE and cleavage of the protein.

mediators (9,10). On release from cells, uPA binds to a specific receptor (uPA receptor) on phagocyte surfaces, where it functions as a cell-associated proteinase. The main action of uPA is to activate the serine proteinase, plasmin from its inactive form, plasminogen. Plasmin lyses blood clots by breaking down fibrin, but also cleaves and activates latent growth factors, latent pro-metalloproteinases (MMP), and protease-activated receptor-1 (PAR-1) on macrophages, which drives macrophage MMP-12 production (11-14). Through this action, it plays an important role in ECM degradation and fibrotic processes in the lung.

Granzymes (GRZ)

These **gran**ule-associated en**zymes** are predominantly expressed by CD8+ T lymphocytes and are stored in the lytic granules (15). The main GRZ family members in human CD8+ T cells are GRZ A and B. Once activated by antigen, the CD8+ T cells commence rapid exocytosis of GRZ and perforin-containing granules. Release of perforin alters the properties of the cell membrane of the target cells, heralding the entry of GRZ into the target cell, and GRZ A and GRZ B then initiate caspase-independent and caspase-dependent apoptosis, respectively.

For this group of proteinases there exists naturally occurring inhibitors. Serine proteinase inhibitors (Serpins) in plasma and interstitial fluids include α_1-anti-trypsin (AAT), α_1-antichymotrypsin, plasminogen activator inhibitors, α_2-plasmin inhibitor, and the universal inhibitor, α_2-macroglobulin (α_2-M), which inhibits all four classes of enzymes (16). Secretory leukocyte proteinase inhibitor (SLPI) and elafin are inhibitors synthesized locally in the respiratory tract by epithelial cells.

2.2 Metalloproteinases

Included in this group of proteinases are the MMPs and the members of the ADAMs family.

MMPs

These proteinases have an NH_2 terminal pro domain, an active site zinc atom, and a COOH terminal hemopexin domain that regulates the binding of the enzymes to their substrates. They are stored in a latent form as the inactive proenzymes or proMMPS, a state maintained by an interaction between the active site zinc atom and the cystein residue in the pro domain. Disruption of this interaction is required for activation of the proMMPs. This is facilitated by the actions of other proteinases and oxidants in the extracellular space (17,18)]. The intracellular serine proteinase, furin, is responsible for activation of some MMPs (19,20).

MMPs can be synthesized de novo by cells activated by pro-inflammatory mediators or growth factors. PMN, however, store preformed MMP-8, MMP-9, and MT6-MMP (MMP-25) in their cytoplasmic granules, and release the enzymes when they degranulate (7). Macrophages express MMPs-1, -3, -7, -9, -12, and -14 (21,22), and lung epithelial cells and fibroblasts produce MMPs-2, -9 and -14.

MMPs are classified into 6 groups based upon a similar domain organization and substrate specificity: 1) the interstitial collagenases (MMPs-1, -8, and -13); 2) the gelatinases (MMPs-2 and -9); 3) the stromelysins (MMPs-3, -10, and -11); 4) matrilysin (MMP-7); 5) metalloelastase (MMP-12); and 6) membrane-type MMPs (MT-MMPs), integral membrane proteinases with either a transmembrane domain or a glycosylphosphatidyl-inositol anchor to the cell membrane (23,24). The interstitial collagenases degrade interstitial collagens. The other subgroups have a broader range of substrates including denatured collagens (gelatins), basement membrane proteins, and pro-inflammatory mediators. MMPs-7, -9 and -12 also degrade elastin (7).

ADAM

This is a family of type I transmembrane proteinases, named ADAMs because they contain **a d**isintegrin and **a m**etalloproteinase domain (25). The metalloproteinase domain of ADAMs plays a role in regulation of inflammation, apoptosis and possibly fibrotic processes by shedding membrane-anchored cytokines such as pro–tumor necrosis factor (TNF-α), other cytokines, growth factors, apoptosis ligands and receptors for these molecules from cell surfaces (25-27). The disintegrin domain is involved in cell adhesion and migration which it accomplishes by binding to integrins (25).

The inhibitors of the MMPs include the universal inhibitor, α_2-M, and the four members of the tissue inhibitors of metalloproteinases family (TIMPs1-4), which are synthesized by connective tissue cells and leukocytes and form non-covalent complexes with MMPs (28,29). Although the inhibitors of ADAMs have not been fully elucidated, it is known that ADAM-17 is inhibited by TIMP-3 but not TIMP-1 or -2 (30,31).

2.3 Cysteine proteinases

This group includes the cathepsins B, H, L, and S, which have been implicated in COPD. Cathepsin S and L are potent elastases *in vitro* (5,6) and contribute to macrophage-mediated ECM degradation. Inhibitors for this group, again include the universal inhibitor, α_2-M, but also the cystatin superfamily and the kininogens (32).

3. Evidence for activities for proteinases in COPD

The proteinase/anti-proteinase hypothesis for the pathogenesis of COPD is not a new concept. It dates back to experimental work done over 50 years ago. The basis of the concept was 2 key observations. The first came from the keen observations by Laurell and Eriksson who noted that deficiency of AAT was associated with early onset, severe panlobular emphysema (33). AAT has since been shown to be the major inhibitor of NE in the lower respiratory tract. The second observation was made when instillation of papain (an enzyme with elastase activity) into rat lungs was shown to cause progressive airspace enlargement (34). Over the years, other elastolytic proteinases have been shown to cause airspace

enlargement when instilled into the lungs of animal models. The concept proposed that the imbalance between proteinases (especially elastases) and their inhibitors lead to pulmonary emphysema. Emphysema, however, does not account for all COPD patients and whereas AAT deficiency is a cause of COPD, AAT deficiency only accounts for approximately 2% of COPD. Other factors have now been implicated in airspace enlargement in COPD, including other classes of proteinases (MMPs and cysteine proteinases), oxidative stress, and apoptosis of lung structural cells. COPD is a clinically and pathologically heterogeneous disease and includes chronic inflammation in the alveolar space, airways, and lung interstitium; mucus hypersecretion; and subepithelial fibrosis in the small airways. Although the proteinase/ antiproteinase concept does not account for all of the complex pathologies that make up COPD it certainly has far-reaching effects, many of which have been investigated in *in vitro* studies, and studies of human samples from COPD patients and animal models of COPD

4. Proteinase biology in cells relevant to COPD pathogenesis

The role of proteinases in COPD has been studied at a cellular level with *in vitro* studies.

Lung inflammation and airspace enlargement

The serine proteinases, NE, CG, PR3, and GRZ, can promote lung inflammation in COPD patients, through their direct action stimulating the release of pro-inflammatory mediators from airway epithelial cells and macrophages in vitro (35,36) and many proteinases also have an indirect action proteolytically cleaving mediators to alter their biologic activities. The metalloproteinases, MMPs-8 and -9, cleave and activate various chemokines *in vitro* (37,38). ADAM-17 and several MMPs shed and activate membrane-associated, latent pro–TNF-α from macrophage surfaces (25,27,39). NE, MMP-12, and MMP-9 cleave elastin, and MMPs cleave AAT, generating fragments of these two molecules that are chemotactic for inflammatory cells (40,41). Serine, metallo-, and cysteine proteinases acting together can degrade elastin, interstitial collagens, and basement membrane proteins *in vitro* (7). The degradation of these ECM proteins leads to the enlargement of lung airspaces.

Airway pathologies

The proteinases play a role in the characteristic airway pathologies of COPD, including increased mucus production, poor clearance of this mucus and resulting bacterial infections and further inflammation. NE, MMP-9, and ADAMs-10 and -17 increase epithelial cell expression of MUC5AC, a major mucin protein, by activating epithelial growth factor receptor (EGFR) through shedding of membrane–bound pro–transforming growth factor (TGF)-α. The released soluble, active TGF-α, activates the EGFR (42-44). The 3 major serine proteinases, NE, CG, and PR3 potently stimulate goblet cell degranulation (45). Tissue kallikrein is a serine proteinase expressed by inflammatory cells and submucosal glands, which also stimulates mucin synthesis in airway epithelium *in vitro* by shedding and activating pro-EGF, another EGFR ligand (46). NE damages epithelial cells (47) and inhibits ciliary beat frequency of lung epithelial cells (48).

Plasmin, MMP-9, NE, and ADAMs may also induce sub-epithelial fibrosis in COPD airways, because they activate latent growth factors such as TGF-β (11,49,50) and insulin-like growth factors *in vitro* (51,52). These growth factors are known to induce fibroblasts to produce and secrete interstitial collagens. It remains unclear whether these proteinases induce sub-epithelial fibrosis in the small airways of human COPD patients.

5. Mechanisms by which proteinases contribute to individual lung pathologies in COPD patients

Most of the evidence for the mechanisms by which the proteinases act in the disease process of COPD comes from studies of clinical samples from human COPD patients and animal models of COPD.

5.1 Human COPD samples

Following on from the initial discovery that lack of inhibition of NE in patients with AAT deficiency was associated with emphysema, studies from Damiano et al further supported crucial activities for NE in pulmonary emphysema (53). They showed that the amount of NE bound to lung elastin is strongly correlated with the degree of emphysematous change and additional studies demonstrated stable binding of active forms of NE to elastin *in vitro* (54). Since then, additional studies have confirmed increased levels of NE in lung samples from COPD patients and demonstrated elevated levels of CG, PR3, uPA, and MMPs -1, -2, -8, -9, and -14 in various lung samples from smokers and COPD patients when compared to healthy subjects (53,55-65).

Inflammatory cells are the main source of these proteinases in COPD but production of proteinases by lung structural cells and immune cells has also been demonstrated. For example, cigarette smoke increases MMP production by lung epithelial cells (64), and fibroblasts (66). T lymphocytes from blood and BAL samples from COPD patients have increased levels of GRZ and perforin compared to samples from asymptomatic smokers and nonsmokers (67). Elevated levels of GRZ B in BAL samples from COPD patients show a correlation with bronchial epithelial cell apoptosis, suggesting that GRZ B promotes epithelial cell death in the lung and contributes to airspace enlargement in COPD patients.

5.2 Animal models of COPD

Animal models of COPD provide the strongest evidence for the roles of proteinases in COPD.

Acute cigarette smoke exposure models

Exposing mice to smoke for up to 30 days leads to an influx of PMN and macrophages to the lung (68). This is due to direct effects of inhaled smoke on lung capillaries, leading to leakage of thrombin and plasmin into the alveolar space (69,70). These proteinases cleave and activate PAR-1 on macrophages, leading to an increased synthesis of MMP-12 by macrophages (13,14). MMP-12 is responsible for shedding pro-TNF-α from activated macrophages, likely leading to an increase in E-selectin expression on endothelial cells (39). This facilitates transendothelial migration of PMNs. The presence of these increased PMNs and macrophages, releasing serine proteinases, increases lung collagen and elastin breakdown. Delivering human AAT to mice acutely exposed to cigarette smoke prevents PMN influx and ECM destruction. This is probably due to AAT inhibiting both PMN serine proteinase-mediated ECM destruction and thrombin- or plasmin-induced increases in macrophage MMP-12 production (14,71). Further evidence for the role of MMP-12 comes from a study showing that the minor allele of a single-nucleotide polymorphism (SNP) in MMP-12, is associated with a positive effect on lung function in adults who smoke and also a reduced risk of COPD in adult smokers (72).

Chronic smoke exposure models

When wild type (WT) mice are exposed to cigarette smoke for 3-6 months they develop pulmonary changes of airspace enlargement, inflammation and small airway subepithelial fibrosis, making this a good model to investigate the role of proteinases in COPD (73,74).

Work with proteinase deficient mice has confirmed the role of MMP-12 and NE in chronic inflammation and airspace enlargement and MMP-9 and possibly MMP-12 in sub-epithelial fibrosis. MMP-12 deficient mice (MMP-12$^{-/-}$mice) when chronically exposed to cigarette smoke show no increase in macrophages and no airspace enlargement, and so are completely protected from the changes seen in the wild type model (73). In the absence of MMP-12 mediated elastin degradation, the remaining elastin fragments attract monocytes (75). T lymphocytes also play a role in these processes with CD8+ T-cell-deficient (CD8$^{-/-}$) showing a blunted response to smoke exposure and protection from emphysema (76). This is mediated by a CD8+ T cell product, interferon gamma (IFN-γ) inducible protein 10 (IP-10), which induces production of MMP-12 and degradation of the lung ECM. Carrying this through to the human disease, there has been demonstration of increased Th1 cells associated with increased levels of IP-10 and MMP-12 in lung tissue from human COPD patients (77).

NE$^{-/-}$ mice are 60% protected from airspace enlargement and have decreased influx of PMN and monocytes into the lung compared to smoke-exposed WT mice [(78); Fig. 3]. NE likely contributes to airspace enlargement directly by degrading elastin and other ECM protein components of the alveolar walls (78).

There is also a direct action of cigarette smoke on the pulmonary airways. When rodent airways are exposed acutely to cigarette smoke, increases in growth factor and collagen production are detectable within 2 hours, and before inflammation occurs in the airway walls (79). This suggests that smoke directly promotes small airway subepithelial fibrosis and that smoke-induced inflammation and proteinase production are unnecessary for this process. However, in guinea pigs chronically exposed to cigarette smoke for up to 6 months, inflammatory cell MMPs amplify this process, since delivering a synthetic dual inhibitor of MMPs-9 and -12 to these animals significantly reduces small airway fibrosis (80). The use of MMP inhibitors in human COPD patients remains to be explored.

Transgenic murine models

These models are used to investigate over-expression of various proteinases, in contrast to the study of a deficiency of a protein in the knock-out murine models. Transgenic mice over-expressing MMP-1 in the lung develop enlarged airspaces (81), which may either reflect abnormal alveolar development or destruction of mature interstitial collagens by MMP-1. Adult transgenic mice over-expressing a Th1 cytokine (IFN-γ), a Th2 cytokine (IL-13), or a cytokine with Th1 and Th2 activities (IL-18) in airway epithelial cells spontaneously develop obvious lung inflammation, increased lung levels of MMPs and cysteine proteinases, and airspace enlargement (82-84). In mice over-expressing IL-13, the metalloproteinases MMPs -9 and -12 play critical roles in promoting airspace enlargement, with MMP-12 also promoting inflammation and driving the increased expression of other MMPs in the lung (85). In transgenic mice over-expressing IFN-γ, cathepsin S stimulates lung epithelial apoptosis, lung inflammation, and airspace enlargement (86).

Alveolar septal cell apoptosis models of airspace enlargement

In patients with COPD there is apoptosis of alveolar septal cells (87,88) and leukocytes (89,90), and apoptosis of the endothelial and epithelial cells that make up the alveolar walls. This leads to the development of emphysema. Septal cell apoptosis and airspace enlargement in the absence of overt lung inflammation can be induced rapidly in experimental animals by: 1) pharmacologic blockade of vascular endothelial growth factor receptors in rodents (91); and 2) transfection of murine alveolar epithelial cells with caspase-3, a pro-apoptotic cysteine proteinase (88). However, increased elastase activity due to acidic proteinases is detected in BAL samples after transfection of alveolar epithelial cells with caspase-3 (88). Thus, proteinases released from dying structural cells may degrade the lung ECM, thereby acting together with septal cell apoptosis to cause loss of alveolar units and airspace enlargement.

6. Regulation of proteinases in the lung

Proteinases are a significant factor in the pathogenesis of COPD, but do not act in isolation. They interact with other mediators and other pathways and are also regulated by inhibitors. Studies of the $NE^{-/-}$ and MMP-$12^{-/-}$ mice chronically exposed to cigarette smoke demonstrated interactions between these two classes of proteinases, with MMP-12 cleaving and inactivating AAT to increase NE-mediated lung injury, and NE cleaving and inactivating TIMP-1 to amplify MMP-12-mediated lung destruction (78). Proteinases also interact with reactive oxygen species (ROS), and ROS production is increased in the lungs of COPD patients. ROS are present in inhaled cigarette smoke itself, or are released by phagocytes activated by inhaled smoke. ROS are known to activate proMMPs *in vitro* and are thought to exacerbate lung inflammation and injury in COPD patients (92). Transgenic mice over-expressing the antioxidant enzyme Cu-Zn superoxide dismutase in the lung are protected from developing chronic lung inflammation, increased lung MMP levels, and emphysema in response to intratracheal instillation of porcine pancreatic elastase, or chronic exposure to cigarette smoke (93). However, mice deficient in a phagocyte-specific component of the NADPH oxidase, which generates superoxide anions (O_2^-), develop greater airspace enlargement in response to cigarette smoke than WT mice (94). This is due to ROS-mediated inactivation of MMPs via oxidative inactivation of residues in the catalytic domain of MMPs (95). Thus, phagocyte-derived O_2^- (and ROS derived from O_2^-) in COPD lungs may constrain rather than promote phagocyte MMP-mediated lung injury (94,96). It is noteworthy that clinical trials have failed to demonstrate protective effects of antioxidant supplementation in COPD patients, and this could be linked, in part, to antioxidants inducing reductions in ROS-mediated inactivation of MMPs (97).

6.1 Inhibitors of proteinases

Proteinase inhibitors are present in the extracellular matrix. To maintain their action, proteinases need to circumvent these inhibitors through inactivation of the proteinase inhibitor, evading them and / or overwhelming them.

6.2 Inactivation of proteinase inhibitors

Serpins can be cleaved and inactivated by MMPs (98-102), NE (103,104), cathepsin B (105), and bacterial proteinases (106). Serine proteinases cleave and inactivate TIMPs (107).

Proteolytic inactivation of AAT and TIMP-1 by MMP-12 and NE occurs in the cigarette smoke exposure model of emphysema in mice (78). ROS present in cigarette smoke or released by leukocytes activated by smoke, inactivate α_2-M, and AAT, and SLPI in vitro by converting the methionine at the active sites of these inhibitors to methionine sulfoxide. This reduces their capacity to inhibit serine proteinases (108-111). It is not clear if oxidative inactivation of proteinase inhibitors occurs in COPD patients. Some studies have detected oxidized AAT in lung samples from COPD patients but others have not (112-114). Also, ROS can inactivate proteinases as outlined above. It is difficult to know if previous work analyzing the oxidation state of proteinase inhibitors in lung samples from COPD patients actually includes events in cellular microenvironments. Adding to the complexity of studying this process is the fact that ROS are short-lived molecules and are active only at short distances from the cells generating them before they are muted by antioxidants.

6.2.1 Evasion of inhibitors

In another effort to preserve their function, proteinases can evade inhibitors by binding tightly to substrates, being released into sequestered microenvironments, or binding to cell surfaces.

Tight binding of proteinases to substrates

NE binds very stably to elastin in an active form, and AAT and SLPI have reduced activity against elastin-bound NE compared to soluble NE (54,115,116). In the lungs of humans with emphysema, NE is bound to interstitial elastin (53) and this lung elastin-bound NE likely retains catalytic activity and takes a major role in the destruction of elastin fibers in pulmonary emphysema (Fig. 4). MMPs-1, -2, and -9 bind to various ECM proteins, which may increase the retention, stability, and bioactivity of proteinases in the lung and aid their roles in extracellular proteolysis (117,118).

Sequestered microenvironments

Inflammatory cells can, via integrin-mediated adhesion to matrix or to cells, form small pockets of microenvironment. Large inhibitors such as AAT (119) and α_2-M (120) cannot enter these sealed pockets (Fig. 4).

Membrane binding of proteinases

MT-MMP and ADAMs are integral membrane proteinases, and some members of these families are resistant to inhibition by physiologic inhibitors. ADAM-17, for example, is resistant to inhibition by TIMPs-1 and -2 but not TIMP-3 (31), and MT1-MMP is resistant to inhibition by TIMP-1 but not TIMP-2 (121). NE, CG, PR3, MMPs-8 and -9 (which lack transmembrane domains or glycosylphosphatidyl-inositol anchors) are also expressed on the surface of activated PMN (122-127). These surface-bound proteinases degrade lung ECM proteins and proteinase inhibitors and induce goblet cell degranulation (122,126-128). The membrane-bound element of these proteinases confers a resistance to their inhibitors when compared to the soluble variety (122-124,126,127).

6.2.2 Overwhelming of inhibitors

A more obvious way to overcome the inhibitors is for the proteinases to overwhelm them with sheer numbers. This can happen with release of massive quantities of enzymes from

large numbers of inflammatory cells, or when high concentrations are released from individual cells (quantum proteolysis).

Brisk recruitment of inflammatory cells in the lung

COPD exacerbations are characterized by an influx of inflammatory cells into the airways. These cells release active forms of NE, MMP-8, and MMP-9 (58,62,129,130). Macrophage clearance of the PMN recruited into the lung under normal circumstances would occur but in the case of the COPD lung this is hampered by a number of mechanisms. First, cigarette smoke impairs expression of recognition molecules for apoptotic PMN on the macrophage surface (131). Second, NE cleaves recognition molecules for apoptotic PMN from the macrophage surface (132). Third, when PMN ingest *Hemophilus influenzae,* which frequently colonizes the respiratory tract of COPD patients, PMN necrosis is rapidly induced (133).

Quantum proteolysis and PiZZ AAT deficiency

NE is present at millimolar concentrations in each azurophil granule of PMN, which is more than 100-fold higher than the concentration of AAT, its inhibitor, in plasma (134). The release of an azurophil granule into the extracellular space is thus accompanied by a transient burst of proteolytic activity as it greatly outnumbers the proteinase inhibitors. This activity fades as the granule contents diffuse, and the proteinase-inhibitor ratio falls below 1:1 (134). In patients with an inherited deficiency of AAT, the proteinase activity lasts longer, leading to more destruction of the lung. Quantum bursts of NE-mediated proteolytic activity associated with PMN migrating on ECM proteins are 10-fold larger in area and 4-fold longer in duration when PMN are bathed in serum from PiZZ patients compared to serum from healthy PiMM subjects (135), due to defective confinement of PMN-derived NE-mediated ECM degradation. The PiZ AAT mutant proteins polymers formed in this disease are also chemotactic for PMN (136,137).

7. Potential strengths and limitations of proteinase inhibitors and anti-inflammatory drugs as new therapeutic strategies to limit proteinase-mediated lung pathologies in COPD

7.1 Proteinase inhibition

Perhaps the most obvious role for intervention in this setting is to replace AAT in patients with COPD who have known severe, inherited AAT deficiency (AATD). Although we do not have conclusive randomized controlled trials, human clinical research has shown that AAT augmentation reduced exacerbation frequency and slows the rate of lung function decline in these patients (138). More recent work has attempted augmentation of AAT through gene therapy. This involves administration of recombinant adeno-associated virus (rAAV) vectors expressing human AAT (rAAV1-CB-hAAT) to patients with AATD (139). These studies are currently in phase 2 clinical trials and have shown increased expression of normal (PiM) AAT in serum occurs safely in patients for up to 90 days. Further optimization of the vector is likely to be required to generate sustained therapeutic AAT plasma levels. The concept of augmentation of AAT in COPD, outside the setting of AATD, is less clear.

Secretory leukocyte peptidase inhibitor (SLPI) and elafin are naturally occurring antiproteinases with anti-NE activity whose roles in COPD are not fully eludicated but may have potential as future treatment options (140). A number of synthetic low molecular

weight inhibitors have been developed and are potential therapeutic agents for COPD. These include irreversible inhibitors such as the peptide chloromethyl ketones (141) and reversible inhibitors such as peptide boronic acids, peptide aldehydes (142), substituted tripeptide ketones (143), or β-lactams (144). One of the problems with the low-molecular-weight reversible inhibitors is that they can release NE, allowing it to destroy tissue. Although the irreversible inhibitors such as chloromethyl ketone have been shown to function effectively *in vivo* in hamsters to reduce many of the effects of intratracheally administered NE, the toxicity of chloromethyl ketones prevents clinical use.

Some support for potential use of these inhibitors comes from *in vitro* studies showing that low-molecular-weight, synthetic inhibitors of serine proteinases and MMPs effectively inhibit both soluble and membrane-bound proteinases (122,123,126,127), and studies of animal models of COPD showing that proteinase inhibitors effectively block both airspace enlargement and lung inflammation. In animals acutely exposed to cigarette smoke, delivery of synthetic or natural inhibitors of serine proteinases and synthetic inhibitors of MMPs blocks PMN influx into the lung and ECM destruction (68,145,146). In other animal work, a therapeutic effect demonstrated with daily oral delivery of synthetic MMP inhibitors to mice. This prevented airspace enlargement and macrophage accumulation in the lungs of mice exposed to cigarette smoke for 6 months (74). In additional experiments in which MMP inhibitor therapy was initiated after mice were exposed to cigarette smoke for 3 months to initiate airspace enlargement, therapy prevented progression of airspace enlargement as smoking continued (74). These results suggested a role for proteinase inhibition in potentially preventing disease progression in human COPD patients. However, it remains unclear which proteinases should be targeted. The counter argument to these theories is that proteinases have been shown to have beneficial as well as deleterious roles in the lung (roles in innate host defense, dampening inflammation, and inhibiting tumor growth and metastasis), which may prove to limit the usefulness of their inhibition.

7.2 Anti-inflammatory strategies

Strategies to reduce the burden of lung inflammatory cells in COPD would thereby reduce the amount of proteinase that they are responsible for releasing. Inhibitors of phosphodiesterase E4, the major isoenzyme in inflammatory cells, decrease inflammatory cell migration, activation, and release of proteinases. Clinical trials of phosphodiesterase E4 inhibitors in COPD have resulted in one selective PDE4 inhibitor, roflumilast (Daxas ®), being approved for use in humans and available in Canada and the European Union in 2011 for the treatment of a specific population of patients with severe COPD (147). Other anti-inflammatory approaches, such as inhibiting NF-κB activation to reduce pro-inflammatory gene expression, could also potentially inhibit proteinase- and oxidant-mediated lung injury in COPD patients.

8. Conclusions

Proteinases have diverse activities in the pathogenesis of COPD. With over 40 years having elapsed, since the initial breakthroughs showed a role for these enzymes in this disease, much work has elucidated many further elements of the roles they play. It is clear that the proteinase-antiproteinase balance is not the sole cause of all the pathology seen, but it continues to be a major contributor and a potential target for future therapies.

9. Abbreviations

ADAM. Proteinase **a d**isintegrin and **a m**etalloproteinase domain; cathepsin G (CG), chronic obstructive pulmonary disease (COPD), epithelial growth factor receptor (EGFR), extracellular matrix (ECM), *granzymes (GRZ), i*nducible protein 10 (IP-10), interferon gamma (IFN-γ), membrane-type MMPs (MT-MMPs), metalloproteinase (MMP), neutrophil elastase (NE), polymorphonuclear neutrophils (PMN), protease-activated receptor-1 (PAR-1), proteinase 3 (PR3), reactive oxygen species (ROS), secretory leukocyte proteinase inhibitor (SLPI), serine proteinase inhibitors (Serpins), transforming growth factor (TGF)-α, tumor necrosis factor (TNF-α), urokinase-type plasminogen activator (uPA), wild type (WT), α_1-anti-trypsin (AAT), α_2-macroglobulin (α_2-M)

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5

Chronic Obstructive Pulmonary Disease – Chaperonopathology

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1. Introduction

Cigarette smoking is clearly associated with the development of chronic airway obstruction pulmonary disease and is responsible for 80–90% of cases. However, only 15–20% of heavy smokers develop clinically significant airflow obstruction. In the rest pulmonary function remains within normal limits. Besides the risk factors that are involved in airway obstruction, the genetic predisposition is also considered a key factor. It modulates lung's response to cigarette smoke inhalation and the development of airway obstruction. In addition to smoke induced emphysema, genetic susceptibility leading to α1-antitrypsin deficiency is associated with the propensity for the development of early-onset, familial emphysema. Thus both environmental and genetic factors contribute to the pathogenesis of emphysema.

The molecular basis for tobacco smoke-induced emphysema is poorly understood. To thoroughly unravel the cellular and molecular events or signaling pathways that may contribute to the pathogenesis of smoke-induced emphysema or COPD, gene expression profiling - serial analysis of gene expression (SAGE) and microarray analysis as well as proteomics have been recently applied. The gene expression profiles of lung tissues from control smokers (GOLD-0) and moderate (GOLD-2) COPD smokers identified numerous classes of genes, the expression of which is altered in COPD patients. These include genes encoding molecules for signal transduction, receptor function, growth factor, nuclear chromatin and DNA binding, adhesion and cytoskeleton, metabolism, matrix, cell cycle, and oxidative stress such as HSP70 protein, heme oxygenase (decycling) 1 (HO-1). The data from proteomics also confirms a large number of proteins related to cigarette smoke induced endoplasmic reticulum stress, repair/injury proteins, heat shock proteins, apoptosis and cell cycle responsible molecules.

COPD is obviously a disease of imbalance of proteins - oxi/antioxidant, protease/ antiprotease, apoptosis/proliferation, acetylases/deacetylases, that can no longer perform their proper function to keep the homeostasis in the new environmental settings of the oxidative stress.

Molecular chaperones provide the functional activity of proteins, they counteract the formation of abberantly folded polypeptides and allow their correct refolding under stress

recovery. Chaperones are responsible for protein folding and allow the functional state of cells to be maintained, by preventing irreversible protein unfolding and aggregation. Numerous studies, over the last decade have investigated the structural and functional characteristics of molecular chaperones, classifying them in families based on size, structure and activity. Today there are more than 25 families of molecular chaperones, with more than 100 proteins participating in the folding events in the mammal. These include a group of proteins, known as heat shock or cell stress proteins.

Heat shock or cell stress response is one of the most evolutionary conserved protective mechanisms in cells. It is stimulated under ''stress'' (thermal, metabolic, oxidative, etc), when the conditions of the cell environment are deleterious and alter the protein folding and their proper biological activity. Cell stress involves the temporary modification of gene expression and synthesis of different heat shock protein family members. They help the cell and the organism to cope with environmental or physiological stress. Some of the heat shock proteins are constitutively expressed in non-stressed conditions and act as intracellular chaperones towards fundamental cellular processes – cytoskeletal architecture, mutation masking, protein transport, translation regulation, intracellular redox homeostasis, protection against spontaneous or stimulated programmed cell death - apoptosis. Others are synthetised in response to stress to prevent protein aggregation, refold damaged proteins. Heat shock proteins could also participate in the protein triage and modulate the ubiquitin – proteasome pathway, promoting the degradation of irreversibly denatured proteins.

While the cellular protein management of heat shock response and stress proteins is well described their role in the immune/inflammatory responses in multicellular organism is still elusive. As an anti-inflammatory effector the heat shock proteins influence cytokine signal transduction and gene expression by inhibiting the translocation of the transcription nuclear factor kappa B (NF-kB) to the nucleus. In this aspect they regulate the synthesis of inflammatory mediators. As proinflammatory mediators, necrotic and non-necrotic release of constitutively expressed or stress induced heat shock proteins, into the extracellular environment, produce a multifaceted immune/inflammatory response. They activate immune effector cells and stimulate cytokine release. Therefore the ability of heat stress response to modulate inflammation is an important aspect of a variety of pathophsysiological states, characterized by dysregulated inflammatory response.

Cell stress proteins are reported to be positively correlated to longevity and capacity for mounting a cell stress response, implying the fact that chaperones are essential adaptive mechanisms for survival. Unfortunately chaperone levels generally decrease or become functionally incompetent with age. The accumulation of misfolded proteins that occurs with senescence results in a chaperone deficiency and leads to the onset of degenerative or age related diseases. A shift in the balance between misfolded proteins and available free chaperones in ageing organisms can bring about defects in signal transduction, protein transport, cellular organization and immune functions.

Age-related post-translation modifications of proteins can seriously curtail or change their functions and thus give rise to proteinopathies of ageing, a hallmark of senescence at molecular level. A normal set of chaperones would potentially prevent the deleterious effect of proteinopathies. However chaperones also are being modified with the passage of time. These acquired chaperonopathies are likely to contribute significantly to senescence and lower the quality of life in the elderly.

In conclusion normal chaperones, particularly cell stress proteins are important in cell physiology at all ages. They are responsible for protein folding, functioning and homeostasis and play critical roles as major cellular anti-stress and anti-disease mechanism. Defective chaperones are most probably an additional factor, accompanying the development and progression of senescence and age-associated diseases (neurodegenerative, cancer, atherosclerosis, COPD) most of which are aggrevated by stress.

In the current chapter we shall represent COPD as a chaperonopathy (proteinopathy). We shall concentrate on the current data from research studies, concerning the molecular pathology of COPD, studies that are shedding light on the participation of stress molecules in COPD initiation and progression. We shall also comment the relation of chaperones to already known pathological mechanisms, their clinical application as diagnostic markers for COPD, as well as markers for NSCLC early detection.

2. Cell stress or heat shock proteins

All organisms respond to potentially harmful environmental factors by an up-regulation of heat shock protein expression. Cell stress or heat shock proteins were first discovered in 1962 by Ritossa who observed a pattern of Drosophila salivary gland chromosome puffs induced under transient exposure to high temperature. It was subsequently described that these highly conserved group of proteins could be induced by many other stress factors. Mammalian heat shock proteins are classified into two groups according to their size: high molecular weight heat shock proteins (HSP) and small HSP - sHSP. The first group includes three major families: HSP90, HSP70 and HSP60. Some of these proteins are constitutively expressed (in-house chaperones), whereas the expression of the others is induced by stressful conditions. High molecular weight stress protein are ATP-dependent chaperones and require co-chaperones to perform their ATP-binding and modulate their conformation. In contrast sHSP are ATP-independent. Heat shock proteins are expressed in both normal and stress conditions and are responsible for:

1) facilitating the proper folding of nascent proteins in cytosol, endoplasmatic reticulum, mitochondria; 2) import of proteins into cellular transport; 3) prevention of protein aggregates, refolding of denatured proteins; 4) degradation of unstable proteins; 5) control of apoptosis.

HSP also participates in the intracellular transport and have been implicated in the loading of immunogenic peptides in histocompatibility complexes (MHC) in the T-cell presentation.

2.1 High molecular weight heat shock proteins

The HSP70 family is the most highly conserved and best studied class of HSP. Human cells contain several HSP 70 family members – constitutively expressed, inducible, mitochondrial – HSP75, and GRP78, localized in the endoplasmatic reticulum. Under normal conditions HSP70 proteins function as ATP-dependent chaperones, assisting the folding of newly-synthesised proteins, participating in intracellular transport of proteins across cellular membranes. Under stressful conditions the synthesis of inducible HSP70 enhances the ability of cells to cope with the increased levels of denatured proteins. HSP70 blocks caspase-depedent and independent activation of apoptosis (Shi et al,1992; Murakami et al,

1988;) It participates in the ubiquitination of proteins through its co-chaperones – BAG1 and CHIP. (Meacham et al, 2001; Luders et al, 2000)

The HSP90 family include ATP – dependent chaperones – HSP90α and HSP90β and GRP94. The two isoforms of HSP90 (HSP90α and HSP90β) that are essential to cells are abundantly expressed under normal conditions (Csermely et al, 1998). HSP90 proteins make up 1-2% of the cytosolic proteins and are additionally synthethised during stress. They participate in cell signalling pathways –ligand dependent transcription factors – Glucocorticoid receptor (Nathan et al, 1995); ligand independent transcription factors – Myo-D, tyrosine and serine/threonine kinases (Hartson et al, 1994; Shaknovich et al, 1992). Their chaperone function is almost entirely limited to these transcription factors and signal transducing kinases. HSP90 family members also have anti-apoptotic functions and stimulate the protein triage (Tsubuki et al, 1994; Lewis et al, 2000).

HSP60 is called chaperonin. It is constitutively expressed, found primarily in the mitochondrial matrix, although up to 15% could be cytoplasmically expressed. It is ATP-dependent chaperone, protecting the mitochondrial proteins and facilitating the proteolytic degradation of misfolded proteins. The chaperone function of HSP60 is regulated by a co-chaperone, known as HSP10 that modulates substrate binding and ATP-ase activity. In the presence of ADP, HSP60 regulates apoptosis, demonstrating both pro- and antiapoptotic functions (Bukau et al, 1998).

2.2 Small heat shock proteins

The small heat shock proteins constitute of a diverse family of ubiquituous intracellular proteins (Arrigo et al, 1998). In human ten different sHSP have been described but only a few of them (HSP27, HSP22 and α-Bcrystallin (HSPB5) are true heat shock proteins expressed in response to stress. sHSP are characterized by small molecular weight (12-43kDa) and a conserved C-terminal domain (the α-crystallin domain). They share the ability to form globular oligomeric structures with molecular masses ranging between 50-800kDa. The dynamic organization of these proteins is essential for performing their biological activity. It depends on their phosphorylated status which is performed by specific signal transduction pathways. It is generally assumed that stress favors the formation of large oligomers associated with unfolded proteins while phosphorylation does the reverse. Large unphosphorylated oligomers of sHSP have greater potentiality to protect cells through their ability to perform chaperone activity. The formation of small phosphorylated oligomers may be required for the binding of unfolded proteins as well as for the recycling of the larger ones (Kato et al, 1996).

2.2.1 sHSP5

α-crystallin, a major structural protein of the vertebrate eye lens, is the most intensively studied representarive member of sHSP family. α-crystallin is one of the three major crystallins of the vertebrate eye lens (Ingolia et al, 1982). However it became a major focus of studies since 1982 when Drosophila sHSP were found to share sequence similarities with α-crystallin. Soon after, it was shown that α-crystallin has other functions, defining it as a molecular chaperone – prevents the thermal aggregation of various proteins, including the lens proteins. In the lens α-crystallin exists as a heteropolymer with the

molecular size of approximately 800kDa, having up to 40 subunits from two gene products – αA and αB. αA is encoded by and constitutes of 173 aminoacids, while αB is encoded by and has 175 aminoacids and both share 57% sequence similarity. In contrast to αA, αB is also constitutively expressed in various tissues with high rates of oxidative stress – skeletal muscles, brain, heart, kidney (Lowe, 1992). Its primary sequence can be organized in three distinct structural regions: an α-crystallin domain of 90 amino-acids in length which is conserved among all sHSP and flanked by an N- and C – terminal domains of variable length and sequence. The conserved α-crystallin domain spans residues 68-148. It is has seven strands, organized in two sheets. The N - terminal domain is highly variable and influences subunit oligomerization and chaperone-like activity, whereas the C-terminal extension stabilizes the global structure and enhances protein/substrate complex (Sun et al, 1997; Bhatacharyya et al, 2002) αB-crystallin is a major structural protein of human lenses that belongs to the family of small heat-shock proteins. It has auto-kinase activity and participates in intracellular architecture and membrane stabilisation (Nicole et al, 2002; Wang K, Spector A, 1996). It acts as molecular chaperone and stabilises proteins in large soluble aggregates in the cytoplasm. The cytoplasmic expression of αB-crystallin is also responsible for the regulation of cyclin-D1 ubiquitination (Liu et al, 2006) and inhibition of pro-apoptotic proteins such as caspase-3, p53, Bax and Bclxs (Mao et al, 2004; Lin et al, 2007)

3. Oxidative stress, COPD and heat shock proteins

Compared to other organs lungs are unique in their exposure to high levels of oxygen. Because of their close contact with the environment the airway epithelium is directly exposed to either exogenous oxidants – (cigarette smoke, airway pollutants), or endogenous ones – generated by phagocytes or other cell types. To keep the balance lungs need efficient adaptive mechanisms that correspond to their physiological functions. If the enzymatic or non-enzymatic antioxidant systems do not provide the corresponding adaptive response oxidative stress occurs.

It is still considered that oxidative stress is one of the triggers, contributing to the enhanced or abnormal inflammatory response, characteristic for COPD patients.

3.1 Oxidative stress, chaperones and epithelial injury in COPD

The airspace epithelial surface is particularly vulnerable to the effects of oxidative stress. The injury of the epithelium is an important early event, following exposure to cigarette smoke. The noxious effects of the cigarette smoke on human epithelial cell monolayers has been demonstrated by cell detachment, decreased cell adherence and increased cell lysis (Jones, et al, 1980; Lannan S t al, 1994). It is supposed that these effects are in part oxidant mediated since GSH appears to be critical for the maintenance of the epithelial integrity following exposure to smoke. It is demonstrated in studies that the direct exposure to smoke condensates is associated with profound changes in the homeostais of glutathione (GSH) (Li et al, 1994, 1996). Concentration of GSH are significantly decreased after exposure to cigarette smoke condensate. This is due to a decrease of the activity of the enzymes, responsible for the keeping the redox-cycle – glutathioneperoxidase, glucose-6-phosphate dehydrogenase. In addition the depletion of GSH alone induces airway detachment and increases its permeability (Li et al, 1995; Rahman et al, 1995).

The small heat shock proteins – HSP27 and αB crystalline have antioxidant ability and increase cell resistance to oxidative injuries (Arrigo et al, 2001). It is reported (Yan et al, 2002) both in cell cultures and whole animals that their expression correlates to decreased levels of reactive oxygen species (ROS) and nitric oxide (NO•).(Preville et al, 1999; Mehlen et al, 1996) Consequently, in cells exposed to oxidative stress, sHSPs lower the levels of lipid peroxidation (Fridaus et al, 2006; Preville et al, 1998). They maintain the mitochondrial potential and provide the production of ATP, thus corresponding to both increased energy needs for stressed cells on the one hand, and ATP supply for the functional activity of the other chaperones on the other (Paul et al, 2000).

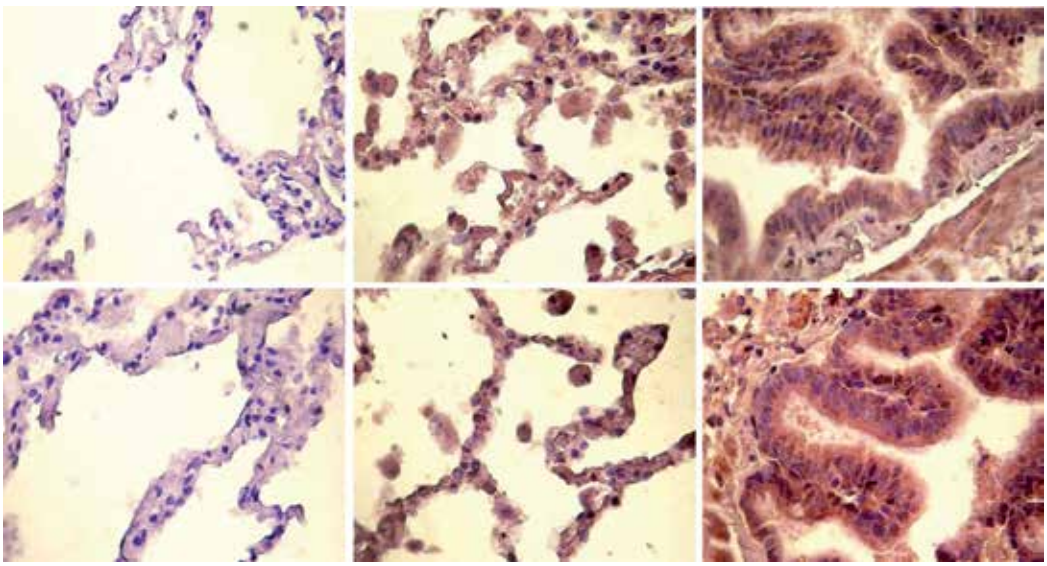
The antioxidant activity of HSP27 and αB crystalline is performed by the increase of the levels of gluthatione. (Mehlen et al, 1996) They induce the up-regulation of glucose-6-phosphate dehydrogenase – the enzyme that provides the reducing power of the cell, by reducing NADP+ to NADPH(H) + . In addition it is recently observed that HSP27 and αB-crystalline expression decreases iron intracellular levels. Thus they prevent the Fenton reaction and the formation of hydroxyl radical (OH•) (Arrigo et al, 2005; Chen et al, 2006).

Ruicheng Hu et al, 2011 performed proteomic analysis and found that the expression of HSP27 was upregulated in smokers, and this upregulation was particularly marked in COPD smokers. The expression of HSP27 between the groups was confirmed by IHC and Western blotting. Based on their results and other studies that have shown a protective role for HSP27 against oxidative stress and apoptosis, it could be suggested that induction of HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis.Their experiments showed that expression of HSP27 was upregulated in the lungs of smokers, and especially smokers with COPD, even though there was no difference in smoking index between smokers with or without COPD. Therefore, it could be suggested that the upregulation of HSP27 expression in smokers is primarily due to oxidative stress and partly due to inflammation, whereas the difference in HSP27 expression between smokers with or without COPD may predominantly be due to inflammation. HSP27 is a multi-functional cytoprotective factor that protects cells from oxidative stress by regulating the activity of several detoxifying enzymes and promoting the degradation of misfolded proteins. HSP27 also protects cells from unfavourable stimuli by playing a role in apoptosis/survival signal transduction pathways (Ito et al, 2003; Bruey et al, 2000). Phosphorylated HSP27 is a ubiquitin-binding protein that binds to 16 polyubiquitin chains and thereby enhances the degradation of ubiquitinated proteins by the 26S proteasome (Jackson et al, 2008) By enhancing the degradation of IκB-α and activating the nuclear factor-κB signal transduction pathway, HSP27 promotes cell survival under conditions of stress. (Yu et al, 2008; Kuoyt et al, 1995)

Another function of the sHSPs that triggers the interest towards their participation in the lung epithelial injury is that both of them are responsible for the protection of cytoskeleton.((Benndorf et al, 1994; Mounier et al, 2002) Small HSPs are involved in the control of cytoskeletal organization during heat and oxidative stress. They maintain the polymerization-depolymerization processes of F-actin and thus are directly responsible for both cell integrity and intracellular contacts. (Jog et al, 2007; Singh et al, 2007; Mairesse et al, 1996). In addition αB-crystalline is a well-known stabilizer of the intermediate filaments and play a major role in cytoskeletal architecture homeostasis (Bennardini et al, 1992). It is demonstrated in epithelial cells that cell signalling pathways, activated by disrupture of

both microfilametns, intermediate filaments and microtubules, lead to the phosphorylation of αB-crystalline, underlying the biological importance of this heat shock protein in preserving the integral cell architecture (Launay et al, 2006).

Cherneva et al, studied the tissue expression of αB crystalline in 28 COPD patients, 14 with age-related emphysema and 23 smokers without COPD. Immunohistochemistry towards αB crystalline was applied. Results were evaluated semiquantitaively. No nuclear staining was present. Only cytoplasmic staining was observed. In most of the cases there was a homogeneous staining among cells. Two patients with COPD had moderate; 26 had intensive staining. In age-related emphysema 5 patients had weak; 2 had moderate and 7 had intensive staining. In smokers without COPD no staining was detected. The clinical implication of our preliminary results needs further investigation (Fig.1).



Non-COPD smoker Age-related emphysema COPD smoker

Fig. 1. Tissue expression of αB-crystalline in lung tissues from non-COPD smokers, age-related emphysema and COPD patients.

The levels of αB-crystalline were measured in the fixed lung sections (3-mm thick) by immunohistochemical staining using rabbit polyclonal anti-αB-crystalline antibody (1:500 dilution) with avidin–biotin–peroxidase complex method followed by hematoxylin counter staining. Brown colour and the variability of its intensity represents the presence of αB-crystalline.The assessment of immunostaining intensity was performed semiquantitatively and in a blinded fashion.

3.2 Oxidative stress, chaperones and neutrophil sequestration in COPD

The oxidant burden in the lungs of smokers can further be augmented by the increased numbers of macrophages (two to four folds) and neutrophils (10-fold). Bronchial biopsies and lung resection studies represent a large number of neutrophils in the lungs and alveolar walls of COPD smokers (Kilburn et al, 1975; Hunninghake et al, 1983).

Neutrophils are first recruited and then sequestered due to size difference between neutrophils and pulmonary capillary vessels (MacNee, 1993). Radiolabelled studies have shown that lungs normally contain a large number of non-circulating neutrophils, which are retained or moving slowly across lung capillary bed (Selby et al, 1991). In comparison to erythrocytes, neutrophils are usually retained and the number of retained cells correlates to their capacity to adapt to the physilologically narrower diameter of the lung capillaries. The less deformable the cells the greater the sequestration of these cells in the pulmonary circulation. The deposition of the cells in microcirculation allows them to interact longer with the endothelium, to adhere to endothelial cells, or transmigrate in the interstitium and alveolar airspaces and respond to the inflammatory cytokines or infections. Thus any conditions that make the neutrophils less deformable create a predisposition to their sequestration in the lung capillary bed.

Studies in humans show that neutrophils and red blood cells are transiently sequesterd in lung capillary bed during smoking and return to the general circulation after smoking cessation. In vitro experiments show that cells exposed to smoke are less flexible. A similar result can be demonstrated in vivo in patients that are actively smoking – their cells lose flexibility. Decreased neutrophil deformability occurs owing to the assembly of the cytoskeleton – polymerization of microfilaments (F-actin), resulting in cell stiffening. It has been suggested that since each puff of cigarette smoke contains > 10^{16} oxidant molecules the effect of cigarette smoking is probably oxidantly mediated. This is confirmed by the fact that the decrease of neutrophil deformability is accompanied by the depletion of GSH (Drost et al, 1992). In addition oxidants affect it by altering cellular cytoskeleton through the polymerization of actin.

The control of F-actin cytoskeleton is mediated by the oligomers of HSP27. It is responsible not only for chemotaxis and cytoskeleton reorganization during migration, but also for processes engaged in exocytosis. It is recently observed that this small heat shock protein regulates neutrophil chemotaxis and exocytosis through the control of actin reorganization. (Jog et al, 2007; Singh et al, 2007)

The role of small heat shock proteins in the maintenance of neutrophil cytoskeleton and their reorganization under oxidative stress and inflammation is not investigated in COPD patients. It could however be speculated that the biological role of this chaperones related to the assembly and dynamics of the cell architecture makes them a target for future research. Since the deposition of neutrophils is triggered by oxidative stress it would be curious to compare the levels of expression of these small heat shock molecules in both COPD smokers, COPD non-smokers and healthy smokers.

3.3 Oxidative stress, chaperones and apoptosis in COPD

It has been proposed recently that COPD is associated with the loss of alveolar endothelial cells as well as lung epithelial cells and that apoptosis can be an essential element of emphysema. Apoptosis in emphysematous lungs is more commonly observed than in nonsmoker's lungs. Peripheral blood leukocytes and lymphocytes of patients with COPD also show increased rates of apoptosis (Tuder et al, 2003; Kasahara et al, 2001). Studies have reported that cigarette smoke induces apoptosis of lung structural cells by oxidative/endoplasmic reticulum stress.When the lungs are insulted with oxidants, consumption of intracellular reductants is increased resulting in aberrant protein folding.

As intracellular chaperones heat shock proteins have anti-apoptotic properties. Gal et al, 2011 showed that cigarette smoke extract stimulates the expression of HSP72 in alveolar epithelial cells, diminishing apoptosis Dimethylarsinic acid exposure also elevated intracellular HSP72 levels, changing the localization of the molecule and suppressing apoptosis of human alveolar cells (Kato et al. 2000). Ruicheng Hu et al, 2011 found that expression of HSP27 and CyPA was upregulated in smokers, and this upregulation was further marked in COPD smokers. HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis. HSP27 inhibits apoptosis by stabilising the mitochondrial electric potential and inhibiting the release of cytochrome C (Bruey et al, 2000; Paul et al, 2002). It promotes survival by activating the mitogen-activated protein kinase (MAPK) signal transduction pathway. Stress has been reported to activate the MAPK signal transduction pathway, including p38 MAPK, which induces expression and phosphorylation of HSP27 through MAPK-activated protein kinase 2 (MK2). So HSP27 inhibits both the intrinsic and extrinsic apoptotic pathways. It can inhibit the release of cytochrome-C or Smac-Diablo from mitochondria as well as act downstream of them preventing the formation of apoptosome. It can also act at the level of caspase-3 activation (Arrigo et al, 2007). At the level of the Fas receptor HSP 27 inhibits the extrinsinc signalling pathway by binding to DAXX (Arrigo et al, 2007). HSP27 is responsible with the Akt signalling pathway and also inhibits its activity (Arrigo et al, 2007). Concerning the structural organization of HSP27 in apoptotic cells it seems that its chaperone activity largely correlates with its anti-apoptotic one as far as in apoptotic cells the large oligomers inhibit caspase activation.αB crystalline also excutes protection against a large panel of apoptotic stimuli. It binds proapoptotic Bax, Bcl-xl and p53 polypeptides and prevents their translocation to mitochondria. It also directly inhibits the proteolytic activation of pro-caspase 3 (Mao et al, 2004; Liu S et al, 2007).

4. Inflammation, COPD and heat shock proteins

Chronic obstructive pulmonary disease is a slowly progressive condition, characterized by airflow inflammation, which is largely irreversible. It is suggested that the main etiological factor - cigarette smoking, produces inflammatory response in the lungs of all smokers and those who develop COPD have an abnormal or enhanced inflammation

4.1 Intracellular heat shock proteins – Antinflammatory molecules

The heat shock response is one of the most evolutionary conserved protective mechanisms in cells. It involves a temporary modification of gene expression. Synthesis of different heat shock proteins helps the organism cope with environmental and physiological stresses. As anti-inflammatory effector the heat shock response modulates signal transduction and gene expression by inhibiting the translocation of transcriptional factor – nuclear factor kappa B to the nucleus and prevents the expression of inflammatory mediators (Wong et al, 1997; Sun et al, 2005; Malhotra and Wong, 2002). Initial observations in animals linked heat shock response to an altered inflammatory response and demonstrate that heat preconditioning confers survival in otherwise lethal endotoxin stress (Snyder et al, 1992; Ensor et al, 1994). Heat conditioned macrophages show decreased secretion of TNF-alpha induced by endotoxins. This decreased secretion was sustained as long as the cells had elevated HSP70 levels. Similar to these studies endotoxin induction of IL-6 was also unchanged in the heat-

conditioned cells. The decrease of cytokine production was associated with a decrease in cytokine mRNA, suggesting that cell stress response regulates cytokine transcription. (Snyder et al, 1992; Ensor et al, 1994)

The liver has been the most extensively studied tissue to delineate the heat stress response in animals, because of the massive accumulation of HSP70 following stress. There is compelling data to support a direct link between liver HSP70 accumulation and altered survival and inflammatory cytokine profile seen in heat-conditioned animals undergoing endotoxin stress. Human peripheral blood macrophages overexpressing HSP70 also inhibited lipopolysaccharide induced production of TNF-α, IL-1β, IL-10 and IL-12 (Ding et al, 2001; Dokladny et al, 2008). These data taken as a whole demonstrates, that HSP70 expression is sufficient to alter proinflammatory cytokine production and increase endotoxin tolerance. A number of potential explanations have been suggested for the HSP mediated inflammatory repression. Immune cells are stimulated by a number of incoming signals from cell surface, including ischaemia, oxidative stress, LPS etc and initiate an inflammatory response by the activation of signalling pathways and transcription factors. The NFκB transcription factors play a pivotal role, altering the expression of cytokines, chemokines, cell adhesion molecules, growth factors, anti-apoptotic proteins and immunoreceptors (Brasier et al, 2006). Inactive NFκB is normally found in the cytoplasm, bound to its inhibitory protein, IκB. NFκB is activated by a number of incoming signals. These activate IKK, which phosphorylates IκB and allows NFκB to translocate into the nucleus and bind its target genes – TNFα, IL-1β, IL-6, IL-12 (Zhang and Ghosh, 2000).

HSP70 interacts directly with the NFκB inhibitor protein – IκB-α, which appears to prevent NFκB dissociation. HSP70 blocks IκB-α degradation and up-regulates IκB-α mRNA. Another mechanism of inflammatory suppression is indirect mechanism - repression of MAPK activation that mediates the inhibition of NFκB cascade. In addition, HSP70 suppresses activation of Jun kinase (JNK) MAPK. This prevents the phosphorylation of c-JUN and the subsequent activation of transcription factor AP-1. (Wang et al, 2002)

High mobility group box 1 protein (HMGB1) can trigger MAPK pathway and subsequent NFκB mediated synthesis and release of inflammatory mediators. HMGB1 is another step in the inflammatory cascade that is regulated by heat shock response. (Tang et al, 2005). HSP 70 overexpression suppressed the release and translocation of HMGB1.

The anti-inflammatory function of the small heat shock proteins is also confirmed. They interfere with the signallingpathway through their ability to protect against oxidative stress (Mehlen et al, 1995) and through modulation of TAK-1 activity (Alford et al, 2007). Another mechanism is the ability of HSP27 to suppress NFκB activation by interaction with IKK-α and IKK-β (Kammanadiminti SJ and Chadee K, 2006). HSP27 is also needed for the activation of TAK1 and downstream signaling by p38 MAPK, JNK (Alford et al, 2007). Both HSP27 and αB-crystalline have crucial roles in the control of inflammatory processes.

Among the pathologies where anti-oxidative defence of HSP27 is important is the airway inflammation in asthma. It is observed that in the airway of asthmatic patients HSP27 has increased expression and generates protection against oxidative stress, accompanying the chronic inflammatory state of this tissue.

In addition to altering of cytokine production heat shock proteins are also capable to influence cell tolerance to cytokines (Kusher et al, 1990, Jattela et al, 1993). The mechanisms

by which HSP confer protection from cytokines are not clear but may involve the interplay of intracellular triggers related to cell survival, stress tolerance and inflammation. It is majorly accepted that this is cell type specific and is executed by the regulation of apoptosis. (Schett et al, 2003; Ran and Lu, 2004)

4.2 COPD and intracellular heat shock proteins – A mechanism of self-defence, a trigger for immune inflammation, or a chaperonopathology

There are a few studies that deal with the levels of expression of heat shock proteins in lung of COPD patients.

Gal et al, 2011 showed for the first time that cigarette smoke extract stimulates the expression of HSP72 in alveolar epithelial cells. One possible mechanism for this could be the activation of cell preservation mechanisms, including decreased degradation of HSP72 in cells exposed to severely damaging substances. Heat shock protein induction is cytoprotective by preventing the onset of apoptosis. HSP72 has been shown to protect cells both from apoptosis and necrosis (Fekete et al. 2006; McConkey 1998). There are studies confirming other noxa - dimethylarsinic acid exposure-elevating intracellular HSP72 levels, changing the localization of the molecule and suppressing apoptosis of human alveolar cells (Kato et al. 2000).

In addition HSP72 siRNA abolished the mRNA and protein increase in cells, in parallel apoptosis increased and less cells survived. These results confirm upregulation of HSP72 in the presence of CSE in order to ensure cell survival, and indicate key protecting role for HSP72 under this cellular stress condition. Moreover these authors show that the anti-apoptotic effects of dexamethasone in alveolar epithelial cells are accomplished only after the upregulation of HSP72 that follows the cigarette smoke exposure. According to these experiments, increase in the inducible form of HSP70(HSP72) following CSE administration might enable proper action of administered dexamethasone, by increasing the assembly of GR and opening the steroid binding cleft. Glucocorticoid receptor (GR) function is dependent on the HSP90/HSP70 chaperone machinery. Initial GR interaction with HSP70 appears to be critical for the triage between HSP90 heterocomplex assembly and preservation of receptor function. HSP70 is required for the assembly of protein - HSP90 heterocomplexes, and the two chaperones interact directly with each other while opening the steroid binding cleft in the GR (Pratt and Toft 2003).

The results of Gal et al, are consistent to those reported by Chao-Jun Li et al, 2007. They found upregulation of HSP70 in human lung fibroblasts exposed to cigarette smoke. Similar is the data represented by Doz et al, 2008 who report that there is an increase in the levels of HSP70 in bronchoalveolar lavage of mice exposed to cigarette smoke. The inducible form of HSP70 is also upregulated in monocytes and endothelial cells of COPD patients. (Balsano, et al, 1999; Ning et al, 2004)

Ruicheng Hu et al, 2011 applied proteomics (MALDI-TOFF) to compare the expression profiles of proteins in cell lysates of lung tissue of 24 COPD smokers (6 in stage I and 18 in stage II, stable COPD), 24 non-COPD smokers, and 24 never-smokers. Age, gender distribution, body mass index did not differ significantly between the groups. Smoking index did not differ significantly between 11 COPD smokers and non-COPD smokers. Key spirometric parameters, including FEV1, FEV1%, FVC%, maximum predicted expiratory

flow rate at 50% of vital capacity (MEF50%), and MEF25% were comparable between the non-COPD smokers and never-smokers.Twenty-four proteins were identified by MS as being differentially expressed among the three groups of subjects. The main functions of these proteins involve basic metabolism, oxidation/reduction, coagulation/fibrinolysis, protein degradation, signal transduction, inflammation, and cell growth/ differentiation/ apoptosis. Proteomic analysis revealed that the expression of HSP27 and CyPA was upregulated in smokers, and this upregulation was particularly marked in COPD smokers. The variation in expression of HSP27 and CyPA between the groups was confirmed by IHC and Western blotting. Based on the results from the present study and other studies that have shown a protective role for HSP27 against oxidative stress and apoptosis, it is suggested that induction of HSP27 protects the lung cells of smokers and COPD patients against oxidative stress and apoptosis.

In contrary to the other heat shock proteins Capello et al, 2006 found a decrease of tissue expression of HSP60 and HSP10 that were parallel to chronic obstructive pulmonary disease progression, but did not correlate to the severity of COPD in smoking patients with NSCLC. They detected a trend for a decrease of intracellular expression of this chaperone that correlated best to the degree of tissue dedifferentiation.

In conclusion most of the studies dedicated to the role of heat shock proteins in COPD pathology are concentrated on tissue cell lysates or epithelium. The up-regulation of chaperones in them seems to be a protective mechanism, providing survival through anti-apoptotic and anti-oxidant role. Little is known about the expression of these cell proteins in neutrophils, lymphocytes and dendiritc cells and the way they influence the immune inflammation.

4.3 Extracellular heat shock proteins – Pro-inflammatory molecules

Intracellular HSP confer anti-inflammatory state because they downregulate inflammatory cytokine production, increase cellular tolerance to cytokines mediated cytotoxicity and attenuate epithelial barrier permeability changes. Alongside heat shock proteins are involved in multifaceted inflammatory reactions when seen by immune effector cells in the extracellular environment. They serve as co-stimulator molecules for immune recognition and are among the major molecules, refered by Matzinger, as "danger" signals (Matzinger, 2002) The release of HSP in the extracellular environment is an area of current research. It is known however that HSP can be released either passively after cell necrosis, trauma of cells or viral infection, or actively – exercise, physiological stress, certain diseases (Basu et al, 2000, Caldwerwood et al, 2007; Hightower et al, 1989). In addition the immune effects of HSPs are different when released by necrotic cells or during physiological response. The immunogenic activity of HSPs is mediated by two main mechanisms: 1) cytokine reponse by the activation of Toll-like receptors 2) facilitation of antigen uptake and formation of HSP-peptide complexes by antigen presenting cells. (Asea, et al, 2007)

Extracellular HSP can bind to cell surface receptors like TLR2 and TLR4 and lead to signaling events and activation of antigen presenting cells (Srivastava et al,1994). This activation leads to a signalling cascade and includes the activation of IL-1 and NFκB signal transduction pathway. HSP70 for example signals through TLR2 and TLR4 with the involvement of CD14 of human monocytes. This is followed by an upregulation of inflammatory cytokine secretion (IL-1β, IL-6, TNF-α).

Extracellular HSP, particularly HSP70, HSP90 and gp96, serve as antigen carriers and facilitate antigen uptake by dendritic or antigen presenting cells. Uptake is mediated by several mechanisms, including the α2-macroglobulin receptor (Binder et al, 2000). The HSP-peptide complex is more efficiently taken up by APCs than antigen alone. In addition HSP also stimulate APC maturation and activate NFκB signal pathways (Basu et al, 2000).

HSP facilitate antigen processing and transfer to MHC – I complex for presentation to cytotoxic T-lymphocytes. They are also expressed on the surface of tumor cells in cell culture as well as in cells infected with viruses.(Multhoff et al,1997) The HSP expression on tumor cells correlates to direct natural killer cell induced cytotoxicity and can be blocked by incubating target cells with antibodies directed against HSP70 prior to NK cell exposure. (Roigas et al, 1998)

4.4 COPD and extracellular heat shock proteins

The molecular mechanisms by which cigarette smoke causes the inflammatory process and pathology of COPD remain poorly understood. Chronic bronchitis and lung emphysema are pathologic characteristics of COPD and both conditions result from progressive and amplified inflammation that destructs and remodels parenchyma. Immune activation is not restricted to the lungs and is systematic. It procedes even after smoking cessation. In contrast to other inflammatory conditions the inflammation in the lungs is severe even in the advanced stages. There is enough evidence that make it reasonable enough to hypothesize that COPD has some kind of autoimmiunity in its pathogenesis. Nowadays it is largely accepted that the oxidative stress, accompanying COPD leads to changes in cell structures that makes them antigenic, thus triggering an autoimmune inflammatory response in patients with a genetic susceptibility.

It has been demonstrated that expression of HSPs is upregulated under stressful events in the lungs. Besides known intracellular chaperoning, it is possible that HSPs may also be released into the extracellular space following massive trauma or stress. This spillage of proteins serves as "danger signal" leading to cytokine transcription and release.

The involvement of extracellular HSP in COPD inflammatory process was described by Chao-Jun Li, et al 2008. They show that lung fibroblast exposed to cigarette smoke extract (CSE) release IL-8. The secretion was HSP70 dependent. Marked induction of HSP70 was observed in fibroblast culture medium in response to CSE. Upon exogenous administration of recombinant HSP70 to CSE treated fibroblasts, IL-8 production also increased. These results suggest that HSP70 is secreted into the extracellular environment via an unidentified mechanism that stimulates the production of IL-8 in primary lung fibroblasts. To examine whether it is the extracellular HSP70 that leads to CSE-induced IL-8 production, they determined CSE-induced IL-8 production in the presence or absence of neutralizing antibodies against HSP70 in the medium. Fibroblasts subjected to neutralizing antibody to HSP70 in the medium, exhibited marked reduction of CSE induced IL-8 production but did not completely abrogate the response. These data suggest that the extracellular HSP70 plays a critical role in mediating CSE-induced IL-8 production but also point to an HSP70-independent pathway of IL-8 production by CSE stimulus. They identified a novel early molecular pathway that mediates chemokine IL-8 release by human primary fibroblasts after cigarette smoke exposure. Early growth factor -1 (EGR-1) can trigger the synthesis of

HSP70. The HSP70 is then secreted into the extracellular environment and activates proinflammatory molecule (such as IL-8) production. They hypothesize that released HSP70 may activate cells through TLR-2, TLR-4, and CD14 and thereby mediate inflammation.

Similar are the results presented by Chase et al, 2007. Through their investigation, they have found evidence that supports the presence and biological activity of extracellular HSP72 in the lung. Chase et al, established that the airway epithelium itself is responsive to extracellular HSP72 and that this cytokine response is regulated through the TLR4 and NF-κB pathways. Their data would suggest that extracellular HSP72 is responsible for inducing and propagating inflammation, a process that is central to the pathogenesis of lung injury. Ganter et al. found extracellular HSP72 to be a marker of improved alveolar fluid clearance and therefore recovery from lung injury. This and other clinical investigations presenting divergent effects of extracellular HSP72 would suggest that the mere presence of HSP72 in the extracellular milieu is not the only factor. Chase et al, hypothesize that there may be a threshold of extracellular HSP72 that is required to maintain adequate signaling, below which the cells are unprepared for the insult, and above which excessive inflammation and therefore increased injury occur.

Doz et al, 2008 show that cigarette smoke exposure of the airways induces acute inflammation in mice. They found that airway inflammation is dependent on Toll-like receptor 4 and IL-1R1 signalling. Cigarette smoke induced a significant recruitment of neutrophils in the bronchoalveolar space and pulmonary parenchyma, which was reduced in TLR4(-) and MyD88 (-), and IL-1R1-deficient mice. Diminished neutrophil influx was associated with reduced IL-1, IL-6, and keratinocyte-derived chemokine levels and matrix metalloproteinase-9 (MMP-9) activity in the bronchoalveolar space. Cigarette smoke condensate (CSC) induced a macrophage proinflammatory response in vitro. The process was dependent on MyD88, IL-1R1, and TLR4 signaling, but not attributable to LPS. Heat shock protein 70, a known TLR4 agonist, was induced in the airways upon smoke exposure, which probably activated the innate immune system via TLR4/MyD88 an resulted in airway inflammation.They concluded that acute cigarette exposure results in LPS-independent TLR4 activation. This led to the IL-1 production and IL-1R1 signaling, which is crucial for cigarette smoke induced inflammation leading to chronic obstructive pulmonary disease.

Considering the presented data little is known about the clinical significance and mechanisms of secretion of extracellular heat shock proteins in COPD. There is scarce information about their relevance to the initiation and maintenance of the persistent inflammation.

4.5 Extarcellular heat shock proteins – A diagnostic marker, a marker of inflammation or disease severity

Hacker at al, 2009 investigated serum levels of heat shock proteins - HSP 27, 60, 70, 90alpha, 20S proteasomes, C-reactive protein (CRP), and interleukin-6 (IL-6). Serum levels were evaluated in healthy non-smoking volunteers (15), smokers without COPD (14), patients with mild to moderate COPD (19) and patients with severe or very severe COPD (16) were evaluated in four study groups. HSP27, HSP70 and HSP90α were significantly altered in patients suffering from COPD as compared to controls.

There was a significant increase of HSP27 in serum samples taken from the peripheral blood flow of patients suffering from COPD as compared to healthy smokers. Hacker et al, demonstrate a continuous increase of serum HSP27 concentrations with disease severity in their study. This effect may be due to increased tissue devastation especially in late stages of COPD and spreading of the inflammatory disease to other organs provoking a systemic spillage of HSP27 into the vascular bed. HSP27 generally acts as antiapoptotic mediator and can be seen as an endogenous immunosuppressive attempt to control excessive inflammation in COPD. Serum contents of HSP27 showed diagnostic potential to determine the occurrence of COPD in a logistic regression model and may serve as a marker for diagnosis and prediction of disease severity. Serum levels of HSP70 were elevated in patients at early and late stages of COPD. There was a four-fold increase in the GOLD I-II group compared to non-symptomatic smokers. Values were highest in the COPD I-II group, indicating a state of vast immune activation primarily at the early stages of the disease. Serum levels of HSP70 showed high sensitivity and specificity to define the occurrence of COPD in a logistic regression model and could serve as diagnostic marker. Because there was no significant difference between the COPD groups, HSP70 in comparison to HSP27 is unlikely to be a suitable marker for disease progression or response to therapy.

Rajagopal et al. characterized HSP90 as central factor in antigen presentation to T lymphocytes via major histocompatibility complex class II molecules (MHCII). Hacker et al, found soluble HSP90α was significantly upregulated in the peripheral blood of COPD groups as compared to healthy non-smokers. They hypothesize that elevated levels of extracellular HSP90α in COPD are essential in the adaptive immune system, triggering a possible autoreactive response to self-antigen. They suggest that HSP90α has immunomodulatory effects through cross-presentation of associated peptides in the context of major histocompatibility complex molecules. According to their results HSP60 is not a key element in the pathogenesis of COPD. Serum concentrations of HSP60 did not correlate with levels of other HSPs. Authors did not analyse whether there was a correlation between extracellular HSP and markers of inflammation – IL-6, CRP. They concluded that there were elevated serum concentrations of soluble heat shock proteins 27, 70 and 90α in patients with COPD. Their spillage into the vascular bed may be caused by continuous activation of the immune system in the deterioration of COPD through endogenous and exogenous trigger mechanisms. Furthermore, HSP27 and HSP70 showed statistical trends to serve as diagnostic markers or markers for disease progression.

Cherneva et al, compared the plasma levels of αB-crystalline in 63 COPD patients, 52 healthy-smokers and 48 smokers with inflammatory lung diseases. ELISA was applied as a method of detection. 43 of the COPD patients had severe disease (GOLD – III-IV) and 20 had mild disease (GOLD – I-II). The age distribution between the three groups was similar with a mean age of 67.24 (±8.06). All the patients had a comparable smoking exposure – 29.58 (±12.28) pack-years. In 26 of the COPD patients plasma levels of MMP-9 and CRP were also evaluated.

The mean levels of αB-crystalline were respectively: COPD patients – 0.352 (±0.12); healthy smokers – 0.291 (±0.07); smokers with inflammatory lung diseases – 0.433 (±0.27). Statistically significant difference was established between the COPD patients and the healthy smoking volunteers - ($p=0.010$) and between smokers with inflammatory lung

diseases and the healthy smoking volunteers (p=0.007). In comparison there was not a significant difference between the COPD patients and those with inflammatory pulmonary diseases - (p=0.158). No relation was established between αB-crystalline plasma levels and hsCRP and MMP-9 levels respectively (p=0.91 and p=0,76)

Authors concluded that αB-crystalline is not a specific diagnostic marker. It rather reflects oxidative stress, and inflammation that accompanies it.

5. COPD – An accelerated lung ageing disease, chaperonopathy or proteinopathy?

Ageing implies a certain period; the passage of time from conception till death. Senesence is a function of ageing and implies the molecular and cellular processes that accompany it. Senesence is the structural characteristics that appear as times goes by. They could either be physiological, or more often deleterious ones that occur in the organism after it has fully developed and affect its molecules, cells and tissues. A distinction should always be made between senescence and ageing, since the structural characteristic at molecular level do not always correspond to the age, depending on the "stress" conditions and the adaptive capacities of the organism.

Different organisms, particularly their cells are challenged to thrive to different environmental and emotional stress factors (physiological stress, emotional stress, oxidative stress, food deprivation, sleep deprivation, hypoxia, ischaemia, etc) for one and the same period of time. They are trying to keep the balance, reaching the homeostasis and getting adapted to the new conditions that they are currently in. This is performed by the stimulation and engagement of a number of evolutionary developed and genetically predetermined pathways:1) inflammatory pathways; 2) unfolded protein response 3) heat shock response 4) ubiquitin proteasome system. The pathways are executed by an armamentarium of chaperones that " take care" and provide functionally available proteins. In case of failure the damaged proteins are cleared by another armamentarium that clear the cells from destructive components.

Chaperonology, the study of chaperones and HSP is emerging as a new area of physiology and molecular biology that could be of importance in both pathology and medicine. Defective chaperone function can contribute to the etiology of pathologic conditions, known as chaperonopathies. Chaperonopathies can be genetic or acquired. Usually the latter are described as quantitative variations in chaperone levels in tissue or body fluids that are accumulated as a result of posttranslational modifications. These variations and modifications have been described for the separate members of the HSP and their association with old age and age-related diseases have been largely reported. However data is scarce to demonstrate neither a direct link between a chaperonopathology and a definitive molecular or cellular characteristic, typical of senescence, nor to attribute certain chaperonopathy to a specific disease, associated with ageing.

Age-dependent damage to proteins is one of the primary molecular features of senescence. The appearance of age-related post-translational modifications of proteins – proteinopathies can seriously disturb or entirely change their cellular function, or make them immunogenic. The accumulation of modified proteins as time goes by could be attributed to several models:

- A cell possessing a normal set of chaperones could potentially counteract the proteinopathies. However chaperones are themselves modified by the passage of time So it could be that both chaperonopathies and proteinopathies start in parallel and independently of one another. In that case chaperones would also play a major role, though not the primary one, demonstrating a failure of the cellular adaptation to maintain the protein homeostasis and functioning during senescence (age-related emphysema);
- There could be an accumulation of damaged proteins due to high levels of stress, which would result in a widespread deficiency of otherwise normal chaperones and thus lead to an accelerated onset of degenerative or age-related diseases. In that case chaperonapathies seem to be a major trigger in the mechanisms of senescence (COPD);
- Another possibility – the presence of genetically defective chaperone system a chaperonopathy that can not counteract and keep the protein homeostasis even when there are no environmental challenges. Chaperone failure in that case would cause the initiation and progression of proteinopathies. Besides this the abnormal chaperones would not perform their physiological roles in cells, thus affecting essential cellular processes (emphysema in COPD);
- Chaperonopathies could be treated as a different characteristic of the process of senescence but not one of its mechanisms. However taking into account clinical, pathological and experimental studies this is hardly reasonable.

Ignoring the dilemma, whether the chaperonapathies or the proteinopathies are first during the process of ageing, the immune system will inevitably response to what is going on in the organism. If this is the chaperonopathy 1) Chaperones could be spilled extracellularly as "danger" signals, activating the antigen-presenting cells; 2) chaperones could be associated with other peptides on the surface of cells announcing for a ''danger''cytotoxicity or; 3) chaperones can simply allow the accumulation of modified proteins that are accepted as self-antigenic.

If this is simply a proteinpathy as Kirkow assumes the defects cause inflammatory reactions, which can themselves exacerbate existing damage, so that inflammatory and antiinflammatory factors can play a part in modulating the outcome of the ageing process. Thus, age-associated inflammation/structural change is a failure of elimination and/or failure of repair (DNA, protein).

Ito and Barnes, 2009 define COPD as accelerated lung ageing disease. They find a lot of similarities between aged lung and COPD lung. These are not mere clinical characteristics – accelerated decline of lung function but a number of molecular ones.

1. Telomere length has been demonstrated to be significantly shorter in patients with emphysema than in asymptomatic nonsmokers. This is confirmed in alveolar type II cells and endothelial cells, (Tsuji et al, 2006) peripheral blood mononuclear cells,(Morla et al, 2006) and fibroblasts.(Muller et al, 2006)
2. There is ample evidence that oxidative stress plays a major role in COPD. Increase in nitrotyrosine deposition is also a feature seen in COPD lung as well as aged tissue. This is the evidence of an increase in nitrative/oxidative stress, and may contribute to the accumulation of nitrated and oxidized proteins. Superoxide dismutase enzyme activity is reported to be lower in long-term healthy smokers and in stable COPD patients than

in healthy adults,(Kirkril et al, 2008) although this is still controversial (Nadeem et al, 2005).COPD patients also have reduced total antioxidant capacity. Furthermore, ferric-reducing antioxidant power is lower in COPD patients, and it had a positive correlation with the severity of airways obstruction (FEV1 percentage of predicted).

3. There are several similarities in inflammation between ageing and COPD, such as neutrophil accumulation, NF-kB activation (Barnes, 2006) and increase in IL-6/IL-8/TNF-α. COPD patients are also corticosteroid insensitive as similar to healthy aged people. Protein turnover system in COPD is also impaired. HDAC2 is markedly reduced in COPD. This reductionis involved in enhancing inflammation is involved in enhancing inflammation and corticosteroid insensitivity.
4. Furthermore, expression of antiageing molecules are reduced in COPD - SIRT1 is a major inhibitory regulator of MMP-9, and reduction in SIRT1 causes structural changes of lung, such as emphysema (Vuppusetty C, et al 2007; Rajendrasozhan S, et al. 2008). SIRT6 loss leads to abnormalities in mice that overlap with ageing-associated degenerative processes, and SIRT6 is a nuclear, chromatin-associated protein that promotes resistance to DNA damage (Meyer et al, 1988).

Analysing the presented data we can assume that COPD could be regarded as a chaperonopathy (proteinopathy), as a model of accelerated ageing, in which the organism can not keep the homeostasis under the conditions of oxidative stress. The immune system is involved, but instead of restoring the balance it augments the oxidative stress, generating a large number of reactive – oxygen species. This leads to the accumulation of modified self-proteins that are recognized as antigenic. Autoimmunity occurs as an epiphenomenon. A vicious circle is created. The environmental and the inflammatory oxidative stress leads to the accumulation of modified proteins. Chaperones are additionally depleted or also modified. Ubiquitin proteasome system is overloaded or also modified.

6. COPD as a risk factor of lung cancer

COPD is currently the leading cause of morbidity and mortality worldwide whose prevalence and burden are projected to increase due to smoker exposure and the changing age structure of the world population, particularly in women (Lopez et al, 2003). The presence of COPD increases the risk of lung cancer of up to 4,5 fold among long-term smokers. COPD is by far the greatest risk factor for lung cancer amongst smokers in 50-90% of smokers with lung cancer (Young et al, 2009). Even a small reduction of FEV1 is a marker of airflow obstruction and is a significant predictor of lung cancer (Wasswa et al, 2005).

Lung cancer accounts for 12% of cancers diagnosed worldwide, making it the most common malignancy other than non-melanoma skin cancer. Approximately over one million die of lung cancer each year (Jemal et al, 2009). Worldwide, tobacco smoking is associated with more of 90% of cases of lung cancer. In less developed countries lung cancer rates are predicited to continue to increase due to endemic tobacco use. In more developed countries, the incidence and mortality rate are generally declining, reflecting previous trends in smoking prevalence (Youlden et al, 2008). Only 15% of life-time smokers develop lung cancer and 10% of lung cancers occur in never smokers especially in women and in Asiatic women in particular, which underlines the role of genetics (Scagliotti et al, 2009).

Lung cancer is also a leading cause of morbidity and mortality in patients with COPD as 33% of patients died of lung cancer over a 14,5 year follow-up (Anthonisen et al, 2005; Yao et al, 2009). Furthermore ≈60% of patients, diagnosed with lung cancer have a spirometric evidence of COPD (Molina et al, 2008). NSCLC accounts for 85% of lung cancer cases in USA and the COPD related cancer type (squamous cell lung cancer) still represents the most common histological subtype of lung cancer in European men (Papi et al, 2004). Despite significant advances in diagnostic approaches, the pathology of lung cancer is still elusive and there has been little improvement in 5-year survival rates (≈ 15% overall; <14% among males and <18% among females) (Youlden et al, 2008).

Two of the leading causes morbidity and moratlity worldwide – COPD and lung cancer are due to the environmental risk factor and cigarette smoke exposure in combination with genetic predisposition.

6.1 HSP and cancer

There is a cascade of molecular events that mediate the transformation of a normal cell to a cancerous one. Several etiological factors and events are recognized as triggering mechanism of cancerogenesis – viruses, radiation, hereditary and non-hereditary mutations, carcinogenic compounds. Most tumors are formed by stepwise progression of normal cell into a cancer one by using alterations in cell physiology, described by (Hanahan and Weinberg, 2000) – self sufficiency in growth signals, insensitivity to growth inhibiton, evasion from apoptosis, limitless replicative senescence, sustained angiogenesis and tissue invasion and metastasis. Heat shock reponse participates in cancerogenesis of both up – and downregulation of specific heat shock proteins. Variations in HSP expression could be found in many tumors and preneoplastic lesions as well. At a histological level the transition from a normal tissue to tumor is accompanied by the increase in HSP expression. HSP are involved in the cancerogenesis and are up-regulated to protect cells from apoptosis and induce drug resistance. Their role in cancer cells is to protect other proteins against aggregation, to solubilize initial protein aggregates, to assist in folding of nascent proteins or refolding of damaged proteins; to target severely damaged proteins to degradation. Overexpression of HSP in cancer cells is beneficial to their survival because they inhibit apoptosis and induce drug resistance. They act as a double-side sword. Some of them - HSP90 maintains chaperoning function in a number of oncogenic molecules and promotes tumor survival. Others – HSP60,70 and HSP72 may sensitize cancer cells to immune attacks by two mechanisms. They may be expressed on tumor cell surface and enhance their recognition by NK-cells or induce antitumor immunity by HSP related tumor vaccines (Calderwood et al, 2006).

6.1.1 HSP and non-small cell lung cancer

Bonay et al, 1994 are the first to study the expression profile of HSP in the normal lung as well as the effect of cigarette smoke on their expression. They provide detailed description of HSP distribution in lung carcinoma, applying both immunohistochemical and immunoflourescent techniques for their investigation. In lung tissue from non-smokers, lung epithelial cells are positive for HSP90 and the inducible form of HSP70. There was also a weak expression of HSP60. Macrophages also expressed these HSPs but weaker in

comparison to bronchial epithelium. However no other parenchymal, immune or inflammatory cell was positive for these heat shock proteins.

Cigarette smoking modifies neither the distribution, nor the intensity of staining in bronchial epithelium in smokers. Macrophages also expressed one or more of the HSP but in low levels. Bonay et al, 1994 have shown considerable heterogenity in the expression of HSP by cells in a given tumor. They explained their observations by different degree of differentiation state of the cells.

HSP90 is required for conformational maturation as well as stability of many proteins, involved in signalling pathways. It is responsible for the functional activity of a lot of oncogenic kinases that drive the signal transduction and proliferation of lung cancer cells. It seems to be upregulated in lung cancer and recently it has been connected with the stabilization of the mutant form of EGFR and one of the mechanisms for the resistance to tyrosine kinase inhibitors.(Shumamura et al, 2005, 2008)

HSP70 is another chaperone of interest in lung cancer. Volm et al, 1995 studied the resistance of lung cancer and its associatation to HSP70 expression. Tumor samples of 90 patients with NSCLC were investigated by immunohistochemistry, and no association between HSP70 and doxorubicin resistance was found. However a trend for an association between glutathione-S-transferase positivity and HSP70 positivity was observed. In addition there was a strong positive association between catalase positivity and HSP70 positivity. These observations show that both heat shock and stress promote intracellular oxidative damage and catalases are necessary for their protection.

Malusecka et al, 2001 studied the expression profile of both HSP70 and HSP27 proteins in 106 patients with NSCLC. They found in the majority of patients (95/106) both cytoplasmic and nuclear positivity to HSP70. In stage I tumors and dysplastic lesions however there was an enhanced nuclear positivity. As for HSP27 a positive cytoplasmic immunostaining in 70% of cases with the highest score for squamous cell lung cancer was found. A positive association for the expression levels of both proteins was also described. HSP27 and HSP70 were indicated as important factors for lung tumor transformation process, as well as for factors for chemoresistance.

Capello et al, 2006 studied the role of HSP60 and HSP10 in lung cancerogenesis. They described the level of expression of these chaperones in 35 patients with spirometirically proven COPD and compared them to the levels of expression in 10 adenocarcinomas and adenosquamous cell lung cancers. In normal bronchial epithelium that was found in 10 of the COPD patients HSP60 and HSP10 were positive in 23% of cases. In basal hyperplasia lesions they were positive respectively in 29% and 26%. Only 3% of squamous metaplastic lesions were positive for HSP60 and only 2% positive for HSP10. Of the dysplastic lesions 3% were positive for HSP60; 2% - for HSP10. Adenosquamos cell lung cancer was negative for both chaperones. The authors showed that HSP60 and HSP10 loss is related to the development and progression of bronchial cancer in COPD patients.

Recently Jackson and Garcia Rojas investigated the role of HSP27 in cellular resistance in lung cells, and reported that HSP27, which is phosphorylated by MAPK pathway protect epithelial cells from oxidant stress.

The role of another small heat shock protein in NSCLC was described by Cherneva et al, 2010.The expression of alpha-B crystalline was explored applying immunohistochemical

analysis on a tissue microarray slide, containing samples from 146 NSCLC patients - 96 squamous cell lung tumours, 10 adenosquamous carcinomas, 35 adenocarcinomas and five broncho-alveolar carcinomas. Tumors were of different grade of differentiation (29 - well differentiated, 56 -moderate and 36 - poor differentiation) and different clinical stage - 37 patients were in stage I, 27 in stage II, 65 in stage III and 17 in stage IV.

αB-crystallin was not detected in the normal alveolar pneumocytes; a few of the peribronchial glands, however, stained faintly but only in the cytoplasm. Although partially, there were a few areas where basal epithelial cells of the normal ciliated bronchial epithelium also showed weak cytoplasmic staining and no nuclear staining. In contrast, the basal layer of the tumours showed intensive cytoplasmic staining and a lack of nuclear staining. Lymphoid cells infiltrating the tumour stroma as well as the macrophages showed no cytoplasmic staining, but the nuclear staining varied from intensive to a lack of staining. Apoptotic and necrotic cells had faint cytoplasmic and intensive nuclear staining. Intensive nuclear staining was also detected in cells undergoing mitosis.

Nuclear staining was detected in 133 tumours (95 squamous cell histology and 38 adenocarcinomas). Cytoplasmic staining was detected in 127 tumours (95 squamous cell histology and 32 adenocarcinomas). Lack of nuclear staining was detected in 44 (33%) cases and intensive nuclear staining was observed in 89 (67%). A total of 26 tumours strongly expressed αB-crystallin in both nucleus and cytoplasm. Most of the tumours showed homogeneous cytoplasmic staining; more than 60% of the cells of the tumour had the same intensity of staining. The heterogeneity was detected up to the level of nuclear staining. The cytoplasmic staining was not of statistically significant correlation to histology. In contrast, the nuclear staining proved to be characteristic for the adenocarcinomas ($p < 0.001$, Contigency Coeff Cramer 0.369).

αB-crystallin was significantly overexpressed in NSCLC. In these tumors, the cytoplasmic expression of αB-crystallin was statistically significantly related to the tumour size (T-factor). This might be due to the fact that αB-crystallin has been reported to serve as a chaperone under stress conditions for other oncogenic molecules (beta-catenin, cyclin D1 and VEGF) or is itself oncogenic (Ghosh J, 2007).

In comparison to breast, renal and colorectal cancers, where only cytoplasmic and membraneous staining was reported, in NSCLC a nuclear staining was observed. The nuclear relocalisation is a characteristic feature for the whole group of small heat-shock proteins and in most cases is triggered under stress conditions (Klemenz R et al 1991, Voorter Ch et al 1992). In the nucleus, αB-crystallin is claimed to be responsible for the stabilisation of the speckled architecture of lamin A/C and is thus involved in the splicing factor compartment (Adhikari A, et al 2004). IJssel et al, 2003 discuss that its fundamental role in the nucleus (transcription, splicing and genomic stability) is difficult to be discerned from its chaperone function in that cellular compartment.

The precise biologic function of both cytoplasmic and nuclear localisation of the protein is obscure and needs other approaches for elucidation. Moreover, the variability of cellular compartment expression is complicated by the fact that in many epithelial tumours the protein is down-regulated and lacks cytoplasmic expression – buccal cancer and head and neck cancer. The importance of αB in NSCLC may be due to the fact that the nuclear staining was characteristic for adenocarcinoma histology and was significantly related to the

tumour stage (p = 0.042). Patients whose tumours had nuclear staining had shorter overall survival time in comparison to those that lacked staining (log-rank test p = 0.002). This supports the hypothesis that the nuclear positivity of the tumours refers to a more aggressive tumour biology. The nuclear positivity of αB in NSCLC stratifies patients from II and III stage in risk subgroups. Keeping in mind that more than 75% of patients are diagnosed at stage III, the introduction and validation of prognostic markers at this stage would undoubtedly help in predicting recurrence and improving clinical prognosis.

To sum up the role of heat shock proteins in NSCLC we can say that the high molecular chaperones are important molecular mechanisms in lung cancerogenesis, probably contributing by their chaperoning abilities related to other oncogenic molecules - (HSP90,70) as well as by performing their role in apoptosis – (HSP60,70). They could be used in cancer treatment as their inhibition (HSP90) is associated with overwhelming of chemoresistance (Shimamura et al, 2005, 2008) – or their induction (HSP70) as a way of sensitizing tumors to chemo- and radiotherapy (Gehrmann, 2006) The small heat shock proteins are probably related to the regulation of apoptosis, cytoskeletal stability, chaperoning of antioxidant enzymes and prevention of oxidative stress. Their clinical significance is related to their applicaton as markers for chemoresistance - (HSP27), or risk stratification and survival (αB-crystallin).

6.1.2 HSP as cancer diagnostic markers in COPD patients

HSP were found to be expressed on the surface of tumor cell and extracellular HSP were reported in plasma of both cancer and non-cancer patients. As HSP are highly immunogenic their cell surface and extracellular expression was employed in the production of vaccines as well as an approach for cancer detection.

Zhong et al, 2003 first decribed the diagnostic significance of extracellular HSP70 and 90 in NSCLC patients. The assay was performed in a group of 49 NCSLC patients and 40 healthy volunteers. The diagnostic utility of the HSP70 expression showed a modest sensitivity 0.74 and specificity 0.73; area under the curve AUC=0.73; while HSP90 antibodies were of poor performance AUC-0.602.

Wang et al, 2010 also tried to characterize the levels of expression of HSP70 and HSP27 in plasma and lymphocytes. They compared the expression of these chaperones in 99 coal miners without NSCLC, 51 coal miners with NSCLC and 42 patients that were not coal-miners. They found higher levels of plasma HSP27 and HSP70 in coal miners, which corresponded to a higher risk for lung cancer. Lymphocytes of coal miners with NSCLC had the lowest levels of intracellular HSP70 compared to coal miners and non-coal miners.

The presence of αB antibodies in NSCLC patients was also reported. Cherneva et al, 2010 compared the levels of expression of αB-crystallin in 51 NSCLC patients, 38 high risk COPD patients and 52 age and sex matched healthy volunteers. They found that the expression of αB crystalline antibodies was significantly higher in NSCLC patients in comparison to age and sex matched healthy volunteers - ($p<0.001$). αB-crystallin antibodies showed sensitivity 62% and specificity 72% in discerning cancer patients among healthy volunteers.

The clinical significance of αB-crystallin antibodies however is limited while comparing the healthy volunteers to the high risk group of COPD patients. A potential explanation of this

could be that the major characteristic of this pathology is the increased oxidative stress and chronic systemic inflammation, predominantly localized in the lungs.This may provoke reactive αB-crystallin protein overexpression in COPD patients, as one of its function is antioxidation (Aggeli et al, 2008).

Analysing the levels of antibodies of αB-crystallin in the plasma of patients with NSCLC and their clinicopathological chracteristic, Cherneva et al, found no significance between pathological parameters and this biological marker. This however was not the issue when concerning the lymphogenic spread of the disease. The levels of antibodies in patients with lymph node metastases was higher compared to those without them. The reason for this remains elusive and requires further investigation. The ROC curve analysis showed decent characteristics in discerning patients with and without metastatic spread –AUC 0.667 (95%CI – 0.515-0.820) sensitivity- 60% and specificity –70% at a cut-off 0.381. It should be however carefully taken in consideration that the clinical staging does not envisage the molecular one and the presence of already spread micrometastatic disease in N0 patients is obscure. Whether the higher rate of antibodies in patients with lymph metastases corresponds to a better immune reactivation and host defence remains a matter of question, since patients should be followed up.

Summarizing, the expression of heat shock proteins in NSCLC patients is of limited significance as a diagnostic approach, either alone or in a combination panel. The presence of αB-crystallin antibodies in plasma of NSCLC patients seems to be due the reactivation of the immune system and is unspecific as far as it is provoked under various stress conditions. The higher levels of the antibodies detected in patients with lymphogenic metastatic spread could be of clinical application as far as they could be used as markers for risk of recurrence and patients' prognosis.

7. Future research in COPD

Although a lot of research is done, unraveling the signalling pathways and the intimate mechanisms of innate and adopted immunity, COPD remains a leading cause of morbidity and mortality worldwide. It seems that the current concepts of COPD are not leading to a solution that can be applied in clinical practice. It refers to both pathogenesis and treatment.

7.1 "Stressing" the oxidative stress, inflammation and autoimmunity

Oxidative stress does exist in the lungs and it is inevitable, having in mind its physiology. Keeping its balance is a kind of self-protection. COPD is obviously a disease of disbalance - oxi/antioxidant, protease/antiprotease, apoptosis/proliferation, acetylases/deacetylases. In most cases COPD develops in smokers that makes us think that it necessarily is related to smoking, but why only 20% of smokers develop COPD? Moreover how can we explain the presence of non-smoker 's COPD? Is this another disease? If not can we say that it is a proteinopathy? A proteinopathy that sets a disbalance in lung cells even in the absence of exogenous noxa; a reason for sending a "danger" signal to the innate immunity that acts on default and instead of helping additionally stresses the already "stressed cells". Could it be that COPD in smokers is also proteinopathy, but acquired? There are not enough studies that let us make certain conclusions about the role of chaperones, particularly of heat shock proteins in COPD. There is only data that let us make hypotheses.

Can we assume that: In smokers' lungs, cells are exposed to additional stress, which acquires the intensive assistance of chaperones to maintain their proteins functionally available. In some individuals the chaperone system cannot adapt to the new set-point. The balance is disturbed. The cells are "stressed" and send signals for help. At the same time, however, there is already a depletion of chaperones and accumulation of abnormal proteins that appear as a result of the disbalance (proteinopathy/chaperonopathy) and this is another reason for sending "danger signals" to the immune system. These "danger signals" could themselves be heat shock proteins as the studies present. The "danger signals" bind to Toll-like receptors, which according to Doz et al, are essential for triggering the inflammation in COPD. Neutrophils are sequesterd in the pulmonary circulation and themselves become "stressed" Obviously something "happens" to their cytoskeleton as they become less deformable and retained. It is highly probable that their small heat shock proteins could not restore the cytoskeletal damage. Their F-actin probably cannot reorganize and form the lamelipodia and fillopodia needed for migration. Thus instead of protectors, neutrophils become generators of oxidative stress but also of inflammatory mediators - that is their cell-type specific response to stress. The immune system is activated. An inflammatory response follows. The innate immunity is triggered. Dendritic cells are already activated through their Toll-like receptors and the adoptive cell response becomes engaged. By that time the accumulation of modified proteins is still going on, the chaperone system and energy balance are overloaded. Instead of resuming the misfolded proteins, HSP – peptide, self-antigenic complexes accumulate in cells. Autoimmunity probably appears as an epiphenomenon. Rather than being resolved the problem persists, accelerated by the induction of an autoimmune inflammation. Despite performing their own cellular functions chaperones are trying to keep the other proteins. The cellular physiology is disturbed and a lot of cells commit a suicide. This leads to the spillage of "danger molecules", modified proteins, and self-antigenic products, that additionally activate the immune system. Inflammation persists despite of the elimination of the primary noxa and is even augmented by the immune cells. The "stressed lung cells" are sending danger signals to the immune system but instead of altruistic response they are put under even severe stress. The disbalance between proteins - acetylase/deacetylase; oxidation/antioxidation; proteases/antiproteases – changes the phenotypic characteristic of lung cells, as well as their environment – tissue remodelling follows. The functional characteristics of lung cells is already irreversibly deteriorated.

It is highly probabale that COPD smokers have chaperonopathy, affecting their cells as a whole but as lungs are the primary organ proteomic analysis of lung cells lysates will probably help most unraveling the puzzle. Microdissection techniques on epithelial cells should be used in patients with GOLD-0 and be compared to GOLD- I-II, GOLD – III-IV. It would be more reasonable to select patients considering phenotypes in COPD.

It would be interesting to find the role of extracellular HSP70 in COPD etiology an progression. The ''danger signal'' that stimulates inflammation through Toll-signalling.

7.2 Exacerbations – A spillage of HSP, or antigenic mimicry?

There is a lot of data about inflammatory and autoimmune (neurodegenerative, degenerative joint diseases, atherosclerosis, diabetes type I) diseases triggered as a result of

cross-reactivity between bacterial HSP and human HSP. Assuming this we can hypotheisize that exacerbations in COPD may reflect antigenic mimicry – a cross-reactivity between the self-antigenic HSP-peptide complexes accumulated in lung cells and the evolutionary conserved HSP of the infectious viruses and bacteria. Determining the levels of HSP65 during exacerbations could give a clue.

Exacerbations could also be a massive spillage of extracellular heat shock proteins that trigger the Toll-like receptors and thus induce immune response.

7.3 Treatment

Despite being or not a chaperonopathy, it will certainly be of interest to know whether the chaperones within the heterocomplex (HSP90/HSP70/GR) of the glucocorticosteroid receptor are related to corticosteroid resistance and if so how could this be modified? How is HSP90 involved in the telomere assembly and how can be manipulated to deter the telomere shortening and lung ageing? If Toll-like receptors and danger molecules as HSP 70 are the primary triggers of the immune response in COPD lung how effective will be their inhibiting? If COPD is a chaperonopathy how can we booster the chaperones instead of restoring the balance at so many levels - acetylase/deacetylase; oxidation/antioxidation; proteases/antiproteases? Is it reasonable to think that resuming one side of the problem as the application of sirtuin agonists, antioxidants, steroids would be a solution? Isn't it more reasonable to think of the natural protectors of these molecules that ''chaper'' (keep) them from alteration?

8. Conclusions

There is scarce data on the role of chaperones in the molecular pathogenesis of COPD. The contemporary techniques – proteomics (MALDI-TOFF) and transcritpomics (SAGE – serial analysis of gene expression) show overexpression of both high molecular weight – HSP70 and small heat shock proteins – hem-oxygenase -1, HSP27 in lung cell lysates. Far more studies are dedicated to the high levels of circulating extracellular heat shock proteins, representing them as a trigger for Toll-like receptor mediated immune response – HSP70, or as a panel of diagnostic markers in COPD – HSP90, HSP70, HSP27. Presuming the biological functions of the chaperone system, its significance in protecting cells from "stress", its large collaboration with the immune system and importance of preserving the proper functioning and balance of the proteome within cells it is undoubtedly necessary to further elucidate their place in COPD etiology and progression.

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Part 2

Clinical Aspects

6

COPD: Differential Diagnosis

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a high prevalent and impact socioeconomic disease. Although, cigarette smoking clearly fulfils all criteria to be classified as the etiology of COPD (Hill´s criteria), the latest version of GOLD does not include this concept clearly, therefore half century of main documents has been culminated and they has never mentioned in the definition, tobacco or cigarette smoking as cause of COPD. American Thoracic Society and European Respiratory Society (ATS / ERS) in the definition of COPD include the phrase "primarily caused by cigarette smoking". Nevertheless, the next page smoking changes from the category of "cause" to simply a "risk factor"; in fact, smoking is included in table 2 as a risk factor under the column of "exposures", together with socioeconomic status, environmental pollution, disease in childhood, or diet, among other. A reason for not to refer to cigarette smoking as aetiology is that only 15% of smokers are susceptible to COPD, a concept wrongly attributed to Fletcher. Although not all subjects exposed to cigarette smoking develop COPD does not preclude such exposure is the cause, just as not all people infected with *Mycobacterium tuberculosis* develop Tuberculosis, but there is no doubt about the etiologic role of mycobacteria. Another reason for nor establish smoking to the category of etiological factor in COPD is the existence of COPD in non-smokers.

Therefore, when the aetiology is not part of the definition of the disease, it is usually replaced by a clinical description and the definition based on clinical findings are very poor, so a new definition of COPD is needed to ensure a more valid an accurate way to manage this worldwide condition.

In the Table 1 shows some of the criteria that different societies, guidelines and organizations have been used to diagnose COPD.

Actually COPD is defined as postbronchodilator FEV1/FVC ratio < 70%. Threshold of FEV1/FVC <70% is agedependent and will probably lead to a significant degree of overdiagnosis of COPD in the elderly and underdiagnose young adults. GOLD guidelines recommend that using the lower limit of normal (LLN) values for FEV1/FVC is a way to minimize the misclassification. But use LLN we also overdiagnose healthy subjects. Although use post-bronchodilator FEV1/FVC ratio <0.70 simplify the diagnosis of COPD, some pulmonologists, ever more, consider a diagnosis of COPD can not be based only on spirometry parameters; it is important to include the presence of respiratory symptoms and exposure to risk factors.

Society	Year	Criteria
ECCS	1983	FEV_1/VC or FEV_1/ FVC<LLN
ATS	1987	FEV_1/ FVC<0.75
ATS	1991	FEV_1/ FVC<LLN
ECCS/ERS	1993	FEV_1/VC or FEV_1/ FVC<LLN
ERS	1995	FEV_1/VC <88% predicted (men) or 89% (woman)
BTS	1997	FEV_1/FVC <0.70 and FEV_1<80% predicted
NLHEP	2000	FEV_1/FVC or FEV_1/ FEV_6/<LLN and FEV_1<LLN
GOLD	2007	FEV_1/FVC<0.70 postbronchodilator
NICE	2004	FEV_1/FVC <0.70 and FEV_1<80% predicted
ATS/ERS	2004	FEV_1/FVC<0.70 postbronchodilator
ATS/ERS	2005	FEV_1/VC <LLN

ATS: American thoracic Society; BTS: British Thoracic Society; VC: Vital capacity; ECCS: European Community for Coal and Steel; ERS: European Respiratory Society; FEV_1/ /FVC: ratio of forced expiratory volume in 1s to forced vital capacity; GOLD: Global Initiative for chronic obstructive lung disease; LLN: lower limit of normal (LLN); NICE: National Institute for health and clinical excellence; NLHEP: National Lung Health Education Program.

Table 1. Spirometry criteria to COPD in some guidelines Society

There are many diseases or processes that show a FEV1 / FVC post-bronchodilator < 70%, these processes constitute the great chapter of what we call "disease" COPD.

Although nosological or semantically, definition of COPD as a syndrome is questionable, recently the term has come to be considered by other authors. Table 2 outlines a long list (not exhaustive) of entities that may be associated with airflow obstruction syndrome or COPD. Most of them are common such as pneumoconiosis and other occupational diseases, Airway obstruction in pulmonary tuberculosis, some clinical forms of asthma, etc and other less common such as lymphangioleiomyomatosis, Bronchiolitis obliterans associated with consumption of Sauropus androgynus among others. This chapter will show a list of differential diagnosis, as complete as possible and some clues for the recognition of these processes vs COPD.

2. Smoking COPD

In 1950, smoking was established as a cause of COPD (chronic bronchitis and emphysema) and Fletcher and Peto corroborated its natural history. The relationship between smoking and COPD, probably influenced by genetic determinants poorly understood, is primarily a dose-effect relationship as demonstrated in multiple studies.

Findings have proven smoking cessation disrupts the natural history of COPD, but there are authors who have more controversial opinions about it and assert that in many cases, inflammation persists despite smoking cessation. The perpetuation of inflammation may be related to other factors, bacterial colonization has been proposed.

In the past, it was considered that only 15% of smokers were likely to develop COPD, when in fact it is known that this percentage is near 50% if they survive long enough. This

COPD from smoking COPD from alpha 1 antitrypsin deficiency COPD in non smokers Chronic Asthma (perennial) Aging Sequelae of tuberculosis Mitral stenosis Cardiac failure Anorexia nervosa Cystic fibrosis in adult Bronchiectasis Ambiental exposure (biomass smoke) Marihuana smoking Sequelae accidental exposure (ammoniac) Occupational exposure • coal miners • pulmonary silicosis • byssinosis • pig farmers • cabinetmakers Others Endovenous exposure (heroin, cocain) Digestive exposure • (Sauropus androgynus) Bronchiolitis • Bronchiolitis obliterans • Diffuse panbronchiolitis • Obliterative bronchiolitis in microwave popcorn plant workers • bronchiolitis by rheumatoid arthritis • Others	Sjogren syndrome Inflamatory bowel disease Wegener syndrome Sarcoidosis Extrinsic allergic alveolitis • Chronic Eosinophilic granuloma Lymphangioleiomyomatosis Neurofibromatosis Tuberous sclerosis Birt-Hogg-Dubé syndrome Scleroderma HIV AIDS (Pneumocistis jiroveci) Placental transmogrification Paraneoplasic Pemphigus Fabry disease Salla disease Amyloidosis Ligth chain deposition disease Relapsing polychondritis Tracheobronchomalacia Tracheobronchopathia achondropasia Ehler Danlos syndrome Tracheal stenosis Cord vocal paralysis Relapsing Polichondritis Traqueal neoplasia Papilomatosis tracheobronchopathia multiple Others

Table 2. Causes of COPD syndrome

percentage of susceptibility increases if others methods, better than simple spirometry, have been used to detect COPD.

Although transfer of carbon monoxide (DLCO) and computed tomography (CT) with high resolution have demonstrated useful in early diagnosis of emphysema, they are underused.

Disadvantages for Chest CT and other imaging techniques are expensive and irradiation exposure.

3. Alpha-1-antitrypsin deficiency

There are excellent reviews about alpha 1 antitrypsin deficiency (AATD). AATD is associated with impaired pulmonary antiproteasas defenses leading to unopposed protease activity. ATTD is the best model of COPD and emphysema. The clinical course is accelerated mainly by the smoking, but also by air pollution, and phenotype well-known. Some cases are diagnosed as asthma or bronchiectasis for clinical manifestations. Others may be diagnosed by hepatologists if the first manifestation is liver findings. In recent years, the characteristics in

subjects over 60 years of age have been described. It is important the determination of the DLCO for evaluation of its prognosis and outcome, not only spirometry.

Heterozygous individuals have a higher susceptibility to develop COPD in the presence of smoking or cigarrete exposure, and should be detected for a better affiliation of the syndrome. Therefore, measurement of alpha-1-antitrypsin should be practiced at least once in all patients with chronic airflow obstruction.

4. COPD syndrome by tuberculosis

Although Tuberculosis is considered as precursor in pulmonary specialty, airflow obstruction in pulmonary tuberculosis has been just considered few years ago. (Figure 1). In 1971, Snider et al had described Obstructive airway disease and pulmonary tuberculosis, and PLATINO study in Latin America has been updated this. In an environment where tuberculosis is or was common, the sequelae of tuberculosis are a major cause of chronic airflow obstruction in individuals who have never been smokers. Airway obstruction in pulmonary tuberculosis:

- do not correlate with the degree of the affected area, could coexist even if area of damage is small
- is presented in patients with treated pulmonary tuberculosis even healthy subjects
- is unusual progresses and
- is irreversible to bronchodilator.

5. Asthma chronic non-reversible perennial

Problems in the differential diagnosis of bronchial asthma not reversible and COPD are well known.

It is accepted that 30% of asthmatic patients are smokers, and this variant of overlap syndrome has been well described by various authors. There is evidence that smokers with asthma are more resistant to treatment. The natural history of patients with COPD asthmic syndrome involves a fast loss of FEV1; they have a decreased life expectancy, though this aspect is not well known because many studies included as non-smokers and former smokers.

6. Aging

In the population over 80 or more years old, up to 50% of individuals may have a FEV1/FVC <70%. Although some analogies between COPD and elderly have already been highlighted, further studies are required. Hence tables with normal spirometric values for the elderly have just been available recently. Nevertheless, the presence of a FEV1/FVC <70% is clinical data that should not be neglected, because there has been a marker of reduced life expectancy, even in old age.

7. Heart disease

Classically, the chronic forms of valvular heart disease could present with airflow obstruction and / or reduction in DLCO. The decrease in the incidence of rheumatic fever and development of new cardiac diagnostic methods avoid this situation as a common

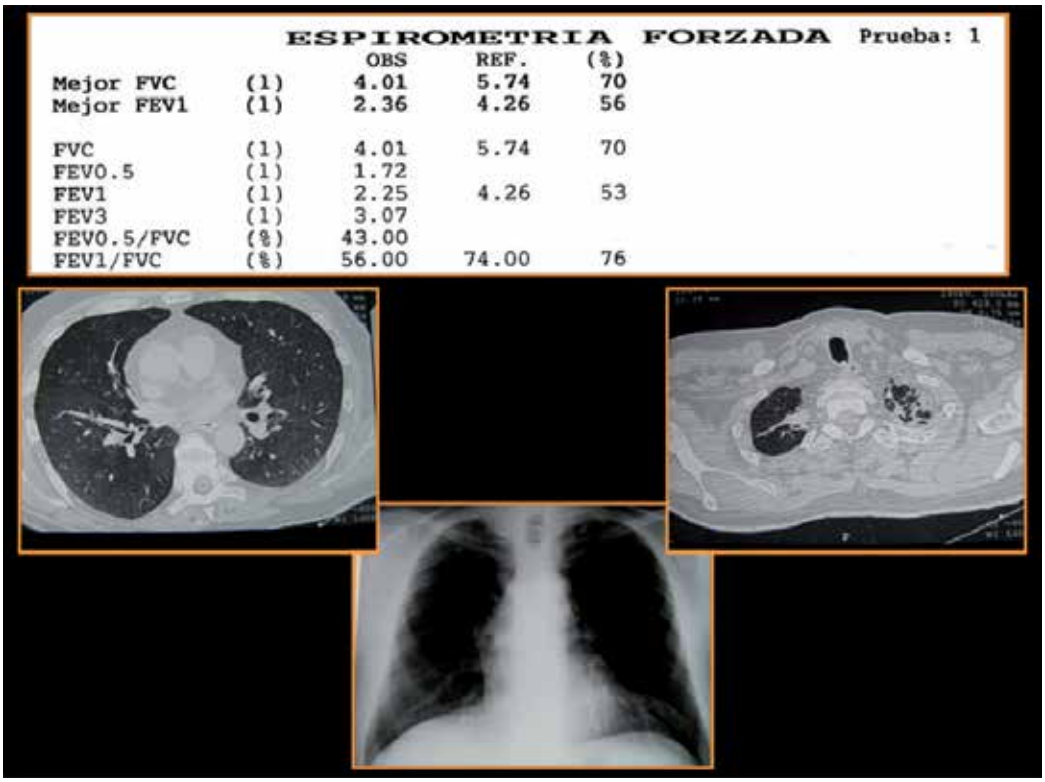


Fig. 1. A 55-year old woman, nonsmoker, was seen as outpatient in a check-up, a spirometry revealed the presence of obstructive patterns. A diagnosis of Pulmonary Tuberculosis had been made 25 year ago and antituberculosis treatment was completed. Diagnosis: sequelae of tuberculosis.

problem of differential diagnosis. The comorbidity of COPD and heart failure caused by smoking, metabolic syndrome, the syndrome of obstructive sleep apnea and aging, could hinder the diagnosis of COPD exacerbation vs heart failure. However, measurement of the natriuretic peptide can help in the differential diagnosis. Once patients have been discharged from hospital, they should be required to filial the impact of both processes. Studio ergometer should be made in outpatients in stable phase, although this is done in rare occasions.

8. No smoking COPD and antitissular antibody

To date, Birringer et al have perhaps been the only ones who have been systematically studied COPD in nonsmokers. Four hundred consecutive patients who visited for 2 years, Birringer found that 25% of them were smokers. Once discarded asthmatics, patients with bronchiectasis and a small subgroup with sputum eosinophilia, they were probably non-reversible asthma, a small percentage of 4% had common characteristics: they were preferably female, with age over 50 years, often with a history of Hashimoto's thyroiditis and / or antithyroid antibodies and / or antitissular antibodies or other features of autoimmune disease. Therefore, the measurement of antithyroid antibodies should be included in patients with features similar.

9. COPD from exposure to biomass smoke

It is considered that the population at risk of inhaling smoke from biomass could reach 3000 million people worldwide, mostly female. Anatopatology, COPD from inhalation of smoke from biomass has been becoming well known, has important similarities with COPD from smoking, but also significant differences.

Although its natural evolution is not well known yet, COPD from exposure to biomass smoke has some features in common with COPD from smoking. Romieu et al designed a study with methodology of trial in a group of Mexican women was divided into two groups: the control group cooking with the traditional open fire and the treatment group cooking with Patsari stove. After 6 years, confirmed a dramatic difference in the evolution of FEV1: the control group decline 62 ml FEV1 per year, while the intervention group only lost half. Orozco et al demonstrated that COPD in Spain by exposure to biomass smoke should be considered especially in older women from rural areas.

The impact of this disease is usually not epidemiologically relevant in developed countries, although some cases have been identified in countries as the United States, for example, in New Mexico, USA reported that 26% of subjects had been exposed to smoke from biomass fuel.

10. Other disease

Bronchiectasis, cystic fibrosis, bronchiolitis and alveolitis extrinsic allergic are disease or syndromes very often manifested with chronic airflow obstruction, as well as occupational exposures. Occupational exposures, in particular, are syndromes that constantly incorporating new disease. An example would be an extrinsic allergic alveolitis called hot tub, which is related to recreational activities whit contact hot water, as in water parks. Mycobacterium avium could have a main role.

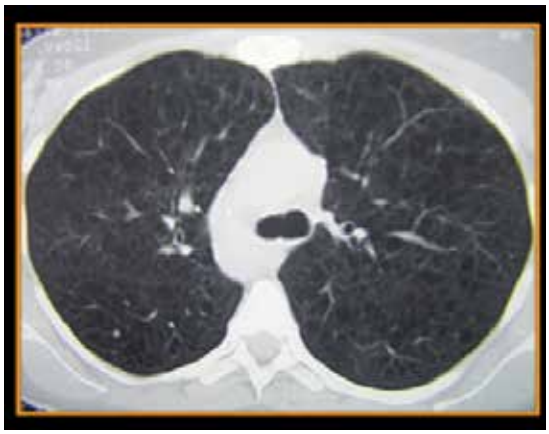
Paradoxically, until recently it discussed the evidence that pneumoconiosis of miners could be the cause of pulmonary emphysema in the absence of smoking, which is currently shown. Other occupational exposure disease is obliterative bronchiolitis in microwave popcorn plant workers. It was observed when the additives to provide flavor was replaced. The patients had a very aggressive clinical course.

Another microepidemic is bronchiolitis obliterans associated with sauropus androgynus, it also led to an extremely aggressive bronchiolitis and is not by respiratory exposure. The intention to lose weight was the reason of ingestion of extract of Sauropus androgynus.

Recently, CT scan has shown that patients with anorexia nervosa could be associated with emphysema. This observation was already known in the Nazi death camps. However, there is not evidence that emphysema cause by anorexia nervosa has airflow obstruction.

Patients infected with the human immunodeficiency virus (HIV) may have some respiratory disorders including pulmonary emphysema with airflow obstruction. It is accepted that the combination of smoking and inflammatory reactions caused by HIV accelerates the presentation of emphysema in 10 to 20 years. Recently it has tended to take an important role to *Pneumocystis jiroveci* in the obstruction of patients with HIV, but even this pathogen has been isolated in patients with COPD from smoking.

The eosinophilic granuloma, lymphangioleiomyomatosis (figure 2), histiocytosis X, tuberous sclerosis syndrome, Birt-Hogg-Dubé and deposition disease heavy chains are some orphan



	Pre-Bro			Post-Bro		
	Actual	Pred	%Pred	Actual	%Pred	%Chng
----- SPIROMETRY -----						
FVC (L)	3,32	3,64	91	3,32	91	-0
FEV1 (L)	1,39	3,00	46	1,51	50	8
FEV1/FVC (%)	42	83	51	45	55	8
FEF 25% (L/sec)	1,34	5,44	25	1,49	27	11
FEF 75% (L/sec)	0,21	1,72	12	0,22	13	5
FEF 25-75% (L/sec)	0,42	3,16	13	0,52	16	25
FEF Max (L/sec)	3,13	6,90	45	3,85	56	23
FIVC (L)	3,32			3,23		-3
FIF Max (L/sec)	3,10			2,92		-6
----- LUNG VOLUMES -----						
SVC (L)	3,41	3,64	94			
IC (L)	2,24	2,23	100			
ERV (L)	1,17	1,41	83			
FRC (N2) (L)	3,80	2,73	139			
RV (N2) (L)	2,62	1,51	174			
TLC (N2) (L)	6,03	4,96	122			
RV/TLC (N2) (%)	44	30	145			
Washout Time (min)	2,29					
----- DIFFUSION -----						
DLCOunc (ml/min/mmHg)	11,27	23,43	48			
DLCOcor (ml/min/mmHg)		23,43				
DL/VA (ml/min/mmHg/L)	2,28	4,72	48			
VA (L)	4,94	4,96	100			

Fig. 2. A 36-year old woman was seen in pneumology clinic because of dyspnea. She had a 5-pack-year history of smoking but had stopped smoking 4 year earlier CT: thin-walled cystic. Pulmonary function testing reveals an obstructive pattern. Lung biopsy: Lymphangioleiomyomatosis.

diseases, most of them genetic disease, they can cause airflow obstruction and pulmonary emphysema.

In half the cases, a rare disease such as vasculitis with urticaria and hypocomplementemia syndrome could present with severe emphysema. Its mechanism is could be local inactivation of alpha-1-antitrypsin.

Systemic diseases such as rheumatoid arthritis, lupus erythematosus, diffuse scleroderma, polymyositis and mixed connective tissue disease can cause bronchiolitis at some point in its evolution. Söjgren syndrome can provide images similar in CT of emphysema and additionally present with airflow obstruction.

Although, Sarcoidosis in advanced stages is present as pulmonary fibrosis, in the initial and/or mild stages is present as mild obstruction because hyperresponsiveness or involvement of the bronchial mucosa.

The connective tissue diseases such as Marfan syndrome and Ehlers-Danlos syndrome, among others, may present with lesions of emphysema, usually paraseptal. Simultaneously, may present with tracheobronchomegaly and hipercolapsabilidad tracheobronchial. CT and test of forced expiration have increased the diagnosis of bronchial hipercolapsabilidad. It probably is one of the main causes of airway obstruction in healthy people.

Likewise, tracheal tumors, the Wegener, vocal cord paralysis and vocal cord dysfunction also cause of airflow obstruction.

11. Special situations

Parentage of a patient with obstruction initially suffered from asthma and who subsequently becomes a smoker can be a clinical problem almost insoluble.

The combination of smoking and disease interstitial chronic or pulmonary fibrosis has been highlighted in several recent publications. A recent epidemiological study in area cardiology, MESA study, performing CT lung, indicates that this match will be anecdotal in the future.

Respiratory bronchiolitis with interstitial respiratory disease (RB / ILD) is another example of interstitial and bronchial disease secondary to smoking. Some patients who do not meet the criteria for COPD are patients with severe by accelerating their natural evolution. (Figure 3)

12. Comorbidity and differential diagnosis

Comorbidity in COPD is controversial. In fact, it has been so exaggerated to name as chronic inflammatory syndrome, including in the same process other systemic manifestations. It is much more common that the vascular comorbidity in patients with COPD due to chronic exposure to tobacco that systemic inflammation secondary to COPD. Table 3 lists the mechanisms of comorbidity and Table 4 summarizes the comorbidities of COPD.

Introduce comorbidity in the differential diagnosis have done difficult in many cases the appropriate affiliation to the patient. In a smoker of 65 years with a cumulative consumption of 40 pack / year comes to a vascular surgeon for a pulsatile abdominal mass is likely to

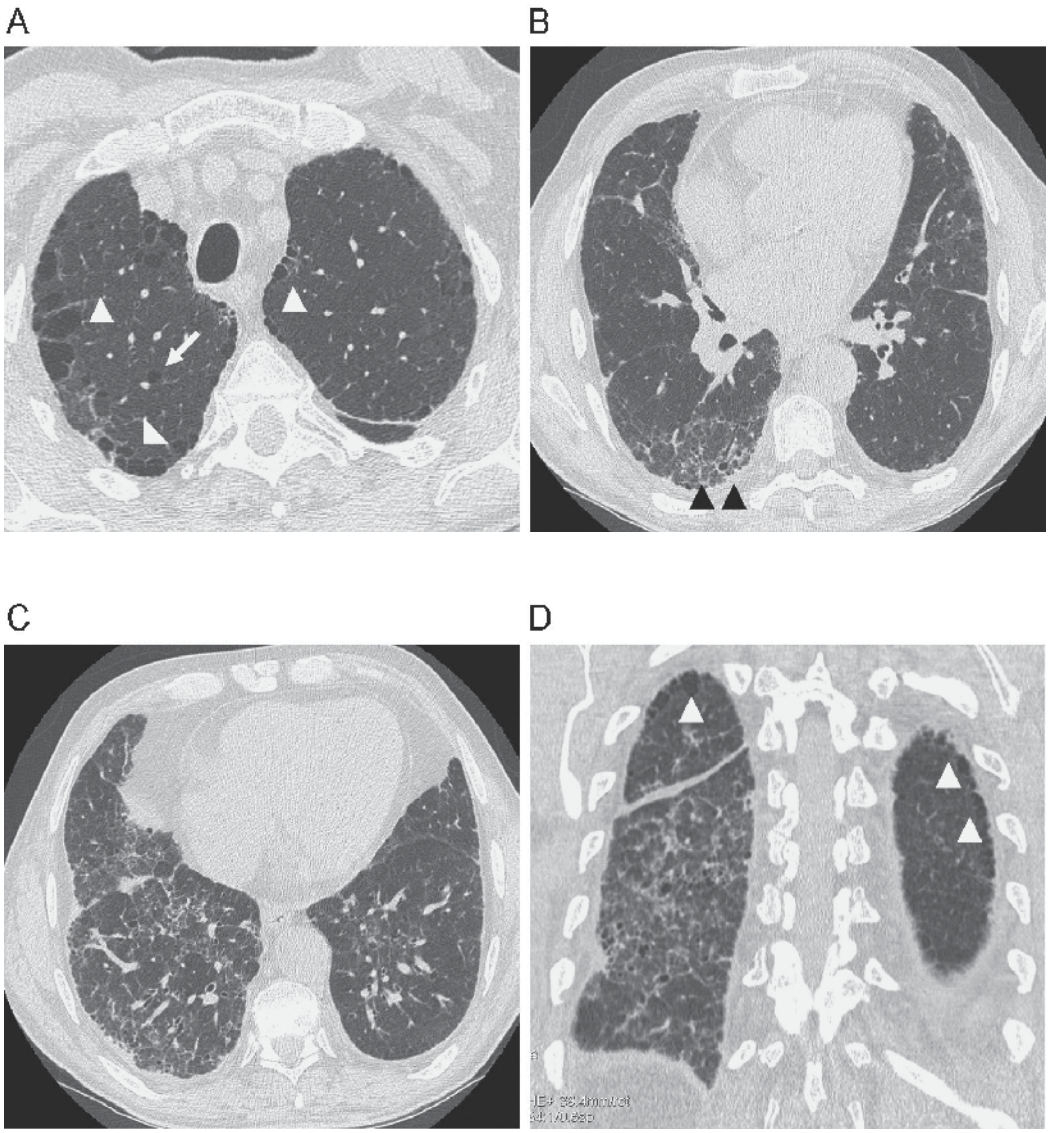


Fig. 3. High resolution computerized tomography (HRCT) of the same patient. A) Presence of paraseptal emphysema and subpleural bullae (white arrowheads) and centrilobular emphysema (arrows) in both upper lobes. B) Reticular interstitial disease with intralobular thickening and images of subpleural honeycombing and traction bronchiectasis (black arrowheads) C) Reticular interstitial disease in middle and right lower lobes, with interlobular septal thickening, subpleural honeycombing and traction bronchiectasis. D) Coronal reconstruction in the posterior regions of both lungs: Bilateral paraseptal emphysema (white arrowheads) and reticular interstitial disease and honeycombing in right lower lobe. (Used with permission MD Portillo)

Systemic features of cigarette smoking
Systemic features of COPD
Comorbidity of COPD
Extrapulmonary feature of COPD

Table 3. Mechanisms underlying the comorbidity of COPD

Respiratory • Bronchopulmonary carcinoma • Pulmonary arterial hypertension • Bronchiectasis • Pneumonia • *Pneumocystis* pneumonia • Obstructive sleep apnea • Invasive aspergillosis • Others **Cardiovascular** • Coronary disease • Auricular fibrillation • Cardiac failure • Carotid stenosis • Arritmia • Aneurysm of thoracoabdominal aorta • *Cor pulmonale* • Others **Neuropsychic** • Depressive disorder • Ictus • Lacunar infart • Anxiety disorders • Orthostatic hypotension • Intracranial hypertension • Cognitive impairment • Others	**Digestive** • Gastroesophageal reflux • Malabsorption • *Helicobacter pylori* infection • Others **Endocrine** • Diabetes • Hypogonadism • Others **Systemic** • Cachexia • Myopathia • Anemia • Osteoporosis • Polycythemia • Facial wrinkles • Hypercoagulable state • Others **Others** • Rhinitis • Cataracts • Inguinal hernia • Nephrotic syndrome • Periodontal disease, etc

Table 4. Comorbidity of COPD

delay the practice of spirometry. Recently, Remy-Jardin et al have made an interesting proposal to TC (dual-energy) for the simultaneous evaluation of pulmonary and vascular damage smoking.

13. When should we suspect that COPD is not secondary to tobacco?

An example, a female patient over 70 who had lived much of her life in a rural area, who had never smoked, presents with cough, expectoration and airway obstruction would be a candidate that her disease was secondary to exposure biomass. We cannot be in accordance with a diagnosis of COPD in individuals who had smoked fewer than 10 packs / year,

unless they had simultaneous deficiency of alpha-1-antitrypsin disease. In obvious cases of airway obstruction in people younger than 40 years, it is unusual that this was secondary to smoking. If the annual decline of FEV1 was greater than 75 ml, an additional cause should be suspected. A familial aggregation might suggest cystic fibrosis in adults. The coexistence of joint, skin or ophthalmic symptoms, mucosal dryness and thyroid disease should alert us about other causes of COPD. Bronchiectasis, mostly in women, with *Micobacterium avium complex* is associated with low body mass index. Finally, laboral and hobbies history should be complete in the first interview in pneumologic specialty. Table 5 shows signs and symptoms to help to exclude COPD.

History of smoking <10 pack-year
Onset before 40 years old
Decline of FEV_1 >75 mL per year
Autoimmune or collagenous disease
Ambiental exposure
Systemic symptoms
Progression of the obstruction years after smoking cessation

Table 5. Signs and symptoms to help to exclude COPD

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7

Current Overview of COPD with Special Reference to Emphysema

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1. Introduction

Pulmonary emphysema is a chronic disease defined pathologically as an abnormal permanent destruction and enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of alveolar walls without predominant fibrosis. Emphysema frequently occurs in overlapping association with chronic bronchitis which is clinically defined as chronic productive cough for three months in each of two successive years in a patient in whom other causes of chronic cough have been excluded. Previously, emphysema and chronic bronchitis was regarded as distinct entities and grouped under the umbrella term Chronic obstructive pulmonary disease(COPD).

COPD is a collection of heterogeneous conditions characterized by persistent expiratory airflow limitation. There is significant overlap and co-existence of conditions like emphysema, chronic bronchitis and asthma(see Figure 1). COPD is heterogeneous clinically and at a pathophysiologic level and its recognition, has led to new initiatives to categorize and define COPD and its subsets.(American Thoracic Society[ATS],1995,2010;British Thoracic Society[BTS],1997)

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) does not emphasise on the distinction between emphysema and chronic bronchitis (GOLD,2006). This report produced by the National Heart, Lung, and Blood Institute (NHLBI) and the World Health Organization(WHO) emphasised on the common feature of altered lung function recognizes both the systemic nature and the heterogeneity of COPD and defines it as follows:

> "Chronic obstructive pulmonary disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation

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that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases."(GOLD,2006)

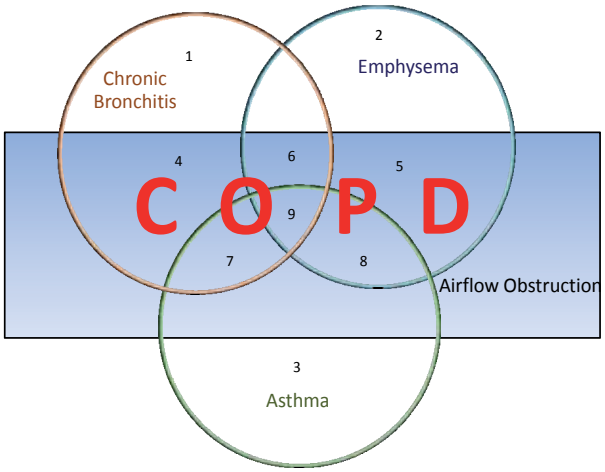

Fig. 1. Schematic Venn diagram of subsets of COPD. This is a non-proportional Venn diagram adapted from *Am J Respir Care Med* (ATS,1995) showing subsets of patients with chronic bronchitis, emphysema, and asthma(circles) and their relationship to airflow obstruction (box). The subsets comprising COPD are 4,5,6,7,8 and 9. Asthma is depicted by subset 3, whose airflow obstruction is completely reversible and are not considered to have COPD.

2. Historical background

COPD and its various subsets have been known in human history since pre-industrialisation era. (Snider, 1992 as cited in Shapiro SD,2010). Badham is known to have first used of the term "chronic bronchitis" in 1808(Badham,1808 as cited in Shapiro SD,2010). Fletcher and Laennec have presented early reviews and studies in 18th and 19th century (Fletcher et al.,1976;Laennec,1835 as cited in Shapiro SD,2010). Reid demonstrated increased mucus gland size in his pathologic studies and led to the development of the "Reid Index" and highlighted the anatomic basis for chronic bronchitis(Reid,1960).

Ruysch described enlarged respiratory air spaces on the surface of human lungs in 1691(Ruysch,1691 as cited in Shapiro SD,2010). Over the next centuries, work of Matthew Baillie, Laennec and Gough helped in describing the pathologic enlargement of airspaces and classified it between centriacinar emphysema and panacinar emphysema(Baillie,1799,1808,Gough,1952;Laennec,1835 as cited in Shapiro SD,2010). Various "hypothesis" were proposed over the years to describe the disease pathogenesis:

i. "Dutch hypothesis" - originated by Orie(Netherlands) proposed that asthma and airway hyperreactivity leads to fixed airflow limitation. (Orie et al.,1961 as cited in Shapiro SD,2010)
ii. "British hypothesis" - suggested the concept that mucus hypersecretion led to airway remodeling and airflow limitation.(Fletcher,1976 as cited in Shapiro SD,2010)

iii. "Protease-antiprotease hypothesis"("Swedish hypothesis") - association of homozygous alpha1 protease inhibitor deficiency with emphysema was discovered by Laurell and Eriksson in Sweden. (Laurell & Eriksson,1963;Snider et al.,1974 as cited in Shapiro SD,2010)
iv. "American hypothesis" - American pathologist Averill Liebow emphasised that altered repair mechanisms contribute to the development of COPD and that deficient maintenance of lung structure, could lead to emphysema.(Liebow,1959;Rennard,2004 as cited in Shapiro SD,2010)

COPD has been described and subclassified on various clinical, etiopathogenetic and pathological basis. Chronic bronchitis has been associated with a "blue bloater" clinical phenotype on basis of the concept that altered airway anatomy would lead to heterogeneity of airflow distribution within the lung, resulting in ventilation-perfusion imbalance, hypoxemia, and right heart failure. On the other hand, emphysema has been described with the "pink puffer" phenotype based on the concept that it primarily causes decreased airflow from the obstruction and less prominent hypoxia.

Pathologic studies delineate inflammation of airway structures (bronchitis) from destruction of the alveolar wall (emphysema). Emphysema has been described as centriacinar, panacinar and paraseptal based on the location of emphysematous airspaces in an acinus. Centriacinar variety is common in cigarette smokers and panacinar emphysema is predominant in proteinase inhibitor deficiency.

3. Epidemiology

COPD is a major public health problem with a high and continually increasing morbidity. The Burden of Obstructive Lung Disease (BOLD) study showed that the worldwide prevalence of COPD (stage II or higher) was 10.1%. This figure varied by geographic location and by sex with prevalence among men at 11.8% (8.6-22.2%) and among women at 8.5% (5.1-16.7%)(Buist et al.,2007). The wide differences noted was partly due to site and sex differences in the prevalence of smoking. The true prevalence is likely higher because COPD is both under-recognized and under-diagnosed. COPD was the sixth leading cause of death worldwide in 1990 and is expected to become the third leading cause of death by 2020(Murray & Lopez,1997 as cited in Shapiro SD,2010).

COPD has higher prevalence in low-socioeconomic population(Fletcher,1976). Commonly, patients present in their fifth decade of life with productive cough or acute chest illness. Alpha-1 Protease Inhibitor (A1PI) deficient patients present earlier than other COPD patients in 3rd-4th decade of life. COPD progresses with age and is more prevalent in elderly populations. In the United States, 15% of the total population aged 55 to 64 will have moderate COPD (GOLD stage 2, FEV1 < 80% predicted), and this increases to over 25% for those older than 75(Stockley et al.,2009 as cited in Shapiro SD,2010).

3.1 Risk factors

3.1.1 Cigarette smoking

Cigarette smoking is the most significant and predictable risk factor in pathogenesis of COPD. Almost 80% of individuals who have COPD and 80% who die from COPD in the

United States are smokers (Mannino et al.,2002 as cited in Shapiro SD,2010). The estimated fraction of COPD mortality attributable to smoking was 54% for men 30–69 years of age and 52% for men 70 years of age or older (Ezzati & Lopez,2003 as cited in ATS,2010). There is a consistent exposure–response relationship which is demonstrated in evidence from cohort studies fulfilling the causal criterion of temporality (exposure preceding onset of disease). Although only 15% of smokers have clinically significant COPD, smoking leads to a predictable dose-dependent loss of lung function in pre-symptomatic phase which accelerates with age and has prognostic implications (Rennard & Vestbo,2006 as cited in Shapiro SD,2010). Smoking has supra-additive effect in worsening lung function and prognosis when combined with other risk factors like A1PI deficiency or occupational exposures(Silverman et al.,2009 as cited in Shapiro SD,2010).

3.1.2 Genetic predilection

Genetically determined deficiency of alpha1–protease inhibitor (A1PI) represents a proven genetic abnormality that predisposes to COPD(Ericksson,1964;Laurell & Eriksson,1963 as cited in Shapiro SD,2010). A1PI is inherited by codominant alleles on chromosomal segment 14q32.1. Early adult-onset emphysema associated with A1PI deficiency occurs most commonly with PiZZ (mutation in *SERPINA1* gene) phenotype. It is prevalent globally but most commonly found in whites of Northern European ancestry. Worldwide there are an estimated 116,000,000 carriers and 1,100,000 individuals with severe α1-AT deficiency.(Boas & Winnie,2011)

Linkage analysis studies in early-onset COPD families have identified another serine proteinase inhibitor(serpin E2) on chromosome 2 as a potential defect site(DeMeo etal.,2006;Wilk et al.,2009 as cited in Shapiro SD,2010). Twin and familial aggregation studies suggest that genetic factors likely influence variation in pulmonary function in nonsmokers, but may not necessarily increase the risk of developing a clinical diagnosis of COPD(ATS,2010).

3.1.3 Occupational and environmental exposures

Farming and occupations with dusty environments increase the risk of developing chronic bronchitis two to threefold and, in combination with smoking, the risk increases to almost sixfold above average population(Melbostad et al.,1997;Salvi & Barnes,2009 as cited in Shapiro SD,2010). Environmental particulate air pollution and indoor smoke from biomass fuels have also been linked to COPD (Tashkin et al.,1984). There is strong evidence of an association between outdoor pollution(particulate matter, O_3,NO_2) and decreased pulmonary function (Gauderman et al,2004,2007; Rojas-martinez et al.,2007 as cited in Shapiro SD,2010). Exposure to air pollutants, occupational exposure, second-hand smoke exposure, fumes from burning biomass fuel, etc can produce deleterious effects on the airway. Oxidative stress, pulmonary and systemic inflammation, reduction in airway ciliary activity, amplification of viral infections, and increases in bronchial reactivity could lead to irreversible loss of pulmonary function over time and COPD(ATS,2010).

3.1.4 Gender

Epidemiological studies shows a male gender predominance related to the higher cigarette smoking habit or other inhaled toxins and occupational exposure among men within a

population(Buist et al,2007). Increase in smoking among women has diminished the difference among gender prevalence. Mortality may be peaking among men in the United States but, among women, mortality continues to rise and deaths from COPD among women now even exceeds those among men (Mannino et al.,2002,Silverman et al.,2000 as cited in Shapiro SD,2010).

3.1.5 Asthma

Accelerated loss of lung function has been noted among asthma patients(Lange et al.,1998; Peat et al.,1989 as cited in Shapiro SD,2010). Functional changes in both the small airways and the alveolar parenchyma have been reported. Many individuals have bronchial inflammation with features of both asthma and chronic bronchitis/emphysema(Gelb & Zamel,2000 as cited in Shapiro SD,2010).

3.1.6 Socioeconomic status

Morbidity and mortality rates have been shown to be inversely related to socioeconomic status(U.S. Department of Health and Human Resources,1984; Mannino & Buist,2007). Relative lack of awareness,diagnostic and therapeutic facilities and poorer health conditions may in part be connected to the socioeconomic status of the affected population.

3.1.7 Developmental events

Impairment in early lung growth and development appears to increase the risk of development of COPD(Weiss & Ware,1996 as cited in Shapiro SD,2010). Maternal smoking, low-birth weight and recurrent childhood respiratory infections have been associated with higher incidence of adulthood COPD.(Marossy et al.,2007; Shaheen,1998 as cited in Shapiro SD,2010)

3.1.8 Dietary factors

Observational studies strongly suggest that dietary factors, such as a higher intake of vitamin C and other antioxidants(carotenoids,Vitamin E,lutein, flavanoids) are significantly associated with better lung function(ATS,2010). Some dietary elements like fruits and vegetables(antioxidants), fish(omega-3 polyunsaturated fatty acids) and Vitamin D seem protective while processed foods like cured meats(nitrites) may be deleterious for lung function preservation(ATS,2010).

3.1.9 Tuberculosis

Pulmonary tuberculosis can lead to scarring and accelerated decline in lung function(Hnizdo et al.,2000 as cited in Shapiro SD,2010). Some population-based surveys(PLATINO and PREPOCOL) reported strong association between previous tuberculosis and a greater risk of COPD(Caballero et al.,2008; Menezes et al.,2007 as cited in Shapiro SD,2010).

3.1.10 Intravenous drug abuse

Emphysema is prevalent in approximately 2% of intravenous drugs abusers which can be attributed to pulmonary vascular damage possibly from the insoluble filler. Bullous cysts

are found in upper lobes of cocaine or heroin abusers whereas basilar and panacinar emphysema are associated with methadone and methylphenidate injections.

3.1.11 Immune deficiency syndromes

Human immunodeficiency virus (HIV) infection was found to be a risk factor for COPD, independent of confounding variables(Crothers et al.,2006 as cited in Shapiro SD,2010). Apical and cortical bullous lung damage occurs in autoimmune deficiency syndrome and *Pneumocystis carinii* infection.

3.1.12 Vasculitis syndrome

Hypocomplementemic vasculitis urticaria syndrome (HVUS) and other associated conditions include angioedema, nondeforming arthritis, sinusitis, conjunctivitis, and pericarditis may be associated with obstructive lung disease.

3.1.13 Connective-tissue disorders

Several connective disorders have been implicated in causation of or co-existence with emphysema and poor lung function. Cutis laxa is a congenital disorder of elastin-tropoelastin that is characterized by premature aging and occasionally emphysema. Marfan syndrome (autosomal dominant inherited disease of type I collagen), Ehlers-Danlos syndrome,Salla disease (autosomal recessive storage disorder with intralysosomal accumulation of sialic acid), Birt-Hogg-Dube' syndrome and familial spontaneous pneumothorax syndrome(mutations in folliculin gene) have been associated with poor lung function, blebs, pneumothorax and emphysema(ATS,2010).

4. Pathology

4.1 Chronic bronchitis

Chronic bronchitis is a clinical entity defined by a chronic productive cough for three months in each of two successive years in a patient in whom other causes of chronic cough have been excluded. It is characterised by an overlapping pathologic process of bronchial wall inflammation. The submucosal glands show dilated ducts and hypertrophy and hyperplasia. Reid index (the ratio of glandular to bronchial wall thickness) as well as goblet cell frequency and airway smooth muscle thickness is increased in chronic bronchitis(Reid,1960). The airway wall contains inflammatory cells predominated by macrophages and CD8+T lymphocytes. Bronchus-associated lymphoid tissue(BALT) is also present in late GOLD stages(Hogg & Timens,2009 as cited in Shapiro SD,2010). Increased numbers of neutrophils are found in the airway lumen and in the glands during episodes of exacerbations(Saetta et al.,1997;Thompson et al.,1989 as cited in Shapiro SD,2010).

4.2 Emphysema

Pulmonary emphysema, a pathological entity defined as destruction and enlargement of air spaces distal to the terminal bronchiole involving respiratory bronchioles, alveolar ducts, and alveoli. Cigarette smoking, inhaled irritants, recurrent infections and proteinase-

antiproteinase imbalance lead to inflammatory cell recruitment, proteolytic injury to the extracellular matrix (ECM), and cell death. Alveolar walls become perforated and later due to incomplete and disorderly repair, become obliterated with coalescence of small distinct air spaces into abnormal and much larger air spaces, which is the pathological hallmark of emphysema (Shapiro & Ingenito,2005 as cited in Shapiro SD,2010). Emphysema has been classically described with absence of interstitial fibrosis to differentiate from restrictive lung diseases. However, scarring of the small airway subepithelial space and collagen accumulation around larger disrupted air spaces has been noted in emphysema.

Various subtypes of emphysema have been described based on location and distribution of the lesions in the acinus (Pipavath et al.,2009 as cited in Shapiro SD,2010). In most patients, however, the process within the lung will be heterogeneous and in advanced stages, distinction becomes blurred. The following three patterns of emphysema are noted:

4.2.1 Centriacinar (centrilobular, proximal acinar) emphysema

Strongly associated with smoking, it begins in the respiratory bronchioles and spreads peripherally, primarily involving the upper and posterior parts of lungs(Leopold & Gough,1957;Thurlbeck,1991 as cited in Shapiro SD,2010). Focal emphysema, a form of centriacinar emphysema, occurs in persons with heavy exposure to inert dust such as coal dust (Morgan & Seaton,1984 as cited in Shapiro SD,2010).

4.2.2 Panacinar emphysema

Involves the entire alveolus uniformly and is predominant in the lower half of the lungs(Snider et al.,1962;Thurlbeck,1963,1976 as cited in Shapiro SD,2010). It is observed in patients with homozygous A1PI deficiency.

4.2.3 Paraseptal (distal acinar) emphysema

It usually involves the distal airway structures, alveolar ducts, and alveolar sacs around the septae of the lungs or pleura(Hogg & Timens,2009;Kim et al.,1991 as cited in Shapiro SD,2010). Apical and giant bullae described in this subtype may lead to spontaneous pneumothorax or compression of adjacent lung tissue.

4.3 Other pathologic variants mimicking emphysema

4.3.1 Air space enlargement with pulmonary fibrosis

It is commonly seen as an inconsequential lesion adjacent to scars but may be extensive arising as a complication of fibrosing diseases such as tuberculosis, silicosis, and sarcoidosis (Reid & Simon,1962;Thurlbeck,1991 as cited in Shapiro SD,2010). The spaces have dense fibrous walls and are mostly lined by bronchiolar epithelium(Akashi et al.,2009 as cited in Shapiro SD,2010).

4.3.2 Bullae

Bullae are marked focal dilation of respiratory air spaces that may result from coalescence of adjacent areas of severe panacinar emphysema, or from a ball-valve effect in the bronchi

supplying an emphysematous area(Reid.1967;Thurlbeck,1976,1991 as cited in Shapiro SD,2010).

4.3.3 Blebs

These are intrapleural collections of air, a form of interstitial emphysema. They may arise from interstitial emphysema of the newborn period or pulmonary barotrauma complicating mechanical ventilation. Ruptured blebs can cause spontaneous pneumothorax.

4.3.4 Cysts

Cysts are air spaces lined by epithelium, which usually have the characteristics of bronchial epithelium., They are classically known as intrapulmonary bronchogenic cysts and usually occur near the tracheal bifurcation, but they may be seen more peripherally in the lung parenchyma(Reed & Sobonya,1974;Rogers & Osmer,1964 as cited in Shapiro SD,2010).

4.3.5 Overinflation

Air space distention with or without alveolar rupture and is often reversible. "Simple air space enlargement" in which there is no destruction or loss of orderly appearance of the lung acinus, occurs in the contralateral lung following pneumonectomy(Compensatory overinflation or emphysema). Air spaces, particularly alveolar ducts, enlarge with advancing age, resulting in what has been termed "senile emphysema". Obstructive overinflation results from partial obstruction of a bronchus or bronchiole, when it becomes more difficult for air to leave the alveoli than to enter; there is a gradual accumulation of air distal to the obstruction, the so-called bypass, ball valve, or check valve type of obstruction. These conditions are listed in Table 1.

Compensatory Hyperinflation: Atelectasis Post-lobectomy
Intrinsic Obstruction of Major Bronchus: Tumor—benign or malignant Postinflammatory stricture Foreign body Amyloid
Extrinsic Obstruction: Congenital Aberrant vessel Extrapulmonary sequestration Tumor Nodes
Congenital Malformation of Bronchus:(Defective Cartilage or Mucosal Fold) "Congenital Lobar Emphysema"
Intrinsic Obstruction of Bronchioles: Unilateral acquired Bronchitis Bronchiolitis Obliterans

Table 1. Conditions with overinflation mimicking Emphysema

4.4 Pediatric conditions with emphysema

Some pediatric conditions display emphysematous pathologic findings resulting from a variety of developmental abnormalities in alveogenesis leading to impaired septation and alveolarization with consequent enlarged air spaces. Some are briefly described as below:

4.4.1 Congenital lobar emphysema

It is characterized by hyperinflation of one or more of the pulmonary lobes resulting from congenital deficiency of the bronchial cartilage, external compression by aberrant vessels, bronchial stenosis, redundant bronchial mucosal flaps or kinking of the bronchus caused by herniation into the mediastinum. The disease usually becomes apparent in the neonatal period but are delayed for as long as 5-6 mo in 5% of patients. The disease primarily involves lower lobes and occurs in familial preponderance. Treatment by immediate surgery and excision of the lobe may lifesaving, but some patients respond to medical treatment or selective intubation of the unaffected lung.

4.4.2 Overinflation of all three lobes of the right lung

Rarely produced by anomalous location of the left pulmonary artery impinging on the right main stem bronchus or occassionally with absent pulmonary valve type of tetralogy of Fallot and secondary aneurysmal dilatation of the pulmonary artery. Some neonates have lobar overinflation while on assisted ventilation, suggesting an acquired cause.

4.4.3 Broncho-pulmonary dysplasia

A form of chronic lung disease that develops in preterm neonates treated with oxygen and positive-pressure ventilation. Injury to small airways and pulmonary vasculature can interfere with alveolarization (alveolar septation), leading to alveolar simplification and reduction in the overall surface area for gas exchange. Premature birth and subsequent events (eg, exposure to oxygen, mechanical ventilation, inflammatory agents, infection) shifts the lung development towards premature maturation with an arrest in development and a loss of future gas exchange area.

4.4.4 Pulmonary interstitial emphysema

Collection of gases outside of the normal air passages and inside the connective tissue of the peribronchovascular sheaths, interlobular septa, and visceral pleura may result from alveolar or bronchiolar rupture commonly in premature infants on mechanical ventilation. It is a radiographic and pathologic diagnosis frequently in conjunction with respiratory distress syndrome, meconium aspiration syndrome, amniotic fluid aspiration and infection.

4.4.5 Acute generalized overinflation of the lung

Usually reported in infants and children secondary to a number of clinical conditions affecting bronchioles, including asthma, cystic fibrosis, acute bronchiolitis, interstitial pneumonitis, atypical forms of acute laryngotracheobronchitis, aspiration of zinc stearate powder, chronic passive congestion secondary to a congenital cardiac lesion, and miliary tuberculosis.

4.4.6 Bullous emphysematous blebs or cysts (pneumatoceles)

They result from overdistention and rupture of alveoli during birth, or as sequelae of pneumonia and other infections. They have been observed in tuberculosis lesions during specific antibacterial therapy.

4.4.7 Subcutaneous emphysema

It results from any process that allows free air to enter into the subcutaneous tissue. It can also result from pneumomediastinum or pneumothorax,fracture of the orbit, following tracheotomy, perforation in the esophagus or laryngopharyngeal area. Rarely, air is formed in the subcutaneous tissues by gas-producing bacteria.

5. Etiopathogenesis

The etiopathogenesis of COPD involves interplay of several over-lapping and co-existing injuries, defects, inflammation and disorganized repair in a vicious cycle, ultimately leading to a chronic progressive impairment of lung function. These processes are shared by other airway and parenchymal diseases of lungs and aggravated by other pulmonary and systemic co-morbidities. The key steps involved are epithelial injury, inflammatory cell activation, protease-antiprotease imbalance, airway inflammation, goblet cell hypertrophy and hypersecretion, recurrent infection, acute exacerbations, attempts to disorganized repair and fibrosis, ultimately leading to chronic progressive permanent airway obstruction(Barnes,2008;Kasahara & Tuder,2000;MacNee & Tuder,2009;Rennard,2003 as cited in Shapiro SD,2010).

5.1 Cigarette smoke induced inflammation

Cigarette smoke initiates inflammation both by direct oxidative-irritative cellular injury and indirectly by recruiting several inflammatory cells in the air space(ATS,1962,Barnes et al.,2008;Shapiro & Ingenito,2005 as cited in Shapiro SD,2010). Neutrophils rapidly accumulate in the lung in response to exposure to cigarette smoke(Rennard,2003 as cited in Shapiro SD,2010). Recruitment occurs via stimulated epithelial cells and macrophages releasing TNF-α and neutrophil chemokines CXCL1 and CXCL8 (IL-8) operating through the neutrophil receptor CXCR2(Barnes,2008 as cited in Shapiro SD,2010). Neutrophils contain proteinases like neutrophil esterase(NE) and Matix metalloproteinases(MMPs), particularly MMP-9, which are preformed and stored in granules and readily released upon activation. In addition to causing matrix destruction, proteinases generate fragments of ECM proteins such as collagen and laminin, which are also chemotactic for neutrophils, leading to a vicious feedback cycle of inflammation and tissue destruction(Adair-Kirk et al.,2003;Gaggar et al.,2008;Mydel et al.,2008 as cited in Shapiro SD,2010).

COPD is characterized by a gradual, progressive accumulation of macrophages in the lung most apparent in respiratory bronchioles which is the primary site of centriacinar emphysema(Niewoehner et al.,1974 as cited in Shapiro SD,2010). Stimulated macrophages produce both neutrophil and macrophage chemokine and cytokines like macrophage chemotactic protein-1 (MCP-1; CCL2), which recruits more monocytes from the peripheral blood(Barnes,2008 as cited in Shapiro SD,2010).Macrophages produce a variety of MMPs,

particularly elastases such as MMP-9 and MMP-12, resulting in lung tissue injury. Degraded elastin fragments are chemotactic for macrophages, thus ensuing a self-propagating cycle(Houghton et al.,2006;Senior et al.,1980 as cited in Shapiro SD,2010).

T cells lympocytes, especially CD8+ cells are increased in airway walls and alveoli of patients with COPD(Saetta et al.,1998 as cited in Shapiro SD,2010). Airway epithelial cells in smokers with COPD have increased expression of CXCL10 (IP-10), the ligand for CXCR3 found on CD8+Tcells and thus may be the activation pathway for macrophages to produce MMP-12(Grumelli et al.,2004;Saetta et al.,2002 as cited in Shapiro SD,2010). Cytotoxic T cells may target epithelial cells and induce cell death, particularly those with (latent) viral infection.

Elastin fragments can serve as autoantigens and immunoglobulin G (IgG) autoantibodies with avidity for pulmonary epithelium have been found in patients with COPD(Feghali-Bostwick et al.,2008;Lee et al.,2007 as cited in Shapiro SD,2010). Increased numbers of B cells and lymphoid follicles in the lung have raised interest in a possible autoimmune pathogenesis of COPD(Curtis et al.,2007;Taraseviciene-Stewart & Voelkel,2008 as cited in Shapiro SD,2010). Interplay of all these inflammatory pathways results in the tissue damage that keeps adding up over the years.

5.2 Proteinase-antiproteinase imbalance

Elastin and collagen is critical to the structural integrity of the lung. Experimental studies in animal lung models with instilled proteinases have replicated emphysematous changes(Gross et al.,1964 as cited in Shapiro SD,2010) resulting in an initial rapid increase in air space size due to direct elastolysis with diminution of lung elastin content at 24 hours followed by rapid restoration of total lung elastin to normal levels(Janoff et al.,1977;Snider et al.,1984 as cited in Shapiro SD,2010). The anatomic arrangement of the restored elastic fibers is grossly disordered (Kuhn & Senior,1978 as cited in Shapiro SD,2010). The tissue displays inflammation with neutrophils and macrophages with subsequent release of endogenous inflammatory mediators including IL-1β and TNF-α with endogenous proteolytic progression of the disease. Based on these studies and the association between A1PI deficiency with emphysema(Laurell & Eriksson,1963 as cited in Shapiro SD,2010), the proteinase-antiproteinase hypothesis proposed that the balance between matrix-degrading proteinases and their endogenous inhibitors determines whether the lung is protected or susceptible to proteolytic injury.

Some proteinases such as NE and MMP-9 are bound to the surface of the neutrophil, they are resistant to complete inhibition by A1PI and tissue inhibitors of metalloproteinases (TIMPs). "Microenvironmental" concentration of proteinases and their proximity to the target site of matrix proteins when released from the neutrophil and macrophages, may explain how the balance tilts towards the proteinase function even in presence of adequate overall anti-proteinase in circulation(Owen & Campbell,1999 as cited in Shapiro SD,2010). There are four classes of proteinases, serine, cysteine, aspartic, and MMPs:

5.2.1 Serine proteinases

The serine proteinase Neutrophil elastase(NE) is suspected to be the major causative agent of tissue injury in COPD after the findings that patients deficient in its endogenous

inhibitor, A1PI, are at increased risk for emphysema and that instillation of NE caused emphysema in experimental models. NE also plays a role in airway disease as a potent secretagogue, facilitator of monocyte transvascular migration. NE is produced mainly by neutrophils, but also to a small degree by monocytes(Shapiro et al.,2003 as cited in Shapiro SD,2010). Cathepsin G (CG) and proteinase 3 (PR3) are other neutrophil-monocyte derived serine proteinases. Other than NE, minor inhibitors are Alpha2 microglobulin, secretory leukoprotease inhibitor (SLPI) and elafin.

5.2.2 Matrix metalloproteinases (MMPs)

MMPs are a family of 24 enzymes that require coordination of zinc at the active site, have overlapping substrate specificity, and are inhibited by TIMPs(Parks & Shapiro,2001 as cited in Shapiro SD,2010). Several MMPs degrade elastin and contribute to emphysema including MMP-2, MMP-9 (gelatinase A and B), MMP-7 (matrilysin), and MMP-12 (macrophage elastase). MMP-1, -8, -13 are also collagenases, and thus degrade another critical matrix component.

Several MMPs have been associated with human COPD including MMP-1, MMP-9, MT1-MMP, and MMP-12(Imai et al.,2001;Molet et al.,2005;Russell et al.,2002 as cited in Shapiro SD,2010). Macrophages have the capacity to produce MMP-1, MMP-3, MMP-7, MMP-9, MMP-12, and MMP-19. MMP-12 is one of the most highly up-regulated genes in macrophages of human smokers(Atkinson & Senior,2003 as cited in Shapiro SD,2010). Role of MMPs in COPD pathogenesis is further supported by the epidemiologic prevalence of polymorphisms of MMP in caucasian COPD patients(Hunninghake et al.,2009 as cited in Shapiro SD,2010).

5.2.3 Cysteine proteinases

Cathepsin L, S, and K are macrophage generated elastolytic enzymes. Cathepsin S also processes antigens in T cells(Riese et al.,1998 as cited in Shapiro SD,2010). Cathepsin K is the most potent elastase and collagenase. Cathepsin B is an epithelial cell product that has proapoptotic properties (Foghsgaard et al.,2001 as cited in Shapiro SD,2010). Cathepsins are inhibited by cystatins like Cystatin C which is the most ubiquitous cystatin found in all human tissues and body fluids. Cathepsins are being studied for their potential to contribute to COPD.

5.3 Cell death

Cell viability requires cell-matrix attachment via integrins, loss of matrix disrupts the contact and predisposes to cell death (termed "anoikis"). Experimental models show that noninflammatory cell death can initiate air space enlargement as demonstrated in studies involving rodent models with inhibition of vascular endothelial growth factor receptor or instillation of active caspase 3 in lung epithelial cell tissues(Aoshiba et al.,2003;Kasahara & Tuder;2000,Tang et al.,2004 as cited in Shapiro SD,2010). Thus cell death by injury or apoptosis can be an initiating trigger for emphysema.

5.4 Disorganised and incomplete repair

Injury is followed by aberrant repair of alveolar cells and matrix resulting in coalesced and enlarged air spaces with depleted and disordered parenchymal elastic fibers and excessive,

abnormally arranged collagen. Although following pneumonectomy, there is compensatory lung growth of the remaining lung in humans, whether the injured lung can ever reinitiate the process of septation and the intricate juxtaposition of matrix, epithelial, and endothelial cells to form functional alveoli during lung development is highly doubtful(Buhain & Brody,1973;Nolen-Walston et al.,2008;Shifren & Mecham,2006 as cited in Shapiro SD,2010).

Elastin is the principal component of elastic fibers, which allows reversible extensibility and elastic recoil to the intercellular matrix of alveoli throughout the respiratory cycle.Elastin synthesis in the lung begins in the late neonatal period, peaks during early postnatal development, continues to slow through adolescence, and stops by adult life probably because of rapid mRNA degradation preventing expression of the protein.(Shapiro et al,1991;Swee et al,1995 as cited in Shapiro SD,2010).

Animal model studies involving intratracheal injection of elastase show an acute depletion of elastin followed by rapid ECM synthesis, although the lungs develop emphysema(Karlinsky et al.,1983;Kuhn & Senior,1978). The newly synthesized elastic fibers appear disorganized, similar to the elastic fibers in human emphysema and thus emphasizing the role of impaired repair in its pathogenesis. Collagen is the other important ECM fiber to play a role in COPD. Total collagen content in the lung is actually increased in humans with COPD(Wright & Churg,1995 as cited in Shapiro SD,2010). Following tissue injury, excessive collagen deposition around the larger coalesced airspaces is noted. Small airway fibrosis is also prominent in COPD.These findings suggest that emphysema is not purely a destructive process but one of aberrant matrix turnover.

5.5 Alpha-1 protease inhibitor (A1PI) deficiency

A1PI , also known as alpha$_1$-antitrypsin, is a serine proteinase inhibitor(serpin) that is produced mainly in the liver and found in the bloodstream and permeates tissues including the lung. A1PI inhibits various serpins including pancreatic trypsin, chymotrypsin but the main target is the neutrophil elastase(Brantly et al,1988;Travis,1989 as cited in Shapiro SD,2010). A1PI is also an acute phase reactant, with its serum concentration rising during pregnancy, during infections, after severe burns, and in the presence of malignant tumors. Smoking elevates the serum A1PI concentration by about 20%.

A1PI is coded by a single gene with two alleles on chromosome 14q32.1 producing a glycoprotein composed of 394 amino acids. The A1PI gene is highly pleomorphic and more than 75 alleles are known, and they have been classified into normal (normal serum levels and normally functioning A1PI), null (undetectable A1PI in the serum), deficient (serum A1PI levels lower than normal), and dysfunctional (A1PI levels are normal but does not function normally)(Brantly et al,1988 as cited in Shapiro SD,2010).

Most variants of A1PI arise point mutations with a single amino acid substitution. The Z variant(most common and severe disease) results from the substitution of a lysine for a glutamic acid at position 342, which changes the charge and the electrophoretic mobility of the molecule(Yoshida et al,1976 as cited in Shapiro SD,2010). The mutant protein polymerizes and the aggregated form causes hepatic cell injury., The Z protein is also incompletely glycosylated, which may interfere with the protein's excretion from the liver into body fluids. (Ekeowa et al,2009;Gooptu & Lomas,2008 as cited in Shapiro SD,2010). The protein looses its physiologic function of inhibiting the NE.

The normal (M) alleles are found in about 90% of persons of European descent with normal serum A1PI levels(150 to 350 mg/dL or 20 to 48 μmol/dL); their phenotype is designated Pi MM. More than 95% of persons in the severely deficient category are homozygous for the Z allele(Pi ZZ) and have serum A1PI levels of 2.5 to 7 μmol/dL (mean, 16% normal)with an estimated prevalence between 1 in 1600 to 1 in 4000.This allele is mostly found in whites of northern European descent.

Rarely observed phenotypes associated with low levels of serum A1PI include the following: Pi SZ and persons with nonexpressing alleles; Pi null, found in homozygous form as Pi null-null and found in heterozygous form with a deficient allele as Pi Z null. Persons with phenotype Pi SS have A1PI values ranging from 15 to 33 μmol/dL (mean, 52% of normal). The threshold protective level of 11 μmol/dL (35% of normal) is based on the knowledge that Pi SZ heterozygotes, with serum A1PI values of 8 to 19 μmol/dL (mean, 37% of normal), rarely develop emphysema. Pi MZ heterozygotes have serum A1PI levels that are intermediate between Pi MM normals and Pi ZZ homozygotes (12–35 μmol/dL; mean, 57% of normal). There appears to be a small increase in risk of COPD in all Pi MZ individuals.

COPD in homozygous A1PI deficiency patients is characterized by premature development of severe panacinar emphysema usually in the basilar regions of lung (Silverman & Sandhaus,2009). The onset of dyspnea occurs at a median age of 40 years about 1-2 decades earlier than rest of the population(Silverman & Sandhaus,2009 as cited in Shapiro SD,2010). However smoking has supradditive effect on poorer prognosis both with earlier onset severity and poorer prognosis of the disease(Janus et al,1985 as cited in Shapiro SD,2010). Radiographically, disease is more prominent in PiZZ patients and worse in basilar regions, sometimes hairline arcuate shadows separating markedly radiolucent areas in the lung bases from the less severely involved upper portions of the lungs (Gishen et al,1982;Hepper et al,1978 as cited in Shapiro SD,2010).

A1PI deficiency is diagnosed by measuring the serum A1PI level, followed by Pi typing for confirmation. However, by the time they develop COPD symptoms, they already have significant liver disease often diagnosed in infancy/childhood with hepatomegaly or hepatosplenomegaly and evidence of cholestasis and elevation of hepatocellular enzymes. Screening for PiZZ in COPD patients is not recommended at present. Augmentation therapy with A1PI supplementation has been proposed for COPD patients with PiZZ genotype as per guidelines issued by ATS(ATS,1995)

6. Natural history of disease

The natural history of COPD as we know, is based on multiple longitudinal studies, although most spanned much shorter duration than the actual length of disease progression(Rennard & Vestbo,2008 as cited in Shapiro SD,2010). The classic study of Fletcher and colleagues and extrapolation of the data of other studies have yielded the "Fletcher-Peto curve," which is basically a plot of FEV_1 versus age (Fletcher,1976). The curve describes the gradually progressive permanent loss of lung function as age advances. Although it doesn't include the concept of COPD in non-smokers and extrapulmonary effects of COPD, it serves a basic guide to understand the clinical course of the disease.

The natural history of COPD probably starts at pre-conception age related to genetics and intra-uterine lung development and growth, extending into early life events such as childhood and adolescent lung growth and injury from infections as well as later events such as adult lung exposures to cigarette smoke and occupational inhalants.

Since the disease progresses slowly over the years, the earlier stages of the disease are often "silent" and mostly unnoticed by the patient. Exertional dyspnea, the earliest symptom, primarily from dynamic hyperinflation from exercise induced tachypnea, results in subconscious preferential sedentary lifestyle and thus avoiding the symptoms till later stage.(O'Donnell et al.,2001 as cited in Shapiro SD,2010)

The intrauterine growth of lung includes development of conducting airways, gas exchange structures, including respiratory bronchioles and alveoli, but branching of alveolar wall continues postnatally for several years and usually complete by the first decade of life(Ten Have-Opbroek,1981 as cited in Shapiro SD,2010). Subsequent growth of the lung is due to increase in alveolar size and increase in airway diameter, but not in number. Maximal lung function is attained in young adulthood and remains relatively constant as a plateu for some years before declining in a slowly accelerating manner in older age(Weiss & Ware,1996 as cited in Shapiro SD,2010). The decline averages 20 mL/yr increasing in an accelerating manner and by age 50, there is an average drop of FEV1 by 1L.

Smoking adversely affects the entire course, with interference in maximal lung capacity attainment if smoking starts in the early growth phase, to shortening of duration of the plateu phase, to rapid decline in lung function in later age(Burrows,1990 as cited in Shapiro SD,2010). This effect is very well depicted in the "Fletcher-Peto curve" shifting the plot downwards and earlier in age (Fletcher, 1976). The average COPD patient who smokes loses almost twice the lung function than usual(about 2 L of FEV1 over 50 years, an average decline of about 40 mL/yr). Acute exacerbations have descending step-ladder like effect with acute drops over short period with incomplete recovery resulting in faster drop of lung function(Burrows,1990 as cited in Shapiro SD,2010). Smoking has a predictable dose-dependent deleterious effect on the lung function and cessation of smoking has beneficial slowing of disease progression if initiated early enough in course of disease (Anthonisen et al,1994;Buist et al.,1976 as cited in Shapiro SD,2010).

Some individuals experience a rapid decline in lung function (Gottlieb et al.,1996 as cited in Shapiro SD,2010). Faster decline in lung function is noted in patients with low baseline lung function, less reversibility to β_2-agonists, more severe bronchial hyperresponsiveness, mucus production, male sex, and frequent exacerbations(ATS,2010). Identification of slow and rapid decliners in longitudinal studies such as the Lung Health Study has allowed exploration of biomarkers to characterize these groups. Importantly, systemic markers of inflammation have been associated with poorer lung function, and, in some, studies, with an increased rate of decline in lung function (Fogarty et al.,2007;Shaaban et al.,2006; Sin & Man,2003 as cited in Shapiro SD,2010).

Although early stage COPD is difficult to diagnose, newer studies have shown a poorer prognosis among these population primarily from adverse cardiac events (Ashley et al., 1975; Mannino et al., 2003 as cited in Shapiro SD,2010). The cardiac events may be linked to the extrapulmonary effects of COPD, especially elevated systemic inflammatory mediators. Identifying and treating this group thus can have valuable prognostic benefit.

With advanced disease, obvious exertional dyspnea, cough and frequent acute exacerbations dominate the picture. Morbidity and mortality increases with declining FEV1. Primary cause of mortality is cardiac events, however with advanced age and disease, pulmonary complications causing death, increase in proportion. Each exacerbation and the following recovery stage makes the patient most vulnerable to adverse outcomes as shown by the SUPPORT (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments), which demonstrated a 49% 2-year mortality rate after hospital admissions with COPD exacerbation with CO_2 retention(Connors et al.,1996).

Various therapeutic and supportive medical and surgical intervention combined with rehabilitative measures help in alleviation of symptoms, slowing of pace of progression of disease and reduction of disability. Individualized plans for each patient based on the characteristic and stage of disease is important to achieve optimal results.

7. Clinical presentation

7.1 History

COPD is a gradually progressive chronic disease presenting with clinically obvious symptoms late in the course, usually in their fifth decade of life with productive cough or breathlessness or acute chest illness. A1PI-deficient patients present earlier than other COPD patients usually in 3rd-4th decades and by then they have significant liver disease, which usually starts in childhood.

Early COPD results in gradual progressive worsening of pulmonary function, which results in patients unknowingly avoiding exertional dyspnea (the most common early symptom of COPD) and fatigue by shifting their expectations and limiting their activity. Patients who have an extremely sedentary lifestyle but few complaints require further evaluation for possibility of underlying COPD as many patients reset their expectations with regard to health, termed "response shift"(Rennard et al,2002 as cited in Shapiro SD,2010). Generalised muscle weakness found in COPD patients can also contribute to this finding.

Most patients usually present in the fifth or sixth decade of life by when they have dyspnea with mild exertion and usually the forced expiratory volume in 1 second (FEV1) has fallen to 50% of predicted. Moderate to severe COPD patients report variability in symptoms over the course of the day or week-to-week; morning is typically the worst time of day. Dyspnea is related to both respiratory (hyperinflation and impaired gas exchange) and extra-respiratory (like muscle dysfunction, heart disease, anaemia and depression) features of COPD.

The chronic cough is characterized by the insidious onset of sputum production, which occurs in the morning initially, but may progress to occur throughout the day. The sputum is usually mucoid, but becomes purulent during exacerbations. Hemoptysis complicating chronic bronchitis usually occurs in association with acute exacerbation. Lung cancer and tuberculosis needs to be ruled out in this scenario(Thompson et al,1992 as cited in Shapiro SD,2010). Wheezing may also be found in some patients due to co-existence of asthma or COPD alone.

Acute exacerbations are characterized by increased cough, sputum, dyspnea, and fatigue, are increasingly frequent as the disease worsens. Each exacerbation may last for a few weeks

and followed by prolonged recovery over months and may be difficult to distinguish from other causes of dypsnea, cough, and/or sputum including pneumonia, congestive heart failure, pulmonary embolism, or pneumothorax(Spencer & Jones,2003 as cited in Shapiro SD,2010).

A history of cigarette smoking or alternative inhalational exposure is usually found in majority of COPD patients. A1PI deficient patients may develop disease without smoking, however presence of smoking significantly worsens the course of disease. Some patients develop COPD without an obvious risk factor. Other historical features that may accompany COPD include certain comorbidities (eg, lung cancer, coronary artery disease, osteoporosis, depression, skeletal muscle weakness). Although most patients are usually obese, weight loss can also occur in COPD and is associated with a worse prognosis.

7.2 Physical findings

Physical findings in early COPD is highly non-specific and unreliable. Early stage patients may have coarse crackles and rhonchi. Wheezing may be found occasionally especially associated with asthma or acute exacerbations.

The hallmark finding is obstruction of expiratory airflow. Measurement of the forced expiratory time maneuver is a simple bedside test and most consistent finding in symptomatic COPD. A forced expiratory time greater than 4 seconds indicates severe expiratory airflow obstruction. Objective measurement of airflow by spirometry, which is simple and accurate forms the basis of staging and follow-up of disease progression(Petty,2001).

As the airway obstruction worsens, physical examination may reveal hyperinflation, decreased breath sounds, wheezes, crackles at the lung bases, and/or distant heart sounds. In addition, the diaphragm may be depressed and limited in its motion, and the anteroposterior diameter of the chest may be increased.

Patients with end-stage COPD may present with barrel-shaped chest, increased span of hyperresonant lung percussion, distended neck veins, full use of the accessory respiratory muscles of the neck and shoulder girdle, purse-lipped breathing, paradoxical retraction of the lower interspaces during inspiration (ie, Hoover's sign), emaciation, and frequently, inguinal hernias. They may adopt positions that relieve dyspnea, such as leaning forward with arms outstretched and weight supported on the palms(Tripod sign). This position stabilizes the shoulder girdle and helps to maximize intrathoracic volume. Late signs may include cyanosis, clubbing, asterixis due to severe hypercapnia, and an enlarged, tender liver due to right heart failure.

8. Complications of COPD

8.1 Pneumothorax

Pneumothorax can precipitate severe dyspnea and acute respiratory failure and may be life threatening since they have only a marginal pulmonary reserve. Presence of giant bullae as part of disease predisposes to this complication. It can be difficult to treat if accompanied by a persistent air leak between the involved lung and the pleural space (bronchopleural fistula).

8.2 Pulmonary hypertension and Cor pulmonale

Both resting and exercise mean pulmonary arterial pressures may be elevated. Prolonged pulmonary hypertension can give rise to chronic cor pulmonale in late stages. Alveolar hypoxia, respiratory acidosis, remodeling of the pulmonary vasculature with medial hypertrophy of muscular pulmonary arteries, increased viscosity of blood due to erythrocytosis, increased blood volume, left ventricular dysfunction and chronic pulmonary thromboembolic disease can all contribute to the pulmonary hypertension(Farber et al.,1982;Fletcher et al,1989 as cited in Shapiro SD,2010). Correction of hypoxia and acidosis by long-term oxygen therapy and pulmonary vasodilators may slow this process.

8.3 Pneumonia

COPD predisposes the lungs to pneumonia as part of acute exacerbation or as discrete event. (Ewing & Torres,1999;Griffith & Mazurek,1991 as cited in Shapiro SD,2010)

8.4 Systemic complications and co-morbidities

Ischemic cardiac disease is more common in COPD and cardiac events are the single largest cause of mortality in this population (Ashley et al.,1975;Mannino et al,2003 as cited in Shapiro SD,2010). Arrhythmia, congestive heart failure and aortic aneurysm are more common. COPD may lead to a hypercoagulable state due to erythrocytosis and systemic inflammation(mediated via TNF-α, IL-6) posing greater risk of stroke, pulmonary embolism and deep vein thrombosis(Bhowmik et al,2000;Wouters et al.,2002 as cited in Shapiro SD,2010). Weight loss, osteoporosis, skin wrinkling, anemia, fluid retention and depression are some of the other systemic co-morbidities commonly associated with COPD. Major chronic diseases (e.g. congestive heart failure, dementia, ischaemic heart disease, stroke, diabetes, cancer, asthma, COPD, depression and hypertension) were associated with at least one of the other diseases in 60–90% of cases(Charlson et al.,2007 as cited in Shapiro SD,2010). A major question is whether coexisting chronic illnesses found in COPD subjects are merely related to common risk factors (e.g. aging, tobacco smoking and genetic predisposition) or are also consequences, at least in part, of the pulmonary and/or systemic inflammation that characterise COPD.

9. Diagnosis and laboratory work-up

9.1 Spirometry

Objective measurement of airflow obstruction is the mainstay of workup for diagnosis, staging and follow-up of COPD(Petty,2001). The most important values measured are the forced expiratory volume in one second (FEV1) and the forced vital capacity (FVC) or the forced expiratory volume after 6 seconds, (FEV_6), which is the recommended substitute for FVC(Enright et al.,2002 as cited in Shapiro SD,2010). COPD is confirmed when a patient, who has symptoms that are compatible with COPD, is found to have airflow obstruction (FEV1/FVC ratio less than 0.70 and an FEV1 less than 80 percent of predicted) and there is no alternative explanation for the symptoms and airflow obstruction (eg, bronchiectasis, vocal cord paralysis, tracheal stenosis). If airflow is abnormal, postbronchodilator testing should be performed. Correction of airflow to the normal range suggests a diagnosis of

asthma and could exclude COPD. Because of variability in the FVC (or FEV_6) measure, the FEV_1/FVC ratio can establish a diagnosis of obstruction but is not useful to monitor disease progression(GOLD,2006). FEV_1/FVC ratio is the basis for GOLD staging of COPD(see Table 2).

Stage	FEV1*/FVC**(in %)	FEV1*(in % of predicted)
I: Mild COPD	<70 %	≥80 %
II: Moderate COPD	<70 %	50 % to <80%
III: Severe COPD	<70 %	30% to <50%
IV: Very Severe COPD	<70 %	<30% or <50% with chronic respiratory failure***

*FEV1: forced expiratory volume in one second; **FVC: forced vital capacity; ***Chronic respiratory failure: arterial partial pressure of oxygen (PaO2) less than 60 mm Hg (8.0 kPa) with or without arterial partial pressure of CO2 (PaCO2) greater than 50 mm Hg (6.7 kPa) while breathing air at sea level.

Table 2. Staging of severity of COPD (GOLD,2006)

Other spirometric findings include decreased inspiratory capacity and vital capacity, accompanied by increased total lung capacity, functional residual capacity, and residual volume are indicative of hyperinflation. The single breath carbon monoxide diffusing capacity (DLCO) decreases in proportion to the severity of emphysema because of the destruction of the alveoli and the loss of alveolar capillary bed.(Bates,1989)

9.2 Arterial blood gas

Arterial blood gases reveal mild or moderate hypoxemia without hypercapnia in the early stages of COPD. In the later stages of the disease, hypoxemia tends to become more severe and may be accompanied by hypercapnia with increased serum bicarbonate levels(Bates,1989). The changes in ABG represent ventilation perfusion mismatch, which may be worsened during exercise, sleep and episodes of exacerbation.

9.3 Alpha1-antitrypsin level

Of the approximately 75 different alleles for alpha1-antitrypsin (AAT) deficiency variants, 10-15 are associated with serum levels below the protective threshold of 11 μmol/dL. The most common severe variant is the Z allele, which accounts for 95% of the clinically recognized cases of severe AAT deficiency. The diagnosis of severe AAT deficiency is confirmed when the serum level falls below the protective threshold value (ie, 3-7 μmol/dL). Specific phenotyping is reserved for patients in whom serum levels are 7-11 μmol/dL or when genetic counseling or family analysis is needed.

9.4 Sputum evaluation

In patients with stable chronic bronchitis, the sputum is mucoid and the predominant cells are macrophages(Miravitlles,2002;Sethi et al.,2002 as cited in Shapiro SD,2010). With an exacerbation, the sputum becomes purulent, with excessive neutrophils and a mixture of organisms visualized through Gram staining. *Streptococcus pneumoniae* and *Haemophilus influenzae* are pathogens frequently cultured during exacerbations.

9.5 Imaging studies

Chest radiographs and CT-scan of chest are the mainstay of COPD imaging. Although not contributing to diagnosis of COPD, they may add valuable information regarding severity, stage and special findings during the course of disease.

9.5.1 Chest X-ray

Radiographic features suggestive of COPD are prominent usually in advanced disease and include:

i. Signs of hyperinflation: Prominent hilar vascular shadows and encroachment of the heart shadow on the retrosternal space, increased radiolucency of the lung, a flat diaphragm, and a long and narrow heart shadow on a frontal radiograph, accompanied by a flat diaphragmatic contour may be seen.
ii. Bullae, defined as radiolucent areas larger than one centimeter in diameter and surrounded by arcuate hairline shadows. They are due to locally severe disease.
iii. Rapidly tapering vascular shadows and cardiac enlargement may become evident only on comparison with previous chest radiographs. These findings are due to pulmonary hypertension and cor pulmonale, which can be secondary to COPD.

9.5.2 Computed tomography

High-resolution CT (HRCT) scanning is more sensitive than standard chest radiography and is highly specific for diagnosing emphysema and outlines bullae that are not always observed on radiographs. CT can visualise whether the emphysema is centriacinar or panacinar(Hasegawa et al.,2006;Nishino et al.,2010;Washko et al.,2008 as cited in Shapiro SD,2010). A CT scan is not indicated in the routine care of patients with COPD but is helpful when the patient is being considered for a surgical intervention such as bullectomy or lung-volume reduction surgery(Fishman et al,2003 as cited in Shapiro SD,2010).

10. Stage of disease severity and progression

The GOLD staging system is based on the FEV_1/FVC ratio(see Table 2). It has been criticized for underestimating the importance of the extrapulmonary manifestations of COPD in predicting outcome(GOLD,2006;Bourdin et al,2009). The BODE index addresses this criticism. The four factors included in the BODE index are weight (BMI), airway obstruction (FEV1), dyspnea (Medical Research Council dyspnea score), and exercise capacity (six-minute walk distance)(see Table 3). This index provides better prognostic information than the FEV1 alone to assess an individual's risk of death or hospitalization due to COPD. However, it is not used to guide therapy.

A component of disease assessment that is used in research studies is to evaluate the impact of airflow limitation on quality of life. The St. George's Respiratory Questionnaire (SGRQ) is a 76 item questionnaire that includes three component scores (ie, symptoms, activity, and impact on daily life) and a total score(Jones et al.,1991). It has been validated in patients with COPD, asthma, and bronchiectasis. Another questionnaire based instrument to assess quality of life is the Chronic Respiratory Disease Questionnaire (CRDQ) (Guyatt et al.,1987).

Parameter	0 Points	1 Point	2 Points	3 Points
Body: BMI	>21	≤21	—	—
Obstruction: FEV1 (% predicted)	≥65%	50–64%	36–49%	≤35%
Dyspnea: MMRC score	0-1	2	3	4
Exercise: 6-minute walk distance (meters)	≥350	250–349	150–249	≤149

BMI, body mass index; FEV_1, forced expiratory volume in 1 second.

Table 3. BODE Index for Staging COPD (Celli et al.,2004)

11. Treatment

Treatment of COPD encompasses health promotion, prevention, control of symptoms and exacerbations, rehabilitation and palliation. Treatment plan needs to be individualized according to the stage and characteristics of the disease, age, co-morbidities in each patient. Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommends that pharmacologic and nonpharmacologic therapies should be added in a stepwise fashion to control symptoms, decrease exacerbations, and improve patient function and quality of life (GOLD,2006). Patient should be educated about the disease and should be encouraged to participate actively in therapy and understand the need of proper dosing and timing of medications as well as proper inhaler technique is essential.

ATS Statement (1995) recommended symptomatic management after the patient presented to the healthcare system with specific complaints(ATS,1995). However, new evidence suggests that the "pre-symptomatic" phase individuals progressively loose lung function in these years and also have poorer prognosis in terms of cardiac outcomes and hence earlier and more aggressive diagnosis and appropriate treatment of these previously unidentified individuals can help, not only by slowing progression but also by improving symptomatic control(GOLD,2006).

Mainstays of drug therapy of stable COPD are bronchodilators, primarily beta agonists and anticholinergics, and inhaled glucocorticoids, given alone or in combination depending upon the severity of disease and response to therapy. Attention to co-morbidities like heart disease, depression, osteoporosis and rehabilitation for acceptable quality of life is also important. Reduction of risk factors like cigarette smoking and occupational exposure should be a central feature of every comprehensive treatment plan. The only medical therapies that clearly reduce disease progression and mortality are smoking cessation and supplemental oxygen (NOTT,1980).

11.1 Cessation of cigarette smoking

Smoking cessation is the single most effective therapy for the majority of COPD patients(Anthonisen et al,1994;Department of Health and Human Services(US),2008). The transition from smoking to nonsmoking status involves following five stages: precontemplation, contemplation, preparation, action, and maintenance. Smoking intervention programs include self-help, group, physician-delivered, workplace, and community programs. Setting a target date to quit may be helpful. Physicians and other

health care providers should participate in setting the target date and should follow up with respect to maintenance. Successful cessation programs should include patient education, target date to quit, follow-up support, relapse prevention, advice for healthy lifestyle changes, social support systems, pharmacological agents.

According to the US Preventive Services Task Force(USPSTF) guidelines, recommends "5-A" approach to counseling that includes i)Ask about tobacco use, ii)Advise to quit through personalized messages, iii)Assess willingness to quit, iv)Assist with quitting, v)Arrange follow-up care and support. Behavioral counseling and pharmacotherapy are most effective when used together.(USPSTF,2009)

Supervised use of pharmacologic agents is an important adjunct as withdrawal from nicotine may cause unpleasant adverse effects during the first weeks after quitting smoking. Nicotine replacement therapies are available in the form of chewing gum and transdermal patches to counter the withdrawal symptoms(U.S. Public Health Service Clinical Practice Guideline,2008). Long-term success rates have been 22-42%, compared with 2-25% with placebos. The use of an antidepressant medication, bupropion (Zyban,150 mg bid) has been shown to be effective for smoking cessation and may be used in combination with nicotine replacement therapy. Varenicline (Chantix), is a partial agonist selective for α4, β2 nicotinic acetylcholine receptors and action is thought to result from partial agonist activity at a nicotinic receptor subtype while simultaneously preventing nicotine binding. Nortriptyline and clonidine have also been proposed to help in cessation of smoking (U.S. Public Health Service Clinical Practice Guideline,2008).

11.2 Pharmacologic treatment

The U.S. Food and Drug Adminstration (FDA) recommends five treatment end points be considered for COPD: improvement in airflow obstruction, providing symptom relief, modifying or preventing exacerbations, altering disease progression (including mortality), and modifying lung structure. Effective treatment of the COPD patient requires effective integration of pharmacologic treatment and nonpharmacologic therapy, most importantly pulmonary rehabilitation.

11.2.1 Bronchodilators

Bronchodilators are the mainstay of any COPD treatment plan. The mechanism of action is primarily by dilating airways and thereby decreasing airflow resistance increasing airflow and decreasing dynamic hyperinflation which is the origin of early stage symptoms. Many patients with COPD will have reduced dyspnea and improved exercise tolerance with bronchodilator therapy, even if improvement in resting spirometry is very modest(O'Donnell,2000 as cited in Shapiro SD,2010). Unlike asthma, COPD patients mostly need bronchodilators both on a chronic basis as well as for "rescue". All symptomatic patients with COPD should be prescribed a short-acting bronchodilator for as-needed basis and a regularly scheduled long-acting bronchodilator should be added if symptoms are inadequately controlled. Bronchodilators include beta agonists, anticholinergics, and theophylline, which is used less often.

The initial choice of agent remains debated. Historically, β2 agonists were considered first line and anticholinergics added as adjuncts. Studies have shown combination therapy

results in greater bronchodilator response and provides greater relief. The degree of bronchodilation achieved by short-acting beta agonists and anticholinergics is additive. The adverse effect profile may help guide therapy.

i. Beta2(β2)-agonists:

This group of medications bind to the β-adrenergic receptor present on airway smooth muscle, resulting in bronchodilation and improvement in airflow. They may also help by increasing ciliary beating frequency and improving mucus transport and may improve endurance of fatigued respiratory muscles(Nava et al,1992;Santa Cruz et al,1974 as cited in Shapiro SD,2010).

Beta agonists are available in short-acting and long-acting inhaled formulations. The short-acting-β agonists(SABA) like albuterol, its racemer levalbuterol, pirbuterol and terbutaline, have a relatively rapid onset of action after inhalation, in about 5 to 15 minutes, and the bronchodilation lasts for 2 to 4 hours. Long-acting β-agonists (LABAs) like salmeterol, formoterol, arformoterol and indacaterol have a longer onset and bronchodilation lasting for up to 12 hours or more. Salmeterol has also shown anti-inflammatory effects, to reduce edema, and to reduce airway epithelial cell injury in model systems.

Inhaled route is preferable owing to more favorable ratio of therapeutic effect to undesirable side effects(Shim & Williams,1983 as cited in Shapiro SD,2010). A metered dose inhaler (MDI), dry powder inhaler (DPI) is the preferred mode to deliver a bronchodilator medication by inhalation as it simplifies therapy, improves compliance, and may reduce extra medication usage and patient cost. Nebulizers may be more effective in patients too weak to use an inhaler device, in those with altered mental status, or in those whose inspiratory capacity is too limited to permit effective inhalation (Tenholder et al,1992 as cited in Shapiro SD,2010).

Benefits of treatment include improvement in airflow obstruction and symptom relief. Although the magnitude of improvement is less and incomplete as compared to asthma patients, 25-30% patients achieve "positive bronchodilator response" as defined by the ATS. Improvement in FEV1(about 200- to 300-mL) and symptoms assessed by SGRQ have been elicited in multiple randomized placebo-controlled trials(Appleton et al,2006;Calverley et al,2003;Rodrigo et al,2008 as cited in Shapiro SD,2010). Modest benefit is noted in prevention of exacerbations with LABAs to the tune of 20-30% reduction in frequency of exacerbations(Appleton et al,2006;Sin et al,2003 as cited in Shapiro SD,2010). However, they have no effect on disease progression and alteration of lung structure(Calverley et al.,2007 as cited in Shapiro SD,2010).

Side-effects commonly include tremor, palpitations, anxiety, and insomnia. Ventricular arrhythmias and hypokalemia may also occur. These effects are dependent on systemic absorption and hence, spacer devices, DPIs,MDIs are preferable. R-enantiomer of albuterol, levalbuterol was promoted widely based on the possibility to have lesser side effects such as tachycardia and tremors as well as lacking the inflammatory effect of the S-enantiomer. The small difference noted in studies has raised doubts of its clinical relevance(Donahue et al,2008 as cited in Shapiro SD,2010).

A significant proportion of COPD patients have concurrent cardiac co-morbidities and although recent studies have failed to show any clinically significant adverse outcome of β2

agonists, caution is warranted.(Cazzola et al,1998;Anthonisen et al,2002 as cited in Shapiro SD,2010)

ii. Anti-cholinergics:

Anticholinergic agents block M2 and M3 cholinergic receptors and result in bronchodilation (Rennard,2000 as cited in Shapiro SD,2010). In airway smooth muscle cells, acetylcholine stimulates the production of neutrophil chemotactic activity and anticholinergics could, theoretically, have anti-inflammatory action (Koyama et al,1992;Wessler & Kirkpatrick,2001 as cited in Shapiro SD,2010).

Short-acting anticholinergic agents like ipratropium and oxitropium improve lung function and symptoms. In double-blinded studies, ipratropium improved lung function, increased exercise capacity, decreased dyspnea, and decreased cough when compared to placebo. Ipratropium produces bronchodilation in 10 to 15 minutes and lasts for 4 to 6 hours.

Tiotropium is a longer-acting anticholinergic because it dissociates from the receptor extremely slowly achieving peak bronchodilator activity after 1 to 2 hours, but duration of action lasts long enough for once daily dosing. When administered chronically, the bronchodilator effect of tiotropium increases with daily dosing and is maximal after 1 week (Hansel & Barnes,2002;Littner et al,2000 as cited in Shapiro SD,2010). It is relatively selective for M3 receptor, and this may have better clinical efficacy as it doesn't alter the M2 mediated feedback inhibition of acetylcholine (On et al,2001 as cited in Shapiro SD,2010). The Understanding Potential Long-Term Impacts on Function with Tiotropium (UPLIFT) trial studied the effects of use over a 4-year period and showed improvements in lung function, quality of life, and exacerbations but did not show a decrease in the rate of decline of lung function (Tashkin et al.,2008).

Benefits of treatment are similar to beta-agonist agents including improvement in airflow obstruction and symptom relief. Tiotropium improves airflow and lung volumes, reduces dyspnea, and improves health status and exercise performance(Tashkin et al,2008). In combination with albuterol tiotropium has been reported to reduce risk of COPD exacerbation to the magnitude of the risk reduction by 20% to 25%(Niewoehner et al,2005;Sin et al,2003 as cited in Shapiro SD,2010). However, as with other bronchodilators, anticholinergics have no effect on disease progression and alteration of lung structure. Reported adverse effects include dry mouth, metallic taste, and prostatic symptoms. Equivocal data exist regarding possible increased adverse cardiac events with chronic use of anti-cholinergic agents.

iii. Theophylline:

Theophylline is the only methylxanthine currently used to treat COPD patients. It has modest bronchodilator activity, but it also has additional potentially beneficial effects including anti-inflammatory, modest inotropic and diuretic effects, and may also augment skeletal muscle strength(Barnes,2003;Culpitt et al,2002 as cited in Shapiro SD,2010). Dose-related adverse effects of theophylline include nausea and vomiting, seizures, and arrhythmias. Its use is also complicated by many drug interactions and concurrent morbidity that affecting liver and cardiac function can alter theophylline levels. The therapeutic index of theophylline is narrow and desired serum levels are 8 to 12 μg/mL. Dose-related adverse effects of theophylline include nausea and vomiting, seizures, and

arrhythmias.Hence, use of theophylline is usually limited to an add-on therapy when symptoms continue in patients with more severe disease despite the use of other treatments.

11.2.2 Choice of bronchodilator and combination therapy

Historically, β2 agonists were considered first line and anticholinergics added as adjuncts. Most patients in GOLD stage I disease will have acceptable relief of symptoms with one short acting bronchodilator used on as needed basis. Combination therapy with short and long acting β2-agonists and anticholinergics is supported by trials indicating greater bronchodilator response and achieving better symptom relief(Cazzola et al,2004;CIAS Group,1994;VanNoord et al.,2000 as cited in Shapiro SD,2010). In patients with GOLD stage II and above, whose symptoms are not well-controlled with a single long-acting bronchodilator, the combination of both an anticholinergic and a β2-agonist long-acting bronchodilator may provide better symptom relief and improve quality of life index(ATS/ERS Task Force,2004;GOLD,2006).

SABA, LABA, anticholinergics have been tried in various combinations and data suggests improved FEV1 and symptom control with combinations than either agent alone. The choice and order of agents can be guided by response, side-effects and co-morbidity profile (ATS/ERS Task Force, 2004; GOLD, 2006). Although clinical responses among individual patients may vary, poor compliance and ineffective use of the device must be considered before changing medications.

11.2.3 Anti-inflammatory medications

COPD is characterized by both airway and systemic inflammation as discussed in the pathogenesis and the primary reason of disease progression. Bronchodilators achieve temporary symptom control but have failed to show any effect on the underlying inflammation. Corticosteroids are by far the leaders of this class of medication, and some newer phosphodiesterases have shown promise.

i. Corticosteroids:

Inhaled glucocorticoids decrease frequency of exacerbations and modestly slow the progression of respiratory symptoms, but appear to have little impact on lung function and mortality. Because of their lack of effect on bronchodilation, inhaled glucocorticoids can be used only as part of a combined regimen, but are not as sole therapy.

Benefits from therapy include reduction in the frequency and severity of exacerbations of COPD by 25-30%, comparable to LABAs(Calverley et al.,2003,2007;Szafranski et al,2003 as cited in Shapiro SD,2010). Effect on improvement of airflow obstruction and symptom relief is minimal, although some additive benefit is reported for combination therapy with LABAs(Burge et al,2000;Pauwels et al,1999 as cited in Shapiro SD,2010). Though the anti-inflammatory effect promises possible alteration of disease progression and slowing of decline in FEV1, studies have failed to show any such benefit(Highland et al,2003;Soriano et al,2007;Sutherland et al.,2003 as cited in Shapiro SD,2010). Some studies have reported equivocal reduction in hospital admission and mortality rates(Sin & Tu,2001;Soriano et al,2002 as cited in Shapiro SD,2010). No study has proven any effect on lung structure remodelling.

Inhaled corticosteroids are only minimally absorbed and therefore systemic adverse effects are limited. Local effects include oral candidiasis and dysphonia(Pauwels et al,1999 as cited in Shapiro SD,2010). Systemic effects include increased bruising and reduced bone density, and possible susceptibility for pneumonia(Calverley et al.,2007 as cited in Shapiro SD,2010). Appropriate caution and monitoring is recommended although the clinical importance of these effects remains uncertain.

Systemic steroids have been widely used in the treatment of acute exacerbation of COPD. A meta-analysis concluded that systemic corticosteroids significantly reduced treatment failure and need for additional medical treatment and increased the rate of improvement in lung function and dyspnea over the first 72 hours(Rice et al,2000 as cited in Shapiro SD,2010). The use of oral steroids in persons with chronic stable COPD is not recommended given the adverse effect profile, which includes hypertension, glucose intolerance, osteoporosis, fractures, and cataracts, among others(Burge et al,2003 as cited in Shapiro SD,2010).

Inhaled glucocorticoids are typically used in combination with a long-acting bronchodilator for patients in GOLD stage III-IV, who have significant symptoms or repeated exacerbations, despite an optimal bronchodilator regimen. Steroids may be introduced earlier if there are signs of inflammation or an asthmatic component to the COPD(Ferguson et al.,2008 as cited in Shapiro SD,2010). In the TORCH (Toward a Revolution in COPD Health) trial involving patients with moderate to severe COPD, salmeterol plus fluticasone significantly improved the lung function, health status, and the rate of exacerbations compared to placebo, salmeterol alone, or fluticasone alone(Calverley et al.,2007). It also minimally decreased mortality compared to placebo (10.3 versus 12.6 percent, hazard ratio 0.81, 95% CI, 0.67-0.98). The Investigating New Standards for Prophylaxis in Reduction of Exacerbations (INSPIRE) trial included 1323 patients with stable, mostly severe COPD and results failed to show significant benefits of combining inhaled steroids with LABAs(Wedzicha et al.,2008).

"Triple inhaler therapy" - with a long-acting beta agonist plus an inhaled glucocorticoid plus a long-acting anticholinergic is often used in refractory COPD patients. This approach is supported by some studies(Tashkin et al.,2008). These data are insufficient to warrant a change in the current guidelines in which the first step is initiation of a long-acting bronchodilator alone and then if response is inadequate or disease advances, to introduce a combination of long-acting beta agonist plus an inhaled glucocorticoid.

ii. Phosphodiesterase inhibitors :

Phosphodiesterase-4 (PDE-4) inhibition decreases inflammation and promotes airway smooth muscle relaxation. Cilomilast and roflumilast are highly specific, oral, second-generation PDE-4 inhibitors being considered for use in patients with asthma and COPD(Gamble et al.,2003;Profita et al.,2003 as cited in Shapiro SD,2010). Recent trials have supported their inclusion in COPD combination treatment plans(Calverly et al.,2009;Chong et al.,2011as cited in Shapiro SD,2010). Several randomized, double-blind, placebo-controlled multicenter trials revealed increased FEV1 ($P < .0001$) and the rate of COPD exacerbations was reduced by 17% ($P < .0003$) in patients who received roflumilast compared with placebo. Additional studies are necessary before PDE-4 inhibitors can be recommended for routine use in patients with stable COPD.

iii. Cromolyn Sodium Nedocromil and Leukotriene Antagonists :

There are no supportive data advocating a beneficial role for cromolyn, nedocromil, or cysteinyl leukotriene antagonists in treating COPD(DeJong et al,1994 as cited in Shapiro SD,2010).

11.2.4 Adjuvant pharmacologic agents

i. Augmentation therapy for A1PI deficiency:

The treatment strategies for A1PI deficiency involve reducing the neutrophil elastase burden, primarily by smoking cessation, and augmenting the levels of A1PI. Available augmentation strategies include pharmacologic attempts to increase endogenous production of A1PI by the liver (ie, danazol, tamoxifen) or administration of purified A1PI by periodic intravenous infusion or by inhalation. Tamoxifen can increase endogenous production of A1PI to a limited extent, so this may be beneficial in persons with the PISZ phenotype.

Intravenous augmentation therapy is the only available approach that can increase serum levels to greater than 11 mmol/L, the protective threshold. Studies show that the infusions can maintain levels of more than 11 mmol/L, and replacement is administered weekly (60 mg/kg), biweekly (120 mg/kg), or monthly (250 mg/kg) (Buist et al,1989;Sandhaus,2009 as cited in Shapiro SD,2010). Uncontrolled observations of patients suggest that the FEV1 may fall at a slower rate in patients who receive A1PI replacement. It seems reasonable to weigh carefully the advantages and disadvantages of augmentation therapy and to reach a decision jointly with elderly persons or with those with severe lung function impairment (FEV1 values < 0.8 L)(Buist et al,1989 as cited in Shapiro SD,2010).

ii. Mucoactive and expectorant agents:

Mucolytic agents like acetylcysteine, dornase(DNAse), guaifenesin reduce sputum viscosity and improve secretion clearance. however, studies have failed to justify a role for these medications in management of COPD(Decramer et al,2005 as cited in Shapiro SD,2010). The role of oral expectorants like guaifenesin in promoting mucous clearance in COPD patients remains controversial.

iii. Antibiotic therapy:

Chronic infection or colonization of the lower airways with *S pneumoniae, H influenzae,* and/or *Moraxella catarrhalis* is common and in later stage disease, with Gram-negative organisms such as *Pseudomonas*. Macrolides like erythromycin, may have additional antiinflammatory effects. Patients whose COPD is associated with, bronchiectasis may benefit from chronic antibiotic therapy. However, at present, chronic antibiotics are not recommended for stable COPD management.

The use of antibiotics for the treatment of acute COPD exacerbations and pneumonias is well supported (Adams et al.,2008). The patients who benefited most from antibiotic therapy were those with exacerbations that were characterized by at least 2 of the following: increases in dyspnea, sputum production, and sputum purulence (The Winnipeg criteria).

iv. Vaccine prophylaxis:

Infection is a common cause of COPD exacerbation and vaccination is the most effective way of prophylaxis. Pneumococcal polysaccharide vaccine should be offered to patients

with COPD who are ≥65 years old, or who are younger than 65 years with a forced expiratory volume in one second (FEV1) less than 40 percent. An annual influenza vaccine should be given to all patients with COPD.

11.2.5 Supportive management

i. Oxygen therapy:

Chronic hypoxemia may develop in patients with severe stable COPD (GOLD stage IV). Two landmark trials, the British Medical Research Council (MRC) study and the National Heart, Lung, Blood Institute's Nocturnal Oxygen Therapy Trial (NOTT) showed that long-term oxygen therapy improves survival by 2-fold or more in hypoxemic patients with COPD(Kvalle,1980;Medical Research Council Working Party,1981). Improved quality of life is also achieved likely due to reduced dyspnea during exercise, which improves performance of activities of daily living. Other benefits include reduction in hematocrit, modest neuropsychological improvement, and some improvement in pulmonary hemodynamics(Heaton et al,1983;Kvalle,1980;Timms et al,1985 as cited in Shapiro SD,2010).

Hypoxemia which is defined as a PaO2 of <55 mmHg or oxygen saturation of <90%. For those whose resting arterial Po_2 is between 56 and 59 mmHg, long-term oxygen therapy is indicated if they demonstrate erythrocytosis (hematocrit ≥ 55%) or evidence of cor pulmonale. Stable ambulatory patients should meet these criteria after being on an optimal treatment regimen for at least 30 days (Petty, 1990;Petty & Snider,1988;Tiep,1990 as cited in Shapiro SD,2010). Exercise-induced hypoxemia is also an accepted indication for supplemental oxygen because it improves exercise performance (Cotes & Gilson,1956;Woodcock et al,1981 as cited in Shapiro SD,2010). Supplementary oxygen during air travel is recommended for only those individuals whose in-flight PaO2 is expected to fall below 50 mmHg since all commercial airline cabins are not always pressurized to sea level(Gong,1984;Schwartz et al,1984 as cited in Shapiro SD,2010). Patients with major bullous disease run a high risk of life-threatening pneumothorax and hence, probably should not fly. Studies have failed to show any benefit arising from nocturnal oxygen supplementation targeted at correcting hypoxemic episodes during sleep (Chaouat et al,1999,2001; Zanchet & Viegas,2006 as cited in Shapiro SD,2010).

The continuous-flow nasal cannula is the standard means of oxygen delivery for stable hypoxemic patients. The cannula is simple, reliable, and generally well tolerated. Each liter of oxygen flow adds 3-4% to the fraction of inspired oxygen (FiO_2). Oxygen-conserving devices function by delivering all of the oxygen during early inhalation. Three distinct oxygen-conserving devices are available, and they include reservoir cannulas, demand-pulse delivery devices, and transtracheal oxygen delivery.

ii. Nutrition:

Patients with advanced COPD and a predominance of emphysema often experience progressive weight loss. The weight loss is multifactorial including a 15% to 25% increase in resting energy expenditure from elevated work of breathing and increased circulatory inflammatory cytokines, higher energy cost of daily activities and a reduced caloric intake(Barnes,2009;Di Francia et al,1994 as cited in Shapiro SD,2010). This leads to reduced muscle strength including weakness of respiratory muscles thus worsening the dyspnea.

Improved nutrition can restore respiratory and general muscle strength and endurance (Wilson,1986,Whittaker et al,1990 as cited in Shapiro SD,2010).

iii. Pulmonary Rehabilitation:

Comprehensive pulmonary rehabilitation has been shown to improve exercise capacity, improve independence quality of life, decrease dyspnea, and decrease health care utilization and it may also reduce mortality (Celli et al.,1995 as cited in Shapiro SD,2010). Although airflow obstruction ($FEV_{1)}$ is not improved, the effects of rehabilitation on health status ("quality of life") are generally much greater than seen with pharmacologic treatments (Finnerty et al,2001 as cited in Shapiro SD,2010). Pulmonary rehabilitation should be considered as an addition to medication therapy for symptomatic patients who have GOLD Stage II, III, or IV COPD.

Pulmonary rehabilitation program usually requires a team approach, including physicians, nurses, dietitians, respiratory therapists, exercise physiologists, physical therapists, occupational therapists, recreational therapists, cardiorespiratory technicians, pharmacists, and psychosocial professionals. This multidisciplinary approach emphasizes on patient and family education, smoking cessation, medical management (including oxygen and immunization), respiratory and chest physiotherapy, physical therapy with bronchopulmonary hygiene, exercise, and vocational rehabilitation and psychosocial support.

Exercise conditioning is the single most important aspect of rehabilitation and comprises of aerobic lower extremity endurance exercises and upper extremity exercise training to improve dyspnea and allow increased activities of daily life(ATS,1987). Breathing retraining techniques (eg, diaphragmatic and pursed-lip breathing) may improve the ventilatory pattern and may prevent dynamic airway compression (Celli,1991;Lotters,2002 as cited in Shapiro SD,2010).

11.2.6 Treatment of respiratory failure

i. Chronic Ventilatory Failure - Intermittent Noninvasive Ventilation :

The use of noninvasive mechanical ventilators is based on the concept that, in patients with severe COPD, the respiratory muscles are at the fatigue threshold. Resting the muscles provides time for "recovery" and prevents small increases in respiratory requirements from precipitating fatigue and perhaps acute respiratory failure. Due to lack of evidence of clinical benefit in several studies, the routine use of this form of support for COPD patients is not recommended at present(GOLD,2006).

ii. Altering Ventilatory Control
   a. Almitrine bismesylate - a peripheral chemoreceptor agonist, significantly improves resting room air arterial pO2 in about 80% of stable COPD patients mainly from improved ventilation-perfusion relationships because almitrine enhances hypoxic pulmonary vasoconstriction by way of sympathetic efferent pathways(Bury et al.,1989;Romaldini et al.,1983;Weitzenblum et al.,1991 as cited in Shapiro SD,2010). Further evidence is needed in its support before it can be recommended for regular use in COPD (GOLD,2006).
   b. Analeptic agents: The benefit of the analeptic agents, like acetazolamide, which stimulates respiration by acidifying plasma and cerebrospinal fluid(Skatruc &

Dempsey,1983 as cited in Shapiro SD,2010), and medroxyprogesterone acetate, which directly acts on brainstem respiratory neurons, is not established for COPD patients. Clinical benefits are not established for these medications and their use to stimulate ventilation in COPD is not recommended(GOLD,2006).

11.2.7 Surgical intervention

i. Lung Volume Reduction Surgery:

Various surgical approaches to improve symptoms and restore function in patients with emphysema have been described. Dr. Otto Brantigan pioneered resectional surgery in 1950s, but it was Cooper et al's work showing remarkable improvement in physical measures and quality of life measures in patients of COPD who underwent lung volume reduction surgery, generated tremendous interest in the procedure and led eventually to the National Emphysema Treatment Trial (NETT,1999). The NETT study found a substantial reduction in mortality and improvements in HRQOL and exercise capacity as a result of lung volume reduction surgery (LVRS) in properly selected patients(Pinto-Plata et al.,2007 as cited in Shapiro SD,2010). Caution is recommended in proper selection of patients as individuals with an FEV_1 less than 20% predicted and either homogenous disease or a diffusion capacity of less than 20% predicted were at very high risk for mortality if treated surgically.

ii. Bullectomy:

Removal of giant bullae has been a standard approach in selected patients for many years. Giant bullae may compress adjacent lung tissue, reducing the blood flow and ventilation to the relatively healthy lung. Giant bullectomy can produce subjective and objective improvement in selected patients, ie, those who have bullae that occupy at least 30%—and preferably 50%—of the hemithorax that compress adjacent lung, with an FEV1 of less than 50% of predicted and relatively preserved lung function otherwise(Kinnear & Tattersfield,1990;Nickoladze,1992 as cited in Shapiro SD,2010).

iii. Lung transplantation:

Despite multiple difficulties and obstacles, single-lung transplant has become most common procedure of choice when transplantation is performed for emphysema. Available data suggest that lung transplantation offers improved function and HRQOL to patients with advanced COPD, but it is not clear that it offers any survival benefit(Marulli & Rea,2008;Stavem,2006 as cited in Shapiro SD,2010). Worldwide, COPD is the most common reason for lung transplantation. Current guidelines by the International Society of Heart and Lung Transplantation recommends referring A1PI individuals with COPD for transplantation in a scenario with the BODE index greater than 5, post-bronchodilator FEV1 <25 percent of predicted, resting hypoxemia(PaO2 <55 to 60 mmHg), hypercapnia, secondary pulmonary hypertension or accelerated decline in FEV1 (Orens et al.,2006 as cited in Shapiro SD,2010).

11.2.8 Management of acute exacerbations of COPD (AECOPD)

GOLD and WHO defines an exacerbation of COPD as an acute increase in symptoms beyond normal day-to-day variation which includes worsening of cough, increase in phlegm production, change in phlegm quality, and increase in dyspnea(GOLD,2006).

Variable decrease in pulmonary function, and tachypnea are typical in acute exacerbations however, severe cases can lead to respiratory failure and death. Higher exacerbation frequency is associated with more loss of FEV_1, impairment in quality of life and increase in dyspnea with time.

AECOPDs occur in clusters and patients with an AECOPD were at an increased risk of another attack in the 8 weeks following their initial episode(Hurst et al.,2009 as cited in Shapiro SD,2010). Viral and bacterial infections and environmental pollutants incite most of the acute exacerbations. The single best predictor of exacerbations was a history of exacerbations. Other predictors of frequent exacerbations were chronic cough and phlegm production, episodic wheezing, pneumonia, active smoking, exertional dyspnoea, lower lung function, advanced age, duration of COPD, history of antibiotic therapy, COPD-related hospitalization within the previous year and having one or more comorbidities (eg, ischemic heart disease, chronic heart failure, diabetes mellitus or gastroesophageal reflux)(Foreman et al.,2007 as cited in Shapiro SD,2010). Important differential diagnosis are heart failure, pulmonary thromboembolism, and pneumonia.

AECOPDs are a major reason for hospital admission for failure of outpatient treatment, marked increase in dyspnea, altered mental status, and increase in hypoxemia or hypercapnia and respiratory acidosis. Mild episodes may be managed as out-patient. Supplemental oxygen is a critical component of acute therapy. It should target an arterial oxygen tension (PaO2) of 60 to 70 mmHg(GOLD,2006). If the episode is severe, the patient may require ventilatory support in the form of either noninvasive or invasive positive-pressure ventilation(GOLD,2006). A Cochrane review showed NIPPV reduces mortality, avoids endotracheal intubation, and decreased treatment failure(Lightowler et al.,2003 as cited in Shapiro SD,2010).

Pharmacological treatment of COPD includes bronchodilators, antibiotics, and steroids. Short-acting bronchodilators are the mainstay of therapy. Oral or parenteral steroids are indicated in the treatment of AECOPD and have been shown to shorten recovery time and improve outcome and reduce hospital stay. Most exacerbations are treated with full dose therapy (eg, prednisone 30 to 40 mg daily) for 7 to 10 days. Antibiotics have been shown to provide benefit in patients who present with dyspnea, increased purulence, and increased volume of sputum(Fagon et al,1990; Iyer & Murphy,2009 as cited in Shapiro SD,2010).

12. Prognosis and follow-up

Several parameters correlate with prognosis in COPD, including forced expiratory volume in 1 second (FEV1), diffusion capacity for carbon monoxide (DLCO), blood gas measurements, body mass index (BMI), exercise capacity, clinical status and radiographic findings on CT scan. A widely used simple prognostication tool is the BODE index, which is based on the BMI, obstruction (FEV1), dyspnea (using Medical Research Council Dyspnea Scale), and exercise capacity (ie, 6-minute walk distance).

The 6-min walk test (6MWT) remains the most popular test for the evaluation of exercise tolerance in COPD patients. It is simple and well standardised, but its interpretation criteria remain controversial. A distance of <361m also predicted mortality in patients with FEV1, 50% predicted. The 6MWT is currently used to evaluate the impact of treatment. The classical 54 m is defined as the minimal significant difference to detect benefit of treatment

(Redelmeir et al.,1997 as cited in Shapiro SD,2010). The shuttle walk test offers the advantages of being perfectly standardized and highly related to peak oxygen consumption.

Health status is mostly impaired by exacerbations on the one hand and dyspnoea on the other, with its negative effect on daily activity. Some extrapulmonary parameters are also correlated with measures of daily activity, independent of GOLD stage and BODE score; they include left cardiac dysfunction (as assessed by levels of B-type natriuretic peptide and echocardiography) and systemic inflammation (C-reactive protein levels) (Watz et al.,2008 as cited in Shapiro SD,2010). Frequent exacerbations have a negative long-term impact on the BODE index, a well known prognostic factor in COPD, and are not purely respiratory episodes but associated with systemic inflammation.

13. Recent advances

COPD is an area of intensive research, reporting important advances in the understanding of and care for the disease. Evidence from recent epidemiological studies have questioned the diagnostic value of the GOLD criteria of fixed FEV1/FVC threshold of 0.7 defining airflow limitation. These studies have demonstrated high false-negative rate in young subjects at risk and the false-positive rate in older patients(Cerveri et al.,2008;Hansen et al.,2007 as cited in Bourdin A,2009). The lower limit of normal(LLN) as recommended by American Thoracic Society(ATS) and European Respiratory Society(ERS) seems to be much more reliable for defining obstruction, particularly for screening purposes(Swanney et al.,2008 as cited in ATS,2010). The criteria of reversibility of airflow obstruction to differentiate COPD from asthma has come under question based on wide range of reversibility demonstrated in UPLIFT trial (Tashkin et al.,2008).

Some consider COPD as a component of a broader syndrome that was called ''chronic systemic inflammatory syndrome''. Patients are diagnosed with this syndrome if they have three or more components of the following: age >40 yrs, smoking history >10 pack-yrs, symptoms and abnormal lung function compatible with COPD, chronic heart failure, metabolic syndrome and increased CRP(Fabbri & Rabe,2007 as cited in Bourdin A,2009).

New evidence has enhanced the understanding of oxidative stress, injury and protective antioxidants such as the glutathione system and the haemoxygenase(HO)-1 pathway. Reduced HO-1 expression has been described in macrophages from lung tissue and bronchoalveolar lavage (BAL) of smokers with COPD(Maestrelli et al.,2003;Slebos et al.,2004 as cited in Bourdin A,2009).Moreover, the subtle molecular regulation of HO-1 and its key protein regulators, such as Nrf2, Bach1 and Keap1, is modified in COPD. Nrf2 protein level is significantly decreased in whole lung tissue and alveolar macrophages and conversely, Bach1 and Keap1 levels were increased in patients with emphysema (Goven et al.,2008 as cited in Bourdin A,2009).

A specific antigen reaction is a hypothesis put forward in order to better understand COPD progression; T-cells(both CD4 & CD8) may play a role in this possible B-cell mediated response. Leptin has been described as a potential regulator of lymphocyte lifespan within the airways of COPD patients(Bruno et al.,2005). The production of RANTES (regulated upon activation, normal T-cell expressed and secreted) is increased, as shown in the sputum of patients with COPD. Regulatory T-cells (Tregs) are special T-lymphocytes that are important for preventing autoimmune reactions by inhibiting T-cell responses (Baraldo &

Saetta,2008 as cited in Bourdin A,2009). The best described population of Treg is CD4+, and expresses CD25 and a transcription factor FOXP3.

Scores used in clinical practice to assess health status have been modified to be useful in primary care setting. The simplified version of the original BODE index, BOD score and a new index called ADO(age, dyspnoea and airflow obstruction) have been studied and found to have similar accuracy for risk prediction(Puhan et al.,2009 as cited in Bourdin A,2009).

Thoracic gas compression during forced expiration is a major event in COPD and a new index of gas compression defined as (NFEV1-FEV1)/NFEV1 (in percent) was demonstrated to be higher at baseline in COPD (32%) than in controls (10%) and it decreased after albuterol only in COPD patients. Shuttle walk test for exercise testing, negative expiratory pressure(NEP) method and forced oscillation technique(FOT) to measure expiratory flow limitation(EFL), single-breath nitrogen washout test(SBN2) for small airway involvement, inspiratory muscle endurance(IME) for monitoring respiratory muscle training, diaphragmatic electromyogram(EMG) for neural respiratory drive, etc include some of the modalities to assess clinical function, response to treatment and some for purely research evaluations.

Newer imaging techniques have recently allowed for the possibility of evaluating pulmonary function as well as anatomy. Although helical CT and HRCT have become the cornerstone of pulmonary imaging, newer modalities such as PET and MRI may soon become critical components in the arsenal of tests used to evaluate pulmonary disease. Newer axial CT is as accurate as fiberoptic bronchoscopy (FOB) and virtual bronchoscopy (VB), or CT bronchography, has received considerable attention and excellent internal images of the tracheobronchial tree can be generated to the level of the 4^{th}-5^{th} generation bronchi.

Increasing evidence in support of therapy with phosphodiesterase-4 inhibitors, antioxidants and augmentation therapy with A1PI in deficient individuals, seem promising. Nonrespiratory treatments of co-morbidities with medications such as proton pump inhibitors, angiotensin-converting enzyme inhibitors, and statins show promise in the management of COPD. Bronchoscopic lung volume reduction (bLVR) is being developed to collapse areas of emphysematous lung in hopes of having the same effect on respiratory function as LVRS, but without the morbidity and mortality of surgery. Safety and effectiveness of minimally invasive approaches like video-assisted thoracoscopy for the treatment of giant bullae is under evaluation.

14. Summary

- Chronic obstructive pulmonary disease is a common condition with a high morbidity and mortality.
- The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines COPD as a preventable and treatable disease characterized by progressive permanent airflow limitation that is not fully reversible. The airflow limitation is associated with an abnormal inflammatory response on exposure to noxious particles or gases particularly cigarette smoking.
- There is substantial overlap and co-existence of emphysema, chronic bronchitis, and asthma. Injury from smoking excites inflammation, which leads to cellular and

extracellular matrix injury which heals with incomplete and disorganized repair mechanisms ultimately leading to permanent progressive airflow obstruction. Deficiency of anti-protease deficiency makes individuals particularly susceptible to this pathologic process with earlier and more severe disease presentation.

- Patients with COPD present late with chronic respiratory symptoms and majority of early stage is asymptomatic and hence needs a high index of suspicion for diagnosis. Some patients present with an acute exacerbation.
- Pulmonary function tests reveal airflow obstruction (ie, a forced expiratory volume in one second [FEV1]/forced vital capacity [FVC] ratio less than 0.70) which is incompletely reversible.
- The GOLD staging system is based on spirometry and is well recognized and commonly used as a guide for management. It has been criticized for underestimating the importance of the extrapulmonary effects of COPD in predicting outcome, which is addressed by the BODE index.
- Cessation of smoking is a central point in treatment of COPD. A short-acting inhaled bronchodilator for use on as-needed basis is a reasonable initiation therapy for early stage disease(StageIA). Addition of a long-acting inhaled bronchodilator and/or glucocorticoid should be considered to improve symptoms, improve lung function, and reduce the frequency of exacerbations in Stage IB disease and onwards.
- Pulmonary rehabilitation is recommended to improve symptoms, exercise capacity, and quality of life. Long-term oxygen therapy is indicated in COPD patients who have chronic hypoxemia.
- All patients with COPD should be advised to quit smoking, educated about COPD, and influenza/pneumococcal vaccines advised as recommended.
- Patients who continue to have significant symptoms despite the above interventions may be candidates for surgical therapy.
- Extensive research is ongoing in various aspects of understanding etiopathogenesis and treatment options for COPD.

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8

Psychosocial Dimensions of COPD for the Patient and Family

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1. Introduction

This chapter will review our current understanding from the qualitative research literature on the experience of COPD for the patient and family. It will provide exemplars from the author's past research to ground these concepts within patient and family experience. Whilst research into symptom measurement, functional and biochemical measurements of lung function and pharmacological outcomes give important insights into the physiological dimensions of COPD, methodologies that explore the psychosocial dimensions are not always well understood. Research output is increasingly valued according to clearly definable 'Levels of Evidence' (National Health and Medical Research Council, 2009). This approach makes visible the rigour of processes that underpin clinical evidence and considers practices confirmed at one extreme by double-blinded, randomised, controlled trials, through to the accepted wisdom of experts in the field. Demonstrable rigour in research is particularly important when evaluating the safety and efficacy of new drugs and interventions. In this case, large sample-sizes, strict control of variables and meticulous monitoring of the research protocol to maintain objectivity means that clinicians can weigh up, with confidence, the therapeutic choices available to them. This quantitative approach to research relies on statistical methods to determine 'truth'.

Clinicians have sought ways to apply quantitative research methods to measure psychosocial dimensions of illness and treatments. Symptom and impact scales such as the Hospital Anxiety and Depression Scale (Zigmond et al., 1983) and the International Continence Society Sex Questionnaire (Blanker et al., 2001), are examples of instruments that can identify the presence and frequency of issues of importance to patients across the COPD population. Instruments like the SF-36 (Mahler & Mackowiak, 1995) and the Sickness Impact Profile (McDowell & Newell, 1987) allow us to determine the influence of disease and interventions on a person's quality of life. Measures of adaption to illness such as the Jalowiec Coping Scale (Jalowiec et al., 1984) quantify the behavioural and cognitive coping strategies people use to deal with social, physical end emotional stressors.

These quantitative measures of psychosocial aspects of COPD are useful in their ease of applicability to large research samples. They provide an aspect of evaluation that goes beyond the purely physiological concerns of health professionals to consider the patient-

perceived impacts of health problems and treatments. What they do not always achieve is firstly, the sensitivity necessary to reveal subtle but important changes in patient experience, and secondly, an explanation of the meanings behind results. Qualitative research methods have a different purpose to positivist, quantitative studies. Rather than seeking objective 'truth', they seek to gain an understanding of the meanings of illness and treatments for people. As such, there is an acceptance of the subjectivity of experience, and an acknowledgement of the context of an experience, rather than trying to control for context.

2. Understanding qualitative approaches in COPD research

Phenomenology is an example of a widely applied method in the qualitative COPD literature. Edmund Husserl, the founder of *descriptive* phenomenology sought a rigorous scientific method, grounded in the experience of people living within their world. Husserl's transcendental phenomenology attempted to strip away what we know and take for granted about a phenomenon to reveal and describe its fundamental essence (Husserl, 1936:1970). He called this the phenomenological reduction and suggested this process required us to suspend or *bracket* our prior knowledge of the subject being studied (Husserl, 1931:1960).

Husserl's student, existential philosopher Martin Heidegger, further developed Husserl's ideas around discovering the essence of an experience to create an *interpretative* phenomenology. He did not agree that we could separate ourselves objectively from phenomena in our world. He saw people, not as passive recipients of information about, and perceptions of, objects in the world, but rather believed that we exist *in-the-world* and are drawn towards and grasp things of significance for us that need to be taken care of (Heidegger, 1927:1996).

In Heideggerian phenomenology, the essence of the experience, for example 'breathlessness in a shrinking-life world' (Gullick & Stainton 2008), acts as a lens through which to view the participants' story. The story is interpreted through language (i.e. transcripts of in-depth interviews) against the background of their personal concerns (perhaps expression of masculinity, earning a wage, social connection), that are aspects of the person's history, culture and family and comprise their *being-in-the-world*. The result is a rich narrative that interprets experiences to describe the meaning behind them for the participants.

Another common approach to qualitative inquiry is Grounded Theory. First described by Glaser & Strauss (1967) and further developed by Strauss & Corbin (1998), this method may use both in-depth interviews and field observations. The resulting data is coded progressively from the first interview by a method of *constant comparison* according to a highly structured framework. During coding, the researcher memos their ideas and these may grow into theories about the phenomena. As these theories emerge they are tested by theoretical sampling; this is a type of purposive sampling that increases the diversity of the sample, seeking participants and pursuing questioning that tests the developing theory until that particular idea is 'saturated' with enough data to evidence it. Traditionally, literature is collected only as it becomes relevant to the data, rather than as a precursor to the study. Past literature is given the same status as data and is treated as data to support the new theory. Many studies use a modified approach to this method.

Good qualitative research is underpinned by a philosophical framework to strengthen the scholarly rigour of the interpretation. Examples include s*ymbolic interactionism* (Blumer,

1969), which is frequently used alongside Grounded Theory to find social explanations for behaviours. Symbolic interactionism sees people as 'pragmatic actors' who constantly adjust their own behaviour in response to others, and we can do this because we have the cultural and social understandings to interpret the meaning of those actions (McClelland, 2000). Maurice Merleau-Ponty's philosophy of the body (Merleau-Ponty, 1945:1962) is an example often applied to phenomenological studies that explore the embodied experience of illness. Merleau-Ponty describes people as perceiving the world through their body which acts spontaneously, in a *taken-for-granted* manner until something goes wrong.

Other modes of qualitative inquiry informing this review include, but are not limited to, content analysis (Krippendorff, 2004) and narrative analysis (Reissman, 1994). As with all research, findings from qualitative studies should be carefully considered according to the pre-determined criteria for rigour within the chosen methodology (Ezzy, 2002).

3. Loss of *taken-for-granted* breathing

Breathlessness is at the forefront of the experience of COPD, and breathing becomes a conscious focus of the person's life. COPD has been described as "a story with no beginning" (Pinnock et al., 2011); the changes in breathing are so slow and insidious that for a long time the decline is normalised; put down to getting older or being less fit. Eventually, the breathlessness begins to impact on the person's ability to conduct their day-to-day activities and is accompanied by other respiratory symptoms and poor exercise tolerance. Petra (63 yrs) had severe COPD before she sought advice from her doctor: *"… to go from my bed to the lavatory and back, I'm huffing and puffing. I thought 'This can't be right' ... I get out of breath all the time".*[1]

Distressing breathlessness can be precipitated by certain body positions, by activities such as walking and climbing stairs and by extremes of emotion. Environmental triggers such as excessive heat or cold, smoke or perfumes exacerbate breathlessness and people may need to anticipate and avoid these triggers. This avoidance of the triggers of breathlessness can isolate people from locations and activities that once that once afforded them pleasure (Gullick & Stainton, 2008). Chris (67 yrs) explained: *"There's lots of things I'd like to do but I just can't… Get out in the garage, make things. Well, I went out the other day to try and sand down our cutting board …there's all the dust, and … forget it!"*

Breathless people experience good days and bad days and this means that despite planning ahead, a bad day may rule out hoped for activities. Certain times of the day can be more problematic, with breathing often worse in the mornings, coinciding with the need to clear sputum and the need to attend to washing and dressing, and at night interfering with sleep. Certain times of the year can also worsen breathlessness due to extremes of temperature (Barnett, 2004). Williams (2011) reported that the person's perception of air movement made a difference to breathing, with fresh 'outdoor air' being easier to breathe.

Acute breathlessness is associated with panic, fear of suffocation, and fear of dying during an attack. People feel helpless and out of control of their bodies at these times (Williams et al., 2011, Avsar & Kasilkci, 2011, Elkington et al., 2005). Strategies can be taught that help

[1] Any unreferenced participant quotes in this chapter are sourced from unpublished interview data from research reported in Gullick (2008). All names are pseudonyms.

bring respiratory distress under control. Breathing techniques such as consciously slowing breathing, diaphragmatic breathing or purse-lipped breathing are reported widely by patients as effective ways to help manage frightening breathlessness (Fraser et al., 2006, Avsar & Kasilkci, 2011, Cicutto et al., 2004).

4. Losing control of the body's *taken-for-granted* functions

When our body works well its functions and understandings are *taken-for-granted*. For well people, the habitual functions of breathing, walking and moving the body in meaningful ways are unconsciously undertaken as the body moves purposefully towards its tasks. The existential philosopher Merleau-Ponty notes the sudden awareness of our bodies as task-orientated when function is disrupted:

> *"I am conscious of the world through the medium of my body. It is precisely when my customary world arouses in me habitual intentions that I can no longer, if I have lost a limb, be effectively drawn into it, and the utilisable objects, precisely insofar as they present themselves as utilisable, appeal to a hand which I no longer have… Our body comprises as it were two distinct layers, that of the habit-body, and that of the body at this moment..." (Merleau-Ponty, 1945:1962)*

Sputum production, uncontrolled coughing and wheezing and urinary urgency (Avsar & Kasilkci, 2011, Gullick & Stainton, 2008) are examples of changed body behaviours that signify a loss of control, displaying the body in socially unacceptable ways. Terry (77 yrs) confided, "*I cough, and I cough, and I cough and… all of a sudden it's on the table. It just comes out, no control* " (Gullick & Stainton, 2008). Because these behaviours draw attention to the person's unpredictable body, they detract from enjoyable participation in family and community activities. This has been described as the 'stigma' of COPD where the illness is visible, and is associated with disability and lack of control (Johnson et al., 2007). The visibility of the illness challenges the person's personal integrity and sense of effectiveness (Leidy & Haase, 1999, Gullick & Stainton, 2008). Marcia explained of her husband Pete (66 yrs): "*He goes to the club for the raffle …and meets a chap he went to school with, just for an hour and a half… But he's on to that oxygen the minute he gets home. See, he's stubborn that way. He wouldn't dare let anybody see him using a bottle, or a wheelchair*" (Gullick, 2008).

Other common manifestations of the failing body in COPD are weakness and fatigue, pain, insomnia, loss of appetite and difficulty with mobility (Elkington et al., 2005, Jones et al., 2004, Gullick & Stainton, 2008, Seamark et al., 2004). Fatigue is strongly linked to levels of breathlessness and depression and has major consequences for functional performance (Kapella et al., 2006). Fatigue is responsive to improved rest and sleep; however, sleep deprivation is part of the experience of living with COPD and the resulting constancy of fatigue makes it difficult to maintain daytime motivation. Focussing on patient perceptions of sleep, the qualitative report of Shackell et al (2007) revealed almost all participants waking more than three times per night to pass urine, and disturbances from pain and breathlessness were frequent. Some people were isolated from their daytime support structures and so felt vulnerable during the night, fearing nocturnal breathlessness and panic and wondering if they would "see the next morning". Those with poorer sleep had poorer lung function and quality of life scores and were more likely to be anxious and depressed. Daytime sleep or sleep whilst in hospital was seen as safer. It is notable that some patient strategies for insomnia actually function as barriers to good sleep including the comfort-seeking activities of drinking tea, late night TV and daytime napping. Whilst people

continue with low expectations for a good night's sleep and remain physically inactive, they remain prone to sleep problems (Shackell et al., 2007).

Anorexia & weight loss are found amongst many COPD sufferers and are associated with worsening breathlessness (Seamark et al., 2004, Jones et al., 2004). The study of Odencrants et al (2005) focussed on the experience of meals and their findings noted a number of barriers to sustainable eating. The problems began in obtaining food, with difficulty parking, breathlessness during shopping and difficulty transporting heavy groceries being contributors. Some people experienced physical challenges when preparing food, particularly if they were rushed, whilst others found it difficult to tolerate cooking odours. Some chose to smoke instead of eat.

The attraction of food is sometimes reduced due to a loss of taste sensation. Fungal infections or a dry mouth resulting from the use of puffers can make chewing painful. Coughing before or during meals can tire the person and reduce the focus on the meal, making food a real challenge during exacerbations (Odencrants et al., 2005). Keith (73 yrs) defended his poor eating to his wife Marcia, "*Do you want me to eat or do you want me to breathe? I can't do both together*!"(Gullick, 2008). People experience bloating, feel full before finishing meals and are often embarrassed by the food left on their plate. They report having their intake watched during mealtimes by family members and experienced feelings of failure, anger or sadness when they are not able to eat (Odencrants et al., 2005).

Eating smaller amounts more often and planning a number of meals in advance on a 'good day' is a common strategy to improve food availability and intake. Of concern was that Odencrants' et al's participants thought positively about their low body weight and this may be problematic given the association between low body mass index and higher mortality in COPD (Yang et al., 2010).

Pain is commonly reported in qualitative studies on COPD (Halpin et al., 2008, Elkington et al., 2005, Shackell et al., 2007) although authors do not tend to elaborate on the nature or location of pain. It is reasonable to assume this pain may in some part relate to reduced mobility, and perhaps, to age. Boueri et al (2001) noted that whilst their participants reported pain, levels were similar to healthy individuals in the community. Pain is particularly noted for people with COPD in the last year of life (Elkington et al., 2005).

5. Loss of the body's spontaneity

People with healthy bodies combine their movements and activities in a fluid manner. They spontaneously act in response to sensory stimuli, or to a perceived need to attend to a particular task, and this rarely requires a conscious appraisal of the body's capacity.

> *"The body is polarised by its tasks, of its existence towards them, of its collecting together of itself in pursuit of its aims". (Merleau-Ponty, 1945:1962)*

People with COPD lose this spontaneous application of the body to its tasks; in fact, a lack of forward planning can leave the person gasping for breath. Chris explained, "*… things you've done all your life, you don't think, and you go to do them again. Picking things up that I shouldn't pick up and carry*". Simple activities such as walking and talking become difficult to combine (Gullick & Stainton, 2009).

Breathlessness requires the person with COPD to consider the task, the steps they need to go through to undertake it and their particular physical effectiveness on that day. They may need to research how far they have to walk, whether there may be stairs and whether a toilet is close. People need to allow more time in order to avoid having to rush or keep up. The use of oxygen bottles takes considerable planning in relation to the cylinder's duration and portability. Even just walking from one room to the next may require rest stops. Patricia (63 yrs) lamented, "*Coming out to the lounge room where the nebuliser is, opening the blinds and curtains, then sitting down to get on my nebuliser. I have to stop about five times just doing that*".

Attending to day-to-day activities means pacing the body and spacing out activities that tax the body's breathing. Pacing of movement and activities with frequent breaks and aligning activities into sequential rather than combined tasks allows the person to recover their breathing along the way. Because of the daily variability in symptoms, people may need to take on a flexible approach to assessing, on the day, outings they have planned in advance (Barnett, 2004). Those who adjust most effectively to their bodily restrictions *listen* to their body, plan, pace, prioritise and balance their activity with capacity on that day, and try hard to achieve a certain level of contribution within realistic parameters (Lindqvist & Hallberg, 2010, Leidy & Haase, 1999, Gullick & Stainton, 2008, Fraser et al., 2006).

6. Changes in personal effectiveness

Leidy & Haase (1999) noted physical effectiveness as a core component of personal integrity that is challenged in COPD. Effectiveness is expressed as 'being able'; the body's predictability in doing what we expect or desire it to do. In sharp contrast, the failing body in COPD is nothing like what is presumed for, or wished of the body (Nicolson & Anderson, 2003). Physical effectiveness is just as much an interpersonal process that includes doing for others, as well as for one's self. This notion of contribution is an important one to most well-socialised adults. When the ability to contribute is lost to ineffectiveness and dependence, then people feel shame, self-blame and perceive the blame of others (Lindqvist & Hallberg, 2010, Barnett, 2004).

COPD symptoms often begin during a person's productive, working life. For many, there is an assumed level of physical adeptness and a physical and aesthetic appearance that has constituted their body as it is known to themselves in its predictability, and known to others in its apparent wholeness and application to visible tasks. Even though women are long established in the workforce, men still tend to perceive themselves as 'the breadwinner' and this forms an important part of their self-concept that becomes threatened in chronic illness. For men, heavier household tasks such as mowing lawns and managing gardens are frequently tied to their own and their family's perception of them in their gendered roles. Mary recalled of her husband Keith, "*…because he's always been the really strong one… He did marvellous things around the house… He doesn't do anything now… He can't, he gets too breathless. … and he's very conscious of this and it upsets him.*"(Gullick, 2008).

For men, these heavier tasks are eventually taken over by another family member, or by paid help. For women with COPD, there tends to be a sense of ownership and obligation towards housework, and they will tolerate significant symptoms to retain these duties. As the disease progresses there are often visible changes such as development of the classic 'barrel chest', significant weight loss and for some, facial and postural changes from

prolonged steroid use. Norman described changes to his wife Catherine (58 yrs), a once, striking woman who ran an exclusive boutique, *"She had this sort of wheezy voice, and she was beginning to get hunched shoulders."*

The net result of this changed capacity and appearance is that people lose a variety of modes of self-expression (Leidy & Haase, 1999). Andy, (57 yrs) explains: "*I had to give up sport, I'm a real sport nut. I had to give up walking… Of course sex was out of the question"*. Each task is considered as to whether the reward, for themselves or others, will outweigh any distressing symptoms. If the real or anticipated discomfort is thought to be greater than the perceived benefit, that task will be avoided. Rewards include either personal pleasure and fulfilment, or a task that is to the benefit or welfare of others (Leidy, 2008, Shackell et al., 2007).

7. Losing independence with body care

Severe COPD sees people coming to terms with their diminishing ability to care for themselves. Early losses in independence may include difficulty with shopping or driving. As the disease progresses, people find that basic tasks such as showering and dressing may become insurmountable, making them feel almost child-like in their dependence on others (Gullick, 2008, Oliver, 2001, Barnett, 2004). Chris was sensitive to his wife's workload around body care. "*I'm nearly an invalid, aren't I? She has to help me up the stairs… shower me… help me get dressed. Basically the stupid things I should be able to do myself*" (Gullick, 2008).

This loss of independence with self care is an enormous threat to people's sense of hope (Milne et al., 2009). Showering causes particular problems because of the effect of steam on breathlessness. Pete explained, "*I panic a little bit when I get in the bath or shower, and then I've got to get out and get dried up. I'm pushing for my breath, … and I dry one leg down to my ankle and …stand there and hang on to something until I get my breath and then I put the other leg up"*. Lifting arms to wash the hair, or bending to dry the feet are movements that cause considerable restriction to breathing, and so may be avoided. For people who live alone, this loss of self care may herald their movement into residential care. For people with family carers, it may alter the existing family relationship dynamics (Barnett, 2004, Gullick, 2008).

8. Changes in personality and mood

There is an important temporal framework to the experience of COPD with the visions of past, present and future selves being held in constant comparison to each other. Nicolson & Anderson (2003) describe how these gradual changes from independence to dependence lead to loss of self-esteem, loss of self-image and loss of power. The disease creates an otherness where the more visible 'medical self' is separate from the real self. Several studies reveal the nature of patient storytelling with past selves portrayed as athletic and vigorous, and present selves being barely able to walk (Bailey & Tilley, 2002, Gullick, 2008). Their future is seen in terms of loss: loss of anticipated retirement, loss of hoped for relationships with children and grandchildren (Nicolson & Anderson, 2003), and loss of 'possible selves' which are no longer conceivable (Gullick & Stainton, 2009).

This loss of independence and loss of family and community roles frequently lead to frustration, irritability and depression (Elkington et al., 2005, Seamark et al., 2004, Wilson et al., 2007). Those with advanced disease may see their life as meaningless. They communicate hopelessness, worthlessness and resignation and this can make death seem

like an attractive option (Ek & Ternestet, 2008, Oliver, 2001, Lindqvist & Hallberg, 2010). Terry (72) recounted: *"As true as I'm sitting here, … I go to bed and I say, 'Tonight would be a nice night to die. Take me.' … Really and truly, what good am I? I can't take my wife down to the shop, I can't walk from here to my barber who's just round the corner…"* (Gullick, 2008).

Despite the extremes of emotions, people try to contain their feelings as emotional turmoil can bring on exacerbations of breathlessness that are difficult to recover from. This has long been recognised and described as living within an 'emotional straight jacket' with both positive emotions such as laughter, and negative emotions such as anger, leading to distressing dyspnoea (Rabinowitz & Florian, 1992, Dudley et al., 1980, Diethorn, 1985). Partners of COPD patients tend to avoid discussing problems, or subjects that could lead to conflict with their ill spouses, for the same reason (Ring & Danielson, 1997, Sexton & Munro, 1985, Gullick, 2008).

Hypoxia may result in cognitive and personality changes that can further isolate people from family and others in the community. These may manifest as hallucinations, confusion, memory loss or unreasonable and unsociable behaviour (Gullick, 2008, Boyle, 2009b). Betty (73 yrs) explains of her husband, Terry: *"It's been hard… He gets very stressed and cranky over nothing… If anything goes wrong, I've done it… I know he's having trouble; he can't get about too much… It makes him more upset"*. For carers, the mood and personality changes of their loved one are often the hardest thing about living with COPD (Oliver, 2001, Wicks, 1997).

We know that rates of depression in COPD are reported at around 40% (Yohannes, 2005, Wilson, 2006) and up to 57% for those on home oxygen (Lacasse et al., 2001). Depression is further tied up in self blame and the perceived blame of others as people acknowledge the burden of their care and their ineffectiveness (Barnett, 2004). Anxiety is suffered by around a third of COPD sufferers, is a predictor of hospital admissions, and impacts significantly on the person's quality of life (Yohannes, 2000, Jones, 1991) Despite our awareness of anxiety and depression, there remains a lack of access to psychology services that could ameliorate these symptoms (Wilson et al., 2007).

9. The confining nature of COPD for the patient and family

People with COPD and their close family members live within a shrinking life-world (Gullick & Stainton, 2008). The physical boundaries of their life are diminished as the sick person begins to avoid taxing outings and spends the majority of their time within their own four walls. Mary explained of her husband, Keith: *"He could just walk on to the verandah and play with the dog a little bit, just in the confines of what you might loosely call the house. And then he just gradually stopped doing that"* (Gullick & Stainton, 2008).

People become socially isolated as they avoid environments and situations that may trigger breathlessness. Their consciousness of the socially unacceptable nature of their coughing and spitting makes them reluctant to enter new social situations. People reliant on home oxygen concentrators may be literally tied to an electrical power source and this increases isolation for the patient and the complexity of care for the family (Boyle, 2009b). People lose shared experiences with family and friends leading to loneliness, sadness and abandonment as they not only avoid social activities but feel they are avoided by others (Ek & Ternestet, 2008, Wilson et al., 2007, Leidy & Haase, 1999). Williams et al (2011) describe this experience as like living within a 'stagnant pool'. The physical stagnation

through loss of mobility is likened to an imprisonment; there is a stagnation and staleness of self that highlights the disparity between what the mind wants to do and what the body is able to do.

The confining nature of COPD extends to the family carer. As the physical effectiveness of the ill person declines, the workload of close family members increases. In the case of older couples, the primary carer may be facing their own health and ageing issues and the role of caring can seem overwhelming. The fear that something may happen to their loved one in their absence means that they become bound, physically to the home and psychologically to the role of caring due to a perceived need for increased vigilance. Their need to closely monitor their loved one leads to the use of phones and intercoms, listening to breathing during the night, watching for early signs of exacerbation and using the current level of breathlessness as a gauge of capacity for tasks (Boyle, 2009b, Gullick, 2008).

The experience of caring differs between spouses and other family members. The reciprocal nature of most marital relationships places caring in a framework of the historical give-and-take between partners and is sealed with the understanding of "for better or worse". Amongst younger caregivers, caring may be challenged by the competing roles of working and parenting and a different level of perceived reciprocity (Gullick, 2008, Nicolson & Anderson, 2003). Children and siblings are more likely to find the caring burdensome, and to note the lack of caring input from other family members (Gullick, 2008). Those carers with a higher level of education may find it more difficult to accept the loss of independence (Nordtug et al., 2010). Family enmeshment also makes adjustment to illness more difficult. When people weave their identities and activities around each another so completely it is difficult for any one member to function independently (Kanervisto et al., 2007).

Carers often feel weighed down by their multiple roles and feel similar losses of shared social experiences (Seamark et al., 2004). The caring role may coincide with a time of both declining health and fitness and increasing heaviness of the work of nursing. Women caregivers in particular are prone to somatic symptoms and anxiety, and although taken for granted, the frequent interruptions to sleep can be wearing (Bergs, 2002, Nordtug et al., 2010, Boyle, 2009b). Whilst some carers manage to integrate caring with employment to provide some personal time and space, others are forced into an unwelcomed, early retirement (Boyle, 2009b, Gullick, 2008). It is known that for people who are unable to leave the home for some sort of personal pursuit, there is a higher perceived burden of care (Boyle, 2009b). These losses of social participation for carers may contribute to a loss of self-identity with some women becoming unable to separate a sense of themselves from their husbands. Their future hopes for meaningful pursuits and achievements, a relaxed lifestyle and personal freedom become lost in the daily grind of their present reality (Boyle, 2009b).

The majority of social interaction for carers is with the ill person; however, males with COPD tend to isolate themselves from conversation, have a reduced interest in things, and as a consequence, have little to talk about (Bergs, 2002). This loss of intimacy through conversation is parallelled with a loss of physical intimacy, including sexual interaction (Gullick, 2008, Sexton & Munro, 1985). Where intercourse is attempted it may be frightening with distressing breathlessness distracting both partners from the

romanticism or eroticism of the moment. Whilst for many couples sex becomes less important, other forms of intimate physical contact is also avoided so that simple loving gestures such as cuddling or kissing may be lost to the caregiving spouse. Carer Claire, (55yrs) explained, *"you get used to not having those sort of things. You get used to being…not touched"* (Gullick, 2008).

Much of the caring literature on COPD focuses on female spouses. However, where both men and women are participants there appears to be a difference in caring styles and responses to caring. Women carers, in particular, take on a micro-management approach, arranging medical appointments and scrutinising diet, medication and exercise compliance and this differs from the more passive and delegatory style of male carers. Women try to play down the ineffectiveness of the sick person by secretly completing heavier jobs or slowing their pace whilst walking. They look for opportunities to promote a sense of effectiveness by leaving available the achievable jobs around the house, and only assisting with body care where it is absolutely necessary. Liz described her approach with her brother, Andy: *"I made every effort so that he didn't see a lot of the things that I did, so that he didn't know that he was incapable of doing it"* (Gullick, 2008). Women try to protect others in the family from seeing how bad things are. There is a sense of wifely duty reported, with women determined to 'walk the road' with their husband until the end. They can't imagine life without their partners after giving such intense care for so long. Women caregivers ignore their own health needs and become sad and worn out (Bergs, 2002).

There are a number of unmet needs amongst family carers in COPD, including the desire for better support with physical care and symptom control, and more useful information about the course of the illness (Currow et al., 2008, Bergs, 2002). Women carers are often too proud to ask for help from other family members (Bergs, 2002), whereas male carers more happily enlist outside help. Because of the intensity of carer engagement in COPD home-management, health professionals must seek the insights of carers during the patient assessment process, and educate and involve them when introducing new therapies. Carers may be the champions of patient motivation, but they are also known to actively eliminate treatment strategies they see as unnecessary or harmful (Boyle, 2009a).

10. COPD and smoking: The meanings of a 'self-inflicted' disease

In developing countries COPD is most often related to exposure to cooking fires. In a small group of people, an inherited alpha-1 antitrypsin deficiency can lead to early onset COPD. For the vast majority of people in the western world, however, COPD develops as a direct result of cigarette smoking (GOLD, 2010). Up to half of all smokers will die from a tobacco related disease (World Health Organisation, 2011). Whilst some manage to give up smoking easily when confronted with a diagnosis, many people continue to smoke. If the issue of smoking is to be dealt with collaboratively, clinicians need some insight into the meanings of smoking for the addicted person.

People with COPD are stigmatised by the self-inflicted nature of their disorder (Johnson et al., 2007). They experience enormous guilt and shame that may cause them to deny smoking as the cause of breathlessness, to hide their symptoms and to delay their engagement with medical services (Gullick & Stainton, 2006, Arne et al., 2007, Robinson, 2005, Earnest, 2002). Smokers have described reduced access to services because they either fear the judgement of

health professionals or because of the actual attitudes of health professionals (Johnson et al., 2007, Burrows & Carlislea, 2010, O'Neill, 2002). For example, current smoking is a contraindication for many elective surgical procedures, including lung volume reduction procedures for emphysema. People are known to have been excluded on this basis without receiving the smoking cessation support that could facilitate their access to such interventions (Gullick & Stainton, 2006). Smokers are less likely to have visited a doctor in the past year (Fisher & Hill, 1990), and smoking is associated with non-adherence to pulmonary rehabilitation (Young et al., 1999).

The context of self-infliction may create an underlying anger and resentment amongst family members, particularly where family have not struggled with an addiction of their own. This anger may make the caring burden harder to accept, but may also be intermingled with guilt over these emotions (Boyle, 2009b, Gullick & Stainton, 2006). Gary (38 yrs) explains of his father: *"They can hardly drag themselves across the room, but they'll still smoke. It makes it tough for families, you're doing everything you can, but you feel, 'What's the use of doing it if he's still smoking?' He tries to blame different things… infection in his lungs… exercise… which is so idiotic. If you had a tape, and … let him hear himself he'd probably go "Oh… Silly!"*

Those who accept the causative role of smoking in their illness experience regret and anger for their past inability to stop (O'Shea et al., 2007). However, only a small proportion of people with COPD attribute cigarettes as the primary cause of their lung disease (Hansen et al., 2007). In a large, early survey of older smokers with or without COPD, 47% didn't think quitting would improve their health and 45% did not believe smoking was harming them (Fisher & Hill, 1990). The fact that there may be COPD amongst other family members is usually explained away as a family predisposition rather than a shared family smoking addiction. Numerous studies demonstrate the widespread denial of smoking as the main cause of breathlessness. Rather, patients attribute occupational exposure, ageing, lack of fitness and 'bad luck' as major contributors (Wilson et al., 2007, Hansen et al., 2007, Burrows & Carlislea, 2010, Gullick & Stainton, 2006). The study of Boyle (2009b) demonstrated that spouses are also inclined to find explanations for the illness that externalise the responsibility from their partners to others. Knowing other smokers who do not have COPD reinforces their beliefs.

The self-talk around the impact of smoking sometimes extends from denial of harm to positive physical, social and psychological benefits (Schofield et al., 2007, Osman & Hyland, 2005). Some research participants report that smoking makes them feel better and eases their breathing and others recall shared social experiences around smoking with affection. The issue of the pure enjoyment of smoking to the addicted individual cannot be ignored. Cigarettes have been described as a 'best friend', providing comfort and companionship (Lindqvist & Hallberg, 2010). Research participant Terry recalls and craves the sensation of smoking: *"I enjoyed smoking, and even now… I'd love a cigarette. My son … goes outside and has a smoke. I say 'Sit in here and I can smell it.' I want the smell of his smoke."* (Gullick & Stainton, 2006).

Smoking is widely utilised tool for stress reduction. It is common for people who have succeeded in smoking cessation to later relapse due to extreme stress or bereavement (Schofield et al., 2007, Burrows & Carlislea, 2010, Gullick & Stainton, 2006). The findings that cessation does not automatically deliver better well-being adds to the problem. All ex-

smokers in the study of Burrows & Carlislea (2010) described feeling worse after quitting due to symptom exacerbation or weight gain. This was the case for Petra after her successful cessation attempt: "*I was under the impression if I stopped smoking I would get better, or I'd stay the same. And I thought, 'I'll give them up immediately' which I did, straight away … and I didn't get any better, I felt as though I was getting worse*". Even clinicians are unable to give reassurance of disease reversal, with slowing of COPD progression the best outcome of cessation. The lack of conviction of smoking as the main cause of illness is profound in its influence on smoking cessation failure (Hansen et al., 2007).

Smokers experience smoking as a "need of their taken-for-granted-body". In long-term smokers, the need to smoke is an embodied and automatic function that is reinforced by triggers of daily routine such as completion of a meal, having a cup of coffee or talking on the telephone (Gullick & Stainton, 2006). For smokers, this places smoking within a framework of ritual behaviour rather than addiction (Lindqvist & Hallberg, 2010). Whilst ever the immediate embodied rewards of smoking are stronger than the longer-term and more abstract possibility of future health gains, cessation success amongst long-term smokers is unlikely (Osman & Hyland, 2005). In the context of denial, merely providing education around harmful effects of smoking is equally unlikely to make a difference. As disability progresses, for the person to continue to smoke whilst accepting smoking as the cause of their illness means they are confronted with ideas of their own inherent foolishness, selfishness or weakness, leading to self-harm and burden to loved ones, and they find this idea of themselves unacceptable. That health professionals understand these meanings of denial around smoking is central to supporting cessation attempts.

A US Clinical Practice Guideline for tobacco dependence (Fiore et al., 2000) proposes the acknowledgement of smoking addiction itself as a chronic disease. By presenting smoking in a disease framework, clinicians can move beyond the issue of patient accountability for cessation failure and create the permission to accept medical, psychological and social support. It may also reduce the anger and resentment of family members arising from the addiction.

A number of disease milestones can act as prompts to stop smoking including being confronted with a diagnosis, the threat of oxygen dependence and serious exacerbations leading to hospital admission. Patient stories frequently link periods of heavy smoking with sudden and life-threatening health events and this may strengthen the person's resolve to stop. Taking the opportunity to communicate the 'right words at the right time' during a period of perceived vulnerability can be a precipitant for the person's eventual decision for cessation (Gullick & Stainton, 2006). West & Sohal (2006) describe this as 'motivational tension', a point at which even small triggers may lead to an unplanned quit attempt, and supportive treatments may be most effective. In their survey of almost 2000 past and current smokers, nearly half the reported attempts at quitting were unplanned and these unplanned attempts succeeded for longer.

The approach clinicians take to smoking advice is important. It is known that smokers will resent 'being told what to do', and need to feel that they have reached the decision for their own reasons (Burrows & Carlislea, 2010). If clinicians seek a partnership with the patient in managing the chronic illness of smoking addiction then this may sit more comfortably in the guilt/shame milieu of smoking experience. Whilst a didactic approach to discussions is not

recommended, it must be noted that there is a strong dose-response association between the intensity of smoking cessation counselling and its effectiveness. Programs that provide person-to-person contact such as face-to-face individual or group counselling or telephone counselling have demonstrated their consistent effectiveness, and effectiveness increases with treatment intensity (Anderson et al., 2002).

11. Living with crises

COPD is often experienced as relatively quiet times interrupted by episodes of serious illness. Episodic crises create the essence of uncertainty that defines the experience of COPD (Boyle, 2009a, Oliver, 2001, Gruffyd-Jones et al., 2007). These episodes are often described by patients and carers as near-death experiences that leave people with a constant sense of their own possible death. This has been described as 'living in the proximity of death' (Lindqvist and Hallberg 2010) and from a Heideggerian perspective, 'being-towards-death' (Gullick, 2008).

Crises may be the result of panic attacks, acute chest infections, allergic reactions or acute emergencies related to comorbidities. The crisis events begin with dyspnoea that does not respond to the usual self-management strategies. Initially, people may feel the need to be on their own during acute breathlessness, sensing that others can't help bring dyspnoea under control and that there is a need to focus internally on breathing and maintaining calm (Fraser 2006). Although the onset of exacerbation is recognised with panic and dread (Leidy, 2008), people are often reluctant to seek help, hoping things will improve and hospital admission will be avoided. Professional assistance is sought only after people are convinced they can't self-manage the event (Gruffyd-Jones et al., 2007, Leidy & Haase, 1999, Bailey, 2001). Gary described his father Jack's frightening experience: "*… he got a bit worried and rang the ambulance and by the time they got there all his vital signs… were starting to break down… they ended up working on him to save his life in the garage*."(Gullick, 2008).

As respiratory distress increases and panic rises, people may change in appearance, may be unable to speak and may experience choking and loss of bladder or bowel control (Bailey, 2001, Gullick, 2008). These understandably terrifying events usually lead to emergency hospital admission. These crises are watershed events that mark a 'before' and 'after' in the person and family's life from which other events are then measured (Bailey, 2001). These crises underline life with COPD as uncertain and unpredictable and people fear each attack could be their last (Boyle, 2009a, Oliver, 2001). The experience reinforces the conviction of carers that they must closely monitor the person for early signs of deterioration, and this vigilance thereafter binds them emotionally and practically to the task of caring (Gullick, 2008). People will often develop emergency protocols that may define triggers for help-seeking and roles for family members that require 'understanding and trustworthiness' amongst those individuals (Bailey, 2001, Leidy & Haase, 1999) .

12. Emotional coping strategies in COPD

COPD is an imposing illness in its effects on normal body functioning, daily management of the body and the home environment and on the lives of family members who give support. People find strategies around 'conscious management of self' to counter the impact of the unpredictability of the disease. Many of these strategies can be found across the literature of other chronic illnesses. These strategies include conscious control of emotions, comparing

oneself to others worse off and learning to 'go with the flow' and make the best of unpredictable symptoms (Gullick & Stainton, 2008, Seamark et al., 2004, Cicutto et al., 2004). For some people, religious faith and spirituality provide an important emotional support that can reduce feelings of powerlessness (Leidy & Haase, 1999, Bergs, 2002, Milne et al., 2009, Seamark et al., 2004, Boyle, 2009b). Coming to a point of acceptance of the disease is named by many, but elegantly articulated by Lindqvist & Hallberg (2010) who describe the process of embodying and making a relationship with the disease. This requires a conscious replacement of the previously known life structure with a new, adapted one. This allows a determination of a reframed identity and normality that includes COPD. Patricia explains "*I've just got to learn to live with it. I call it 'me and my friend'*." Part of this acceptance lies in finding different foundations upon which to build hope; from cure to coping; from old dreams to new, realistic goals; and by discovering hope in the 'rewards of the moment' (Milne et al., 2009). People find simple and meaningful pleasure in realising skills, in having a good day, in being able to achieve a walk in the park or a shopping trip or in remembering past experiences with affection (Milne et al., 2009, Ek & Ternestet, 2008, Seamark et al., 2004).

Perhaps the most significant recognition for both the carers and people with COPD is of their family as 'the best thing in life' (Gullick & Stainton, 2008). Family is not only a practical support structure, but a reason for surviving and enduring, and through children and grandchildren, embodies an important source of meaningful connectivity and joy (Leidy & Haase, 1999, Cicutto et al., 2004, Bergs, 2002, Barnett, 2004).

13. The impact of pulmonary rehabilitation for the patient and family

Pulmonary rehabilitation is a valuable treatment option in chronic lung disease and is directed towards reversing the downward spiral of disability. People with COPD tend to use their body within the limits of worsening breathlessness so that they gradually decrease their body's activity. Patricia confided: "*They say you should go out for a walk, but I just can't be bothered because I just get too tired. You know, to me it's not worth it*".

Pulmonary rehabilitation programmes aim to reduce symptoms and disability and to reduce the person's reliance on acute health care systems by improving their understanding of the disease and encouraging active involvement and self-management. Current clinical practice guidelines (Ries et al., 2007) advise a multidisciplinary team approach, individualised patient assessment and the setting of realistic, patient-centred goals. A well-rounded rehabilitation program pays attention to the psychological, emotional and social dimensions of the patient experience, whilst trying to optimise the person's physical function by monitoring best-practice medical therapy.

Programmes usually offer a mixture of upper and lower body strength and aerobic exercise and expose the person to 'safe' breathlessness. Education sessions are an important component and typically discuss use of puffers and spacers, management of exacerbations and panic attacks, access to services and benefits, psychosocial support and understanding of Advanced Care Directives and No Resuscitation orders (Milne et al., 2009, Wilson et al., 2007). The duration and intensity of pulmonary rehabilitation programmes seem to impact on outcomes. People with mild to moderate COPD may see benefits from short to medium term participation, whilst people with severe COPD do best with programmes of at least six

months (Salman et al., 2003). The physical effectiveness gains also appear to be tied to the frequency of sessions per week (Gullick, 2008).

Having a specific COPD class means participants are empathetic towards others with symptoms of breathlessness and sputum production, and so are less self-conscious about their bodies' unpredictable behaviours (Gullick, 2008, Arnold et al., 2006). Under supervision, people become more comfortable exerting their body and are less likely to become panicked by exertional dyspnoea (Williams et al., 2010). Chris learned to manage his panic through the classes: *"The most helpful was avoiding panic attacks… It changed my outlook… I probably looked at it from the aspect 'Well, Bugger it! I can do these things' and I'd have a go at whatever it might be."* The increased sense of disease control due to greater confidence with managing medications and breathing techniques leads to a reduced likelihood of presentation to hospital (Camp et al., 2000).

Perceived physical gains include improved muscle strength, balance and mobility, reduced breathlessness and fatigue, and an improvement in joint mobility and pain management for those with musculoskeletal comorbidities. The result is that daily tasks are more achievable and require less pacing to complete (O'Shea et al., 2007, Gullick, 2008). Pulmonary rehabilitation has led to improvements in health related quality of life even where no significant improvement in lung function is demonstrated (Haave et al., 2007, Camp et al., 2000). This is in part, due to the reduction in social isolation and improved opportunities for expression of 'self' (Gullick, 2008, Toms & Harrison, 2002); patients describe feelings of enhanced well-being and hope (Milne et al., 2009, O'Shea et al., 2007), have higher self-esteem and mood (Arnold et al., 2006) and, following rehabilitation, are more likely to talk about their abilities rather than their limitations (Williams et al., 2010). Pulmonary rehabilitation can lead to a change in physical appearance and in turn, body image, and creates a sense of pride, satisfaction and achievement (O'Shea et al., 2007).

The intrinsic motivation of the person with COPD is important in determining the most successful approach to exercise training. Home-based programs may not be so successful for people who live alone or who do not have high internal levels of motivation. The notion of locus of control (Rotter, 1966) is a useful construct to predict those who may be most successful. People with a higher internal locus of control are more likely to seek information about their circumstances. They perceive a greater power to influence events through their own activities and behaviours and are more likely to believe that their labours will be successful. Those with a lower internal locus of control tend to see events as influenced by their environment, powerful others or fate. People with COPD who describe a higher intrinsic drive demonstrate more active engagement with rehabilitation and seem more successful with continuing on a home-based maintenance routine. Petra had severe COPD, but was carrying on a home-based exercise program more than a year after her initial rehabilitation: *"I have a walker… I only have to look at that and I'm at it. Never, ever will I fail! But I have two days off … Wednesdays comes my cleaning lady… Sunday… I entertain… So all the other days, that's exercise. That's like going to a job"* (Gullick, 2008). Those whose motivation is linked to exercising with others are less likely to benefit from a home-based rehabilitation (Milne et al., 2009). Jim (60 yrs) found maintaining a home-based program challenging: *"It's pretty right what they say – 'In a group you'll do it', whereas a lot of times you'll put it off at home."*

Pulmonary Rehabilitation itself can foster subsequent patient empowerment and a higher internal locus of control by demonstrating to the participant, the positive effects of self monitoring and management of their clinical status (Cafarella & Frith, 2001). Whilst breathlessness is still a feature of the person's experience after rehabilitation, it is the change in the way breathlessness is perceived that is most important, resulting from increased confidence and a loss of fear of physical exertion. With an increased sense of control over breathing, people often find panic and anxiety are reduced or eliminated and they increase their activity levels as a result (Williams et al., 2010, O'Shea et al., 2007).

14. The impact of volume reduction interventions for the patient and family

The major limitation to exercise tolerance, and therefore to functional performance in COPD, is dynamic hyperinflation (O'Donnell & Webb, 2008). Surgical procedures, such as Lung Volume Reduction Surgery (LVRS) and Endobronchial Valve Insertion (EBV™) have expanded the therapeutic possibilities for people with emphysematous hyperinflation. The procedures aim to reduce the amount of space taken up by hyperinflated lung tissue to improve elastic recoil, and chest wall and diaphragm dynamics. LVRS is an invasive procedure that requires the resection of between 20-40% of the total volume of each lung. It is safest and most effective for people with an FEV_1 greater than 20% of predicted and a heterogenous rather than diffuse pattern of emphysema (NETT, 2001). LVRS is not a first-line treatment, but should be considered where optimal medical management and pulmonary rehabilitation fails to improve the person's clinical status (Ries et al., 2005). LVRS is known to result in significant improvements in quality of life, exercise performance and lung function, and the best results occur where surgery is complemented with an extended period of pulmonary rehabilitation (Criner et al., 1999).

In response to the potential morbidity and mortality following the major surgical procedure of LVRS, minimally invasive alternatives have been developed, and these are usually targeted towards upper zone, heterogenous emphysema. To date, the most commonly utilised approach is to insert one or more one-way endobronchial valves (EVB) to allow air to escape from hyperinflated zones and to prevent the return of air to those zones.

Whilst some patients are known to benefit from this procedure, only a minority (these tend to be those with the most hyperinflation at baseline) experience long term improvements in lung function (Kotecha et al., 2011). This improvement comes at a cost of more frequent hemoptysis, pneumonia distal to the valves and more frequent exacerbations of COPD in the few months after valve implantation (Sciurba et al., 2010). These results are confirmed in the only qualitative study of lung volume reduction procedures to date, demonstrating sustained wellness amongst most of the LVRS participants in contrast to a gradual decline in effectiveness for those who had endobronchial valve insertion (Gullick & Stainton, 2009).

Importantly, FEV_1 as the hallmark of COPD measurement, frequently does not predict the person-centred outcomes of surgery (Gullick & Stainton, 2009, Leyenson et al., 2000, Moy et al., 1999). Patients and families who accept surgical intervention for COPD feel the need to 'take a chance' on a procedure, even if they perceive that to be high-risk decision. Whilst COPD leads to shrinking of the boundaries of the self, for some, undergoing a surgical intervention allows an increase in physical effectiveness and a regaining of self. Gail

explained of her husband Jim after EBV insertion, "*He can dig in his garden…he's got a lovely veggie garden at the moment. There's lots of things he wouldn't have been able to do had he not had it done*". Claire, (52 yrs) describes her husband Sam's regaining of self after LVRS: "*It was important for all of us to get back what he wanted; his mobility, his freedom, his right to choose what he wants... It was a chance for Sam to continue being Sam, and the surgery achieved that. He could go on being the same person that he was – he was able to continue being himself.*"

15. COPD at end-of-life

End-of-life planning in COPD is an important concept that allows goal-setting for patients and families, and facilitates a peaceful and dignified death. Specialist referral to palliative care services, in combination with a partnership approach with patients and families, allows the person to retain control over aspects of the experience of dying in the context of an otherwise uncontrollable illness course.

One of the great difficulties of planning the timing of end-of-life discussions is the uncertain disease trajectory in chronic respiratory conditions. COPD has not only an insidious onset, but also, an unchartable end-stage. We know that compared to patients with lung cancer, COPD patients have more Emergency Department admissions, more anxiety & depression, and report a lower quality of life. Compared to cancer patients, financial support comes later in the disease process and patients feel in greater need of aids and appliances, and of information on services and benefits (Gore et al., 2000, Crawford, 2010). COPD patients are less likely to receive prognostic information, less likely to know they are dying, or know they are dying for less time and they are more likely than lung cancer patients to die in hospital. Relatives of COPD patients are less likely to be present at the time of death, although, we know most would like to be present (Edmonds et al., 2001).

Many General Practitioners (GPs) may not think about COPD as a terminal disease and so may not consider a palliative management plan (Halliwell et al., 2004). They do not tend to talk about what dying may be like or how long that may take (Curtis et al., 2004). The recently revised Initiative on Chronic Obstructive Pulmonary Disease guidelines (GOLD, 2010) gives brief mention of end-of-life discussions and advance directives, yet gives no strategies for these considerations or for palliative management of COPD.

A nurse participant in the study of Crawford (2010) described COPD patients as having "*nine lives… you see them sick and think they won't get through this and then they do.*" This tendency for people to bounce back has led to practitioners considering how best to define the time for end-of-life discussions and interventions. For clinicians, the final phase of life may be suggested by an FEV_1 less than 30% of predicted, frequent exacerbations and admissions to hospital, and the presence of right heart failure. The need for mechanical ventilation and long-term oxygen therapy dependence also signal serious disease (Halpin et al., 2008). However, such markers are not always reliable predictors of the terminal phase of COPD (Seneff et al., 1995). Patients may have their own interpretation of the time when treatment is no-longer worth the burden that continued life presents. Scenarios that include prolongation of inevitable death, dependence on machinery, functional and cognitive impairment, unmanageable symptoms and a burden on loved ones have been noted as unacceptable by patients (Fried & Bradley, 2003).

Another complicating factor is that what is acceptable to patients may change over time as they adjust to severe illness and this may influence discussions and the willingness of GPs to initiate advance care planning (Halpin et al., 2008). It is typical of people to normalise their experience of even severe day-to-day symptoms and see themselves a sick only during acute exacerbations. This may be, in part, a coping strategy, but is also a result of the long illness trajectory. Whilst in cancer narratives, there is a definite beginning and developing plot to the 'cancer story', COPD is more likely to be insidious in its beginning and intertwined with the person's 'life story'. The unpredictability of exacerbations creates a chaotic component to the person's experience of illness, yet they may have a sense of relative wellness between these crises (Pinnock et al., 2011). Whilst people may feel that each acute exacerbation may be their last (Oliver, 2001) the threat of death recedes after a COPD crisis, or perhaps the threat of death is also normalised. The result is that death is less likely to be considered imminent and so wishes are rarely discussed with professional carers, friends or family (Pinnock et al., 2011). Where end-of-life discussions do occur, they may be poorly documented and so patient wishes may not be visible to family or other members of a multi-disciplinary team (Crawford, 2010).

Having end-of-life discussions with COPD patients and families constitutes significant emotional work for clinicians and requires 'conscious emotional management'. This comes with experience as professionals learn to feel their way with an individual, and apply emotional intelligence and empathetic skills in their discussions (Crawford, 2010). Some ways to approach these difficult conversations include beginning discussions early in the disease course, using the uncertain disease trajectory to ease discussions and building a caring and respectful relationship with patients. It is useful to have a team approach with recognition of the collective responsibility of GPs, respiratory nurses and physicians to proactively identify and use opportunities to talk about prognosis (Halliwell et al., 2004).

The aim of good end-of-life discussions is to inform without removing hope, and to bring to the forefront the wishes of the patient and family. Research participant, Mary, described how she appreciated the honesty and sensitivity of the discussions after her husband had an ICU admission: *"The doctor did tell us the dangers of intubation …then when he was moved to ward said, 'You've come through this okay…Perhaps in the future it might happen again…You need to think what you want done, you and your family.' Just nicely … And I thought this is great"*.

Discussing prognosis broadly in terms of a diagnostic population rather than directing it at the individual leaves room for hopeful possibilities. Physicians can foster hope by giving a 'commitment to non-abandonment', by addressing people's fears, such as fear of pain at end-of-life, and by having a management plan that addresses their changing situation (Curtis et al., 2008). Helping people to identify realistic goals and discussing their concerns about day-to-day living can also be useful (Clayton et al., 2005). The ideal is for a formal Advance Care Plan to be documented early. Again, the uncertain disease course of COPD makes this more complex, and means physicians are less comfortable with initiating such plans (Halpin et al., 2008). Fins et al (2005) point out that the process can be simplified by creating possibilities for revision of the plan, and by trying to understand and be true to the patient's core values whilst remaining flexible around practical details such as where they would prefer to die.

One marker of the end-of life stage may be the point where maximal therapy no longer provides relief of symptoms. Symptoms in the last year of life are characterised by constant breathlessness, weakness and fatigue. Pain, insomnia, depression, anxiety and panic attacks also shape the patient experience at this stage (Elkington et al., 2005). This requires a change in priorities of care, with symptom management needing the greatest focus. For example, in late-stage disease opioids may be central to dealing with dyspnoea, dyspnoea-related anxiety and pain. Clinician concerns around respiratory depression may lead to the underutilisation of opoids (Halpin et al., 2008). This may require a change in our understanding of what is 'good' or 'safe' for patients at different stages of their illness experience.

The COPD journey is a long and consuming one both for the person with the disease, and for the family carer. Whilst this may set up challenges for clinicians in understanding and supporting psychosocial concerns, it also creates possibilities for true management partnerships with our patients and their families. If we embrace these possibilities we may achieve real meaning in the care we provide, and we are more likely to locate the humanity within our practice.

16. References

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9

Alpha-1 Antitrypsin Deficiency – A Genetic Risk Factor for COPD

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1. Introduction

Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder characterised by low circulating levels of the key antiprotease alpha-1 antitrypsin (AAT) and is associated with the development of chronic obstructive pulmonary disease (COPD), often by the 3rd or 4th decade, and liver disease. The two most common SERPINA1 mutations associated with AATD are the Z and S mutations, and the vast majority of AATD individuals diagnosed with COPD are ZZ homozygotes. AATD is an under-diagnosed condition with the majority of cases misdiagnosed as COPD. The World Health Organisation (WHO) and the American Thoracic Society/European Respiratory Society (ATS/ERS) advocate a targeted screening approach for the detection of AATD in patients with COPD, non-responsive asthma, cryptogenic liver disease and first degree relatives of known AATD patients (Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting 1997; American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). It is our contention that a diagnosis of AATD gives the clinician a vital and unique opportunity for early medical intervention and the possible prevention of COPD in both the affected individual and first-degree relatives. Unfortunately, despite huge strides in awareness and understanding of this condition, this opportunity is too often missed.

2. Alpha-1 antitrypsin deficiency (AATD)

2.1 Alpha-1 antitrypsin (AAT)

AAT is a secretory glycoprotein produced by the liver and is the most abundant serum antiprotease in circulation (Kueppers 1971). While the majority of AAT in the body is hepatocyte-derived, it is actively transcribed and secreted by other cell types including monocytes (Carroll et al. 2010), macrophages (Mornex et al. 1986), neutrophils (Bergin et al. 2010), intestinal epithelial cells (Perlmutter et al. 1989), and various epithelial cells in the lung (Hu and Perlmutter 2002; Venembre et al. 1994; Cichy, Potempa, and Travis 1997), albeit in smaller quantities. In keeping with its role as an acute phase reactant, the

hepatocyte expresses approximately 200 times more AAT mRNA than other cells (Rogers et al. 1983) and serum levels can rapidly increase by between two- and five-fold during infection, trauma, surgery and burns (Kossmann et al. 1995; Voulgari et al. 1982; Sandford et al. 1999; Jeschke, Barrow, and Herndon 2004). The AAT released by inflammatory cells is more relevant to the local inflammatory milieu, serving to limit local degradation of the extracellular matrix by proteases released from migrating immune cells (Knoell et al. 1998). In a similar fashion, the range of cells producing AAT locally in the lung underlines its key role in protecting the fragile lung parenchyma against proteolytic damage. AAT was originally named because of its ability to inhibit pancreatic trypsin (Schultze, Heide, and Haupt 1962) and while its principal function as an antiprotease has been well established, AAT has significant anti-inflammatory properties affecting numerous cell types, and has been implicated in areas as diverse as apoptosis (Petrache, Fijalkowska, Medler, et al. 2006), chemotaxis (Bergin et al. 2010), tissue repair (Dabbagh et al. 2001), innate immunity (Bergin et al. 2010), and cell signalling (Koulmanda et al. 2008).

The gene encoding AAT is the SERPINA1 gene, found on the long arm of chromosome 14 at q32.1 and is composed of four coding exons (II, III, IV, V), three untranslated exons (Ia, Ib, and Ic) in the 5′ region and six introns. The hepatocyte transcription start site resides within exon Ic, while the mononuclear phagocyte transcription start sites reside within exons Ia and Ib. The SERPINA1 gene encodes a 418 amino acid protein, which includes a 24 amino acid signal peptide. The mature form of the protein is a 394 amino acid, 52 kDa glycoprotein with three asparagine-linked carbohydrate side chains (Carrell et al. 1982). Serum concentrations of AAT are genetically determined by the two alleles of the SERPINA1 coding gene, expressed co-dominantly, and normal serum concentrations of AAT are in the range of 1.04 g/L to 2.76 g/L (20 – 53 μM) (Brantly et al. 1991). Over 30 mg/kg body weight of AAT is secreted into the circulation daily, and the glycosylated protein has a serum half-life of 4 – 5 days (Jeppsson et al. 1978; Jones et al. 1978).

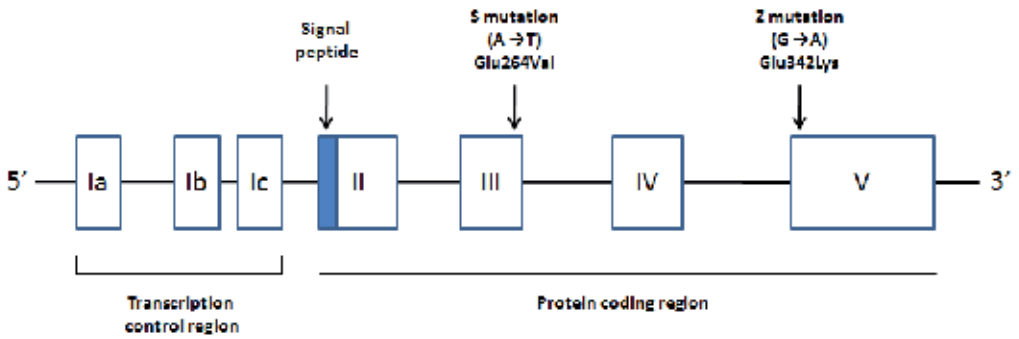

Fig. 1. SERPINA1 gene encoding the alpha-1 antitrypsin protein. The protein coding region is within exons II-V, with the S and Z mutations arising in exon III and exon V respectively.

Although AAT diffuses though all tissues of the body its main site of action is the lung, where it protects the fragile alveolar, connective, and epithelial tissues from proteolytic attack by the omnivorous serine protease neutrophil elastase (NE) (Bieth 1986). In this regard, AAT is the archetype of the serine protease inhibitor or serpin super-family, whose primary function is to inhibit a family of proteolytic enzymes with serine at the active site.

Other prominent serpins include alpha-1 antichymotrypsin, alpha-2 antiplasmin, antithrombin, plasminogen activator inhibitor I, and C1 inhibitor (Law et al. 2006). The serpins act like molecular snares or mousetraps, existing in a metastable state, until they entice the unsuspecting cognate protease into their trap, and their energy is released by cleavage of their reactive centre loop (Carrell and Lomas 2002). AAT irreversibly inhibits serine proteases by presenting its inhibitory amino acid sequence as a mobile, exposed reactive centre loop with a methionine residue at its centre (Elliott et al. 1996). This reactive centre loop is a pseudosubstrate for target proteases, such as NE, and upon docking of the protease AAT undergoes a conformational change that results in the irreversible inhibition and destruction of the protease (Huntington, Read, and Carrell 2000). Although AAT can regulate the activity of many other serine proteases including trypsin, chymotrypsin, plasmin, thrombin, plasminogen, cathepsin G and proteinase 3 (Silverman et al. 2001; Travis and Salvesen 1983), it exhibits the highest association constant for NE which is 25 times greater than for any other protease (Beatty, Bieth, and Travis 1980). In addition, NE is the most relevant protease in the lung setting as it is released in large amounts by activated neutrophils (Gadek 1992).

2.2 Alpha-1 antitrypsin deficiency (AATD)

2.2.1 History and clinical features

AATD is a hereditary monogenic disorder caused by mutations within the SERPINA1 gene, previously known as the "protease inhibitor" or "PI" gene. The condition was first reported in the early 1960s by Carl-Bertil Laurell and Sten Eriksson at the University of Lund in Sweden when they noticed the absence of the alpha-1 globulin band on routine serum protein electrophoresis of five patients (Laurell and Eriksson 1963). It was already known that over 90% of the alpha-1 band comprised a single protein that was capable of inhibiting the proteolytic enzyme trypsin (Jacobsson 1955; Schultze, Heide, and Haupt 1962). Following a review of the five patients, three were found to have developed "a degenerative pulmonary disease" at a young age and the condition we now know as AATD was born (Eriksson 1964). Interestingly, the oldest reported case is thought to have been a young girl who had remained undiscovered in the Alaskan permafrost for over 800 years (Zimmerman, Jensen, and Sheehan 2000). More recently there has been speculation that the composer Frédéric Chopin suffered from AATD with his physician failing to confirm pulmonary tuberculosis instead discovering "a disease not previously encountered" on autopsy (Kuzemko 1994; Kubba and Young 1998).

AATD is associated with a substantially increased risk for the development of COPD, often by the third or fourth decade, and is also associated with risks for development of liver disease (Sharp et al. 1969; Lieberman, Mittman, and Gordon 1972; Sveger 1976), cutaneous panniculitis (Edmonds, Hodge, and Rietschel 1991), bronchiectasis (King et al. 1996), vasculitis (Lewis et al. 1985), and Wegener's granulomatosis (Barnett, Sekosan, and Khurshid 1999). AATD is characterised by misfolding of the AAT protein and belongs to a class of genetic diseases underpinned by aberrant protein folding collectively termed conformational disorders (Gooptu and Lomas 2009; Greene et al. 2008). In addition, AATD is a paradigm of the subclass of conformational diseases known as the serpinopathies (Carrell and Lomas 1997). Serpins have a highly conserved structure of three β–sheets, nine α–helices, and an exposed reactive centre loop (Gettins 2002). Serpinopathies are

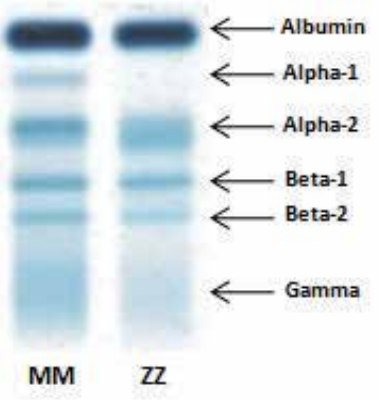

Fig. 2. Serum protein electrophoresis comparison between MM and ZZ individuals. Note the complete absence of the alpha-1 globulin band in ZZ individual as first described by Laurell and Eriksson in 1963.

characterized by mutations that subvert this serpin primary structure to permit the formation of intracellular polymers and this can have pronounced deleterious consequences. In the rare condition familial encephalopathy with neuroserpin inclusion bodies (FENIB) polymerisation of the neuron-specific serpin neuroserpin, caused by point mutations in the shutter domain region of the protein, favours the accumulation of intraneuronal aggregates and eventually leads to early onset dementia (Davis et al. 1999; Belorgey et al. 2002). In contrast to this gain of (pathological) function causing dementia, a loss of (physiological) function, specifically a lack of circulating serpin in individuals with C1 inhibitor, antithrombin, and alpha-1 antichymotrypsin deficiency facilitates unchecked activity of cognate proteases and the development of angioedema, thrombosis, and COPD, respectively (Lomas and Mahadeva 2002).

2.2.2 Molecular pathophysiology

The SERPINA1 gene is highly pleiomorphic with over 120 allelic variants, including 80 deficiency alleles, identified to date (DeMeo and Silverman 2004). The majority of individuals carry two copies of the normal AAT allele, termed M, and are designated M homozygous (Fagerhol and Laurell 1970). The technique of starch gel electrophoresis originally used to separate AAT variants is responsible for the nomenclature used to identify the earliest described variants. These variants were originally designated according to how fast they migrated on starch gel, for example M (medium), S (slow), and F (fast) (Fagerhol and Laurell 1970). As detection techniques advanced and proteins began to be separated on the basis of their isoelectric point (for AAT between pH 4 – 5), the nomenclature system was revised so AAT variants were designated with earlier letters of the alphabet if exhibiting anodal migration and later letters of the alphabet if exhibiting cathodal migration. Furthermore, as the letters of the alphabet were exhausted, place of origin names are used in addition to the letter of the closest anodal allele (Cox, Johnson, and Fagerhol 1980). More precisely the birthplace of the first described individual to carry a novel allele is used, for example Q0cairo was used to describe a novel Null mutation found in the first recognised case whose birthplace was Cairo (Zorzetto et al. 2005).

Mutations in the SERPINA1 gene that confer an increased risk for the development of COPD and/or liver disease are those in which deficiency or Null alleles are combined in homozygous or heterozygous states, and encode AAT plasma levels below a putative protective threshold of 11 μM or 0.57 g/L (Brantly et al. 1991). The two most common mutations associated with disease in populations of European descent are the Z (Glu342Lys) and S (Glu264Val) mutations, and both are caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the mature protein, respectively. In general, AAT alleles can be classified according to plasma levels and function and divided among four broad groups:

a. Normal

Normal alleles are most commonly M subtypes which account for 95% of gene variants and are characterized by normal plasma levels in homozygotes (greater than 1.04 g/L or 20 μM). Other normal variants include V, T, and Lfrankfurt (Faber et al. 1994).

b. Deficient

The Z allele is the most common deficiency variant, with plasma levels of ZZ homozygotes in the range of 0.10 – 0.52 g/L (2 – 10 μM). The S variant is also common but is only clinically significant if inherited with Z or other severe mutations (Mmalton, Null etc.). For example, SZ individuals have AAT plasma levels in the range of 0.33 – 0.98 g/L (6 - 20 μM).

c. Null

The class of SERPINA1 mutations termed silent or "Null" cause a complete abrogation of AAT production (Lee and Brantly 2000) and while ultra rare, confer a particularly high risk of emphysema (Fregonese et al. 2008). As these mutations do not cause the AAT protein to polymerise they pose no risk of liver disease. Most frequent among this class are those mutations that introduce a premature stop codon, for example Q0cairo (Zorzetto et al. 2005).

d. Dysfunctional

Like Null variants, dysfunctional alleles are extremely rare. For example, the single amino acid change caused by the Pittsburgh mutation (Met358Arg) at the active site of the AAT molecule converts it from an elastase inhibitor to a thrombin inhibitor and was discovered in a child who died from an episodic bleeding disorder (Owen et al. 1983).

The Z mutation (Glu342Lys) in AAT is an excellent example of the destabilizing effect of a mutation near the critical reactive centre loop of the protein. The proximity of the Z mutation to the loop and its location at the head of strand 5 of β–sheet A causes the AAT molecule to adopt a more open and promiscuous conformation (Gooptu et al. 2000). Thus, the β–sheet A of one molecule can readily accept the reactive loop of another AAT molecule to form a dimer. Elongation of this dimer leads to the formation of loop-sheet polymers in which the reactive centre loop of one AAT molecule sequentially inserts into the accessible β–sheet of another (Lomas et al. 1992; Sivasothy et al. 2000; Ekeowa et al. 2010). This mechanism of polymerization forms the basis for the liver disease observed in ZZ individuals, and the plasma deficiency arising from the intracellular accumulation of entangled polymers of AAT in the liver forms the basis for the lung disease observed in ZZ individuals. The Siiyama (Ser53Phe) and Mmalton (deletion of 52Phe) alleles predispose to polymerisation of the AAT molecule through a similar mechanism of loop-sheet formation.

Variant	Mutation	Effect	Disease Risk
Z	GAG – AAG, Glu342Lys	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Siiyama	TCC – TTC, Ser53Phe	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Mmalton	ΔTTC, ΔPhe52	Polymerisation, impaired secretion and severe plasma deficiency	Lung & liver
Null	Mutations causing gene deletion, premature stop codon or mRNA degradation	No AAT production	Lung
S	GAA – GTA, Glu264Val	Impaired secretion and mild plasma deficiency	Lung & liver (in compound heterozygotes e.g. SZ)
I	CGC – TGC, Arg39Cys	Impaired secretion and mild plasma deficiency	Lung & liver (case reports in compound heterozygotes e.g. IZ)
F	CGT – TGT, Arg223Cys	Defective neutrophil elastase inhibition	Lung (case reports in compound heterozygotes e.g. FZ)

Table 1. Molecular basis of the most common SERPINA1 variants associated with disease.

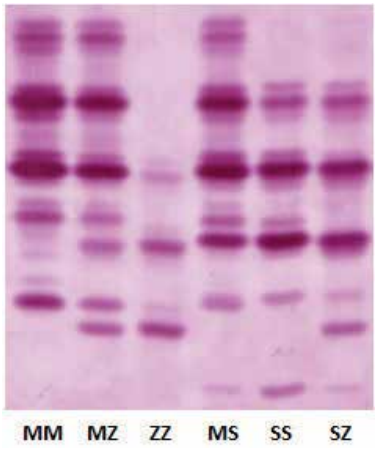

Fig. 3. Phenotype analysis of the most common deficient AAT variants by agarose gel isoelectric focusing followed by immunofixation (Sebia).

The Siiyama allele is the most common cause of AATD in Japan (Seyama et al. 1995), while the Mmalton variant (also known as Mcagliari and Mnichinan) is the most commonly found allele in Sardinia (Ferrarotti et al. 2005).

The formation of rogue AAT polymers can also be caused by the S (Glu264Val) and I (Arg39Cys) alleles, which are associated with milder plasma deficiencies and less risk of disease (Curiel et al. 1989; Graham et al. 1989). The point mutations underlying these variants cause less disruption to β–sheet A when compared to the Z mutation. Thus, the rate of polymer production for each variant is much slower than Z AAT, leading to less retention of protein within liver cells, milder plasma deficiency, and a negligible risk of disease in heterozygotes. However, there is a risk of disease in compound heterozygotes. If a mild, slowly polymerising I or S variant of AAT is inherited with a rapidly polymerising Z (or potentially Mmalton) variant, the two variants when co-expressed can interact to form heteropolymers within hepatocytes, leading to cirrhosis and plasma deficiency (Mahadeva et al. 1999). In addition to the pathophysiological effects of polymer formation and plasma deficiency, the Z AAT protein that does make it out of the liver and is available to defend the lungs is a poor inhibitor of NE. It has been shown that Z AAT is five times less effective at inhibiting NE when compared to normal M AAT (Ogushi et al. 1987). The S AAT protein is also a slightly less efficient inhibitor of NE when compared to M AAT, but the reduction is marginal in contrast to Z AAT (Ogushi et al. 1988).

The final deficient mutation that warrants mention is the F (Arg223Cys) allele. Like the Z, S, and I variants, the anodal F variant was first described by starch gel electrophoresis (Fagerhol and Braend 1965) but the molecular basis for the allele was not identified until much later (Okayama et al. 1991). The point mutation in this variant introduces a cysteine instead of an arginine, and the same amino acid substitution occurs in the I variant. The normal AAT molecule possesses a single cysteine residue and the introduction of a second cysteine potentially favours the formation of disulphide bonds intramolecularly and intermolecularly with other AAT molecules. Interestingly, and possibly a reflection of the extra cysteine residue, the major F bands run as doublets on IEF gels. In the disease context, the inhibitory activity of the F AAT protein against NE is reduced, with the *in vivo* inhibition time for FZ three times longer than for MZ (Cook et al. 1996). This would suggest that individuals who co-inherit the F allele with another severe deficiency allele such as Z or Null would have a high risk for the development of COPD. The rate of polymerisation of the F variant has not been investigated but it may well exhibit a higher rate of polymerisation. A case report described finding hepatomegaly and globules positive for AAT in a liver biopsy from an FZ individual with emphysema (Kelly et al. 1989). Unfortunately, there have been no reports published to date describing F homozygotes (or I homozygotes) as these might shed some light on the polymerigenicity of the F protein and associated risk of disease.

2.2.3 Epidemiology

Since the first description of AATD as a distinct clinical entity by Laurell and Eriksson almost 50 years ago, a wealth of data pertaining to the epidemiology of AATD has been accumulated. However, although a large number of groups have been investigated, the majority of studies have been biased to varying degrees as they investigated cohorts of symptomatic individuals, blood donors, soldiers, seamen, hospital staff, pregnant women, rheumatic disorders or other diseases, newborn infants and work-based employees (Blanco,

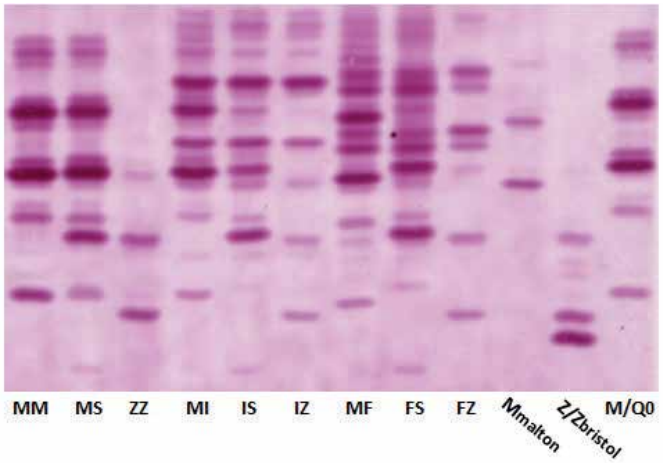

Fig. 4. Phenotype analysis of rare deficient AAT variants (I, F, Mmalton, Zbristol, and Q0) by isoelectric focusing followed by immunofixation. Reference MM, MS, and ZZ standards are included for clarity. The Mmalton variant depicted is from an individual homozygous for this mutation.

Fernandez, and Bustillo 2001). Allele frequencies collected from patients with pulmonary disease cannot be included as they would yield disproportionately high allele frequencies. Findings from several investigations of healthy control cohorts are also unreliable as these by their definition exclude symptomatic ZZ, SZ, and MZ individuals, and this leads to underestimation of the allele frequencies in a general population. Studies of blood bank donors also risk underestimating AATD as these donors tend to be healthy individuals. To our knowledge, a survey carried out in Asturias (Spain) is the only truly representative investigation of AAT allele frequencies in a general population as the subjects were of all ages and randomly selected from a municipal population register (Blanco et al. 1999).

The small sample size and the less accurate isoelectrofocusing methods employed in many of these population studies represent significant limitations. To illustrate this point, a small study was performed in Ireland in 1992 to determine if an association existed between AATD and coeliac disease (Bourke et al. 1993). AAT phenotypes in 111 coeliacs were compared to those in 250 blood donor controls including some hospital staff. Allele frequencies for Z and S in the blood donor group were determined to be 0.008 and 0.04, respectively, using a polyacrylamide gel (pH 4 – 5) isoelectric focusing method. We subsequently carried out a larger analysis of 1,100 individuals randomly selected from the national electoral register and genotyped this group for Z and S mutations. In contrast to the 1983 data, we found allele frequencies for Z and S to be 0.022 and 0.054, respectively (Carroll et al. 2011). The reason for this disparity is most probably due to the small sample size of the original cohort. Methodological limitations may also be a factor. We have previously employed the same phenotyping method (Pharmacia) as was used in the 1983 study and found the MZ phenotype was often difficult to correctly identify and was uncomfortably similar to the M2 subtype, compared to the more accurate and specific Sebia method used in the more recent study. Similar to the revised findings on the prevalence of AATD in Ireland, many of the studies in other countries may have significantly underestimated the frequency of the Z and S mutations due to small sample size and/or methodological limitations.

Moreover, as current genotyping methods only identify Z and S alleles it is worth considering that allele frequencies described in population studies using genotyping methods will potentially underestimate AATD as the rarer SERPINA1 mutations such as I, F, and Mmalton are missed by genotyping methods.

Across Europe the frequency of the Z and S mutations varies widely between countries, geographic regions, and ethnic groupings. It is estimated that approximately 3 - 4% of northern Europeans carry the Z allele and 6% carry the S allele (Blanco, de Serres et al. 2006). The frequency of the Z variant is highest in northern and western European countries with a mean gene frequency of 0.014, peaking in southern Scandinavia with a gene frequency of greater than 0.02, and in general declines from west to east and north to south throughout the continent (Luisetti and Seersholm 2004). The highest frequency for the Z allele recorded to date of 0.045 was found in western Latvia in a region that has seen centuries of immigration from mainland Sweden (Beckman et al. 1999). Haplotype analysis has estimated that the Z mutation first arose from a single origin 66 generations or 2,000 years ago (Cox, Woo, and Mansfield 1985; Byth, Billingsley, and Cox 1994) and its high frequency in southern Scandinavia suggests that the mutation arose in this area and was subsequently dispersed by migration patterns, for example the Viking colonisation of north-western Europe between 800 and 1200 AD (Hutchison 1998).

The region with the highest frequency of the S allele described to date is the Iberian Peninsula with a mean gene frequency of 0.0564. The highest reported frequencies of the S allele in Europe are in the region of Galicia in north-western Spain with 149 alleles found per 1000 (0.149) (Carracedo and Concheiro 1983) and in a Portuguese study with 152 alleles found per 1000 (0.152) (Santos Rosa and Robalo Cordeiro 1986). The allele frequency for S appears to decrease along south to north and west to east gradients, and is extremely low in Serbia and Russia (Blanco, Fernandez, and Bustillo 2001). In general high S frequencies are found all along the western Atlantic seaboard but accepting the premise that the higher the allele frequency in a particular country, the more likely it is to have arisen there first, it would appear the S allele arose in the north-western corner of the Iberian peninsula between 10,000 and 15,000 years ago (Seixas et al. 2001).

The only other AAT alleles whose origins can be reliably predicted are the Siiyama mutation, which appears to have arisen exclusively in Japan, and the Mmalton mutation, which is remarkably common in central Italy and on the island of Sardinia (Ferrarotti et al. 2005). Interestingly, the same mutation underlying Mmalton appears to have also arisen independently in the Japanese population (Matsunaga et al. 1990). Undoubtedly, more surveys relating to the epidemiology of AATD are necessary as there is a remarkable absence of AAT allele frequency data in many regions of Europe, particularly in eastern countries, as well as the lack of data pertaining to the frequencies and origins of rarer AAT alleles such as the I and F mutations.

Upon reviewing the European allele frequencies for the Z and S mutations one is compelled to ask the obvious question: why are these two mutations so common? Could there possibly be an advantage to having one of these mutations? This would then provide the selective pressure to retain these mutations. An intriguing theory put forward by David Lomas in an effort to explain the high prevalence of AATD in European populations surmised that the Z and S mutations conferred a survival advantage on heterozygotes in the pre-antibiotic era

(Lomas 2006). The hypothesis describes how Z and S mutations could favour the generation of polymers at sites of inflammation. Polymers of Z and S could then help focus and amplify the host inflammatory response and help eradicate invading infectious organisms. Heat is known to induce Z and S polymer formation and AAT can be produced locally in the gut and the lung, the most common portals for invading pathogens. Febrile episodes in the infected individual could generate large quantities of polymer at the site of infection and recruit protective neutrophils. Thus, the pro-inflammatory response driven by Z polymers was probably hugely advantageous to a population living in the preantibiotic era. However, in the modern era increased life span and cigarette smoking have transformed a previously protective allele into a harmful one. In support of this theory polymers of Z AAT protein isolated from lung lavage have been shown to act as neutrophil chemoattractants and were found localised with neutrophils (Mulgrew et al. 2004; Parmar et al. 2002; Mahadeva et al. 2005). Furthermore, increased neutrophil numbers and IL-8 concentrations have been demonstrated in sputum from MZ heterozygotes (Malerba et al. 2006). It is also possible that the intermediate levels of AAT in MZ and MS individuals are associated with more exuberant protease activity and this allows for faster and more potent protease-dependent killing of harmful pathogens. Comprehensive studies in MZ and MS heterozygotes are required to provide additional evidence for this attractive theory.

2.2.4 Treatment and management

Although there is still no cure for AATD the accurate and early diagnosis of the condition remains imperative for improved health outcomes in AATD individuals. As soon as a diagnosis is established, AATD individuals can benefit from behavioural changes, careful management, and more targeted therapies. Effective management is guided by smoking cessation, vaccine administration and aggressive management of respiratory exacerbations (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Augmentation therapy, while prescribed in many countries, remains to be conclusively proven to benefit AATD patients (McCarthy and Dimitrov 2010). Specialised referral centres for AATD are urgently needed to ensure the best care and management of AATD patients. The national referral centre for AATD in Ireland has a rapid access weekly AATD clinic for newly-diagnosed ZZ, SZ, and MZ individuals. The clinic is coordinated by a dedicated clinical research nurse and AATD individuals are seen by a multidisciplinary team of doctors, nurses, and physiotherapists with international best practice standards of care followed. Similar models are being followed or implemented in several European countries but not all.

To examine the importance of early diagnosis and mode of ascertainment in AATD we collected spirometry data from 73 ZZ individuals enrolled in the Irish National AATD Registry. After stratification for mode of ascertainment, the mean % predicted FEV_1 (forced expiratory volume in one second) was significantly higher in ZZ subjects diagnosed through family screening, compared to ZZ subjects who were diagnosed because of symptoms (Fig. 5). While impaired lung function would be expected in the symptomatic group, the preserved lung function in the family screened cohort, despite similar age and smoking history, highlights the importance of family screening as a tool for early detection and possible prevention of COPD in ZZ individuals. The findings from the Irish registry are

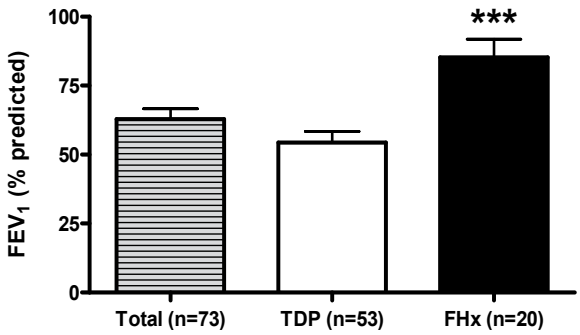

Fig. 5. Mean FEV_1 (% predicted) in ZZ individuals diagnosed by symptomatic screening in a targeted detection programme (TDP) versus those diagnosed via family screening (FHx) enrolled in Irish National AATD Registry (***p < 0.0001, t-test). The mean age of the TDP cohort was 46.1 +/- 1.4 years compared to 41.2 +/- 2.8 years in the family screened cohort, while 77% of the TDP cohort was ever-smoking compared to 65% of the family screened cohort.

supported by a study from the Danish registry which found that non-index ZZ individuals had longer estimated life expectancies when compared to index (symptomatic) cases (Seersholm, Kok-Jensen, and Dirksen 1994). More recently, data from the Swedish registry showed that ZZ individuals identified by family screening had longer median survival times compared to ZZ individuals detected by symptomatic (respiratory and non-respiratory) screening (Tanash et al. 2010).

Many of the early guidelines for AATD screening advocated testing younger COPD patients and this is to the detriment of the larger COPD population of all ages. The age at which manifestations of airway obstruction, pulmonary emphysema, or chronic bronchitis appear in ZZ individuals is highly variable (Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group 1998). While a common presentation of AATD is indeed early onset COPD, a subset of ZZ patients do not develop symptoms until much later in life, particularly if non-smokers (Campos, Alazemi, Zhang, Salathe, et al. 2009). In fact, among ZZ never-smokers the risk of liver disease increases with age (Tanash et al. 2008; Willson, Seow, and Zimmerman 2004). Numerous case reports have described AATD in elderly individuals with COPD who were lifelong never smokers (Jack and Evans 1991). Taken together, it is clear that screening for AATD should be performed in all patients with COPD regardless of advanced age or smoking history, especially as failure to do so has clinical repercussions for undiagnosed family members.

2.2.4.1 Smoking cessation & occupational exposures

While risk factors such as male gender and asthma can contribute to lung disease risk (Demeo et al. 2007), cigarette smoke is by far the single most important risk factor for the development of COPD in AATD patients (Janoff and Carp 1977; Mayer et al. 2006; Seersholm and Kok-Jensen 1995). It has been shown that smoking can reduce the life expectancy of a ZZ patient by up to 25 years (Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. The Alpha-1-Antitrypsin Deficiency Registry Study Group 1998). All AATD patients, including ZZ, SZ, and MZ phenotypes, need to be

educated about the harmful effects of cigarette smoke. Smoking cessation and the avoidance of occupational and environmental exposures (for example particulate matter, chemical vapours, and agricultural dusts) is paramount in AATD patient education (ATS/ERS guidelines, 2003). AATD patients without apparent lung disease should also be encouraged to quit smoking as this cohort offers the most realistic chance of delaying or in some cases preventing the development of COPD. Another important benefit in diagnosing a COPD patient with AATD is that he/she is twice as likely to attempt to quit smoking compared to an AAT-replete, smoking-related COPD patient (Carpenter et al. 2007). Carpenter *et al.* demonstrated that knowledge of AATD motivates smokers towards cessation when compared with COPD patients.

To illustrate the effect of smoking on lung function in AATD patients we collected spirometry data from 70 ZZ patients enrolled in the Irish National AATD Registry and stratified the group according to smoking history. The contribution of occupational exposures was not taken into account; nevertheless, the mean FEV_1 (% predicted) was significantly higher in ZZ subjects who never smoked compared to ZZ subjects who ever smoked, with no difference in mean age between groups (Fig. 6). This clearly demonstrates the major role of tobacco consumption in the pathogenesis of COPD in ZZ individuals.

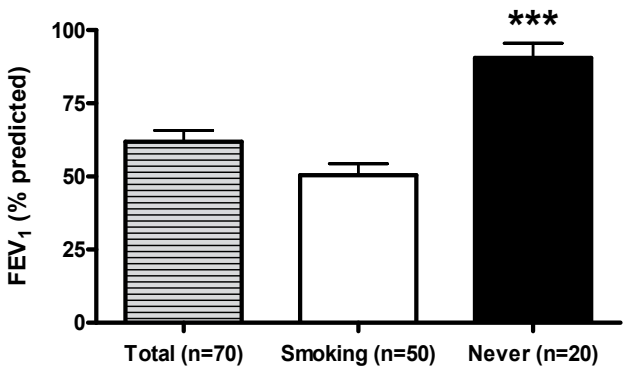

Fig. 6. Mean FEV_1 (% predicted) in ever-smoking versus never-smoking ZZ individuals enrolled in Irish National AATD Registry (***$p < 0.0001$, t-test). Mean age of ever-smoking cohort (50.9 +/- 1.4 years), mean age of never-smoking cohort (50.0 +/- 2.9 years).

However, even in the absence of significant smoking history there exists a significant risk for COPD. The first study to investigate a non-smoking ZZ cohort observed marked variability in both clinical course and lung function decline (Black and Kueppers 1978). Another US study showed that exposure to second-hand tobacco smoke in childhood can accelerate the onset of symptoms in ZZ AATD individuals (Mayer et al. 2006). A study from the Swedish registry demonstrated that, while non-smoking ZZ individuals may not develop COPD until later in life, this cohort still displays a decline in lung function (FEV_1) with age, especially after the age of 50 (Piitulainen, Tornling, and Eriksson 1997). A follow up study by the same group found that an agricultural occupation was associated with decreased lung function in non-smoking ZZ individuals (Piitulainen, Tornling, and Eriksson 1998). Passive smoking was associated with an increased frequency of chronic bronchitis, but not with impaired lung function. Anecdotally, several of the ZZ individuals with COPD attending our clinic are farmers, specifically poultry and grain. A number of the other

occupations reported in our ZZ COPD cohort include welders, chemical factory workers, painters, and firemen.

In the Irish health system there are dedicated smoking cessation officers both in the hospital setting and in the community, as well as another grade of health promotion officers, whose remit also includes smoking cessation. Exposure to second-hand cigarette smoke is also harmful to the lungs of AATD patients and the most important development in the area of lung health in Europe since the introduction of smokeless fuels has been the adoption in many countries of the ban on smoking in the workplace. After many decades of anti-smoking legislation and campaigning the Irish government implemented a law banning smoking in the workplace in 2004 (McElvaney 2004). This was the first law of its kind in Europe and paved the way for a host of other European countries to introduce similar laws (Rada 2010). The positive impact on Europe's lung health may not be significantly visible for several years, but already cigarette consumption in Ireland has dropped to 23.6% (Office of Tobacco Control, www.otc.ie) and this can only be a good thing for AATD individuals.

2.2.4.2 Vaccinations

Influenza and pneumococcal vaccinations are recommended for all individuals with AATD (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Campos et al., (2007) investigated the practice of vaccinations and respiratory outcomes in AATD individuals in the United States and showed over 80% of AATD individuals had received adequate influenza and pneumococcal vaccinations during the influenza season (Campos et al. 2008). However, there was no significant difference in severity or rate of exacerbations between vaccinated and unvaccinated individuals but the authors concluded that the vaccinated group may represent 'sicker' AATD individuals. Influenza and pneumococcal vaccinations in COPD patients are documented as being effective and beneficial for patients (Halpin 2004; Fromer and Cooper 2008) and therefore vaccinations remain highly recommended for AATD individuals (American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). Further studies are necessary to ascertain the benefits of vaccinations for AATD individuals, especially whether protection is afforded during the influenza season.

2.2.4.3 Exacerbation management

Exposure to bacterial and viral infections can potentially result in a respiratory exacerbation. Symptoms typically include increased dyspnoea, cough, and production of sputum (Hoogendoorn et al. 2010). Aggressive treatment of infections is recommended with antibiotics and specific treatments as symptomatically required as per ATS/ERS guidelines. This is particularly important as exacerbations adversely affect COPD patients and frequent exacerbations have been shown to be related to worsening health-related quality of life (HRQoL). Predictors for frequent exacerbations in COPD patients include symptoms of wheeze, cough and sputum production (Seemungal et al. 1998). Needham and Stockley (2005) investigated health status in AATD individuals over 12 months and recorded exacerbations, lung function and HRQoL. The authors concluded exacerbations occur commonly in AATD patients and correlate to worse health status. Exacerbations were associated with a decline in the gas transfer of the lung for carbon monoxide over time

(DLCO), but not FEV_1 (Needham and Stockley 2005). Interestingly, a study investigated exacerbation frequency in AATD patients with COPD who were receiving augmentation therapy and found subjects with frequent exacerbations had the worst baseline HRQoL scores, as well as more physician visits and hospitalizations. Unfortunately, AATD patients not receiving augmentation therapy were not included for comparison (Campos, Alazemi, Zhang, Wanner, Salathe, et al. 2009).

A recent longitudinal study by Campos et al., (2009) undertaken in the United States, evaluated the effectiveness of a disease management and prevention program for AATD individuals, involving 905 individuals, over a 2 year period. The program comprised of written educational material for self-study and individualised treatment plans for exacerbations. This study illustrated improved patient compliance in the use of bronchodilators, oxygen therapy, and steroids during exacerbations. The management program significantly reduced medical visits and showed a considerably slower deterioration of HRQoL during an exacerbation (Campos, Alazemi, Zhang, Wanner, and Sandhaus 2009). A follow-up study would be beneficial by providing additional evidence to evaluate the long-term benefits of an AATD disease management program.

2.2.4.4 Replacement therapy

Replacement therapy is a specific therapy for AATD, and the therapy comprises of intravenous administration of AAT derived from human plasma (Stoller and Aboussouan 2004). At present, this treatment is available in a number of European countries and the United States (Chapman et al. 2009). Some AATD individuals may be candidates for AAT replacement therapy; however, the efficacy of this treatment remains controversial (McCarthy and Dimitrov 2010). Uncertainty persists concerning the therapy's effectiveness and ongoing randomised clinical trials are being performed to definitively assess the efficacy of the treatment. Previous trials have been under-powered and have mostly shown only biochemical efficacy with AAT levels restored to above the putative threshold in the blood and lung. There is some evidence that augmentation therapy can slow lung function decline in patients with AAT deficiency, however, patients with moderate obstruction are most likely to benefit (Modrykamien and Stoller 2009). The therapy comprises of weekly or fortnightly intravenous infusion of an AAT preparation that augments existing levels of circulating AAT in the blood.

3. Alpha-1 antitrypsin deficiency (AATD) and COPD

3.1 COPD

The World Health Organisation definition of chronic obstructive pulmonary disease (COPD) is a lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and is not fully reversible. The more familiar terms 'chronic bronchitis' and 'emphysema' are still in common use, but are to be included within the umbrella term COPD. COPD is not simply a "smoker's cough" but an under-diagnosed, life-threatening lung disease. A diagnosis of COPD should be considered in any patient who has symptoms of cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease. Diagnosis is confirmed by spirometry, however, even with the ready availability of a simple test, COPD is largely under-diagnosed (Mannino and Braman 2007). Despite being a very common disease which affects 5% of the US population and the fourth

leading cause of death in the United States (Eisner et al. 2010), COPD often is a silent and unrecognised disease, particularly in its early phases (Mannino et al. 2000). Globally, COPD is a growing cause of mortality and will become the third biggest killer by 2020 according to the WHO (WHO 2004).

3.2 What we have learned about COPD from AATD

In the mid-1960s there were two major discoveries that led to an exponential increase in our knowledge of COPD and the development of the proteolytic hypothesis of lung disease. These were the discovery of AATD and its association with COPD, and the induction of emphysema by intratracheal instillation of a protease (Gross et al. 1965). While numerous different elastases were subsequently shown to cause emphysema in the same model, none of the elastases known at that time had access to the human lung. This changed in 1968 when a potent elastase was discovered within human neutrophilic leukocytes, the primary acute inflammatory white blood cell in the body (Janoff and Scherer 1968). Called neutrophil elastase or NE, it was found to be exquisitely sensitive to inhibition by AAT. It was then discovered that certain products of cigarette smoke were able to destroy the anti-elastase properties of AAT (Johnson and Travis 1979). While AAT is an excellent inhibitor of NE, the Met358 amino acid at its active site is easily oxidised by cigarette smoke and oxidants released by immune cells (Carp et al. 1982; Hubbard et al. 1987). On foot of these revelations, it was proposed in the 1970s that all COPD could be due, at least in part, to a deficiency of AAT (Gadek, Fells, and Crystal 1979). In the majority of individuals who develop COPD due to smoking, the deficiency is functional and due to the inactivation of AAT by cigarette smoke and the influx of inflammatory cells. In individuals with AATD, the deficiency is genetic.

3.3 The ZZ phenotype as a genetic risk factor for COPD

While smoking-related COPD is acquired, AATD is a form of inherited COPD and is responsible for approximately 1 – 3 % of COPD cases. A US study in 1986 investigated 965 COPD patients and found 1.9% were ZZ and over 8% were MZ (Lieberman, Winter, and Sastre 1986). Another US study investigated 969 patients with diagnosed with emphysema, asthma, or chronic bronchitis and found 1 ZZ case in every 31 samples, which is a case detection rate of over 3% (Brantly M 2003). Moreover, the contribution of SERPINA1 heterozygosity to COPD, while controversial, may account for over 10% of COPD cases if one includes ZZ, SZ and MZ phenotypes (Carroll et al. 2011). The classic pulmonary presentation of lung disease in AATD is severe, early onset panacinar emphysema with a basilar predominance in adults (Gishen et al. 1982). Evaluation of the lungs in ZZ individuals often shows diffuse destruction of the alveoli, first in the lower lung zones, and eventually throughout the entire lungs (Parr et al. 2004). This contrasts with the classic pattern of emphysema observed in smoking-related COPD, which is centrilobular (centriacinar) (Kim et al. 1991). However, emphysema in ZZ individuals may also occur in a diffuse distribution or predominantly in the upper lobes (Parr et al. 2004). Bronchiectasis, with or without accompanying emphysema, is less frequent (Parr et al. 2007). The most prominent early symptom is dyspnea, particularly upon exercise (McElvaney et al. 1997).

COPD is characterized by neutrophil-dominated airway inflammation and elevated protease levels in the lung (Abboud and Vimalanathan 2008). The main physiological role of AAT is to protect fragile alveolar lung tissue from attack by proteases, in particular

neutrophil elastase. NE is a powerful protease and can degrade most protein components of the extracellular matrix (Taggart et al. 2005), several complement proteins and immunoglobulins (Tosi, Zakem, and Berger 1990; Fick et al. 1984), antimicrobial proteins (Britigan et al. 1993; Hirche et al. 2004), and other antiproteases such as secretory leucoprotease inhibitor (SLPI) and elafin (Weldon et al. 2009; Guyot et al. 2008). NE can also induce mucin production and inflammatory gene expression in the lung (Fischer and Voynow 2002; Kohri, Ueki, and Nadel 2002; Carroll et al. 2005). In addition, there is the prospect that NE is situated at the apex of a hierarchical tree of cysteine and metalloproteases, acting as a master regulator of several classes of tissue-degrading proteases (Geraghty et al. 2007). The role of AAT in regulating NE activity *in vivo* is underscored by the fact that inhaled AAT therapy reduced MMP-2 and cathepsin B activity in lavage fluid from ZZ patients (Geraghty et al. 2008).

The traditional protease-antiprotease imbalance theory which explains COPD in ZZ individuals by a loss of function mechanism, while certainly attractive, is not the only explanation for the development of COPD. There are a host of gain of function effects caused by mutations within the SERPINA1 gene discussed in more detail elsewhere (Carroll, McElvaney, and Greene 2010; Greene and McElvaney 2010; Ekeowa, Marciniak, and Lomas 2011). Evidence is mounting to suggest other pathways contribute to tissue injury and inflammation. For example, rogue Z AAT protein can form polymers, and these polymers are present in the epithelial lining fluid of the lung. Polymers of Z AAT made by lung cells or reaching the lungs through the blood can cause the local release of chemokines and the recruitment of immune cells to the lung, contributing to the neutrophilic inflammation characteristic of COPD (Parmar et al. 2002; Mulgrew et al. 2004; Mahadeva et al. 2005). In addition, the expression of Z AAT in immune cells can lead to a more exuberant immune response. Monocytes from asymptomatic ZZ individuals with preserved lung function produced more pro-inflammatory cytokines and chemokines than MM individuals, and this inflammatory phenotype may explain some of the predisposition to COPD (Carroll et al. 2010).

Additional pathways leading to tissue injury highlighted recently include a role for AAT in apoptosis and in the regulation of chemotaxis. Wild-type AAT protein prevents lung alveolar endothelial cell apoptosis, possibly by inhibiting caspase-3 (Petrache, Fijalkowska, Medler, et al. 2006) and reducing oxidative stress (Petrache, Fijalkowska, Zhen, et al. 2006). These pro-survival benefits are lacking in ZZ individuals and could favour structural cell apoptosis and contribute to the development of emphysematous changes, particularly as the COPD lung is an environment with high levels of oxidative stress (Yao and Rahman 2011). A novel anti-inflammatory role for AAT as a "brake" on immune cell chemotaxis was also described. Wild-type AAT was shown to regulate neutrophil chemotaxis by both binding the chemokine IL-8 and preventing shedding of the immune receptor FcγRIIIb (Bergin et al. 2010).

The data accumulated unequivocally demonstrates that the ZZ phenotype is a major risk factor for COPD and this is thought to be mediated by at least four pathological mechanisms:

1. Increased protease activity in the lung,
2. Polymer formation locally,
3. Loss of anti-apoptotic properties of AAT,
4. Loss of anti-inflammatory properties of AAT.

3.4 The SZ phenotype as a genetic risk factor for COPD

Historically there has been a widespread acceptance that the SZ genotype confers increased susceptibility to COPD, particularly in smokers. This could explain why the susceptibility of SZ subjects to COPD has not been the subject of as many studies compared to MZ subjects. In addition, the typical SZ serum AAT level of approximately 11 μM is also deemed the putative protective threshold above which there is presumed to be no increased risk for emphysema in individuals with AATD and it is this level at which augmentation therapy levels are aimed (Wewers et al. 1987). Unfortunately, as a result there have been few studies aimed at assessing COPD risk in SZ individuals and most of these have been underpowered. The first study examined 25 cases, 14 of whom were index cases and concluded the SZ phenotype was of much less importance than the ZZ type in the development of emphysema (Hutchison, Tobin, and Cook 1983). Another study concluded that only a small percentage of SZ individuals are at increased risk of developing emphysema and that in non-smoking individuals the SZ phenotype conferred little or no added risk of developing COPD. However, it was noted that cigarette smoking correlated more strongly with airflow obstruction in SZ rather than ZZ subjects. Again this was a relatively small study of 59 individuals with no specific distinction between index and non-index subjects (Turino et al. 1996). In 1998 a Danish group investigated a cohort of 94 SZ individuals on the Danish AATD Registry and came to the same conclusion that a small proportion of SZ individuals are at increased risk of emphysema (Seersholm and Kok-Jensen 1998). A meta-analysis in 2005 examining COPD risk in the SZ group sought to shed some light on the issue and calculated that there was a three-fold elevated risk of COPD (Dahl et al. 2005). Unfortunately, due to the limited number of subjects with accurate smoking information, it was not possible to calculate separate odds ratios for SZ smokers and non-smokers. The most recent study was an audit of SZ individuals on the UK AATD registry. SZ subjects showed less emphysema on CT scans and less abnormal spirometry test results, but equivalent health status impairment compared to matched ZZ subjects (Holme and Stockley 2009). Like the MZ genotype, attempts to explain the risk of COPD in SZ subjects have stopped at the decreased AAT levels, and other pathological mechanisms have not been explored.

3.5 The MZ phenotype as a genetic risk factor for COPD

It is well established that MZ heterozygotes have moderately reduced serum levels of AAT, but whether they have an increased risk of COPD remains an area of some controversy. Over the last 40 years, over 100 studies have attempted to assess the risk of lung disease in MZ individuals with discordant and contentious results. A meta-analysis of 22 of these studies was published in 2004 (Hersh et al. 2004). Six of the 16 studies examining the categorical outcome of obstructive lung disease found significantly increased odds ratios (OR) for COPD in MZ heterozygotes compared to MM individuals. In nine other studies, the OR was increased, but not significantly. The individual study ORs ranged from 0.15 to 16.78. In summary, the study found that the OR for COPD in MZ compared to MM individuals was elevated at 2.31 (95% CI 1.60 to 3.35). Since this meta-analysis another US study has shown that MZ individuals exhibit accelerated decline in diffusing capacity of the lung for carbon monoxide (DLCO) in a large prospective population-based study of 1,075 individuals (Silva et al. 2008). More recently, a 2010 study

demonstrated that MZ heterozygosity was associated with airflow obstruction in two large populations (Sorheim et al. 2010). In general, studies comparing COPD cases with healthy controls have found an excess of MZ individuals among COPD cases, but many studies comparing FEV_1 (% predicted) in MZ and MM subjects from population-based samples have not found significant differences. There are several factors that undermine many of these conclusions. Firstly, a lack of correction for active and passive cigarette smoking exists in many studies. Secondly, the use of spirometry in defining lung disease is flawed, as subjects with normal spirometry values can have evidence of emphysema on high-resolution CT (Spaggiari et al. 2005). However, it is clear that the weight of evidence is now in favour of a risk of COPD in MZ individuals, but explanations of this risk are limited to the decreased antiprotease levels in subjects, and have not taken into account alternative mechanisms such as Z polymer generation. In the setting of disease management, an individual with smoking-related COPD who is informed of a diagnosis of MZ AATD may be further motivated towards smoking cessation (Carpenter et al. 2007).

3.6 Other AATD phenotypes as genetic risk factors for COPD

Early studies that examined the relatively common MS phenotype found no increase in COPD risk but an increase in bronchial hyperreactivity among MS individuals (Townley et al. 1990) but this was not replicated in a larger study (Miravitlles et al. 2002). In a recent meta-analysis of case-control and cross-sectional studies examining COPD risk in MS individuals a small but significantly increased risk was found (Dahl et al. 2005). However, after correction for smoking the MS phenotype was not associated with elevated risk for COPD. Moreover, studies that measured pulmonary function did not find a difference between MS and MM individuals. Another study did attempt to address the risk of COPD in SS individuals but found no increased risk of obstructive lung disease and was limited by small sample size (McGee et al. 2010). The effect of the I and F mutations on the AAT molecule has been described earlier, but to date any mention of COPD risk associated with these mutations is limited to case reports describing compound heterozygotes (Kelly et al. 1989; Baur and Bencze 1987).

3.7 Conclusion

It is well established that ZZ individuals have a high risk of developing COPD. However, MZ and SZ individuals also have significantly reduced levels of AAT, and are at risk of developing COPD. Anecdotally, a significant number of MZ and SZ individuals (both smokers and non-smokers) from our AATD clinic have severe COPD at a young age. The risk of COPD in heterozygotes has traditionally been explained by the weakening of the antiprotease shield in the lung. However, we know the Z mutation confers harmful gain of function properties on the AAT protein. While the ZZ genotype is relatively well-studied, there is little information regarding MZ, SZ, and other less common genotypes and to date there have been no investigations into the functional consequences of AATD heterozygosity. This is a vital clinical and public health question, as there are predicted to be over 6.6 million MZ and 230,000 SZ individuals in the US alone (de Serres, Blanco, and Fernandez-Bustillo 2010) and the total economic costs of COPD in the US were estimated to be almost $50 billion in 2010 (National Heart 2009). From a basic research perspective, a careful

examination of AATD heterozygosity may lead to a new appreciation of this understudied area and the development of new therapies.

4. Testing for alpha-1 antitrypsin deficiency (AATD)

4.1 Who should be tested?

Guidelines issued by both the World Health Organisation and the American Thoracic Society/European Respiratory Society (ATS/ERS) recommend the establishment of targeted screening programmes for the detection of patients with AATD (Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting 1997; American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. 2003). The biggest problem in the area of AATD is under-diagnosis with most cases misdiagnosed as COPD or non-responsive asthma. As a result, long delays between presentation of first symptoms and correct diagnosis are commonplace and prevent optimal management of the condition, despite education and awareness efforts (Stoller et al. 2005; Campos et al. 2005; Kohnlein, Janciauskiene, and Welte 2010). Compared to population-based studies, which are difficult and expensive to perform on a large scale, targeted detection programmes offer a much higher rate of AATD detection, are easier to perform, and are more cost-effective. However, as they target symptomatic groups the possibility of missing asymptomatic individuals remains. For this reason, comprehensive screening of family members of known AATD individuals is crucial as it offers the most realistic prospect of detecting asymptomatic relatives (Hogarth and Rachelefsky 2008). In the Irish targeted detection programme first-degree relatives of not only ZZ, but SZ and MZ individuals are recommended for testing.

Data from several countries suggests that less than 10% of individuals with severe AATD have been recognised clinically (Aboussouan and Stoller 2009), and improving detection rates is the most urgent issue in the coming years. Several barriers to testing for AATD in

ATS/ERS Recommendations for Diagnostic Testing
Adults with symptomatic emphysema or COPD
Adults with asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
Adults with necrotising panniculitis
Siblings of individuals with AATD
Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly

Table 2. ATS/ERS recommendations for diagnostic testing for AATD (type A recommendations).

the COPD population exist, including a fear of genetic discrimination, financial concerns, and privacy concerns (Stoller et al. 2007). Fears of genetic discrimination have been allayed in recent years with preventative legislation enacted in several countries, including Ireland and the US. Barriers to testing among physicians include lack of awareness and knowledge of AATD, lack of access to testing methods, and testing fatigue among physicians who do not encounter AATD initially and give up testing. An element of therapeutic nihilism can also exist, with the mistaken belief that identifying AATD in a COPD patient offers no immediate clinical benefit. Initiatives to increase detection rates might include automatic physician alerts suggesting AATD testing on pulmonary function test reports of patients with fixed airflow obstruction (Rahaghi et al. 2009), better medical and patient education in the area of AATD (Fromer 2010), and a red flag to recommend testing for AATD on laboratory reports of patient with low levels of AAT. The strategy of electronic prompting offers the greatest potential, and has been trialled in several regional hospital laboratories in Ireland to great effect.

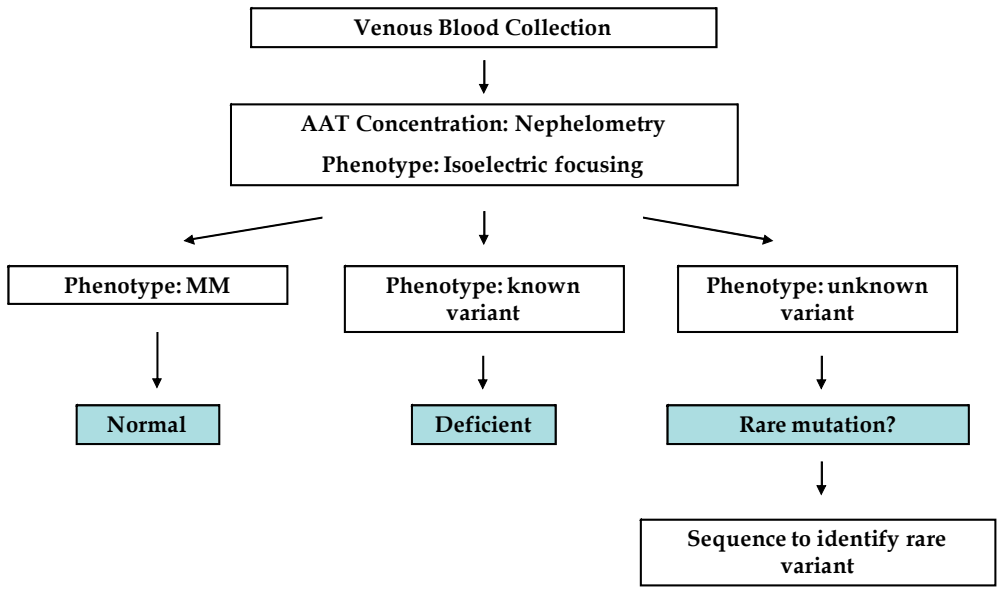

Fig. 7. Diagnostic algorithm for testing of whole blood utilised in the Irish National AATD Targeted Detection Programme (TDP). All IEF results are correlated to the quantification of serum AAT.

The advent of finger-prick tests using dried blood spots (DBS) as a source of DNA has allowed home testing for AATD, with easier transportation of samples to the laboratory (Costa et al. 2000). This method of testing eliminates the fear of needles for the patient, and is also cheaper as the test does not require a visit to a general practitioner. Identification of a deficient variant should be confirmed with serum or plasma AAT quantification, as genotyping of DBS sample can miss rarer alleles such as Mmalton (Rodriguez-Frias et al. 2011). For this reason, finger-prick kits are used in the Irish detection programme only for screening family members of index cases who possess Z or S alleles, with whole blood preferred as this allows identification of rare AAT variants. Several laboratories have developed methods of quantifying AAT from DBS which enhance the diagnostic options

available (Miravitlles et al. 2010). The traditional gold standard for the diagnosis of AATD has been phenotype analysis by isoelectric focusing but there has been a move in the last few years to a combination of genotyping and quantification (Snyder et al. 2006). Ultimately, while there are pros and cons to both methods of sampling, the decision to collect whole blood or DBS is often guided by cost considerations.

4.2 Quantification of AAT

The World Health Organization has recommended that AAT levels should be measured at least once in all COPD patients and this position was supported by the American Thoracic Society (ATS) and the European Respiratory Society (ERS). The most important consideration when quantifying AAT is the fact that, as an acute phase reactant, AAT can be markedly elevated during infection and inflammation. This is especially relevant if testing COPD patients during an exacerbation. While AAT levels in ZZ individuals are so low that any increase is marginal, circulating AAT levels in heterozygotes (both MZ and SZ) can be "falsely" elevated to levels similar to those observed in MM individuals (Fig. 8). A pronounced acute phase response in many individuals is observed in the MM, MS, and MZ groups; however, the acute phase response in SS, SZ, and ZZ groups is blunted. For this reason, quantification of AAT is no substitute for phenotype or genotype analysis, which is not influenced by the acute phase.

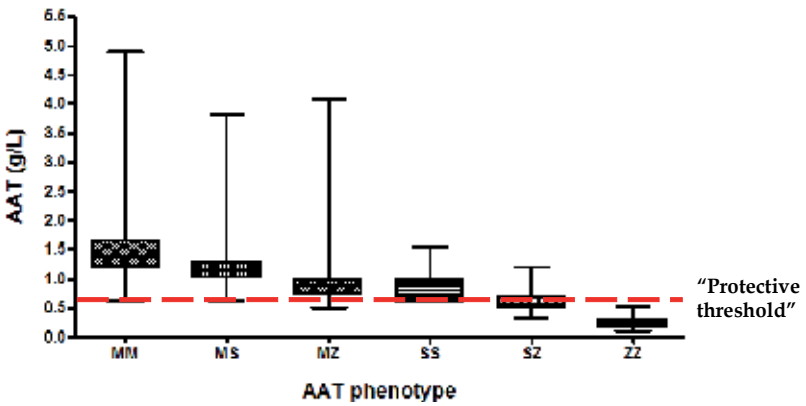

Fig. 8. AAT levels among the various phenotype classes identified in the Irish National AATD Targeted Detection Programme (TDP).

AAT levels are routinely measured by immunoassay techniques such as nephelometry and turbidimetry, or less commonly by radial immunodiffusion (RID) (Viedma et al. 1986). These methods are based on the use of a specific antibody which binds the AAT in a serum sample. Discrepancies can exist when comparing these methods for serum AAT quantification. For example, nephelometric methods can overestimate AAT concentrations due to haemoglobin or lipid interference, while RID-based methods have been shown to overestimate AAT concentrations by as much as 35 – 40% (Brantly et al. 1991) and are less precise than nephelometric methods with higher coefficients of variation (Alexander 1980). Moreover, the lower sensitivity inherent to the RID method because of the high lower limit of detection (0.33 g/L) becomes a factor when testing severely deficient ZZ individuals, as the majority have AAT concentrations < 0.33 g/L.

Phenotype	N	Mean AAT (g/L)	AAT range (g/L)
MM	3621	1.49 +/- 0.01	0.62 – 4.90
MS	568	1.21 +/- 0.02	0.62 – 3.82
MZ	802	0.89 +/- 0.01	0.50 – 4.08
SS	29	0.86 +/- 0.04	0.62 – 1.54
SZ	87	0.61 +/- 0.02	0.33 – 1.20
ZZ	90	0.24 +/- 0.01	0.11 – 0.52

Table 3. AAT phenotypes and serum AAT concentrations analysed as part of the Irish national targeted detection programme (TDP). Some unusually low AAT concentrations in the MM, MS, and MZ groups were measured in patients with chronic liver disease. Data presented as mean AAT (g/L) +/- standard error of the mean (SEM).

An important consideration in optimising screening programmes for AATD is what cut-off level of AAT is suitable to adopt, below which samples should be investigated further by phenotype or genotype analysis. The choice of cut-off level has obvious financial implications and can cause unnecessary anxiety in a patient if inappropriate follow-up testing is performed. Moreover, cut-off values do not apply when testing family members of known AATD individuals or when testing paediatric cases of liver disease. Phenotype or genotype analysis should be performed in these cases regardless of AAT concentration. This can depend on whether the screening programme aims to detect severely deficient individuals (for example ZZ, Z/Null, Z/Mmalton), or if the aim is to also detect MZ heterozygotes. For example, a cut-off of 1.0 g/L may confidently detect 100% of severely deficient AATD cases, but may miss some SZ cases and large percentage of MZ cases (Kok, Willems, and Drenth 2010). A Swiss-Italian study has shown that MZ individuals with a C-reactive protein (CRP) level of > 0.8 g/L had higher mean AAT concentrations than MZ individuals with lower CRP levels, reflecting the acute phase nature of AAT production (Zorzetto et al. 2008). The cautionary note in using CRP levels to correct for systemic inflammation and an acute phase response when measuring AAT is that CRP is also liver-derived. Thus, in patients with chronic liver disease the ability of the liver to produce either AAT or CRP, or both, may be severely impaired. The choice of cut-off for AAT will reflect the goals of the screening programme and depends on the cost of extra testing and budgetary constraints. If financially permitted, we would advocate the phenotypic or genotypic analysis of all COPD patients.

4.3 Phenotyping of AAT

In the manner of Laurell and Eriksson, it is possible to detect ZZ individuals by careful visual inspection of electrophoretic patterns on routine serum protein electrophoresis, particularly in ZZ subjects where the absence of the alpha-1 globulin band is so striking (Malfait, Gorus, and Sevens 1985). However, this diagnostic method is not guaranteed to detect all ZZ cases, and will not detect SZ and MZ phenotypes with any confidence (Slev et al. 2008). For this reason, the gold standard for the diagnosis of AATD is isoelectric focusing, which is based upon the isoelectric point of the AAT protein and separates the various isoforms of AAT based on their migration in a specific pH gradient. Each isoform migrates to the position within the pH gradient where the overall charge of the molecule is zero. Qualitative detection and characterisation of AAT variants is carried out in our laboratories by isoelectric focusing using the Hydrasys electrophoresis platform (Sebia) and the

Hydragel 18 A1AT isofocusing kit (Zerimech et al. 2008). This isoelectric focusing (IEF) method on agarose gel has an added immunofixation step which utilises a specific antibody to AAT, rendering it superior to traditional IEF techniques, and improving the resolution and reproducibility. The AAT phenotype is determined by visual inspection by at least two independent observers and by comparison with reference standards. There are some unusual cases where a phenotype analysis will not lead to correct diagnosis. M/Null, S/Null, and Z/Null individuals will appear as MM, SS, ZZ phenotypes, respectively, although genotyping will not detect Null mutations either. Another example is of a ZZ individual who has received a liver transplant (Hackbarth et al. 2010). This would result in an MM phenotype on serum analysis by IEF. The transplant recipient would have normal AAT levels as a result of the donor liver (presumably MM) but any offspring would still inherit the Z mutation.

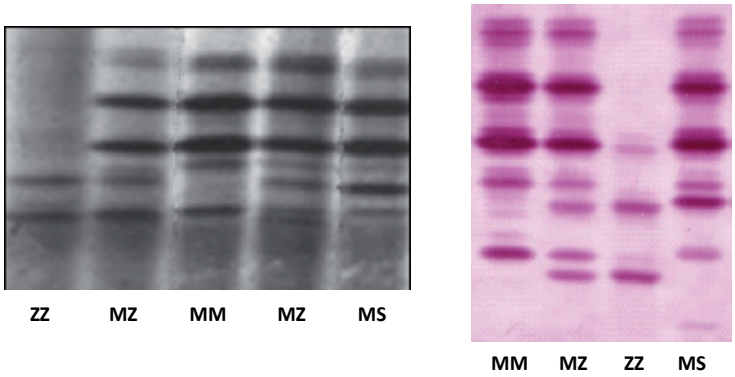

Fig. 9. Technique of isoelectric focusing by polyacrylamide gel electrophoresis (left) compared to isoelectric focusing by agarose gel electrophoresis followed by immunofixation (right).

A novel method for the simultaneous quantification of AAT and identification of the Z and S mutations in a single sample has been recently described which uses liquid chromatography/tandem mass spectrometry (Chen et al. 2011). This has the potential to combine quantification and phenotyping in a single step but a feasibility and cost analysis study is required, and rare AAT mutations are not detected.

4.4 Genotyping of AAT

Genotyping assays are typically performed using PCR-based restriction fragment length polymorphism (RFLP) analysis or by melting curve analysis on real-time PCR instruments with specific primers and probes designed for the Z and S mutations (Ferrarotti et al. 2004; Rodriguez et al. 2002). RFLP methods, although cheaper, are time-consuming and have been superseded by the faster and more efficient melting curve methods (Aslanidis, Nauck, and Schmitz 1999; Bartels et al. 2009).

The advantage of genotyping assays is that they allow the rapid screening of DNA collected by dried blood spots and are not as prone to errors in interpretation as the IEF method. The inherent limitation of the genotyping assay is that most laboratories include only primers for the deficient mutation. This means that in an assay for the Z mutation, a rare mutation will

be mistakenly classified as M, unless specific primers for M AAT are used. This rare mutation should of course be termed "non-Z" but this important distinction is not always made. To avoid any errors in diagnosis, any genotype result should be correlated to the AAT concentration. For example, a Null/Z individual will be classified MZ on a typical genotyping assay, however, consideration of the AAT concentration in this individual should prompt further investigation by sequencing.

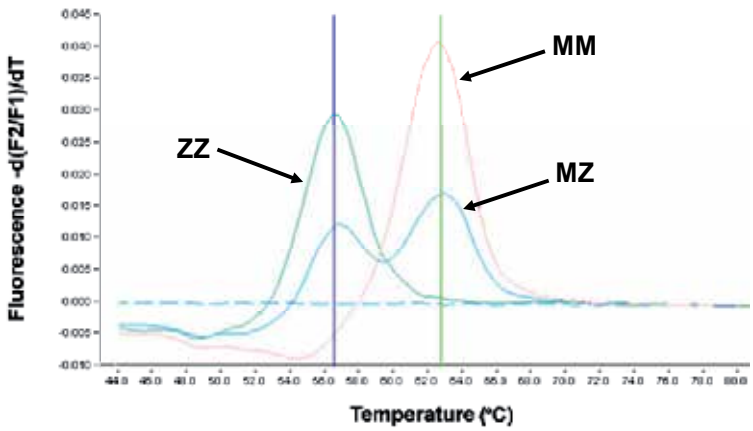

Fig. 10. Genotyping assay for the Z mutation by melting curve analysis on a real-time PCR system (Roche LightCycler 480).

5. Conclusion

The fact that cigarette smoking is often a coincident historical finding in the assessment of COPD has probably contributed to the remarkable global under-diagnosis of AATD. For example, of the 2,000 estimated ZZ individuals in Ireland, only 200 or 10% have been diagnosed (unpublished data). Unfortunately, COPD in a patient with a history of cigarette smoking is often assumed to be purely environmental and testing for AATD is not considered. This is one of a host of misconceptions that have surrounded AATD and prevented optimal management of AATD patients (Stoller and Aboussouan 2009). We strongly believe that increased testing for AATD is desperately needed in the COPD patient community, and stress once again the clinical guidelines that all patients with COPD should be screened for AATD.

The model for COPD diagnosis, assessment and management must include testing for AATD as one of the first steps. Unfortunately, AATD is often relegated to a footnote in many clinical guidelines for COPD. A summary of the ATS and ERS document outlining standards for the diagnosis and treatment of patients with COPD published in 2004 mentioned AATD once, stating that "patients presenting with airflow limitation at a relatively early age (4th or 5th decade) and particularly those with a family history of COPD should be tested for alpha-1 antitrypsin deficiency" (Celli and MacNee 2004). This narrow definition is damaging to efforts at identifying all cases of AATD. Large variability exists in the clinical course of lung disease in AATD and therefore all COPD patients should be tested for AATD, regardless of age or smoking history. Any management strategy for COPD must include testing for AATD. The under-diagnosis of AATD in the COPD population is a situation that cannot be allowed to continue.

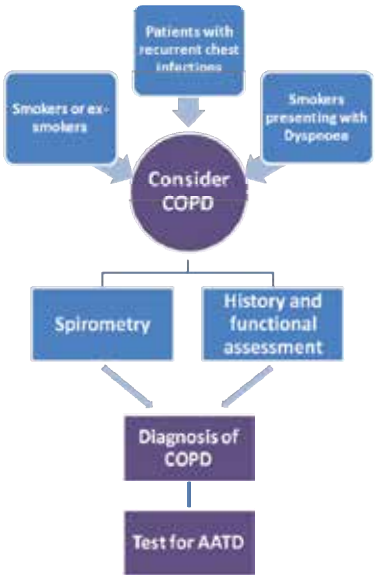

Fig. 11. Model for diagnosis of COPD and AATD.

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10

The Six-Minute Walk Test on the Treadmill

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1. Introduction

Human physical efficiency is shaped by several factors amongst which the functional condition of the heart, lungs and arterial circulation in lower limbs are regarded to be of key importance. Several tests are applied to evaluate human physical efficiency, however, the six-minute walk test (6MWT) deserves special attention due to its usefulness and simplicity. The test builds on the patient's walking capacity, i.e. the natural physical activity. Hence, the performance of the test does not require any individual preparation. The only and at the same time simple evaluation factor in the test is the distance covered within 6 minutes on a flat area. During the six minutes, the patient adjusts the pace of the walk to his or her fatigue, dyspnoea or pain in the lower limbs. According to the recommendations formulated by the American Thoracic Association in 2002, the test should be performed in subjects with at least moderate heart failure, respiratory failure or arterial circulatory failure in lower limbs.

The six-minute walk test is used by physicians as it enables the impartial evaluation of physical efficiency and treatment effectiveness. It also plays a prognostic role in evaluating the life span of patients with heart failure. It is easy to perform the test in a hallway. However, it is difficult to compare the results of the tests in various medical centres because they are performed in hallways which differ in length. In order to overcome this limitation, ATS 2002 guidelines state that the standard length of the hallway should be 30 m and its standard width – 3 m. The hallways in some medical centres comply with the set standards for the 6-minute walk test. However, even in these centres the test cannot be performed because the hallway has been designed for internal circulation purposes. Since several centres do not have a hallway with the required length, the six-minute walk test is performed in halls of non-standard length. Hence, a situation occurs when the right ATS guidelines cannot be complied with for technical reasons.

The idea to circumvent the limitation resulting from various hallway lengths was brought up during a visit to a cardiac rehabilitation centre with very well equipped physical efficiency laboratories in which the patient performing the six-minute walk test disappeared behind the hallway corner changing the direction at a right angle. This rather bizarre situation gave rise to the idea of doing the six-minute walk test in optimal conditions, i.e. in a physical efficiency laboratory, on the treadmills used there. After all, it is enough to adapt the speed of the

treadmill belt to the pace of the patient's walk by means of the appropriate program and sensors. In this way, a situation equivalent to a hallway walk will be reconstructed where the patient slows down or even stops if tired. The team of constructors from ITAM (Institute of Medical Technology and Equipment) in Zabrze started research into this idea. As a result of their work, a treadmill was constructed which adapts to the walking capacity of a patient suffering from chronic obstructive pulmonary disease (COPD), heart failure (HF) or arterial circulation failure in lower limbs. The algorithm designed to control the speed of the treadmill belt is based on precise, wireless determination of the patient's position on the belt.

The second part of the chapter demonstrates the results of the engineers' work on the construction of the treadmill in order to perform the walk test safely. The third part of the chapter contains the evaluation of adjustment of the treadmill to the walking pace of healthy volunteers, as well as a comparison of the distance covered during the 6-minute walk on the treadmill and in the hallway.

The obtained results demonstrating the advantages of the treadmill, have encouraged us to perform a 6MWT for patients with heart failure in the II-III NYHA classes (unpublished trial). The treadmill test was tolerated equally well by the patients as the hallway test. The fact that a similar distance was covered in both tests demonstrates that the technological barrier preventing us from obtaining credible results of the six-minute walk test on the treadmill, has been overcome. Thus, the possibility of performing 6MWT on the treadmill for patients suffering from obstructive airways disease, heart failure, or intermittent claudication, has appeared.

2. The treadmill controlled by a patient's walk

The American Thoracic Society (ATS) in the report issued in 2002, appreciates the advantages of the 6MWT on a treadmill as it saves space and allows constant monitoring during the exercise (ATS, 2002). However, ATS has not approved the use of a treadmill to determine the six-minute walking distance (6MWD) because so far, patients have been unable to pace themselves on an ordinary treadmill.

The popularity of the 6MWT in clinical practice (Stevens et al., 1999; Montogomery & Gardnem, 1998; Roul et al., 1998; Zugck et al., 2000; Rostagno et al.,2003), problems with the performance of the test on the treadmill, as well as the differences between the 6MWT in the hallway and on the treadmill encouraged us to develop a treadmill which applies the algorithm of the safe speed adjustment to the walking capacity of the patient. The purpose of our work was to construct a treadmill which enables the patients to move at their own pace during the walk, as well as to check if such a treadmill would be sufficient to perform the 6MWT.

The paper describes the reasons and a series of works which have led to the development of a new treadmill type adapted to the performance of the 6MWT. The new treadmill, which allows the patients to walk at their own pace, could be useful in rehabilitation, evaluation of physical efficiency, sport training and recreation.

2.1 Preliminary works

The common treadmill forced patients to adjust their walking speed to its belt speed so as to prevent them from being pushed off the belt. The new treadmill changes its belt speed with

the patient's changing walking speed. It is done quickly enough to keep the patient still on the treadmill. At the beginning, the preparation of such a treadmill seemed difficult for us and a different solution was chosen. We decided to combine the speed of the belt with the patient's position on the treadmill. When the patient is close to the front of the treadmill, the maximum speed of the belt is achieved, when he is close to the end of treadmill, the belt stops. While increasing the walking speed, the patient moves toward the front of the treadmill and the belt speed increases, when he slows down, the belt moves him backwards and the belt speed adjusts again. In order to realize such an algorithm, precise measurements of the patient's position on the treadmill are necessary. Already at the beginning, a decision was made to measure the position wirelessly because all the other methods were rather inconvenient.

First of all, the use of the ultrasound wave, reflected by the patient on the treadmill, was chosen to measure the distance from the patient to the front of the treadmill. Due to parasitical echoes from the other objects around, measurements were uncertain and the method turned out to be inconvenient. Because transmitting and receiving ultrasonic waves is simple and cheap in practice, we decided to continue using ultrasound after some modification. In the new method, the patient was carrying a transmitter which produced simultaneously a short impulse of ultrasound wave and infrared beam (about 100 milliseconds long). Both signals were received by the receiver at the front of the treadmill and the distance between the transmitter and receiver was calculated from the time delay between the received signals. Distance measurements turned out to be accurate (error less than 10 mm) and due to the shortest direct way of the ultrasound signal, parasitical echoes did not interfere with the measurements.

Fig. 1 shows the idea of the patient's position measurement using a mixed ultrasound/ infrared method. Carrying a transmitter seemed slightly uncomfortable for the patient, but

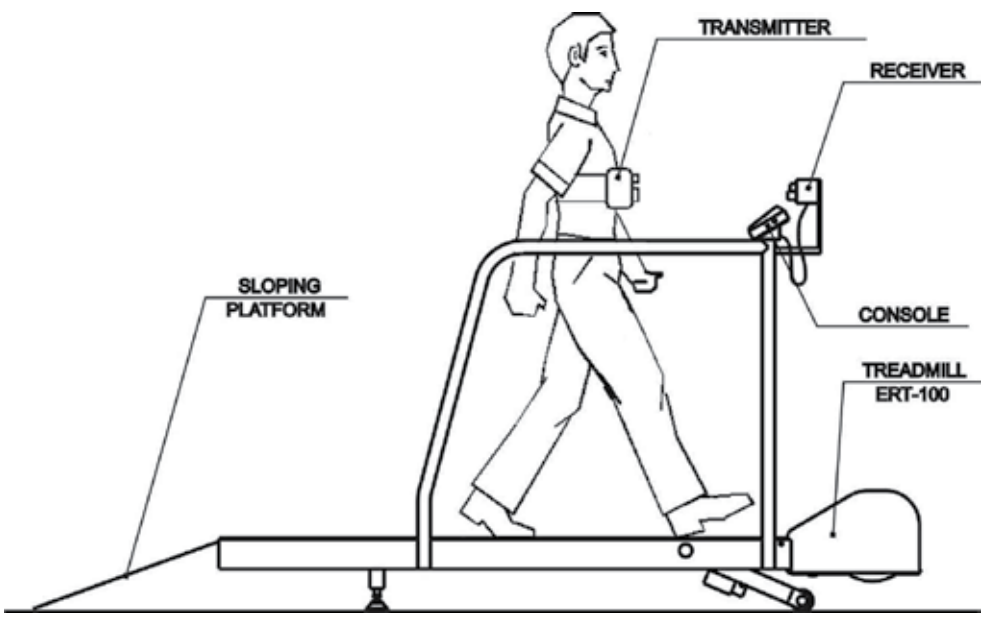

Fig. 1. Six-minute walk test on the treadmill

safe, because only the person carrying the transmitter could operate the treadmill. Additionally, an infrared beam was used to transmit the patient's heart rate (HR) displayed at the control panel of the treadmill.

As soon as the position measurement system with the transmitter and receiver was ready, we began to prepare the treadmill to control the speed. We adapted the treadmill ERT-100 constructed by ITAM, by connecting the receiver and introducing a new control program to its console. By using "the 6-minute walk test program" in the control panel, the operator could input the maximum speed of the treadmill belt and start the test. After 6 minutes from the start of the test program, the test came to an end and displayed the distance covered by the patient. The treadmill belt achieved its maximum speed when the distance between the receiver and transmitter was less than 30 cm and stopped when the distance was longer than 120 cm. Between those two distances, the speed was changing proportionately from 0 to the maximum value which could not exceed 10 km per hour. The ERT-100 treadmill, modified as described above, was examined by a group of 6 healthy volunteers, the employees of ITAM in Zabrze.

The volunteers carried out the 6MWT on the treadmill and in the hallway on two separate days, according to the protocol described in (Lipkin et al., 1986). Distances covered by the volunteers in both tests were similar and the participants found the treadmill test more comfortable than the hallway test. Although the results were satisfactory (Redelmeier et al., 1997), some drawbacks of the treadmill and control algorithm appeared:

- the treadmill ERT-100 was too short for such a control algorithm; an additional sloping platform was necessary for the patient's safety, the patient's rapid stop could be dangerous for him;
- the range of position change was narrow, which caused restless movement of the belt; at the same time, slight changes caused perceptible change of speed;
- the belt speed change ramp was too slow and the treadmill reacted too slowly to rapid changes of the patient's speed;
- the illusion of similarity to a hallway walk was partial, the patient always had to pay attention to maintaining the desired speed.

It was obvious that we could not change the treadmill to eliminate all of these disadvantages, so we had to prepare a new control algorithm instead.

2.2 Main results

A new idea of treadmill control is shown in Fig.2 as a block diagram. The distance measurement method has remained the same, however, the receiver has got some additional functions. As opposed to the former version, the console is used only for communication with the operator.

The treadmill control algorithm is realized by a microcontroller in the receiver, while the console transfers only the speed and slope signal to the treadmill.

The belt speed change ramp, previously programmed in the console, can now be changed as required for the test. We decided to return to the idea of a treadmill that changes its belt speed along with the patient's changing walking speed – one that does it quickly enough to keep the patient still on the treadmill. The microcontroller in the receiver maintains the

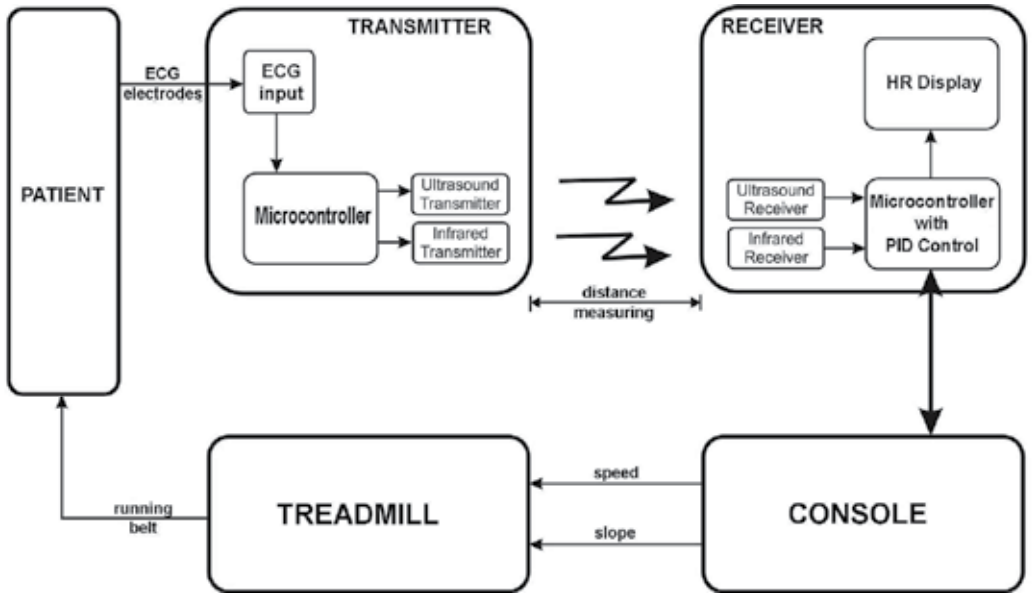

Fig. 2. Block diagram of a treadmill controlled by the patient's walk

patient's stable position on the treadmill regardless of the walking speed by means of a controller utilizing a PID algorithm (proportional – integral – derivative). The algorithm takes into consideration the maximum allowed belt speed set in the console by the operator and the maximum allowed distance from the receiver in which the belt stops. This distance lets the patient stop without a risk of falling off the treadmill. Fig. 3 shows a block diagram of the PID control algorithm.

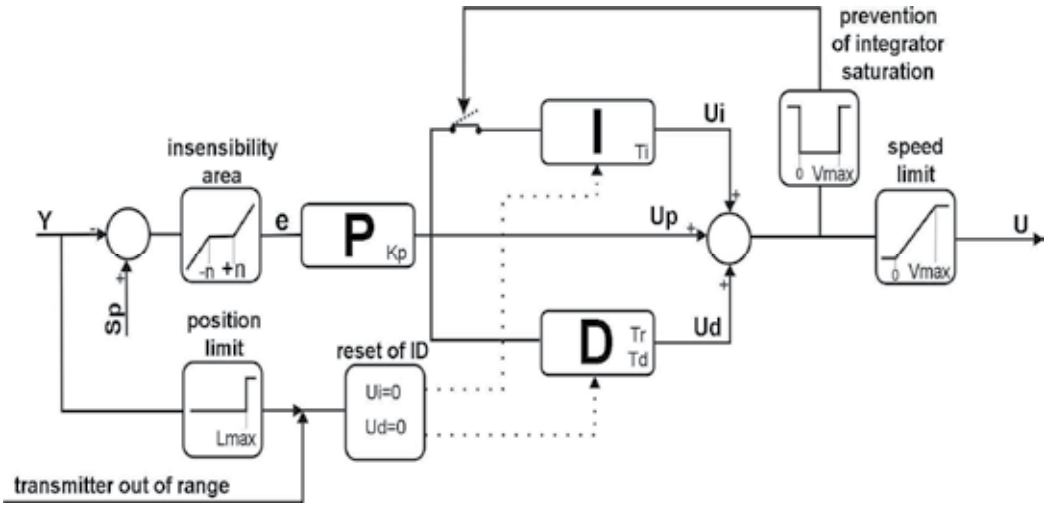

Fig. 3. PID controller algorithm

Several blocks have been added to the standard PID controller structure. First, the insensibility area block prevents the PID's operation when the difference between the

patient's position and the preset position is negligible. Second, the speed limit block prevents the patient from achieving belt speeds that could be dangerous. The limit is preset by the treadmill operator. Third, the position limit block stops the treadmill belt when the patient's position is outside the controlling range. The same happens if the transmitter signal is lost, e.g. when the transmitter is out of range or inoperative. Another block which prevents the integrator from saturation, stops integration when the belt speed is out of range. The last block resets the controller's integral and derivative blocks.

The above diagram uses the following symbols:

Y	–	the distance between the transmitter and receiver
S_p	–	the preset patient's position on the treadmill
U	–	the treadmill belt speed
E	–	error, difference between the preset position and current distance
K_p	–	the controller proportional gain
T_I	–	the controller integral time
T_r	–	the controller derivative time
T_d	–	the inertia time base

The equations which describe each part of the PID controller are as follows:

P: $Up(s) = k_p \cdot e(s)$ D: $U(s) = Up(s) + Ui(s) + Ud(s)$

I: $Ui(s) = k_p \cdot \frac{1}{s \cdot T_i} \cdot e(s)$ U: $Ud(s) = k_p \cdot \frac{s \cdot T_r}{1 + s \cdot T_d} \cdot e(s)$

The treadmill control using the PID controller described above has been simulated with the use of LabView software. The simulation enables us to specify rough parameters of the controller. Fig. 4 shows the LabView screen during a simulation of the treadmill belt speed control. A program for the PID controller was implemented, with constants specified during simulation, into the receiver's microcontroller and checked by volunteers walking on the treadmill at varying speeds. During the tests, the PID controller parameter was being tuned to achieve the best results.

The personnel performing the tests often had different subjective views on the best set of controller constants. Each person had his own favourite algorithm. The differences seemed to be negligible at the time, however, they will become more meaningful if there are differences between patients (due to age, incapacity, disability).

At the end of the test round, an additional test was carried out, using the treadmill's ability to change its elevation. During the 6MWT, after covering part of the route preset in the program, the treadmill changed its elevation in accordance with the value specified in the program. The test showed that elevation changes did not affect the performance of the test.

As a result, we have developed an algorithm that makes the 6-minute walk test on the treadmill much safer than at the beginning and very similar to a classic hallway test. It has allowed us to prepare a commercial version of the ERT-100 treadmill equipped with a transmitter and receiver. The treadmill has passed the CE certification procedure.

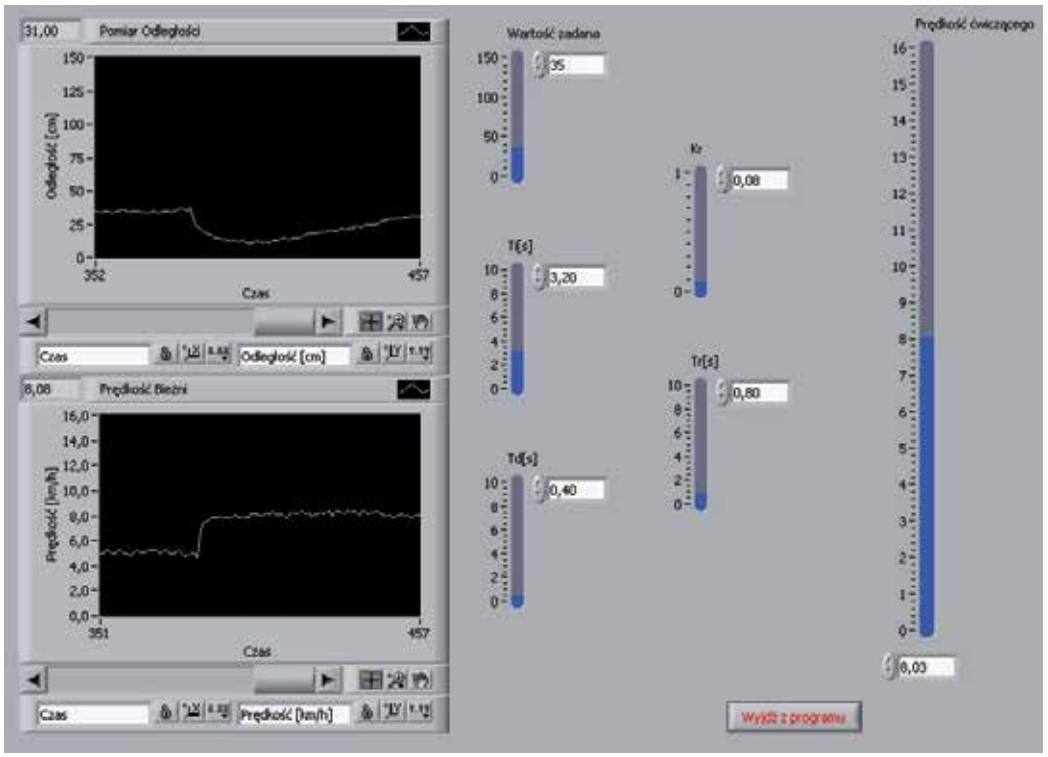

Fig. 4. LabView simulation of treadmill belt speed control

Probably the treadmills should be equipped with several algorithms, each of them focused on a different group of persons (elderly or young, fit or unfit). A treadmill with a CE certificate allows us to choose the best control algorithm for each group of people through research carried out on a large population.

The "6-minute walk test" is not the only application of the treadmill controlled by the patient's walk. It is easy to imagine many other tests based on the same principle, for example a "2-kilometer walk test" where the principle of 6MWT is inverted and the time elapsed after covering the distance is the final result. By adding elevation changes of the treadmill to the walk, we obtain a system which simulates a cross country test, unknown in medicine but well known in sport training.

ITAM is currently working on a new family of treadmills with new features and enhanced characteristics (e.g. enhanced dimensions, higher speed). The new treadmills will be equipped with an algorithm which controls the belt speed not only while the patient is walking, but also while he is running. Position measurements are essential if the treadmill is controlled by the patient's walk. Works are in progress on a new family of treadmills using other methods to determine the patient's position. The achieved results seem to be the same or even more promising than before.

3. A six-minute walk test on a special treadmill: Primary results in healthy volunteers

The guidelines approved by the American Thoracic Society (ATS) in 2002 definitely recognize the six-minute walk test (6MWT) as a useful tool for the evaluation of physical efficiency in individuals with at least moderate chronic obstructive pulmonary disease, heart failure and intermittent dysbasia (ATS, 2002). In order to compare the results obtained in various research centres, the guidelines recommend that the test be performed in a hallway 30 m in length and at least 3 m in width. As a result, some centres without hallways of this area have a limited possibility of carrying out this simple test.

The authors of the ATS report appreciate the advantages of the 6MWT on a treadmill as it saves space and allows constant monitoring during the exercise.

So far, ATS has not approved the use of a treadmill to determine the six-minute walking distance (6MWD) because patients are unable to pace themselves on a treadmill. The divergence between the distances covered on the treadmill and in the hallway was pointed out. To support this point of view, a study of patients with severe lung disease was presented where the mean distance walked on the treadmill was shorter by 14% when compared with the standard 6MWD using a 30 m hallway (Stevens et al, 1999). In particular, doubts were expressed regarding the wide range of differences, with patients walking between 120÷390 m on the treadmill and 360 m in the hallway.

The popularity of the 6MWT in clinical practice (Montogomery & Gardnem, 1998; Roul et al., 1998; Zugck et al., 2000; Rostagno et al., 2003; Lipkin et al., 1986; Redelmeier et al., 1997), problems with the performance of the test on the treadmill and our first positive results with the use of a modified treadmill adapting to the pace of the patient's walk, encouraged us to check if our version of the algorithm enables healthy volunteers to cover a similar walking distance both on the treadmill and in the hallway.

3.1 Methods

3.1.1 The population tested

29 healthy volunteers, full-time and extramural students of the Academy of Physical Education in Katowice, took part in a test. The volunteers were 28 years old (21÷48) on average. The order of taking the 6MWT on the treadmill and in the hallway was established at random. The tests with the use of both methods were performed at a 7-day interval.

3.1.2 The 6MWT in the hallway

For each individual, the 6MWT was performed along a corridor 22 m in length, according to Lipkin protocol (Lipkin et al., 1986). The participants were told to walk the distance of 22 m back and forth, at their own speed, in such a way that they would cover the longest possible distance within 6 minutes. The volunteers were allowed to slow down or stop, but at the end of the test they were expected to have the impression that they could not walk any further within 6 minutes.

3.1.3 The 6MWT on the treadmill

The software adjusting the ERT-100 treadmill belt speed to the patient's walking speed in the range of 1÷10 km per hour was applied for the six-minute walk test. The treadmill was

in a horizontal position and the belt speed was controlled by constant measurement of the patient's position on the treadmill.

After 6 minutes from the start of the test, the program ended the test and displayed the distance covered by the patient.

The test on the treadmill was preceded by a training session lasting a few minutes on the day before the actual test. During the training session the participant learned how the treadmill worked and walked a distance of 100 meters at a changeable pace, as well as practiced stopping and restarting the walk.

The participants were informed about the treadmill test in an identical way as about the hallway test.

3.1.4 Analyzed parameters

The comfort of the test and the distance covered in metres were subject to evaluation in both cases. The evaluation scale for comfort included the question of which type of test was less problematic during performance or whether the comfort of both tests was so similar that the differences were negligent. The number of indications to a given type of test was calculated. The treadmill was also monitored from the point of view of smooth speed adjustment to the individual's sudden slowdown without affecting his or her balance.

The pulse and blood pressure were measured before and after each test in order to assess the hemodynamic impact of both 6MWT varieties.

3.1.5 Statistical analysis

The aim of the statistical analysis was to compare the values of the distance covered, obtained in both 6MWT varieties. Also the heart rate and blood pressure before and after the test were compared using the Student's test for matched pairs for independent trials.

Multidimensional statistical research was conducted, as well the T2 test was applied for vectors of the expected values for both varieties in order to verify whether the compared research leads to similar hemodynamic consequences.

3.2 Results

The comfort of the treadmill test was indicated as better by 18/29 of the participants, the hallway test was indicated as better by 4/29 of the participants and both tests were evaluated as identical in terms of comfort by 7/29 of the participants.

During the test, healthy volunteers were walking most frequently with the speed of 7 km/hour (4÷10). The average distance covered on the treadmill was 683.0 m and was usually 57.1 m longer on average than in the hallway (Table 1). This difference turned out to be statistically significant. The participants covered 29 laps on average during the hallway test (19÷36).

No considerable difference could be seen in the heart rate before the tests. Also, the resulting accelerated heart rate after both types of tests did not show any marked difference (Table 2), just like blood pressure (Table 2).

Way of performing the 6MWT	Mean distance [m]	SD	Difference between the means [m]	P
Hallway	625.9	94.6	57.1	< 0.009
Treadmill	683.0	65.2		

Table 1. The distance covered during the six-minute walk test on the treadmill and in the hallway

	Before the test						After the test					
Way of performing the 6MWT	Hallway		Treadmill		Difference between the means	P	Hallway		Treadmill		Difference between the means	P
	Mean value	SD	Mean value	SD			Mean value	SD	Mean value	SD		
Heart rate [bpm]	76.9	12.9	81.4	13.3	4.5	<0.19	96.8	19.5	103.2	22.5	6.4	<0.25
Systolic pressure [mm Hg]	125.9	13.4	123.0	12.5	-2.9	<0.4	134.2	16.2	135.8	18.5	1.6	<0.73
Diastolic pressure [mm Hg]	81.4	7.8	80.2	8.0	-1.2	<0.57	83.7	11.8	82.6	9.6	-0.9	<0.71

Table 2. The heart rate and blood pressure before and after the test performed on the treadmill and in the hallway

The Hotteling T2 test was used to assess the equality of vectors of the expected values for seven analyzed parameters of the 6MWT.

The obtained result: T2=11.7 and F=7.3<<53 (where 53 stands for the threshold of the hypothesis at significance level <0.05) gives a clear evidence about the identical hemodynamic effects of both testing methods.

3.3 Discussion

The literature on the 6MWT does not provide any comparative material for our results obtained during a walk along a hallway 22 m in length performed by healthy individuals, 28 years of age on average. The mean distance covered by our volunteers amounting to 625.9 m may only be compared with the distance obtained by other researchers, walked by healthy subjects over 40 years of age. In the work by Enright et al. [11], the distance was equal to an average of 535 m, while in the work by Troosters (Troosters et al., 1999) – to 631 m. In comparison with the distance covered by the subjects in the study by Enright & Sherill (Enright & Sherill, 1998), our volunteers covered almost 100 m more. The recorded difference is very likely to be related to our volunteers' young age (29 on average). In the study by Troosters et al., the average distance in a 50-metre-long hallway was a few meters longer than that covered by our healthy volunteers who were two decades younger. Almost identical distances in completely different age groups may only be explained by the fact that older patients had to do two times fewer turnarounds in a 50-metre-long hallway.

ATS guidelines approved in 2002, which specify the length and width of the hallway, will certainly make it possible to compare the 6MWT results obtained in various centres. Another way of comparing the results obtained in various places is the proposed return to the idea of using the treadmill.

In our study most, i.e. 86% of the healthy volunteers who had participated in the test, evaluated the comfort of a treadmill test as better than or the same as the hallway test. Hence, the applied design solutions and algorithm may be regarded as appropriate and flexible in terms of adjusting the speed of the treadmill belt to the walking speed of a healthy individual.

In the paper by Stevens et al., the participants could put in motion, speed up and slow down the treadmill by means of a special switch (Stevens et al., 1999). Although Stevens et al. enabled the participants to adjust the treadmill to their walking speed "by hand", the distance covered on the treadmill turned out to be shorter indeed than the distance walked in the hallway because the participant could hardly adjust to the speed of the treadmill belt.

We applied a treadmill which adjusts its speed to the walking capacity of the individual. As a result, the persons taking part in the test covered a distance 57**.**1 meters longer on average than in the hallway. The distance covered on the treadmill is longer than that walked in the hallway both due to flexible adjustment of the treadmill belt to the walking speed and due to avoiding multiple turnarounds and hence the need to speed up and slow down in the hallway test.

The multi-aspect analysis of the results including the distance covered, blood pressure and pulse measured before and after the 6MWT, shows similar hemodynamic consequences for both methods. Thus, it can be inferred that in the future it will be possible to determine the conversion rate, at least for healthy subjects, facilitating the comparison of the results obtained during a test in a 30-m hallway with the results on an adjustable treadmill.

As opposed to conditions in the hallway, the 6MWT on a moving treadmill creates possibilities of easy monitoring of heart rate and arterial blood pressure. This enables the hemodynamic surveillance that is necessary for the safe test performance in patients with cardiac insufficiency.

The attempt by Stevens at al. to use the treadmill for a 6MWT in patients with respiratory failure did not meet the expectations as it shortened the distance in comparison with a hallway test. Our results show the expected elongation of the distance compared with the hallway test, although they cannot be currently referred to patients with intermittent dysbasia, heart failure or severe lung disease. Patients suffering from such diseases are less fit, which may affect their ability to perform the 6MWT on a modified adjustable treadmill. We are aware of the fact that the decision whether our modified treadmill meets the expectations of physicians who use the 6MWT in their medical practice and research can only be made after performing the tests in these groups of patients. In finished, but yet not published trial we assumed that the quality of the algorithm version adjusting the speed of the treadmill belt to the pace of the patient's walk during the six-minute walk test (6MWT) on a moving treadmill, checked for healthy volunteers, makes it possible to perform the test safely in patients with heart failure (Szczurek et al., 2006; Prochaczek et al., 2007).

The work was intended to compare the distance covered, the level of exertion and hemodynamic effects in a hallway test and in a test on a modified treadmill for patients with

heart failure in NYHA functional class II-III. Twenty people with diagnosed heart failure and tolerance of physical exercise in NYHA functional class II-III took part in the tests.

The analysis of the performance and results of the test indicates that during the six-minute walk test on a modified treadmill, HF patients in NYHA class II-III may slow the treadmill down safely or stop, depending on their exertion level. Our research has demonstrated that a walk test performed on a treadmill, controlled by means of the pace of the patient's walk, is equally well tolerated and generates exertion (Borg Scale 11,87 ± 2,90) that is similar as in case of the classic hallway test (Borg Scale 11,87 ± 2,90).

The fact that we have proved that there are no statistical differences in the distance covered (treadmill 317,36 ± 133,92, hallway 312,43 ± 117,76) and in the hemodynamic effects of the test performed on a treadmill compared to a hallway test, enables us to use a modified treadmill to replace the hallway test and vice versa, in order to evaluate patients with HF or chronic obstructive pulmonary disease. The fact that a similar distance was covered in both tests demonstrates that the technological barrier preventing us from obtaining credible results of the six-minute walk test on the treadmill, has been overcome. The availability of a treadmill adapting its pace to the patient's capacity, makes it possible to start treatment assessment or rehabilitation in patients with HF or COPD both inside and outside the hospital. If the results of our work are confirmed by other authors, the centres specializing in exercise tests will certainly be able to perform six-minute walk tests, while the hallways in hospitals and medical centres will be used for internal circulation purposes, as designed.

The modified treadmill providing solutions that are not offered by any other companies, may be additionally recommended for fitness purposes because the person using the treadmill may avoid dyspnoea by adjusting his or her walking speed.

4. Conclusions

The paper describes reasons and a series of works which have led to the development of a new treadmill type adapted to the performance of the 6MWT in patient with heart failure, chronic obstructive pulmonary disease or arterial circulation failure in lower limbs.

The common treadmill forces patients to adjust their walking speed to its belt speed. The new treadmill changes its belt speed while the patient changes walking speed. The preparation of such a treadmill needed to combine the speed of the belt with the patient's position on the treadmill. While the patient increasing the walking speed, the patient moves toward the front of the treadmill and the belt speed increases, when he slows down, the belt moves him backwards and the belt speed adjusts again. In order to realize such an algorithm, precise measurements of the patient's position on the treadmill are necessary. The idea of the patient's position measurement was based on using a mixed ultrasound/infrared method.

According to the new method, the patient was carrying a transmitter which produced simultaneously a short impulse of ultrasound wave and infrared beam (about 100 milliseconds long). Both signals were received by the receiver at the front of the treadmill and the distance between the transmitter and receiver was calculated from the time delay between the received signals. Distance measurements turned out to be accurate (error less than 10 mm) and due to the shortest direct way of the ultrasound signal, parasitical echoes

did not interfere with the measurements. As a result, we have developed an algorithm that makes the 6-minute walk test on the treadmill much safer than at the beginning and very similar to a classic hallway test. It has allowed us to prepare a commercial version of the ERT-100 treadmill equipped with a transmitter and receiver. The treadmill has passed the CE certification procedure.

The second part of the chapter contains the evaluation of adjustment of the new treadmill to the walking pace of healthy volunteers, as well as a comparison of the distance covered during the 6-minute walk on the treadmill and in the hallway. A better comfort of the 6MWT and a longer distance covered on the treadmill compared with the distance covered in the hallway may indicate that the algorithm of adjusting the speed of the treadmill to the walking capacity of the tested individual has been properly selected.

The obtained results demonstrating the advantages of the treadmill in healthy volunteers have encouraged us to perform a 6MWT for patients with heart failure in the II-III NYHA classes. In yet not published material it has been shown that the treadmill test was tolerated equally well by the patients as the hallway test. The fact that a similar distance was covered in both tests demonstrates that the technological barrier preventing us from obtaining credible results of the six-minute walk test on the treadmill, has been overcome.

The availability of a treadmill adapting its pace to the patient's capacity, makes it possible to start treatment assessment or rehabilitation in patients with HF or COPD both inside and outside the hospital.

5. Acknowledgements

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11

COPD Due to Sulfur Mustard (Mustard Lung)

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1. Introduction

Sulfur Mustard (SM) is a potent toxic alkylating agent that has been used as a chemical warfare gas during the World War I and in the Iran-Iraq conflict between 1983 and 1988(1). SM can cause serious organ damages especially ocular, neurologic, cutaneous, bone marrow, and pulmonary complications (1). The previous studies have shown that the respiratory complications are the most common late complications of SM toxic exposure including chronic obstructive pulmonary disease (COPD), chronic bronchitis, bronchiolitis oblitrans, bronchiectasis, airway hyperresponsiveness, and lung fibrosis (2-6).The COPD which occur after SM exposure is known as "Mustard lung" (7). Since about 45000 patients are now suffering from long term complications of SM toxic exposure, the evaluation of its pathogenesis and finding the possible ways for treatment is necessary. During the last decade, especial attention to the possible underlying mechanism of COPD due to SM intoxications has been applied. Our previous studies have shown that in COPD patients due to SM exposure inflammatory markers (highly sensitive CRP, interleukin 6) are elevated and these markers have direct association with the severity of disease (8,9). The finding which recommends the role of systemic inflammation in the pathogenesis of COPD due to SM intoxication like the COPD due to other causes. In this chapter the historical points, probable pathogenesis, clinical manifestation, and diagnosis of mustard lung will be discussed.

2. Historical background

Mustard gas was possibly developed as early as 1822 by César-Mansuete Desperetez (1798–1863) (10). SM was used as the late 1880 for treating minor tumors (11). Mustard gas is the most widely – used vesicant chemical war agent in the past century (2).Unfortunately SM was first employed effectively as a weapon in World War by the Germans on the British at Ypres (3, 11). It was then used during the Iran-Iraq war (1983-1988) and more than 100 000 military and civilian people were injured by SM gas (12).Now, over 45000 patients are suffering from the late complications of SM exposure (11, 12) and now SM is included as a threat to both military and civilians (13).

3. Sulfur mustard

SM or [bis-(2-chloroethyl) sulfate] (Fig.1) is also known as" Yperite" (Ypres was the name of the place which it was first used), "Lost" (the initials of two German chemists), and "yellow

cross" (it was a symbol means "skin damaging agent" during world war I) (11,12) .It is a colorless to yellowish- brown oily liquid at room temperature that converts to a gas with weapon system delivery (3,11).It has the odor of mustard, garlic, or onion (3).

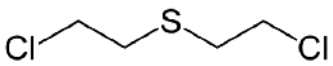

Fig. 1. Molecular structure of sulfur mustard

SM is absorbed by different ways: inhalation, through the skin, eyes, and gasterointestinal tract due to consumption of contaminated food (3).In large amount exposure, it can cause damages in rapid proliferating tissues particularly bone marrow (3).SM can cause different biochemical reactions and alterations in DNA structure (1).

4. Classification

Following the SM exposure, acute and late (long term) complications may occur. The long term complications of SM exposure are much serious than acute effects .Generally the long term effects of SM exposure occur several years after a mild contact and are totally different from continuous longtime exposure (mainly occupational exposure) (12, 13).Several studies in Iran demonstrated that the most common late complication are respiratory problems, including chronic obstructive pulmonary disease (COPD), chronic bronchitis, bronchiolitis obliterans, bronchiectasis, airway hyperreactivity, and lung fibrosis (3-6).Ophthalmologic and cutenous problems are also seen in this period. Unfortunately, the respiratory problems usually exacerbate over time (12).A unique form of COPD known as" Mustard lung" is frequently seen as a long term complication (7).

5. Pathogenesis

The exact mechanism of late pulmonary complication of SM exposure are not fully defined (14).Although the pathogenesis of COPD has completely determined and is mainly dependent on chronic inflammation and oxidative stress following activation of airway inflammatory cells, but there are few studies about the inflammatory basis of mustard lung (8,9,15).The pathological studies have shown that mustard lung is a neutrophilic / lymphocytic disorder (16,17).Also bronchiolar disease with varying degrees of inflammation as the main pathological finding, was demonstrated in a recent pathologic study in patients with sulfur mustard injury (18). According to the previous pathological studies, it seems that activation of inflammatory cells and generation of reactive oxygen species resulting in oxidative stress be involved (16, 17). It has been shown that decreased glutathione and increased serum malondialdehyde levels in mustard lung patients can be an indicator of oxidative-antioxidative system imbalance (16). The previous animal model studies have mentioned that the activation of inflammatory cells are involved in the pathogenesis of SM lung injury (19, 20).It is well documented that oxidative antioxidative imbalance may result in oxidative stress and triggering inflammatory process (21).

Despite the accepted role of inflammatory cytokines in acute pulmonary complications of SM, there are limited studies on the level of cytokines in the long term complications of SM

injury .Our recent studies (8,9) on 50 stable mustard lung patients with all stages according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) classification (Fig.2) (22),showed that despite the exclusion of smoking, cardiovascular diseases, infections, and other important inflammatory conditions, serum level of highly sensitive c reactive protein (hs-CRP) and interleukin 6 (IL-6), as inflammatory markers, are elevated in mustard lung patients in comparison of normal controls and are directly related to severity of COPD according to spirometry findings (Fig.3,4)(8,9). It is clearly documented that IL-6 has an important part in reduced forced expiratory volume in one second (FEV1), impaired functional capacity, and worsening the underlying inflammatory condition by release of acute phase proteins (23,24).Also the previous studies in COPD patients have shown that the serum hs-CRP is related to severity of airflow obstruction (25,26).

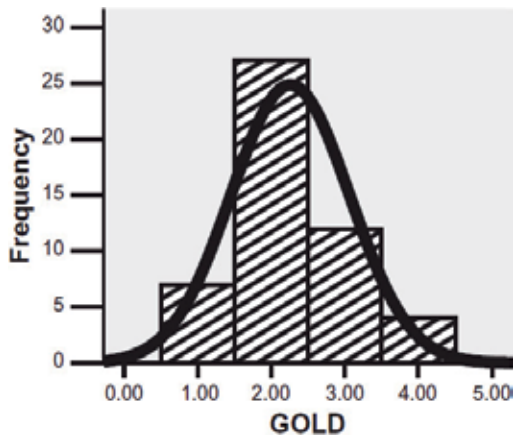

Fig. 2. The frequency of GOLD stages in mustard lung patients (9)

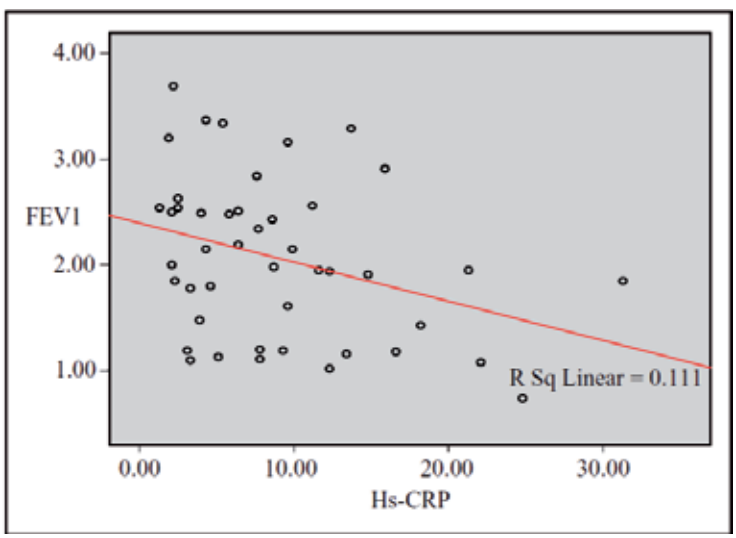

Fig. 3. The correlation of FEV1 and hs-CRP in mustard lung patients (8)

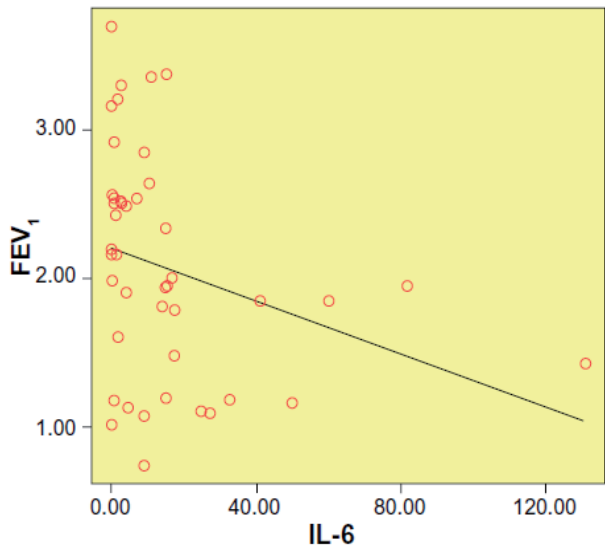

Fig. 4. The correlation of FEV1 and IL-6 in mustard lung patients (9)

Furthermore, the BODE (body mass index, obstruction, dyspnea, and exercise capacity) index, that is more reliable parameter of COPD morbidity and mortality, was significantly correlated with the serum IL-6 level (Fig 5) (9).

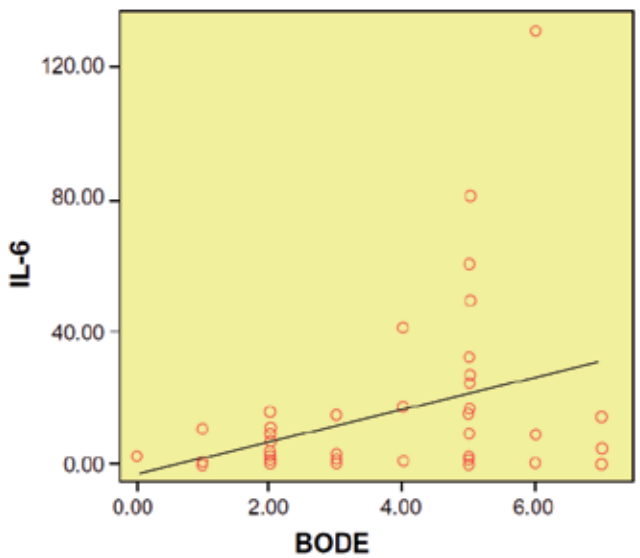

Fig. 5. The correlation of BODE index and serum IL-6 (9)

Additionally increased levels of IL-8 in the bronchoalveolar lavage fluid of patients with sulfur mustard poisoning and late pulmonary complications have been demonstrated (15) Despite these studies, few studies reported that inflammatory mediators probably do not have any major role in the pathogenesis and persistence of pulmonary complications of sulfur mustard exposure (27).

These findings provide evidence of the possibility of an inflammatory basis for the late pulmonary complications of sulfur mustard exposure and is in accordance with previous studies in other COPD patients which pointed out that, even during the stable phase of COPD, serum levels of inflammatory markers, including IL-6, may be raised (8,9)

6. Clinical manifestations

The most common compliant of mustard lung patients is chronic cough (12).A study showed that a triad of cough, expectoration and dyspnea was found in more than 80% of Iranian veterans 3 years after the exposure (1,28).

On physical examination, crackles, wheezing, and rhonchi depending to the state of the patient, can be seen (12, 29).The attacks of COPD exacerbation with increasing the severity of dyspnea, cough, and discoloration of sputum, is a common clinical presentation (12, 30).

The late pulmonary complications of SM injury may occur in patients who had not developed acute symptoms (12).A study on patients who did not have acute symptoms showed that 38% of these patients had air trapping on high resolution CT (HRCT) of chest (31).

Our previous studies on exercise tolerance of mustard lung patients have shown that the mean exercise capacity of these patients measured by 6 minute walk distance test (6MWD) has been decreased in comparison of normal population (8,9).Also the evaluation of quality of life in mustard lung patients showed significant impairment in this assessment by saint George respiratory questionnaire (SGRQ) (8,9,32).Additionally ,in our study the BODE (body mass index, obstruction, dyspnea, and exercise capacity) index had significant correlation with the serum level of inflammatory markers (8,9).

7. Diagnostic evaluation

Pulmonary function tests

Spirometry is a common diagnostic way for staging the severity of pulmonary impairment. Like to COPD due to other causes, generally an obstructive pattern is present in patients. A study showed that FEV1 is decreased at a rate of 50 ml/year in mustard lung patients (11, 33).

In body plethysmography, total lung capacity (TLC) and residual volume (RV) are markedly increased and Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) remains normal (11, 34).

Chest x-ray

Since the majority of mustard lung patients have normal or near normal chest x-ray (CXR) , some authors believe that CXR is not a reliable diagnostic imaging modality in these patients (12,35). Increased bronchovascular markings, and hyperinflation, pulmonary hypertension can be seen in CXR (12, 36).

HRCT

Chest HRCT has became a imaging modality of choice in SM patients (12).Air trapping and airway abnormal wall thickening are the most common HRCT finding (12,37).

Treatment

Unfortunately, there are no cure for mustard lung disease (11).Bronchodilators, inhaled steroids, long- term oxygen therapy, and pulmonary rehabilitation are different therapeutical strategies which are used in these patients (11).The combination of long – acting beta agonists (LABAs) and inhaled steroids has been shown to be effective (11,38,39).

In mustard lung patients, systemic steroid is only recommended during exacerbations (11).The prolonged use of systemic steroids should be avoided because of severe complication (39).

As we mentioned earlier, the oxidative – antioxidative imbalance may be an underlying mechanism in mustard lung patients. So the potent antioxidant agents are tried for this purpose. N-Acetylcysteine is an antioixidat and mucolytic drug that in double- blind clinical trial, improved dyspnea, cough, and sputum after 4 months of treatment (11,40).

8. Conclusion

SM can cause serious late pulmonary complications. A unique form of COPD, known as mustard lung, is frequently encountered in patients .Systemic inflammation may be involved in pathogenesis of mustard lung. Unfortunately there is no cure for these patients.

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12

Chronic Obstructive Pulmonary Disease and Diabetes *Mellitus*

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1. Introduction

The progressive increase in the average age of the population leads to chronic diseases that are increasingly important. Chronic conditions are large in number, the prevalence of each one is high and so does the annual cost of their care. Moreover, clinicians alert about the impact of one disease on the development and severity of others. Among chronic morbidities the most prevalent are cardiovascular disease (CV), cancer, diabetes *mellitus* (DM) and chronic obstructive pulmonary disease (COPD) (Chillón et al., 2009). Noticeably, a 25% of patients older than 65 years have two chronic conditions and this figure rises to 40% in population over 75 years old (Chatila et al., 2008).

The following text focuses on two of these pathologies: COPD and DM. Our group provided data on COPD pathophysiology, particularly about hypoxia and related oxidative stress, the effect of nutritional status, physical exercise and sleep disorders (Álvarez-Sala R, 2010; García-Río et al., 2009, 2011; Braghiroli & Álvarez-Sala, 2010; Alcolea et al., 2007). In addition, the sleep apnea hypopnea syndrome (SAHS), its association with metabolic syndrome (MS) constituents and the sum of SAHS plus COPD in the so called "overlap syndrome" were studied (Santiago-Recuerda et al., 2007; De Miguel et al., 2002). We made a search in PubMed including articles published during the last ten years about COPD and DM, in order to review how one disease influences the onset, evolution, treatment and prognosis of the other one.

2. Chronic obstructive pulmonar disease and diabetes *mellitus* definitions, epidemiology and comorbidities

COPD is defined as a preventable and treatable entity caused by toxic gases, mainly tobacco. Its main feature is poorly reversible obstruction of airflow that is progressive and is associated with a systemic inflammatory response (Álvarez-Sala, 2010). This proinflammatory state may lead to extrapulmonary manifestations (Global initiative for obstructive lung disease [GOLD], 2008) in the majority of patients have a negative effect on the overall prognosis of the disease (Peces Barba et al., 2008). Its prevalence sharply

increases with age and tobacco consumption, and is estimated at around 4-10% globally (Mathers, 2008). Nowadays this disease is considered the fourth leading cause of death and the WHO assessed that in 2020 ranked third in terms of mortality and the fourth in prevalence. In Spain there have been two major studies on the prevalence of COPD. On the one hand we have the IBERPOC study that estimated a 9.1% in patients aged between 40 and 70 years. Most recently EPI-SCAN obtained a 10.2% in subjects between 40 and 80 years (Álvarez-Sala, 2010). In recent years, COPD is considered a disease that goes beyond the lungs involvement. The high morbidity associated with this condition makes some authors (Álvarez- Sala,2010; Sevenoaks et al., 2006; Oudijk et al., 2003) think that pulmonary disease is just an expression of a multisystemic inflamatory disease. The main comorbidities associated with COPD are diabetes, hypertension, ischemic heart disease and heart failure. In addition, other illnesses converge such as malnutrition, osteoporosis, anemia, endocrine disorders, depression or anxiety (Moussas et al., 2008). Most of the comorbidities influence prognosis and length of hospital stay for these patients. One example is low weight, defined as a body mass index (BMI) below 18.5 kg/m^2, and considered a predictor of poor prognosis in patients with COPD. In particular, loss of muscle compartment is the most affected in the body and its measurement is a better predictor of mortality than total body weight. Another important aspect is osteoporosis, which is present in up to 68% of patients with severe COPD, with a consequent increase in fracture risk. There are several risk factors that may influence the development of osteoporosis in these patients: age, malnutrition, weight loss, smoking, hypogonadism, sedentary lifestyle or the use of glucocorticoids.

In addition, patients with more severe lung disease, have endocrine alterations, the most frequent is exogenous hypercorticism that associated with hyperglycemia, infections and cardiovascular complications (Chillón et al., 2009).

With reference to DM, it is a frequent consequence of corticosteroid therapy in individuals with advanced COPD and those receiving high and continued doses. However, coincidence with primary diabetes predominates in COPD patients, even if we assume there is no linkage between both diseases. There are two types of primary diabetes, type 1 is characterized by absolute insulin deficiency secondary to an autoimmune cause in 90% of cases or idiopathic destruction of pancreatic beta cells. These patients require insulin to survive. Type 2 DM is far more frequent in COPD. The natural history of type 2 begins with insulin resistance with a compensatory hyperinsulinemia that maintains normal glucose tolerance at the outset. Persistent insulin resistance facilitates the final expression of a latent β cell dysfunction thus resulting in hyperglycemia and frank diabetes. Diabetes has become one of the most prevalent health problems in recent years, according to some authors, affect over 366 million people worldwide in 2030 (Wild et al., 2004).

3. Links between chronic obstructive pulmonary disease and diabetes *mellitus*

At this point, the question arises about the relationship between both disorders. To answer this question, we will refer to the so called cardiovascular risk of COPD. COPD and DM are associated with an enhanced cardiovascular risk profile. COPD patients have a two to three-fold cardiovascular related mortality when compared to the general population rates. Cardiovascular disease is the second cause of death among COPD patients and the first one

among patients with DM. COPD predisposes to pulmonary hypertension, right ventricular dysfunction and arrhythmias. DM is often accompanied by systemic hypertension, left ventricular dysfunction and congestive heart failure. Carotid and peripheral atherosclerosis are also macrovascular complications of DM. Finally, both COPD and DM converge in a higher occurrence of coronary events and sudden death (Falk et al., 2008).

Probably all these comorbidities are influenced by the inflammatory and oxidative stress in these patients after exposure to tobacco (Lavi S et al., 2007). One hundred million people will be affected by tobacco during the XXI century. The tobacco is currently responsible for five million and six hundred thousand deaths each year worldwide. It acts synergistically with other risk factors and may increase cardiovascular mortality by 20, but after leaving tobacco for two or three years, the risk is superimposed to non-smokers.

The prognostic significance of hyperglycemia in these patients has been evaluated in several studies, especially during exacerbations. It seems that the poor glycemic control increases hospital stay, the isolation of gram-negative bacteria in sputum, increased pulmonary artery pressure and the risk of death (Archer & Baker, 2009; Gudmundsson et al., 2006; Makarevich et al., 2007; Sicras et al., 2007; Parappil et al., 2010). Moreover, it seems that sustained hyperglycemia may have other effects that worsen the prognosis. The vascular damage should be highlighted in the first place. Microvascular diabetic disease may affect the alveolus-capillary barrier. Pulmonary microvascular involvement may worsen respiratory function in patients with COPD and DM. Pulmonary diffusing capacity in patients with type 1 or type 2 DM is decreased and this decrease may be more pronounced in those with other microvascular complications.

It is known that early diagnosis and treatment of COPD and its comorbidities, including DM, have prognostic implications. However, the association and interactions between COPD and DM are not completely understood. Under the current evidence, coincidence is more plausible than a causative connection. Whether causality exists or not, the high rate of simultaneity in general population will give ground for concern. We consider that DM affects 1.6 to 16% among subjects with COPD. DM prevalence increases in relation with pulmonary impairment, older age and BMI of 30 kg/m2 (Lavi et al, 2007).

Pathogenic links between COPD and DM have been hypothesized in the setting of population-based and clinical observational studies. The Atherosclerosis Risk Assessment in Communities (ARIC) and the Fremantle Diabetes Study (FDS) found a lung vital capacity declining in persons with type 2 diabetes (Yeh et al., 2008; Davis et al., 2004). Lung dysfunction was predominantly restrictive, while COPD is an obstructive disorder. Excessive weight could be an explanation as mean BMI of diabetic patients (30.9 ± 5.7 kg/m^2) significantly exceeded BMI of the non diabetic group (27.2 ± 4.8 kg/m^2) in the ARIC study. DM patients who subsequently developed COPD also had a higher BMI in data by Ehrlich et al. (Ehrlich et al., 2010). A theoretical risk for COPD in a diabetic environment is based on several mechanisms: glycation of proteins of lung parenchyma and bronchial tree, thickening of basal lamina, increased susceptibility to infections and a modified sarcolema with subsequent skeletal muscle weakness (Weynand et al., 1999; Dalquen, 1999). Nevertheless, hyperglycemia has mostly been associated with a modest restrictive defect due to diabetic microangiopathy that thickens the epithelial and capillary basement membrane. The result is an increased extracellular matrix and connective tissue and an altered alveolar diffusion capacity of the lungs (Popov & Simioescu, 1997).

Conversely, development of DM once COPD has been diagnosed was also shown by Mannino et al. (Ford & Mannino, 2004; Mannino et al., 2008). Again, more than 60% of patients with COPD and DM were overweight or obese. Stronger evidence of the COPD-DM association comes from the Nurses Health Study (NHS) that involved 97,245 30-55 year old female nurses, 1,342 of whom reported COPD (Rana et al., 2004). The risk of DM among COPD patients was statistically significant (RR 1.8, 95%CI 1.1-2.8) despite the scarce number of incident diabetes cases (n = 19) and after exhaustive adjustment for covariates. It has to be said that a detection bias can not be ruled out in NHS and other cited studies. Besides, among other limitations, data from NHS could only be generalized to median-age Caucasian women. Nevertheless, this study provides the best evidence available due to the homogeneous anthropometry and lifestyle habits of the nurses enrolled including smoking, dietary and exercise, and because of the long-term prospective follow-up.

Beyond diabetes itself, glycemic exposure seems to be relevant. Severity of hyperglycemia was a negative predictor of a reduced lung volume in the FDS. With reference to COPD, a complementary analyses by Ehrlich et al. (Ehrlich et al., 2010) showed the disease was more prevalent among poorly controlled diabetic patients, with an hazard ratio of 1.03 (95%CI 1.01-1.04) per each unit increase in baseline glycated hemoglobin (A1C). To the date, diabetes has not been proven to be a determinant factor for COPD exacerbations, but poor glycemic control is a risk factor of pneumonia related hospitalization in type 1 and type 2 diabetes *mellitus* (Kornum., 2008). Consistent with this findings, in vitro studies under hyperglycemic conditions have shown an abnormal neutrophil function such impaired chemotaxis, phagocytes and bacterial killing (Pozzilli, 1994, as cited in Ehrlich et al., 2010).

We can assume there is a high proportion of undiagnosed glucose intolerance, obstructive and restrictive lung disorders. Thus, one possibility is that untreated diabetes contributes to pulmonary dysfunction and that non diagnosed decreased lung function favors diabetes development in predisposed patients (Davis et al., 2004). Once diabetes is evident, a vital capacity loss was found in the ARIC study. An 8% different FVC in diabetic compared to nondiabetic subjects was found in the Copenhagen City Heart Study (Heindl et al., 2001). The baseline difference was similar in ARIC, but further declining linked to diabetes was not found after 15 years of follow-up. In contrast, more rapid declines of FVC and FEV1 were observed in patients with higher baseline A1C in FDS. Tobacco may contribute to explain these differences. A secondary analysis of diabetic individuals in the Framingham Cohort Study found that the decrease in pulmonary function, with a restrictive pattern, was greater in smokers than in never smokers, inferring that diabetes may increase susceptibility to the adverse pulmonary effects of smoking. A similar interaction was proposed in the NHS (Rana et al., 2004; Walter et al., 2003).

To add complexity, sleep apnea hypopnea syndrome is often added in many of diabetic patients. SAHS is mainly secondary to obesity and is also associated with an increased insulin resistance. There have been several studies linking SAHS and DM. This relationship could be based on a common point such as obesity. In this sense, members of the Wisconsin Sleep Cohort were followed for four years. It was demonstrated that patients with an AHI ≥ 15 had an increased risk of developing diabetes type 2 (odds ratio 2.3 [1.28 to 4 11], adjusted for age, gender and body habitus) (Watz et al., 2009). In the same line, longitudinal follow-up of the cohort of Affairs Connecticut Healthcare System Veteran concluded an independent association between SAHS and incidence of new cases of diabetes type 2

(hazard ratio: 1.43 [1.10 to 1.86], adjusted for age, gender, race, fasting glucose, BMI and weight change) (Reichmuth et al., 2005).

A further step would be the association of COPD and SAHS in the same individual or "overlap syndrome". The prevalence of overlap varies depending on SAHS clinical or subclinical definition. The latter identifies individuals with at least 5 hypopneas or apneas per hour during a polysomnography or polygraphy whom diurnal sleepiness does not necessarily occur. The prevalence of SAHS is estimated to be 1-4% in general population. The percentage of overlap is 3-11% among subjects with SAHS and 16-20% among COPD patients (Owens & Malhotra, 2010; Zamarrón et al., 2008). COPD clinics is characterized by cough, sputum production and dyspnea. Most common symptoms of SAHS include loud snoring, excessive daytime sleepiness, personality changes and deterioration of quality of life. Overlap syndrome is characterized by older, more hypoxemic and hypercapnic patients with higher mean pulmonary pressure and similar or less BMI as compared with single SAHS. Thus, the overlap syndrome is a singular entity that may allow a deeper knowledge of the interactions between COPD, SAHS and glycemic-metabolic related disruptions.

4. Chronic obstructive pulmonary disease and diabetes *mellitus* related pathogenesis

COPD and DM share relevant features in their genesis and course. Hypoxia, insulin resistance, oxidative stress and inflammation are the basis of a common pathogenesis. Concomitant factors such as tobacco, obesity and sleep disorders merge in endothelial dysfunction and atherosclerosis leading to a high cardiovascular risk of both conditions (Figure 1).

Inflammation is a well recognized phenomenon in COPD and DM pathogenesis. In COPD, inflammation and oxidative stress require an energy expenditure that exacerbate the pre-existing hypoxia. In a parallel way, inflammatory cytokines exacerbate insulin resistance through diverse mechanisms. Impaired function of the type 1 insulin receptor substrate (IRS-1) is a key, direct mechanism. Thus, there is a chronic, subclinical inflammation at the background of COPD and DM. The question about its significance in patients with simultaneous COPD and DM is then arised. Being not fully clarified, we propose the following sequence of events: common COPD and DM related pathogenesis would start by hypoxia and insulin resistance followed by systemic inflammation, oxidative stress and a final coexistence of endothelial dysfunction and subsequent cardiovascular events.

4.1 Hypoxia

Hypoxemia and also hypercapnia, though in a less extent, are a stimulus for the hyperactivation of the sympathetic nervous system. In this setting, the activity of the sympathetic system is sustained in a non-resting anomalous way (Ashley et al., 2010; Heindl et al., 2001; Raupauch et al., 2008). Sustained hypoxia in COPD is an important central sympathetic system drive. A higher and long-lasting muscle sympathetic nerve activity (MSNA) is seen in COPD patients. Its direct consequence is a permanent vasoconstriction of the muscle vessels. Ashley et al. did not only show a sympathetic burst of multiple neurones, but they also graded the intensity of the response. The method used was the measurement of the firing probability and mean firing rates of single muscle vasoconstrictor

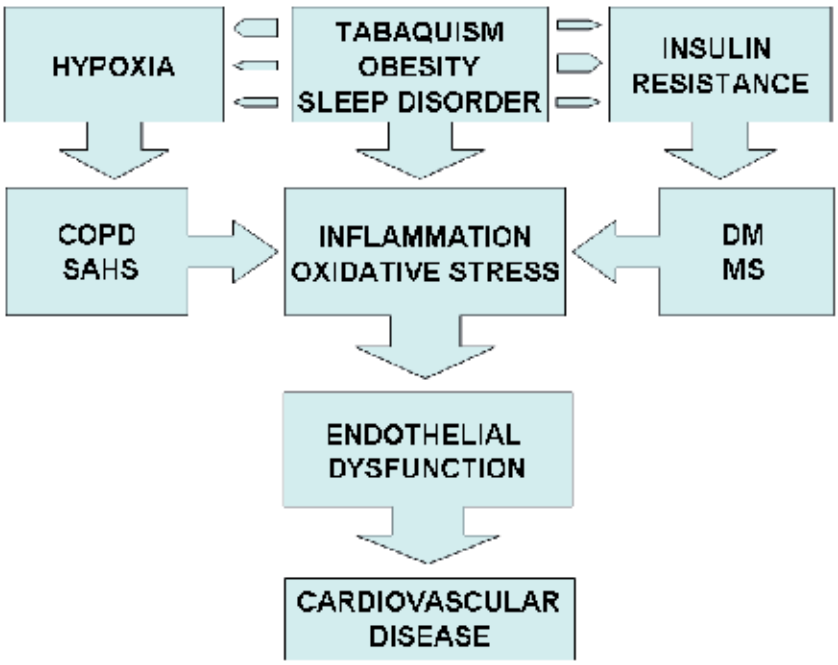

Fig. 1. Convergent pahogenesis of chronic obstructive pulmonary disorders, diabetes *mellitus* and metabolic syndrome. COPD: Chronic obstructive pulmonary disease; SAHS: Sleep apnea-hypopnea syndrome; DM: Diabetes *mellitus*; MS: Metabolic syndrome.

neurons. These authors observed a general and markedly higher sympathoexcitation in COPD patients when compared to SAHS, bronchiectasis or healthy subjects. The individual neurone firing probability and mean firing rate were comparable to those recorded in SAHS, but higher than those observed in the healthy group. This finding suggests that muscle vasoconstrictor response is sustained long-after intermittent hypoxia, as it would occur in SAHS patients. Permanent vasoconstriction causes further resistance to the airway flow in any chronic obstructive disorder that is not completely reversed by normoxia. With respect to DM, the noradrenalin liberation of spontaneously active neurons has also been observed in the isolated disease.

Obesity also increases the MSNA burst incidence, but at lower levels than those seen in COPD or SAHS. Multiple firing of single-unit neurones has not been shown in obese subjects. Advanced age neither seems to be an explanation for the MSNA hyperactivity linked to COPD.

4.2 Inflammation

Systemic inflammation is a common bound between COPD and DM. Both conditions are a proinflammatory state characterized by transcription and expression of hypoxia-induced factor 1 (HIF-1) and increased levels of serum inflammatory cytokines such as C-reactive protein (CRP), interleukin (IL) 1, IL-6 and tumor necrosis factor α (TNF-α) (Archer & Baker 2009). C-reactive protein (CRP) and the nuclear factor NF-κβ pathway are important mediators of the inflammatory response in this context (McNicholas, 2009).

CRP is a type I phase protein with the ability to bind the bacteria surface facilitating the fixation of complement that mediates bacterial killing and/or phagocytosis. CRP stimulates further cytokine production mainly through macrophages activation. TNF-α, IL-1 and IL-6 stimulate CRP synthesis by inducing its hepatic gene expression. NF-κβ is the master regulator of TNF-α, IL-8 and other cytokines transcription and synthesis. TNF-α and other cytokines are produced by monocytes and leukocytes and are enhanced by hypoxia in vitro studies (Takabatake et al., 2000 in Sevenoaks & Stockley, 2006).

TNF-α pathway is related with the deterioration of accessory muscles involved in ventilation. TNF-α induces loss of fat-free mass in COPD patients with subsequent loss of skeletal muscle function. Muscle wasting is also directly mediated by nuclear factor-κβ (NF-κβ) that inhibits the MyoD gene expression. MyoD regulates myofibril synthesis and repairs. Secondly, TNF-α interaction with its receptor can activate muscle and other cellular apoptosis. Reduced IGF-1 and testosterone levels are also adjuvant factors leading to muscle wasting (Sevenoaks & Stockley, 2006).

CRP is a marker of COPD exacerbations and elevated pulmonary pressure in the stable disease (Zamarrón et al., 2008). If we look at the intermittent side of the obstructive disease, CRP is not identified as a prognostic marker of SAHS after adjustment for BMI. TNF-α pathway is related with muscle wasting and pulmonary hypertension commonly developed in COPD disease. NF-κβ and HIF-1 pathways are closely related and may have a differential role in chronic and intermittent hypoxia. Indeed, HIF-1 seems to have a predominant role in COPD, while NF-κβ pathways may predominate in the intermittent hypoxia of SAHS. TNF-α levels can be predicted by the oxygen desaturation index in SAHS. Its levels are increased with independence of obesity (McNicholas, 2009). The prognostic value of these markers in overlap syndrome is unknown.

4.3 Oxidative stress

Now considering the cellular immune response, the leucocitary drive in COPD is a fountain of reactive oxygen species (ROS). The oxidative response advocates a protein, lipid and DNA damage within the cell. Oxidative stress influences NF-κβ cascade through mitogenesis activating protein kinases (MAP-k) perpetuating inflammation this way.

On the inverse loop, TNF-α stimulates ROS production. Indeed, an additional ROS-related mechanism seems to exacerbate TNF-α and NF- κβ effects on muscle wasting (Oudijk, 2003). TNF-α and ROS act in multiple ways. They have a common source in circulating leukocytes. Activation and dysfunction of leukocytes is shared by COPD, SAHS and DM. The activated leukocyte enhances the expression of adhesion molecules such as CD11b and CD18. This effect predominates in lung tissue if we look at COPD, while systemic endothelium is the main target in SAHS. In addition, exacerbations of COPD may deteriorate the antioxidant response, while ROS are particularly enhanced in SAHS when hypoxia is intermittent in a similar way to injury reperfusion syndrome (McNicholas, 2009). Because ROS production is also a direct effect of hyperglycemia, oxidative stress can be posed as a link between SAHS, DM, overlap syndrome and metabolic syndrome. MS is not so clearly identified in the particular case of COPD. A specific oxidative response in SAHS/ overlap may well account for this difference.

Another effect of neutrophil dysfunction is the inactivation of antiproteases leading to airspace ephitelial damage and mucus hypersecretion. As we see, TNF-α / neutrophil axis is

a key in maintenance of the lung COPD phenotype. Finally, advanced COPD stages are characterized by cachectic patients who have an impaired metabolism of proteins, lipids and carbohydrates that is thought to be the maximum expression of systemic inflammation and oxidative stress.

The different pathways involved are complex and the available knowledge on their confluence is still limited. We need to clarify if the mentioned markers of hypoxia, inflammation and oxidative stress have a predictive value of the highest morbidity and mortality in patients with COPD and DM. Moreover, COPD and DM cannot be fully understood if we do not consider classical risk factors, mainly tabaquism in COPD genesis, and obesity in DM. The influence of sleep architecture is an emerging aspect related to DM and hardly studied in COPD with the exception of the overlap syndrome (Zizi et al., 2010). The effect of age and physical activity should also be considered in the COPD and DM interaction. Among these factors, lately research has focused on obesity, sleep disorders and their consequences on respiratory and metabolic environments.

4.4 Insulin resistance and obesity

The inflamed adipose tissue is a well recognized trigger of insulin resistance. A theoretical link between COPD related inflammation and DM could be the switch to a protein depleted and more adipose skeletal muscle. This change of composition inside the sarcolema induces peripheral insulin resistance in the human organism. Such impairment is not necessarily reflected in a visceral fat excess or in obesity (Festa et al., 2002). If present, both conditions aggravate the risk for diabetes.

Then, obesity could be the cause of insulin resistance in patients with lung obstructive disorders. However, obesity does not seem to be the only mediator of insulin resistance. We need to consider a reduction of glucose uptake mediated by hypoxia. Under hypoxemic conditions, insulin resistance has been proved in both obese and lean mice (Polotsky et al., 2007). An explanation could be the hypoxia mediated sympathetic hyperactivity. But sympathetic hyperactivity does not seem to be the unique source of insulin resistance either. Indeed, pharmacologic blockage of autonomic nervous activity did not reverse the insulin resistance under intermittent hypoxia in animal models (Tasali et al., 2008). As we previously mentioned, oxidative stress is a plausible link between the cyclic hypoxia-reoxygenation phenomenon in SAHS and insulin resistance. A first argument is that oxidative stress entails glucose and lipid peroxidation. Secondly, it enhances the inflammatory status through activation of NF-κβ and reduction of nitric oxide bioavailability. Oxidative stress helps to understand why a link between hypoxia and DM seems to be stronger in SAHS than in COPD despite a more sustained and profound hypoxia in the latter. Nevertheless, experimental data about hypoxia and glucose metabolism under mimic SAHS and COPD conditions are still very limited.

The role of obesity in COPD is not established. However, and despite not being the only pathway, obesity is a clear line of causality between SAHS and type 2 DM. Indeed, obesity is the main risk factor for SAHS and type 2 DM. In data from the American National Sleep Foundation, high risk for SAHS is present in one out of four adults and in 57% of obese individuals. The proportion of mild or mild to moderate SAHS attributable to excess weight is 58% (Tasali et al., 2008). Some controversial results have been obtained regarding obesity.

Two epidemiological studies have suggested that obesity is the unique or main cause of insulin resistance in SAHS patients (Reichmuth et al, 2005; Stoohs et al., 1996). However, there is growing evidence about alternative links between SAHS and type 2 DM (Tasali et al., 2008). In terms of SAHS severity, only one study had a prospective design and assessed SAHS by polysomnography. An independent relationship between SAHS severity and glucose intolerance was not found after adjustment for body habitus, although the duration of follow up was only four years (Reichmuth et al., 2005).

SAHS itself aggravates obesity through several mechanisms that also enhances insulin resistance: neuroendocrine dysregulation and physical inactivity. Neuroendocrine dysregulation includes an enhanced ghrelin and leptin secretion. Hyperleptinemia has been proposed as a previous step to insulin resistance even in the absence of weight gain. Indeed, leptin was the only upregulated gene affecting glucose uptake in both obese and lean mice exposed to intermittent hypoxia (Polotsky et al., 2007). Hyperleptinemia may also be a marker of SAHS severity (Pillar & Shehadeh, 2008). Physical exercise increases after CPAP treatment of SAHS. However, the reversal of the insulin resistance by CPAP is controversial (Pillar & Shehadeh, 2008).

Ten out of thirteen clinical based studies suggested a body habitus non-related association between SAHS and insulin resistance or glucose intolerance (Tasali et al., 2008). Three studies considered waist-to-hip ratio because central fat distribution seems to be a more relevant mediator of insulin resistance than BMI (Sharma et al., 2007; McArdle et al., 2007; Tassone et al., 2003). There was a positive association in two out of three. In a similar fashion, three studies found higher HOMA-IR and fasting glucose after adjustment for visceral fat measurements (Kono et al., 2007; Makino S et al., 2006; Vgontzas et al., 2000).

Deposit of neck and abdominal fat alter the regular mechanics of ventilation. The most relevant accumulation of neck fat is located inside upper-airway muscles of the pharynx, this way changing the lumen to an oval shape. Also, abdominal fat exerts a mass effect that reduces the distension of the chest walls resulting in a decreased thoracic and tracheal traction during inspiration (Pillar & Shehadeh, 2008).

To conclude with, the impact of obesity on COPD disease is not as clear as the impact on SAHS. An overall role for obesity in SAHS is a common finding despite the diversity of ethnic and geographical origins of the studied subjects. We also have reasons to think that obesity, with its mechanical and metabolic effects, may impair COPD course, particularly in initial GOLD stages and/or overlap syndrome.

4.5 Sleep disorders

As an additional mechanism, obesity favors insulin resistance and SAHS development through sleep disturbance. Sleep curtailment, sleep fragmentation and a subsequent disrupted signalling lead to unbalanced energy expenditure and far too much appetite. Recent research proposes impaired sleep as a source of metabolic disturbances in SAHS and overlap syndrome patients.

Sleep disturbances are an invariable feature of COPD and SAHS patients. Both chronic and intermittent hypoxemia get worse during sleep. Sleep influences ventilation even in normal subjects due to: a reduced response to the hypoxic drive, a reduced ventilatory efficacy of

hyperrelaxed accesory muscles and upper-airway dilators and, finally, because lung residual capacity is reduced during sleep and so the pharyngeal traction is. Then, normally decreased nocturnal oxygen saturation becomes a challenge in COPD and SAHS patients. A more blunted chemical response to hypoxic drive is seen in both diseases. A diminished ventilation/perfusion quotient results from an hyperinflated lung, less activity of intercostal muscles and a dissociated diaphragmatic and intercostal activity in COPD patients. A collapsible pharynx is the main cause of hypoxia and sleep disturbance in SAHS. Associated symptoms, comorbid diseases, drugs and sedentary lifestyle also reduce sleep efficacy.

Evidence from extent population-based prospective and experimental studies links short and/or poor sleep and type 2 diabetes (Tasali et al., 2008). The sleep-related diabetes is not necessarily explained by apneas (Ayas et al., 2003; Mallon et al., 2005). Two laboratory studies performed in healthy young lean adults obtained an enhanced insulin resistance and a diminished insulin secretion related to sleep deprivation (Knutsonet al., 2007; Spiegel et al., 1999). Measurements of insulin-glucose homeostasis were based on intravenous glucose overload and minimal model technique respectively. Minimal model (Bergman, 2005) resulted in a glucose disposition index (DI) 40% lower than after sleep recovery. A low DI reflects an insulin secretion that is insufficient to compensate for insulin resistance. A low DI indicates a high risk for type 2 DM. Subjects underwent a relative short sleep restriction (4 h for 6 or 2 nights) however inducing a pre-diabetic state similar to the habitual in older adults. Reduction of slow-wave sleep and sleep fragmentation were assessed in another laboratory set-up (Tasali et al., 2008), resulting a similar marked decrease in insulin sensitivity without a balanced insulin secretion. The decrease in insulin sensitivity was correlated with a rise in heart rate variability as a measure of the daily sympathetic activity. In addition, insulin resistance was more related to sleep slow wave suppression than to sleep fragmentation. These experimental procedures have not been reproduced in specific SAHS and COPD settings.

We conclude that, in addition to hypoxia, sleep curtailment enhances sympathetic activation. Noradrenalin is a counter-regulatory hormone that reduces insulin release and function. There is also a decreased glucose uptake by muscle cells favored by high evening cortisol levels and extended duration of elevated growth hormone (GH) levels at night. Another relevant effect of short sleep is upregulation of appetite. A hormonal deregulation of appetite has been observed in the mentioned laboratory studies (Knutson et al., 2007). Ghrelin and leptin are hormones that exert respective hunger and satiety effects. Leptin inhibits appetite, modulates fat distribution and increases energy expenditure. Sleep debt shortens the adequate time that leptin levels require to balance the previous onset of a ghrelin peak. The ghrelin peak occurs during the first half of the night. An attenuated function of leptin due to leptin-CRP boundage has also been hypothesized (Chen et al., 2006). It is plausible in an inflammatory scene such sleep debt.

In diabetic patients, sleep duration and quality was associated with a poorer glycemic control in data from a cross-sectional study on African-American adults with type 2 DM (Knutson et al., 2006). Sleep characteristics were self-reported. Interestingly, sleep quality was associated with poorer glycemic control only in patients with chronic complications of diabetes. A theoretical explanation would be an impaired autonomic response at the background of those diabetic subjects. They would be more susceptible to a less-quality sleep. The cited results were adjusted for age, gender, insulin treatment and BMI. Central

obesity and respiratory conditions were not initially considered. Regarding these items, the authors found an association between A1C levels and sleep duration and quality that remained stable after excluding patients at high SAHS risk. Of note, the highest mean A1C was observed in those with higher versus lower risk for SAHS (9.7% vs. 7.9%, $p < 0.01$).

In an inverse direction, poor glycemic control and obesity are associated with a less quality of sleep. Intervention studies are needed to precise the sense of causality. To take into account, as a final insight sleep dept is a novel habit that could influence the exponential increase of diabetes, obesity and SAHS in our worldwide societies.

5. Metabolic syndrome

We can consider three group of factors in COPD patients: respiratory exacerbations and lung function, nutritional and muscle disorders and finally metabolic syndrome. There are several definitions of MS, but a common element is that all the components are related to the existence of insulin resistance, which will lead to glucose intolerance, abdominal obesity, elevated triglycerides, decreased HDL cholesterol and hypertension. It is estimated that 40-50% of individuals over 60 years have MS in industrialized countries. In Europe there is a prevalence of 15% (Hu et al., 2004; Botros et al., 2009). In a study of 170 patients with COPD and 30 with chronic bronchitis, Sicras et al. (Sicras et al., 2007) observed that the frequency of MS was 53%, 50%, 53% 37% and 44% in patients with chronic bronchitis, COPD I, II, III and IV respectively. They explained the lower incidence in the latter stages of the disease would be related to weight loss.

As previously mentioned, insulin resistance and the development of type 2 diabetes is the key point of MS. In this sense, we have discussed that hypoxia, obesity and sleep disturbances reduce the insulin sensitivity. We could say that the association between SAHS and DM resembles the clustering of metabolic diseases found in MS. The components of MS keep bidirectional links, such insulin resistance and obesity, that are plausible between SAHS and DM. Similarly, the sum of SAHS and DM may result in multiplied cardiovascular effects.

We discussed that the association between COPD and MS is far less clear than the parallel course of SAHS and MS. Due to hypoxia, a change towards multiple firing of vasoconstrictor neurons will increase noradrenalin levels, so we could expect at least arterial hypertension in COPD. Surprisingly, patients were not hypertensive in data by Ashley et al. (Ashley et al., 2010), and the authors posed tempering vascular factors that might balance the hypertensive drive. The links between COPD, metabolic syndrome and cardiovascular disease are largely unknown. Most of the data available deals with the association between SAHS, endothelial dysfunction and subsequent cardiovascular morbidity (Zamarron et al., 2008). There is also recent evidence of an increased mortality in overlap patients without CPAP therapy as compared to COPD (42.2 vs. 24.2%, $p < 0.001$) (Marin et al., 2010). Death was most commonly due to cardiovascular disease. A poorer quality of life was also demonstrated, even in patients without diurnal sleepiness.

The COPD, SAHS and DM shared inflammatory state perpetuates these chronic conditions and have a cardiovascular impact. Hypoxia induced factor (HIF-1) triggers inflammation and angiogenesis inside the atherosclerotic plaque this way facilitating the entry of phagocytes, red blood cells and lipoproteins. CRP is also directly related to atherosclerosis.

CRP interaction with Fcgamma receptor (Fcγ R) possibly increases the monocyte chemokine MCP-1 production, leading to monocyte adherence on to the arterial wall (Sevenoaks & Stockley, 2006). CRP also facilitates the production of foam cells that give shape to the atherosclerotic plaque. The "Third National Health and Nutrition Examination Survey" (NHANES III) denoted an association between CRP and myocardial ischemia. CRP levels higher than 3 mg/dl are significantly related to future cardiovascular events (Pai et al., 2004). This level is commonly surpassed in COPD patients. NF-κβ and TNF-α pathways leading to cardiovascular disease deserve a thorough research in COPD, SAHS and overlap syndrome. TNF- α induces the expression of CRP in the liver, being at the core of the process. TNF- α also has an active effect on macrophages migration, adhesion and differentiation within the atheroma plaque (Sevenoaks & Stockley, 2006). During COPD acute exacerbations, a further rise in CRP levels is also followed by a rise in fibrinogen as the expression of a thrombosis risk. Of note, cardiovascular mortality is particularly enhanced within and following hospital admission for an acute exacerbation (Sevenoaks & Stockley, 2006; Smeeth et al., 2004).

Briefly, the common consequence of COPD, SAHS, MS and DM is an inflammatory status that culminates in endothelial dysfunction leading to cardiovascular events. A novel explanation for the convergent endothelial dysfunction is a depletion or low response of bone marrow stem-cells. This phenomenon determines a reduction of circulating endothelial progenitor cells (EPC). Hyperglycemia, obesity, hypertension and dyslipidemia have been associated with a reduction of circulating EPC. Moreover, a synergistic reduction of EPC has been associated to the clustering of metabolic disruptions (Fadini et al., 2007; Werner et al., 2005, as cited in Tiengo et al., 2008).

6. Interactions of chronic obstructive pulmonary disease, sleep apnea hypopnea syndrome and diabetes *mellitus* treatment modalities

The treatment that has shown to increase survival in COPD is smoking cessation. This is the only measure that slows the accelerated decline in lung function in these patients. COPD therapeutic approach is based on: inhaled bronchodilators, inhaled and systemic corticosteroids, pulmonary and muscular rehabilitation, anti-inflammatory drugs, oxygen and palliative symptomatic treatment in the latter stages of the disease. In a greater or lesser extent, these treatments can influence the glycemic control of DM.

To begin with, systemic corticosteroids clearly alter the metabolism of carbohydrates. Corticosteroid treatment increases upperway resistance due to fluid retention in addition to myopathy and metabolic alkalosis. In addition, corticosteroids may predispose to SAHS by promoting central obesity. Among the most widely used, methylprednisolona is the one that worsens glycemic control the most, followed by hydrocortisone. Deflazacort has less effect on diabetic control.

We can not ignore the possible effect of inhaled corticosteroids on glycemic control. Many DM patients follow an inhaled drugs schedule for their coexistent COPD. Although considered a safe treatment, some systemic effects have been described. Cataracts and suppression of the hypothalamic-pituitary-adrenal are possible effects when maximum dose are given (Faul et al., 2009). In addition, some studies have shown (Faul et al., 1998) a significant increase (1.0%) in glycated hemoglobin and the persistence of glycosuria in

patients with DM 2 who used high-dose inhaled fluticasone (2 mg / day). Other study (Slatore et al., 2009), shows that high dose of inhaled corticosteroids are associated with small changes in glycemic control that are detectable but not clinically relevant as they would not be a criteria to stop or change the treatment.

One shared mainstay of COPD and DM treatment is physical exercise. Physical activity improves lung function and provides a better tolerance of the obstructive disease. It also reduces the risk of type 2 DM (13) and improves glycemic control with a lower dose of antidiabetic agents.

Weight loss can clearly be of benefit for patients with SAHS, obesity and/or DM. Probably, a benefit can be obtained in not advanced COPD stages with excessive weight. Weight loss improves SAHS but does not cure it. In a meta-analysis about bariatric surgery and SAHS, the baseline AHI was reduced from 54.7 to 15.8 events per hour, the latter indicating a moderate to severe SAHS still remaining (Greenburg et al., 2009). Patients should be alerted that they will probably need to continue SAHS treatment after surgery. Clinicians should also be aware that weight loss is associated with increased mortality in COPD. There is no evidence to recommend weight loss in overlap syndrome.

In an indirect way, the oxygen prescribed in advanced lung disease may also influence the management of diabetes. Unfortunately we lack solid studies to verify it. The hypothesis is that control of hypoxia may improve glucose tolerance and the associated MS. CPAP treatment of SAHS has not shown to improve metabolic syndrome in obese patients (Vgontzas et al., 2008), whereas it reduces visceral fat in non obese patients (Chin et al., 1999).

As we have described how hyperglycemia may worsen COPD outcome, we could pose if diabetes treatment can improve respiratory function. Being type 2 the most prevalent DM among COPD patients, insulin sensitizers could improve the lung function. This hypothesis was tested by Kim and colleagues (Kim et al., 2010) in a retrospective cohort study. After adjustment by weight, height and glycemic control, they found an improvement of FVC in subjects treated with insulin sensitizers compared to other DM treatments, with no significant changes in FEV1 or in FEV1/FVC.

We wonder if the new anti-inflammatory drugs (anti-phosphodiesterase 4) may have an effect on control of DM trying to improve the chronic inflammation of COPD. Modulators of the oxidative process such as methyl-bardoxolona are a possibility to be explored in both chronic conditions.

7. Conclusion

We think that we should estimate the risk of diabetes in a COPD patient and *vice versa,* given the frequent simultaneity of both conditions and the confluence of common related factors.

Definitely, prospective population-based and experimental evidence is needed to elucidate the crucial pathways between chronic hypoxemic status, insulin resistance and their contributing factors, mainly tabaquism, adiposity and disordered sleep. Of note, the architecture of sleep is of growing importance in DM. Understanding the clustering of these disorders and its cardiovascular prognosis may have an epidemiological impact on the tandem increase of COPD, DM and related conditions. Probably, lifestyle interventions on tobacco, diet and sleep habits are the key to keep the individual's health and long term well-being.

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13

Evaluation of Dyspnea and Fatigue Among the COPD Patients

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1. Introduction

Chronic diseases are diseases of long duration and generally slow progression. Chronic diseases, such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are by far the leading cause of mortality in the world, representing 63% of all deaths (World Health Organization(WHO), 2010a). Rapid improvements in health and longevity are dramatically changing the burden of illness throughout the world. In developed countries changes in lifestyle and improvements in the treatment of major causes of mortality have aged the population and increased the prevalence of chronic diseases. Poor countries that have achieved gains in life expectancy are also experiencing an increase in chronic disease (Davis et al.,1999). According to Centers for Disease Control and Prevention (CDC) more than 1.7 million Americans die of a chronic disease in 2005. Chronic disabling conditions cause major limitations in activity for more than one of every 10 Americans, or 25 million people (CDC, 2005). In Turkey, chronic diseases accounted for 79% of all deaths in 2002(WHO, 2010b). Notification to national reports, 305.467 dies of total 430.459 was related to chronic diseases in Turkey (Turkey Health Ministry National Burden of Illness Report 2004).

Chronic obstructive pulmonary disease (COPD) is one of the important chronic diseases. COPD is characterized by airflow obstruction with related symptoms such as chronic cough, exertion dyspnea, expectoration, and wheeze. (Edelman et al., 1992; Mannino, 2003). COPD is a highly prevalent, usually progressive illness associated with disability and early death (WHO, 2008). COPD is a major cause of chronic morbidity and mortality throughout the world. It is a growing cause of morbidity and mortality worldwide (Mannino, 2003; Mannino & Braman, 2007; Tatlıcıoğlu, 2000). According to the World Health Organization, 80 million people worldwide have moderate to severe COPD. More than 3 million people died of COPD in 2005, which is approximately 5% of all deaths worldwide (WHO, 2008). COPD is currently the fifth leading cause of death and disease burden globally (O'Donnell et al., 2008). In the Turkey, COPD is the third leading cause of death. Although there has been significant decrease in other mortality causes; there has been an increase by 163 % in COPD mortality (Turkish Thorasic Society, 2010). COPD will be third leading cause of death globally by 2020(WHO, 2008). COPD is the tenth leading disease burden, expressed in disability-adjusted life-years (DALYs), and causes about 2% of the burden of disease

worldwide. Overall, COPD was estimated to have resulted in more than 26 million DALYs in 2000 (Lopez et al., 2006).

COPD characterised by progressive airflow obstruction which is mainly irreversible. COPD is associated with significant comorbidities and extrapulmonary manifestations (Baghai-Ravary et al., 2009). Living with COPD can be challenging, as the disease dramatically impacts patients' daily life. When disease symptoms especially dsypnea affects the performance of daily activities, the potential exist for important changes to occure in individual's overall quality of life (Meek et al., 2001). COPD is associated with increased risk for anxiety, depression, and other mental health disorders (Dowson et al., 2004; Singer et al., 2001). Psychiatric disorders are at least three times higher in COPD patients compared to the general population (Laurin et al., 2007). As the disease progresses, any kind of physical activity or social interaction may prove difficult. COPD is a significant disease which affects the individual physically, emotionally, and socially and leads to an increase in the social support needs of the patients (Aras & Tel, 2009).

Dyspnea and fatigue are occurred many chronic disease. COPD, congestive heart failure, and fluid build-up in renal failure can cause dyspnea. (Ramasamy et al.,2006). Fatigue is almost a universal complaint in patients with autoimmune deficiency syndrome (AIDS), congestive heart failure, myocardial infarction, and progressive neurologic disorders such as multiple sclerosis, and autoimmune diseases such as rheumatoid arthritis, and dialysis patients receiving either hemodialysis or peritoneal dialysis, and cancer (Appels & Mulder, 1988; Brunier & Graydon, 1996; O'Brain & Pheifer, 1993; Tel et al., 2011).

Dyspnea and fatigue are the two most common symptoms experienced by patients with COPD (Meek & Lareau, 2003; Oh et al., 2004; Tel, 1998). The most important complaint of patients with COPD is dyspnea. Dyspnea is identified as a perception or observation of abnormal and disturbing sensation of breathing. Dyspnea is the perception and experience of labored, uncomfortable breathing, and may produce secondary physiological, emotional, cognitive, and behavioral responses (American Thorasic Society, 1999).

Another accompanying important symptom of dyspnea is fatigue in COPD. Fatigue is an unpleasant subjective symptom that prevents individuals from performing his functions and using his normal capacity, affects whole body and changes from a slight exhaustion to unbearable fatigue (Swain, 2000). Fatigue is poorly understood and believed to have a significant subjective component strongly associated with dyspnea, although the nature of the relationship remains unclear. Fatigue has been defined as "the multidimensional sensation of tiredness that the individual experiences when perceiving the reduced capacity to function normally" and it often varies with respect to daily pattern, triggers or contributing factors, and responsiveness to interventions (Kapella et al., 2006). In contrast with a prevalence rate of 18.3%–25% in the general population (Lewko et al., 2009; Pawlikowska et al., 1994) fatigue is "almost always" experienced by 43%–58% of persons with COPD (Kinsman et al., 1983; Walke et al.,2007).

Fatigue was reported by patients with COPD as the second most important symptom of COPD, after dyspnea (Blinderman et al., 2009; Janson-Bjerklie et al., 1986; Walke et al., 2007). Peters et al. (2010) found that fifty percent of patients with COPD had abnormal fatigue. Guyatt et al. (1987) report that fatigue ranks second to dyspnea as a symptom contributor to decreased quality of life in COPD patients.

Dyspnea and fatigue are a subjective experinces that can only be measured from the patient's perceptions, because every person have different thresholds for noticing, reporting, and rating the severity of these symptoms (Victorson et al., 2009). In severel studies were found out that there is a significant correlation between dyspnea, fatigue and physical activity and that fatigue levels increase when dyspnea intensifies and physical activity levels reduce (Breslin et al., 1998; Theander & Unosson, 2004; Woo, 2000a). Individuals with COPD undergo a high amount of activity restriction and dependency due to dyspnea or fatigue or both symptoms (Akbal, 2003; Woo, 2000b; Yıldırım, 2006). McCarley (2003) explored that there was a moderate correlation between dyspnea and fatigue experienced by the patients with COPD. Reishtein (2005) reported that there was a moderately negative correlation between dyspnea, fatigue and functional capacity among COPD patients. It is reported that there is a complicated correlation in COPD between fatigue and other disease-related symptoms such as dyspnea, anxiety, depressed emotions and sleep quality (Kapella et al. 2006). Breslin et al. (1998) suggested that physical dimensions of fatigue correlated with an increase in the severity of pulmonary impairment and reduction in exercise tolerance.

1.1 Assessment of dyspnea and fatigue

Because dyspnea and fatigue are subjective symptoms, they are assessed through the use of standardized symptom reports or questionnaires (Guyatt et al., 1993; Victorson et al., 2009). Implementation of many interventions to patients with COPD, measurement and evaluation of dyspnea and fatigue is very important part of this patients care. The two purposes of measuring dyspnea are to differentiate between patients who have less dyspnea and those who have more dyspnea (discriminate), and to determine whether dyspnea has changed over time and/or as a result of treatment (evaluate) (Mahler, 2006). For the most part, questionnaires used to measure dyspnea as an outcome of pulmonary rehabilitation are evaluative instruments and each of this instruments measure different aspect of dyspnea (Meek & Lareau, 2003; Meek, 2004).

The Medical Research Council Scale (MRC); The MRC categorizes the individual based on whether dyspnea is associated with specific tasks and situations (ATS, 1999; Meek, 2004). Patients are assigned to one of five grades, based on their difficulty with mobility, from Grade 1, "never troubled by breathlessness except on strenuous activity," to Grade 5, "too breathless to leave the house or breathless after undressing." The MRC does not uniquely measure dyspnea, since the level of dyspnea is evaluated related to activities. The MRC, is easy to administer and is useful for general screening and categorizing of patients (ATS, 1999; Mahler, 2006).

The Oxygen Cost Diagram (OCD); This scale was developed in an effort to match a range of tasks with the occurrence of dyspnea (ATS, 1999). The OCD is a 100-mm vertical visual analog scale with 13 activities listed at various points along the line corresponding to increasing oxygen requirements for their completion, ranging from sleeping (at the bottom) to brisk walking uphill (at the top) (McGavin et al.,1978).

The Basaline Dyspnea Index(BDI); BDI is a rater evaluation of dyspnea associated with activities (Mahler et al.,1984). The rating includes the magnitude of the task and the effort required to perform the task. Each category is rated on a 0 to 4 grade and summated for a total score. The BDI also has a transitional score, the transitional dyspnea index (TDI), that measures the change in dyspnea associated with activities following an intervention. (Foglio

et al., 1999; Meek & Lareau, 2003). The most widely used multidimensional instruments include the Baseline (BDI) and Transition (TDI) Dyspnea Indices , which consider three components (functional impairment, magnitude of task, and magnitude of effort) (ATS, 1999; Mahler et al.,1984).

The University Of San Diego Shortness Of Breath Questionnaire (SOBQ); The University of San Diego Shortness Of Breath Questionnaire (SOBQ) is a 24-item measure that assesses self-reported shortness of breath while performing a variety of activities of daily living (Eakin et al.,1998). Patients are asked to rate their dyspnea associated with the 21 different activity, from 0 = "not at all" to 5 = "maximally or unable to do because of breathlessness." Three additional questions about limitations due to shortness of breath, fear of harm from overexertion, and fear of shortness of breath are included for a total of 24 items. [Eakin et al 1998; Ries et al., 1995).

The Borg Scale; The Borg scale a category-ratio scale, is commonly used to evaluate the effects of exercise on dyspnea. The original and modified scales have ratio properties ranging from 0 = nothing at all to 10 = very, very severe, with descriptors from 0 to 10. The Borg scale has been used in pulmonary rehabilitation programs to evaluate dyspnea before, during, and after progressive exercise (Foglio et al.,1999).

The Visual Analog Scale (VAS); The VAS is usually a 100 mm line anchored at either end with descriptors, such as "none" to "very severe." When used to measure dyspnea, these anchors are qualified to read "no shortness of breath" to "maximum shortness of breath," or some similar variation (Gift, 1989). The VAS can be used to quantify a number of aspects of symptoms besides the sensation of dyspnea, such as effort and distress with dyspnea. The visual analogic scales and the Borg scale are the simplest tools available; both are completed by the patient, and allow a follow-up of the impact of treatment on dyspnea (Janssens et al., 2000).

The Chronic Respiratory Questionnaire (CRQ); The Chronic Respiratory Questionnaire (CRQ), a 20-item, disease-specific, quality-of-life questionnaire (ATS,1999; Guyatt et al.,1987), has been used extensively in pulmonary rehabilitation settings. The CRQ consists of four domains (dyspnea, fatigue, emotional function, and mastery), rated on a seven-point scale. The dyspnea component of the CRQ asks patients to identify five activities of importance to them. These same activities are rated with 1 = most dyspnea and 7 = least dyspnea, before and after a pulmonary rehabilitation program. (Meek, 2004). The CRQ has a fatigue subscale consisting of five items, scored on a 7-point scale. The CRQ fatigue domain is reliable, valid with the same clinically important differences as the other components. To determine the outcomes of pulmonary rehabilitation, it is safe to say that the CRQ is the most widely used and tested instrument that measures both dyspnea and fatigue (Meek & Lareau, 2003).

The Pulmonary Functional Status Scale (PFSS); The Pulmonary Functional Status Scale (PFSS) is a 53-item, self-administered questionnaire measuring physical, mental, and social function. The dyspnea subscale evaluates dyspnea related to activities, as well as dyspnea independent of activities(Weaver et al.,1998).

The Pulmonary Functional Status And Dyspnea Questionnaire (PFSDQ); The Pulmonary Functional Status And Dyspnea Questionnaire (PFSDQ) is a 164-item, self-administered questionnaire that evaluates dyspnea and activity levels. The pulmonary functional status

and dyspnea questionnaire-modified version (PFSDQ-M), measure dyspnea, fatigue, and activity levels (Lareau et al.,1994). PFSDQ-M has been used to measure fatigue in COPD patients (Meek et al. 2001). The PFSDQ-M comprises three domains: influence of dyspnea on ADLs, influence of fatigue on ADLs and change experienced by the patient in ADLs. The patient reports to what degree dyspnea and fatigue affect 10 specific ADL items, assigning a score from 0 to 10 for each activity as follows: 0 (no interference); 1-3 (mild); 4-6 (moderate); 7-9 (severe); and 10 (extremely severe). Higher values on the scale indicate greater ADL limitation. The five general questions in the dyspnea and fatigue domains are informative and qualitative, and the answers are not calculated in the questionnaire score (Lareau et al.,1998).

The St George's Respiratory Questionnaire (SGRQ); The Saint George Respiratory Questionnaire (SGRQ) is the best-known and most frequently used disease-specific health related quality of life (HRQL) questionnaire for respiratory diseases (ATS,1999; Jones et al.,1992). The SGRQ is a standardized, self-administered questionnaire for measuring impaired health and perceived HRQL in airways disease. It contains 50 items, divided into three domains: Symptoms, Activity and Impacts. A score is calculated for each domain and a total score, including all items, is also calculated. Each item has an empirically derived weight. Low scores indicate a better HRQL(Jones et al.,1992; Ståhl et al. 2005).

Multidimentional Fatigue Inventory (MFI); The MFI consists of 5 subscales: general fatigue; physical fatigue; reduced activity; reduced motivation; and mental fatigue. Each subscale has 4 items with a 5-point Likert scale (1 -no, that is not true, 5 - Yes, that is true), thus the total score for each subscale ranges from 4 to 20. The overall score of fatigue is calculated by adding all subscales, so that the overall score ranges from 20 to 100. A higher score implies more severe fatigue (Breslin et al., 1998; Lewko et al 2009; Meek & Lareau, 2003; Oh et al., 2004).

The Profile Of Mood States (POMS); POMS is a broader measure that has been used in investigations of individuals with COPD (Janson-Bjerklie et al., 1986; Woo,2000b). The POMS is a 30-item questionnaire composed of 6 subscales (tension/anxiety, depression/dejection, anger/hostility, vigor/activity, confusion/bewilderment, and fatigue/inertia); the POMS-F subscale consists of 7 items. Subjects are asked to indicate the degree or intensity of feelings in the past few days on a 5-point Likert scale (0 = not at all to 4 = extremely). The POMS-F presents another possible way to measure fatigue in the COPD population (Meek, 2004).

The Multidimentional Assessment Of Fatigue (MAF); MAF (16 items) was originally designed for arthritis patients (Belza, 1993;Tack, 1990), It has been used in cancer patients (Meek et al., 2001) and with chronic pulmonary disease (Belza et al., 2001). The MAF surveys four dimensions: severity, measured by items 1 and 2; distress, item 3; degree of interference in activities of daily living, items 4 through 14; and, finally, timing (frequency of occurrence and changeability), items 15 and 16 (Belza, 1993; Tack, 1990).

1.2 COPD and pulmonary rehabilitation

Dyspnea and fatigue are closely related symptoms in chronic lung disease that are consistently encountered in the clinical setting. Pulmonary rehabilitation is an essential, basic component of an integrated approach to managing chronic lung disease (Nield, 2003).

When disease' symptoms affect the patient's performance of daily activities, the potential exists for overall quality of life to be decreased. If these symptoms continue to limit daily activities and the intensity of the symptom increases, patients to become deconditioned. This results in an interrelationship of symptoms affecting activities, and vice versa, often referred to as the "dyspnea spiral" or cycle of deconditioning. Pulmonary rehabilitation is one of the few interventions believed to break this cycle of progressive symptoms limiting activities (ATS,1999). Most patients are referred for pulmonary rehabilitation in order to improve the symptom of dyspnea. Nevertheless, patients with high fatigue derive significant benefit from pulmonary rehabilitation. Research of Baltzan et al. (2011) has shown that high levels of fatigue are common in patients entering pulmonary rehabilitation. Fatigued patients benefit from pulmonary rehabilitation, with improved exercise performance as well as improved health status. Lacasse et al. (2006) concluded that rehabilitation relieves dyspnea and fatigue, improves emotional function and enhances patients' sense of control over their condition. The primary measurable benefits of pulmonary rehabilitation to date have been a decrease in symptoms, and an increase in exercise endurance. A pulmonary rehabilitation program is to assess and treat activity limitations associated with symptoms of COPD including dyspnea in order to maximize patients' ability to participate in activities of daily living, leisure, and vocational pursuits (Migliore, 2004). Dyspnea and fatigue are important symptoms associated with COPD that improve with pulmonary rehabilitation (Meek &Lareau, 2003).

Fatigue and dyspnea are important symptoms requiring evaluation and management in patients with COPD. Nurses perform crucial responsibilities for supporting coping-skills against dyspnea and fatigue complaints of COPD patients. Investigating the correlation between dyspnea and fatigue will contribute to coping behaviors against dyspnea and fatigue and the quality of life of the patients. Because of the high prevalence of this symptom and the severity of suffering that can be associated with it, clinicians need to become familiar with available methods for the alleviation of dyspnea.

2. Aim

The present research was conducted in order to investigate dyspnea, fatigue-experience and the correlation between dyspnea and fatigue.

3. Material and methods

The research was consisted of COPD patients who were ambulatory examined and checked at the pulmonary clinics of a state hospital between February and June 2009. The sample of the research was made up by 300 patients with COPD who accepted to participate. Participants were selected according to the following criteria; had been diagnosed of COPD, aged 18 years or older, understand, and communicate in Turkish, did not have any communicational and psychiatric problems. Written approvals from the hospital and oral consents from the patients were obtained. The data of the research were collected using face to face interview technique, personal information form, Medical Research Council Dyspnea Scale (MRC) and Brief Fatigue Inventory (BFI).

Data were entered into SPSS software (v. 14.0; SPSS Inc.,Chicago, IL) and recoded as required according to the questionnaires' scoring instructions. The data analysis was

performed through percentage distribution, ANOVA, t test and Pearson's Correlation Analysis and $p \leq 0.05$ was accepted as statistically significant.

3.1. Personal information form

Personal information form includes sociodemographic characteristics such as age, gender, marital status, educational level and disease characteristics such as disease length, disease severity, health condition, repeated hospitalization. COPD severity was defined by The GOLD criteria classify COPD into four stages (ATS, 1991; GOLD, 2006).

3.2 The Medical Research Council (MRC) dyspnea scale

Dyspnea perception during daily activities was measured using the MRC dyspnea scale. Modified MRC chronic dyspnea self-administered questionnaire consisting of five questions about perceived breathlessness. Grade 1, "never troubled by breathlessness except on strenuous activity," to Grade 5, "too breathless to leave the house or breathless after undressing (Bestall et al., 1999; Stenton, 2008).

3.3 The Brief Fatigue Inventory (BFI)

The BFI was used to assess the severity of fatigue and the amount of interference with function caused by fatigue in this study. The BFI has 9 items that were designed to provide a measure of fatigue. Three items in the BFI ask patients to rate their fatigue during the past 24 hours at its "worst," "usual" or "average," and "now," with "0" being "no fatigue," and "10" being "fatigue as bad as you can imagine." Additional items assess how much fatigue has interfered with different aspects of the patient's life during the past 24 hours. The interference items included in the present study were mood, daily activity, walking ability, eating, relations with other people and enjoyment of life. Each interference item is scored on an eleven point rating scale from "0" (does not interfere) to "10" (completely interferes). A mean BFI score is calculated as the mean of the intensity and interference items (Çınar & Olgun, 2010).

4. Results

It was found out that mean age of the patients was 66.03 years (SD= 11.33), 50.7 % was male, 58.7% belonged to ≥ 65 age group, 72.0% was married, 49.7% was illiterate, 46.3 % was housewives, 27.9% was retired, 74.3% had a moderate income level. It was explored that 30.7% of the patients had the disease for ≥12 years (disease length ≥12 years), 38.6% moderate COPD, 77.3% was repeatedly hospitalized and 89.3% said to use their medications regularly and 47.0% identified their health condition as bad.

Table 1 demonstrates dyspnea severity and fatigue-experience of the patients. All of the patients said to have dyspnea and the analysis made using MRC dyspnea scale revealed that 73.3%of the patients had severe dyspnea. 99.3% of the patient told to experience fatigue. It was explored that 49.0% of the patients had always fatigue experience.

Their total fatigue score'mean was 60.36 ± 20.57, mean score of activities of affected by fatigue was 40.22 ± 14.37, and MRC dyspnea mean score was 3.59 ± 1.31.

Characteristics	n (%)
Dyspnea Complaint	
Mild	12(4.0)
Moderate	68(22.7)
Severe	220(73.3)
Fatigue Complaint	
Yes	298(99.3)
No	2(0.7)
Frequency Of Fatigue Experience	
Sometimes	60(20.0)
Often	93(31.0)
Always	147(49.0)

Table 1. Dyspnea severity and fatigue experience of the patients

It was concluded in our research that there was a positive correlation between dyspnea and fatigue ($r=0.636$, $p<0.01$) and as dyspnea scores increased so did mean fatigue scores. Also, there was a significant negative correlation between the measured FEV_1 values of the patients and dyspnea scores ($r=-.341$ $p<0.01$) and fatigue scores ($r=-.260$ $p<0.01$).

Table 2 demonstrates mean scores of dyspnea, fatigue levels and levels of the daily activities affected by fatigue accoding to some socio demographic and disease characteristics.

It was explored that there was not any statistically significant difference between fatigue levels, levels of the daily activities affected by fatigue and dyspnea scores in terms of age and sex($p>0.05$). It was found out that there was statistically significant difference between fatigue level and levels of the daily activities affected by fatigue and disease length (year) ($p<0.05$).

It was found out that there was statistically significant difference between fatigue level and levels of the daily activities affected by fatigue and dyspnea according disease severity, the number of repeated hospitalization, patients' perception about their health condition and frequency of fatigue-experience. High fatigue score and score of the daily activities affected by fatigue were presented by those who had the disease for ≥ 12 years. High fatigue score and score of the daily activities affected by fatigue and dyspnea score were presented by who fourth stage of COPD, who were repeatedly, had hospitalized for ≥ 4 times a year and who identified their own health condition as very bad and frequency of fatigue as always.

5. Discussion

Chronic obstructive pulmonay disease is characterised by significant physical and psychosocial challanges. Dyspnea and fatigue are the two most common symptoms experienced by patients with COPD (Blinderman et al., 2009; Gift & Shepard, 1999; Kinsman et al., 1983). Dyspnea is predominantly related to a reduction in vital capacity of lungs. Dyspnea is the most commonly experienced complaint of the COPD patients (Rabe et al., 2006; Tel & Akdemir, 1998; Wong et al., 2010). Fatigue may be affected by dyspnea and is frequently told by the patients (Janson-Bjerklie et al., 1986; Reishtein 2005). Wong et al.(2010) found that fatigue was experienced by almost all participants with COPD. Çınar and Olgun (2010) were reported that 97% of patients with COPD experienced high levels of fatigue. The

Characteristics	Mean Scores		
	Fatigue X ± SD	Activities affected by fatigue X ± SD	Dyspnea X ± SD
Age			
≤ 44	51.93±22.22	35.60±13.17	2.73±1.48
45-64	58.30±22.15	38.60±15.68	3.38±1.34
65+	62.35±19.18	41.61±13.49	3.79±1.23
F, p	2.66 >0.05	2.30 >0.05	6.89 >0.05
Gender			
Female	61.72±20.00	41.00±14.17	3.64±1.29
Male	59.03±21.09	39.46±14.58	3.54±1.33
t, p	0.156 >0.05	0.001 >0.05	0.828 >0.05
Disease Length			
1-3 Years	53.05±22.81	35.23±16.20	3.29±1.36
4-7 Years	59.57±19.61	40.61±13.44	3.46±1.35
8-11Years	62.77±17.46	42.00±12.01	3.90±1.28
12+ Years	66.51±18.57	43.77±13.21	3.76±1.20
F, p	7.358 <0.05	6.187 <0.05	3.493 >0.05
Disease Severity			
Stage I(Mild)	52.58±21.65	54.46±15.28	2.96±1.28
Stage II Moderate)	56.30±21.25	37.64±14.75	3.26±1.27
StageIII(Severe)	67.21±17.22	44.97±12.19	4.14±1.13
Stage IV(Very severe)	69.78±15.60	46.43±10.43	4.34±1.11
F, p	10.615 <0.05	10.413 <0.05	17.928 <0.05
Repeated Hospitalization			
No	52.29±23.52	34.57±16.26	3.11±1.37
Once	55.76±19.89	36.63±14.05	3.22±1.24
Twice	61.31±21.28	40.96±14.60	3.82±1.23
Three times	67.77±15.82	45.40±11.03	3.97±1.15
Four times and more	71.42±11.41	48.40±7.92	4.22±1.17
F, p	9.695 <0.01	10.631 <0.01	8.931 <0.01
Health Condition			
Good	47.00±23.35	32.96±16.48	3.04±1.33
Normal	58.39±21.27	38.19±15.02	3.45±1.29
Bad	62.02±18.90	40.91±13.49	3.63±1.31
Very Bad	76.60±9.50	50.25±6.52	4.86±0.52
F, p	8.986 <0.01	5.953 <0.01	8.054 <0.01
Frequency Of Fatigue Experience			
Sometimes	46.50±23.90	31.58±16.61	2.93±1.36
Often	57.31±17.58	37.45±12.25	3.16±1.20
Always	67.95±17.27	45.49±12.39	4.13±1.13
F, p	29.305 <0.01	26.231 <0.01	2.864 <0.01

Table 2. Mean scores of dyspnea, fatigue levels and levels of the daily activities affected by fatigue accoding to some characteristics

rates of the patients who experienced dyspnea and fatigue were higher in our study too; which concurred with literature.

Reishtein (2005) found out that means scores for dyspnea and fatigue were moderately high in patients with COPD. In this study, it was found that the mean scores of fatigue and dyspnea of the participant patients were high.

It was explored that there was not any statistically significant difference between fatigue levels, levels of the daily activities affected by fatigue and dyspnea scores in terms of age and sex. Kapella et al. (2006) reported that fatigue complaint was significantly correlated with age. Skumlien et al. (2006) reported that 82% of the women and 70% of the men had dyspnea complaint and there was not any difference among the sex in terms of dyspnea number and dyspnea scores. Gift and Shepard (1999) reported that men and women did not differ in their level of fatigue. Oh et al. (2004) and Kapella et al. (2006), reported that there were small differences between women and men; however, these difference were not statistically significant. It was observed in our research that although the dyspnea scores and fatigue scores of the women were higher than those of men, it was statistically insignificant.

High fatigue score and score of the daily activities affected by fatigue were presented by those who had the disease for ≥ 12 years. High fatigue score and score of the daily activities affected by fatigue and dyspnea score were presented by who fourth stage of COPD, who were repeatedly, had hospitalized for ≥ 4 times a year and who identified their own health condition as very bad and frequency of fatigue as always. Several studies show that fatigue is a common symptom in COPD and it has been associated with reduced health status and dyspnea (Breslin et al., 1998; Guyatt et al.,1987). We were found out that dyspnea and fatigue scores were higher in patients which health status is very bad. This result was statically significant. Hospitalization rates in the patients with COPD are high, and increase with age. Baghai-Ravary et al (2009) suggested that increased fatigue was related to dyspnea, exacerbation frequency, health status and time spent outdoors. In this study, we found that the hospitalization rates in the patients with COPD were high and these patients'dyspnea and fatigue scores were also high.

Baghai-Ravary et al.(2009) and Wong et al.(2010) explored that they did not find a correlation betweeen severty of COPD and fatigue. Breslin et al.(1998) reported that physical dimensions of fatigue correlated with an increase in the severity of pulmonary impairment and reduction in exercise tolerance. In this study we found that fatigue score was higher in patients with very severe COPD. These data show a relationship between fatigue and pulmonary function in COPD.

Previous studies have noted significant relationships between dyspnea and fatigue (Baghai-Ravary et al., 2009; Janson-Bjerklie et al.,1986; Kinsman et al.,1983; Peters et al.,2010, Reishtein, 2005; Theander et al., 2009). As in earlier studies, we found correlations between fatigue and dyspnea. It was concluded in our research that there was a positive correlation between dyspnea and fatigue ($r=0.636$, $p<0.01$) and as dyspnea scores increased so did mean fatigue scores. This result is consistent with previous research reports. Also, there was a significant negative correlation between the measured FEV_1 values of the patients and dyspnea scores ($r=-.341$ $p<0.01$) and fatigue scores ($r=-.260$ $p<0.01$). McCarley (2003) discovered that there was moderately significant correlation between dyspnea and fatigue

experienced COPD patients whereas Kapella et al. (2006) reported that there was significant correlation between dyspnea and fatigue scores among the COPD patients. Reishtein (2005) reported that there was moderately negative correlation between dyspnea and fatigue and functional lung capacity among the COPD patients. Baghai-Ravary et al.(2009) found that fatigue was related to change in FEV_1. Breslin et al.(1998) found that there was a significant negative correlation between general and physical fatigue and predicted FEV_1 values and that physical aspect of fatigue was associated with the severity of pulmonary deterioration. In the light of these findings, patients undergo dyspnea and fatigue more as lung capacity decreases.

6. Conclusion

According to the results of the present research which was conducted in order to investigate dyspnea, fatigue-experience and the correlation between dyspnea and fatigue; all of the patients experienced dyspnea and almost all of them had fatigue. Mean scores of fatigue and dyspnea of the women were higher than those of men. Dyspnea severity and fatigue was more intensified among those who belonged to ≥ 65 age group, who had the disease for ≥ 12 years, who had fourth stage of COPD, who were repeatedly hospitalized and fatigue scores increased as dyspnea severity increased and there was significant negative correlation between FEV_1 values and dyspnea and fatigue scores. As a result, it was recommended that nurses who care COPD patients should assess dyspnea and fatigue-situations and the complaint severity of the patients using scales; should plan and practice the appropriate nursing interventions considering the linear correlation between dyspnea and fatigue; should perform personal care plans for those COPD patients who belonged to ≥ 65 age group, who had longer disease length, who had advanced stage of COPD, who were repeatedly hospitalized due to the fact that the rates of severe dyspnea and fatigue were higher. Dyspnea and fatigue should be evaluated in usual care with a questionnaire that corrects for them in order to tailor treatment to patients' need. Dyspnea and fatigue is an important symptom requiring evaluation and management in patients with COPD.

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Part 3

Treatment

14

Adherence to Therapy in Chronic Obstructive Pulmonary Disease

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is a major public health problem for both industrialised and developing countries (Viegi et al., 2001). The prevalence of COPD is increasing worldwide, resulting in a substantial economic burden, including direct and indirect health-care costs (Chapman et al., 2006).

Non-adherence in COPD is common and poses a significant barrier to optimal disease management. According to the World Health Organization (WHO), adherence to long-term therapies averages only 50% (WHO, 2003). Patient adherence in chronic diseases can result in poor health outcomes and increased health-care expenditures (WHO, 2003). Discontinuation of COPD therapy contributes to increasing the frequency of exacerbations, the number of hospitalisations and the mortality rate (Bourbeau & Bartlett, 2008; Regueiro et al., 1998; Vestbo et al., 2011).

Clinical trials may overestimate the level of adherence to medication regimens. Adherence rates in clinical trials have been expected to be approximately 70%–90% among patients with COPD (Kesten et al., 2000; van Grunsven et al., 2000; Rand et al., 1995). In clinical practice, these rates are only in the range of 20%–60% (Agh et al., 2011; Bosley et al., 1994; Dolce et al., 1991; Krigsman et al., 2007a). This difference reflects the fact that patient adherence may be an important explanatory factor of the difference between the efficacy of treatment under experimental conditions and the real-world effectiveness of the treatment (Revicki & Frank, 1999).

Non-adherence in patients with COPD has a number of causes, including factors related to the characteristics of the patient, the disease, the therapies and the health-care provider–patient relationship (Baiardini et al., 2009; Restrepo et al., 2008; WHO, 2003). Physicians should understand the factors and the strategies that facilitate adherence to improve the effectiveness of the therapy.

The goals of this chapter are as follows: to highlight the importance of adherence in the management of COPD; to introduce the reader to the concepts of adherence, compliance and persistence; to address different methods of measuring adherence; to identify factors related to adherence; and to emphasise strategies to enhance adherence in patients with COPD.

2. General overview of adherence

There are a number of terms used to describe the extent to which a patient undertakes the recommendations (medication regimens, lifestyle changes, etc.) of health-care providers. The most commonly used terms are compliance, adherence and persistence.

2.1 Terminology: Compliance, adherence and persistence

The definitions used to describe the concepts of compliance, adherence and persistence are not standardised, which causes many difficulties when comparing or combining results of different studies. The definitions from the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) and the WHO are the most widely accepted in the literature.

Medication compliance, as defined by the ISPOR, "refers to the act of conforming to the recommendations made by the provider with respect of timing, dosage and frequency of medication taking" (Carmer et al., 2008). Compliance is expressed as an index number, which is typically given as a percentage and refers to a specified time interval. One of the most commonly used models for calculating medication compliance is the medication possession ratio (MPR). In the model of the MPR, the number of days of medication supplied within the refill interval is divided by the number of days in the refill interval (Peterson et al., 2007). Medication compliance may also be reported as a dichotomous variable, classifying patients into good and poor (or non-) compliance categories (Table 1). The cut-off point of compliance should be determined according to medication and type of disease. However, it is generally set independently at 80%, whether this compliance rate is adequate for disease control or not (Carmer et al., 2008).

Drugs	**Months** (1 month = 30 days)												**Supply** (days)	**MPR**
A	x	x	x	x	x	x				x	x	x	9x30=270	270/360=0.75
B		x	x	x	x		x	x	x	x			8x30=240	240/360=0.66
C			x		x		x	x		x	x	x	7x30=210	210/360=0.58

∑ **MPR**: ((270+240+210)/3)/360 = 0.66 → 66%*
*:Patient is non-compliant (cut-off point: 80%).
|x|: medication supplied, | |: medication not supplied

Table 1. Calculation of medication compliance: medication possession ratio (MPR)

According to the definition of ISPOR, medication persistence may be described as "the duration of time from initiation to discontinuation of therapy" (Carmer et al., 2008). Persistence analyses must also define a permissible gap period, which specifies the maximum allowable time period between refills without discontinuation of the therapy. Persistence may be counted in days. However, it can also be given as the percentage of the number of persistent patients at the end of a predefined time period (Patricia et al., 2006) (Table 2). A patient's drug taking behaviour can best be quantified using both parameters: medication compliance and persistence (Carmer et al., 2008).

Although most research in the field has focused on medication compliance and persistence, therapeutic adherence certainly includes other non-drug therapeutic recommendations

Patients	Months (1 month = 30 days)												Days persistent (gap: 30 days)*	Persisted 180 days**
A	x	x	x	x			x	x	x				120	no
B	x	x	x	x	x	x				x		x	180	yes
C	x	x				x	x			x		x	60	no

*: Patients persisted an average of 120 days ((120+180+60)/3)
**: 33% (1/3) of the patients were persistent for 180 days
|x|: medication supplied, | |: medication not supplied

Table 2. Calculation of medication persistence

(following diets, executing lifestyle changes, etc.) as well. Explanation of adherence by the WHO also reflects this concept. The WHO definition of adherence is the following: "the extent to which a person's behaviour—taking medication, following a diet, and/or executing lifestyle changes—corresponds with agreed recommendations from a health-care provider" (WHO, 2003). This definition accurately highlights the importance of the patient's active role in their own health-care, which emphasises that the relationship between the patient and the health-care provider should be based on a partnership, instead of a one-sided paternal relationship.

Recently, medication adherence has become the preferred term instead of medication compliance. The primary difference between compliance and adherence is that compliance reflects the patient as a passive recipient of medical advice. Furthermore, compliance has also been viewed as a judgmental term when applied to patient behaviour. Thus, medication adherence will be the preferred term from this point forward.

2.2 Methods of measuring adherence

Most studies in adherence research have focused on medication-taking behaviour. Therefore, the following is a brief overview of the methodology of the assessment of medication adherence in COPD. There are a number of ways to assess adherence; nevertheless, there is not a gold standard because each method has strengths and limitations (Table 3).

The easiest way to assess medication adherence within clinical settings is to collect information from the patient themselves through questionnaires or patient diaries (Agh et al., 2011; Dolce et al., 1991; George et al., 2005, 2006a; Laforest et al., 2010). However, it should be mentioned that self-reporting methods may overestimate a patient's drug-taking behaviour (Dompleing et al., 1992; Rand et al., 1992, 1995). Using postal administration can help to obtain data that are more objective because patients are normally intimidated by their health-care providers and tend to give them the expected answers (Agh et al., 2011). Another commonly used method is the analysis of electronic pharmacy records (Breekveldt-Postma et al., 2007; Cramre et al., 2007; Jung et al., 2009). Retrospective database analysis is rapid and inexpensive. Nevertheless, this approach may also be inaccurate. It evaluates the prescriptions written by physicians or the prescriptions filled by patients, but not the medication intake directly.

	Advantage	Disadvantage
Indirect methods		
Patient self-report: adherence questionnaire, patient diary	Easy to obtain	Unreliable
Pharmacy refill data	Rapid Inexpensive	Inaccurate: • Pharmacy database can be incomplete • No indication of ingestion
Pill count, inhaler weighing	Easy to obtain Inexpensive	Inaccurate: • No indication of ingestion • "Dumping"*
Electronic adherence monitoring	Accurate measure of dosing history	Expensive No indication of ingestion
Therapeutic outcome	Easy to obtain	Clinical outcomes can depend on other factors
Direct methods		
Direct observation of the medication intake	Accurate indication of the ingestion	Unpleasant for the patient Require large human resources
Biological assay	Confirm drug use	Expensive Unpleasant for the patient Limited information regarding use over time Insensitive to inhaled drugs

*"Dumping": removing most of the medication at one time.

Table 3. Methods of measuring adherence

Pill count (Dompleing et al., 1992; van Grunsven et al., 2000) and canister weighing (Rand et al., 1995; Simmons et al., 2000) are widely used methods of adherence assessment in clinical trials. Pill counts are limited to oral medications, but canister weighing can also be used to monitor inhaled drugs. These approaches assess only the quantity of the medication removed from the canister without indication of ingestion, dose or dose frequency. Electronic compliance monitoring devices can provide more objective information about medication use than the aforementioned methods (Corden et al., 1997; Simmons et al., 1996, 2000). The cap of the pill bottles can be equipped with a microchip that stores data about each opening. Electronic recording devices (chronologs) can be fitted to metered-dose inhalers and nebulisers as well. Electronic monitors provide an accurate measure of dosing history but also cannot confirm ingestion. The major disadvantage of this method is the price; it is relatively costly.

Medication compliance can also be estimated based on direct assessments, such as direct observation of the medication intake or evaluation of blood levels or urinary excretion of the drug or its metabolite or drug-marker (Clark et al., 1996; Hatton et al., 1996). These methods are unpleasant for the patient and expensive. Interestingly, therapeutic drug monitoring may overestimate the actual adherence rate because patients tend to comply shortly before the drug test but not during the whole observation period. Another limitation is that a biochemical drug test is insensitive to inhaled medication.

The assessment of therapeutic adherence seems to be more complicated. However, clinical outcomes can be used to evaluate adherence, as these depend largely on the extent to which a patient undertakes the recommendations of health-care providers.

3. Adherence with COPD therapy

3.1 Adherence to medication

Medication non-adherence can take many forms: failure to fill prescriptions (primary non-adherence) or overuse, underuse or alteration of schedule or doses of medication (secondary non-adherence) (Bourbeau et al., 2007; George et al., 2007; Rand et al., 2005).

Only a limited number of studies have evaluated adherence in patients with COPD. Jung et al. (Jung et al., 2009) examined medication adherence and persistence among a sample of COPD patients during their last year of life. The study reviewed the use of inhaled corticosteroids (ICS), long-acting β_2 agonists (LABA), anticholinergics (AC) and methylxanthines (MTX), alone and in combination. The overall MPR to COPD medication was 44%. Approximately 30% of the patients persisted with the therapy, and the overall time to discontinuation was 94.2 days. These rates of cooperation are much lower than the drug-taking rates in other chronic diseases. Adherence in hypertension, dyslipidaemia and diabetes is, on average, 72% (MPR), and persistence is 63% (Cramer et al., 2008). In the previously mentioned study, Jung et al. (Jung et al., 2009) found differences between the mean MPRs of COPD drug classes (MTX: 52%, AC: 38%, ICS: 35%, LABA: 34%). Medication adherence was the highest with MTX. One possible explanation of this finding could be that elderly patients may have more difficulty using inhaled medications; therefore, they prefer oral drugs.

Breekveldt-Postma et al. (Breekveldt-Postma et al., 2007) evaluated medication persistence among COPD patients in the first therapy year; new users of tiotropium, ipratropium, LABA and a fixed combination of LABA and ICS (LABA + ICS) were included in their study. The persistence was the highest, 37%, with tiotropium. The COPD patient's drug-taking behaviour was found to be significantly lower with other inhaled medications (ipratropium: 14%, LABA: 13%, LABA + ICS: 17%). Subgroup analysis of persistence data in patients with prior hospitalisation for COPD indicated that hospitalisation may have an enhancing effect on patient cooperation. The one-year persistence rates were increased by 2–3 times in the first year after hospitalisation (tiotropium: 61%, ipratropium: 37%, LABA: 41%, LABA + ICS: 33%). A similar study by Cramer et al. (Cramer et al., 2007) examined trends in patient persistence with inhaled COPD medication. They monitored the refill data of ipratropium, ipratropium + salbutamol, formoterol, formoterol + budesonide, salmeterol, salmeterol + fluticason and tiotropium in a cohort of 31,368 COPD patients. The one-year persistence was considerably higher with tiotropium (53%) compared with other treatments (7%–30%). The significant differences in levels of adherence and persistence between inhaled medications could be partially the result of dosing frequency.

All of the aforementioned studies examined primary adherence, which is based on prescription refill rates. These results represent the maximum possible level of patient cooperation because refill adherence cannot confirm ingestion and does not provide any information on the frequency of medication use. Studies evaluating secondary adherence can provide data about medication use that is more reliable.

The Lung Health Study (Rand et al., 1995) was a double-blind, multicentre, randomised, controlled trial on smoking intervention and bronchodilator therapy (ipratropium or placebo) as early interventions of COPD. Satisfactory adherence was reported by 70% of the participants at the first 4-month follow-up visit, but this rate declined to 60% over the next 18 months. The overall adherence estimated by canister weighing was 72% in the first year and 70% in the second year. Nevertheless, in the first year, only 48% of the participants were classified as adherent with both methods. In an ancillary study within the Lung Health Study, medication adherence rates measured by both self-report and canister weighing were compared with data from electronic medication monitoring (Rand et al., 1992). This study found that self-reporting and canister weighing significantly overestimate adherence: only 15% of the participants used their inhaler 2.5 or more times per day (when three puffs per day were prescribed). In addition, 14% of the patients seemed to be "dumping" medication prior to the clinic visit by removing most of the medication at one time (i.e., actuating inhaler more than 100 times in a 3-h interval) to hide non-adherence. The level of adherence with the prescribed medication regimen was best immediately following each follow-up visit and declined during the interval between follow-up visits. The adherence after each visit was lower for each successive follow-up. These trends could be observed only with electronic medication monitors; self-reporting or weighing could not detect these changes (Simmons et al., 1996).

Studies also suggest that while the underuse of medication seems to be one of the largest problems in the management of COPD, overuse is also common. Symptom-relieving drugs, such as short-acting β_2 agonists (SABA), are more often overused than maintenance therapies (Dekker et al., 1993). Krigsman et al. (Krigsman et al., 2007a) evaluated the primary adherence in patients with asthma and COPD. The obtained results indicated that 53% of the patients underused and 18% overused their prescribed medication regimens. In another study by Krigsman et al. (Krigsman et al., 2007b), it was found that 59% of COPD patients had an undersupply and 12% had an oversupply of ICS medication.

Eighty-four percent of COPD patients have one or more co-morbidity (Yeo et al., 2006). For this reason, a question arises about whether the level of a patient's adherence is the same with therapies for different chronic diseases. Krigsman et al. (Krigsman et al., 2007c) investigated refill adherence in patients who suffered from diabetes and COPD. Participants showed higher adherence for their diabetes drugs (68%) than their COPD medications (42%).

Long-term oxygen therapy (LTOT) plays an important role in the management of COPD (Würtemberger & Hütter, 2000). The daily duration of oxygen administration is crucial in the effectiveness of LTOT. Pepin et al. (Pepin et al., 1996) found that only 45% of the COPD patients who were prescribed oxygen therapy for an average of 16 hours per day (16±3 h/d) used oxygen for 15 hours or more per day. Another study reported that 23% of the patients who had been prescribed LTOT refused to use liquid oxygen away from home and that 12% underused their oxygen (Würtemberger & Hütter, 2000).

Immunisation with both the influenza and pneumococcal vaccines may produce a number of acute exacerbations, hospitalisation and COPD mortality (Nichol et al., 1999; Varkey et al., 2009). However, the vaccination rates in patients with chronic lung diseases are low (Nichol et al., 1999; Tuppin et al., 2011), and the willingness to vaccinate differs by age group. The influenza vaccination status is significantly higher in patients aged 65 years or older (86.2%) than in the younger population (65.7%) (Mehuys et al., 2010).

3.2 Adherence to non-drug therapy

Adherence to non-drug therapies, such as respiratory rehabilitation, exercise programs, healthy lifestyle or smoking cessation, is crucial in the management of COPD. Approximately 60% of the patients refuse to take part in rehabilitation programs, and out of those who join, 30% fail to complete the program (Nici et al., 2006). The most important barriers to rehabilitation adherence include exacerbations and progression of COPD (Bourbeau et al., 2007; Brooks et al., 2002). The literature in this field is quite weak; there is a clear need for further research to find out more about the suboptimal adherence to non-drug therapies in patients with COPD.

4. Factors associated with adherence in patients with COPD

Non-adherence in patients with COPD is a multidimensional phenomenon. The factors include the characteristics of the patient, the disease, the therapies and the health-care provider–patient relationship; many of these are potentially modifiable (Baiardini et al., 2009; Restrepo et al., 2008; WHO, 2003) (Table 4).

COPD	**Treatment**
• Progressive nature of the disease ↓*	• Polypharmacy ↓
• Poor prognosis ↓	• Higher dosing frequency ↓
• Lack of clinical symptoms ↓	• Higher medication cost ↓
• Disease severity —	• Side effects ↓
• Lung function —	• Oral administration ↑
Patient	**Health-care provider–patient relationship**
• Gender -	• Higher quality of communication ↑
• Demographic factors: old age ↑	• Type of caregiver: specialist ↑
• Improved quality of life ↓	• Closer follow-up ↑
• Social support ↑	• Hospitalisation ↑
• Psychiatric co-morbidities ↓	

*Influence on adherence: decrease (↓), improve (↑), no effect (—)

Table 4. Factors associated with adherence in patients with COPD

4.1 Factors related to the characteristics of COPD

COPD is a progressive chronic disease. Adequate cooperation with COPD therapy can improve the patient's quality of life and reduce the frequency of exacerbations but cannot fully control the disease symptoms. A progressive decline in lung function is often interpreted by patients as the medication not helping, so they stop following the recommendations (Chambers et al., 1999). In contrast, a lack of clinical symptoms could also be a reason for suboptimal adherence (DiMatteo, 2004). As implied above, the negative impact of COPD severity or lung function on a patient's adherence is not obvious. Prior studies have shown that disease severity or the post-bronchodilator forced expiratory volume in one second (FEV_1) percentage may be either not (Agh et al., 2011) or negatively (Turner et al., 1995) related to adherence. The pathologic characteristics of COPD influence

adherence to non-drug therapy as well; a poor COPD prognosis has been identified as one of the most demotivating factors to quit smoking (George et al., 2006b).

4.2 Factors related to the characteristics of the patient

Most prior studies have found that gender does not influence the level of patient cooperation (Agh et al., 2011; Apter et al., 1998; Corden et al., 1997; Turner et al., 1995). Adherence differences between men and women reported in the literature may be caused by psychological factors (Laforest et al., 2010). The prevalence of anxiety and depression are higher in women with COPD, and these psychiatric comorbidities have been independently linked with non-adherence (Bosley et al., 1995; DiMatteo et al., 2000).

In general, drug-taking behaviour is related to age; older patients seem to be more adherent. Patients of advanced age are more likely to adhere to therapy that requires adjustments in daily life (Agh et al., 2011). However, memory loss and cognitive impairment, which are associated with both age and COPD duration, may adversely affect adherence (Incalzi et al., 1997).

Social support can also influence patient adherence. Stable family life has been found to improve adherence to medication regimens (Tashkin, 1995; Turner et al., 1995). Furthermore, the study by George et al. (George et al., 2006b) indicates that patients with a good relationship with family and friends may live longer and may quit smoking with a higher success rate.

Better quality of life has been considered a trigger for non-adherence (Agh et al., 2011). Decision-making regarding patient adherence is a personal trade-off between the efficacy of the therapy and the negative effects that it generates. Adherence to COPD therapy can reduce the clinical symptoms and improve the patient's quality of life. However, COPD treatment regimens require adjustments in daily life, such as smoking cessation and exercise programs, and can cause side effects as well. Therefore, the interruption of drug therapy can temporarily also increases the patient's quality of life. Therapy in newly diagnosed COPD patients may significantly improve quality of life; however, the change in quality of life may be much smaller in patients treated previously for longer durations (Soumerai et al., 1991). From the patient's perspective, the benefits from the increase in the quality of life during the complication-free period can outweigh the effects of the worsening disease symptoms (Agh et al., 2011).

4.3 Factors related to the characteristics of the therapy

The number of medications and the dosing frequency have been linked with adherence. According to our evaluations, the dosing frequency of respiratory drugs is one of the most important factors affecting non-adherence in patients with COPD (Agh et al., 2011; WHO, 2003). As a partial result of the daily drug doses, a significant difference has been shown in the adherence rates between the different respiratory drug classes (Apter et al., 1998; Breekveldt-Postma et al., 2007; Laforest et al., 2010). Tiotropium, a once-daily inhaled drug, may enhance adherence compared with other inhaled respiratory medications that are dosed more times daily. We also found that polypharmacy is another common cause of poor adherence (Agh et al., 2011); complicated treatment regimens may frustrate the patients, which may lead to non-adherence (van der Palen et al., 1999).

Patient cooperation is better with oral medication than with inhaled drugs (James et al., 1985; Tashkin et al., 1991). Adherence with inhaled drugs may be compromised by inadequate inhaler technique (Garcia-Aymerich et al., 2000; Shrestha et al., 1996). Furthermore, better adherence with oral theophylline can also be due to the simplicity of the dosing regimens (Kelloway et al., 1995).

Other factors, such as adverse effects and medication costs, are also important. Medication cost is one of the greatest barriers to achieving adequate adherence (Cramer et al., 2007, Jung et al., 2009). Side effects or concerns about side effects from medications can reduce adherence as well (Dolce et al., 1991; Rand et al., 1995). For example, patients with COPD often confuse the side effects of ICS with those of anabolic steroids, which may decrease their cooperation willingness (Boulet, 1998).

4.4 Factors related to the characteristics of the health-care provider–patient relationship

Effective COPD management requires a good relationship between health-care providers and the patients. Quality of communication is related to adherence. Adherent patients report better overall communication with their providers (Blais et al., 2004). Education during the consultation and providing more information about the therapy may improve adherence (Raynor, 1992), as it reduces the risk of forgetting the providers' recommendations and the likelihood of misunderstandings between providers and patients. Previous studies suggest that immediately after the consultation, patients recall less than 50% of the information conveyed by their provider (DiMatteo, 1991).

The type of caregiver also influences adherence. Medication adherence may increase if the prescribing physician is a specialist instead of a general practitioner (Lau et al., 1996). Furthermore, periodic visits, closer follow-up and hospitalisation may also have increasing effects on patient cooperation (Breekveldt-Postma et al., 2007).

5. Adherence enhancing interventions

Strategies for improving patient adherence have to be formulated based on factors related to adherence. Seventy-six adherence interventions were evaluated in the systematic review by Petrilla and Benner (Petrilla & Benner, 2003).

They identified the following main categories of adherence-enhancing interventions:

- coordination of healthcare: improved linkages between primary care physicians, clinicians and other health professionals;
- live consultation and education;
- changes to the therapy dose, dosage and packaging to enhance the drug-taking convenience;
- patient education materials;
- disease management programs by clinicians;
- reminders: medication refill reminders delivered by mail or telephone;
- self-monitoring;
- social support programs;
- and combinations of these interventions.

Successful adherence-enhancing programs include simplified treatment regimens, facilitation of the physician–patient relationship and patient education methods (Petrilla & Benner, 2003).

While many studies have evaluated strategies to enhance adherence, few of these have focused on COPD. Strategies for improving adherence in COPD include simplifying treatment regimens, improving communication between providers and patients, disease education, optimising inhaler technique, reinforcement and self-management (self-monitoring of symptoms and medication use).

It may be important to prescribe drugs with a fixed combination and/or a low dosing frequency to enhance adherence to COPD medication. Furthermore, the recommended treatment should fit into the patient's limitations and lifestyle. Because many COPD patients are elderly, with the dual risk of cognitive impairment and complex medication regimens, the use of dosing aids and adherence devices, such as medication lists, dosette boxes and timers, should be promoted.

Health-care providers must help their patients understand the progressive nature of COPD and the goals of the comprehensive treatment regimens. Physicians should actively involve patients in decisions regarding their therapy and give strong weight to their personal preferences and concerns. Periodic monitoring, understanding the patient's beliefs and positive reinforcement could also enhance adherence to therapy (Dunbar et al., 1979).

6. Conclusion

Suboptimal adherence to medication regimens and to other non-drug therapies are both major problems in the management of COPD. Poor adherence poses a significant health and economic burden in patients with COPD. Non-adherence seems to be influenced by many individual reasons, such as factors associated with the characteristics of the disease, the patient, the therapy and the physician-patient relationship. Among other things, simplified treatment regimens, adequate patient education methods and better communication between caregivers and patients have been found to be critical for overcoming the barriers of poor adherence. However, further research is needed to identify factors related to patient cooperation to develop more effective strategies that can improve adherence.

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15

Management of Acute Exacerbations

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1. Introduction

American Thoracic Society (ATS) and European Respiratory Society (ERS) define an exacerbation as an acute change in a patient's baseline dyspnea, cough, or sputum that is beyond normal variability, and that is sufficient to warrant a change in therapy(Celli MacNee 2004). Exacerbations have a negative impact on mortality and morbidity and as the disease progress, the frequency and severity of exacerbations increase, leading to a fall in the quality of life of COPD patients. There is no standard method or tool for the diagnosis of an exacerbation. The changes in the clinical status of the patient should be taken into account.

The most important parameters predicting mortality in patients who are hospitalized due to an acute exacerbation are; severity and stage of COPD, advanced age, co morbidities such as diabetes mellitus or cardiovascular disease, need of intubation and mechanical ventilation, high APACHE II score, presence of sepsis and multi organ failure (Groenewegen et al. 2003).

2. Etiology of exacerbations

Tracheobronchial infections (40-50% bacterial, 30-40% viral, 5-10% atypical bacteria) are involved in 50-70% of COPD exacerbations. Another factor is air pollution that is thought to be involved in 10% of exacerbations. In about 30%, the etiologic factor cannot be identified(Sapey Stockley 2006). Other medical problems, such as congestive heart failure, nonpulmonary infections, pulmonary embolism, and pneumothorax, can also lead to a COPD exacerbation.

Infections:

Bacterial: (Streptococcus pneumonia, Haemophilus influenza, Moraxella catarrhalis, Chlamydia pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus)

Viral: (Rhinovirus, influenza, adenovirus, parainfluenza, coronavirus, respiratory cincitial virus)

Environmental factors:

Indoor and outdoor air pollution

Patients who are known to have COPD are defined as an exacerbation when they are admitted to the emergency departments with increased dyspnea during fall and winter. The main issue is the underestimation of non-infectious causes such as pulmonary embolism, pleural effusion, pneumothorax, thoracic traumas, inappropriate use of sedatives, narcotics and beta blockers, arrhythmias, cardiac failure or problems in the use of long term oxygen therapy. Therefore, in a COPD patient with increased dyspnea, first the diagnosis of exacerbation should be established correctly and then the etiology should be identified as infectious or non-infectious.

Potentially pathogen bacteria are identified in 30% of sputum cultures in mild exacerbations, while this rate can be up to 70% in severe exacerbations in patients who need ventilatory support (Sapey Stockley 2006; Siddiqi Sethi 2008).

3. Initial evaluation of an exacerbation

There are two main steps in the evaluation of a COPD exacerbation. The first step is the determination of severity of the disease that will guide the physician about the treatment approach and hospitalization decision. The second step is the identification of the etiologic cause and decide whether to initiate antibiotherapy or not.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) classifies COPD exacerbations as mild, moderate or severe, based on the intensity of the medical intervention required to control the patient's symptoms (Table 1)(Rabe et al. 2007).

Clinical history	**Mild** (home treatment)	**Moderate** (hospital treatment)	**Severe** (ICU treatment)
Comorbidity	+	+++	+++
Frequent exacerbation history	+	+++	+++
COPD stage	Mild/Moderate	Moderate/Severe	Severe
Hemodynamic status	Stable	Stable	Stable/Unstable
Accessory respiratory muscle use, cyanosis, paradoxal breathing, cyanosis, tachypnea	No	++	+++
Change in neurologic status	No	No	Yes
Symptoms of right heart failure	No	++	+++
Persistence of symptoms despite drug therapy	No	++	+++

+: probably doesn' t exist, ++: probably exists, +++: strongly may exist, ICU: intensive care unit

Table 1. Classification of COPD exacerbations

Another classification approach is suggested by Anthonisen and colleagues (Anthonisen et al. 1987). According to this approach, severe exacerbations requiring antibiotheraphy are characterized by the presence of increase in all of the 3 criteria: dyspnea, sputum production and sputum purulence. Moderate exacerbations show only the 2 of these criteria, while in mild exacerbations, only one of these criteria is present with a recent history of upper airway infection or fever or symptoms like wheezing, cough, tachypnea and tachycardia.

Diagnostic evaluation of a suspected COPD exacerbation varies whether the patient will be treated in the hospital or at home. Routine sputum culture evaluation is not recommended for mild exacerbations. In case of a severe exacerbation, oxygen saturation must be measured by a pulse oxymeter. Patients who are referred to a hospital must be evaluated by advanced diagnostic tests such as arterial blood gas analysis, chest x-ray, sputum gram staining and cultures, electrocardiography and blood drug levels if possible (Table 2).

Diagnostic procedures	**Mild**	**Moderate**	**Severe**
Oxygen saturation	Yes	Yes	Yes
Arterial blood gas analysis	No	Yes	Yes
Chest X-ray	No	Yes	Yes
Blood tests *	No	Yes	Yes
Serum drug concentrations**	If possible	If possible	If possible
Sputum gram staining and cultures	No	Yes	Yes
ECG	No	Yes	Yes
BNP †	No	No	Yes
Cardiac enzyme measurement ‡	No	No	Yes

*: blood cell count, serum electrolytes, urea, creatinine, liver function tests.
**: theophylline, warfarin, carbamazepine, digoxin
†: One third of dyspnea in chronic lung disease may be attributable to congestive heart failure.
‡: Cardiac ischemia (myocardial infarction is underdiagnosed in patients with COPD).

Table 2. Diagnostic evaluation of patients with suspected COPD exacerbation

About 50% of COPD exacerbations are not reported to physicians(Seemungal et al. 2000). This suggests that half of the exacerbations are mild and do not require hospitalization. Indications for hospitalization of a patient with COPD exacerbation are as follows:

1. Onset of new physical signs such as cyanosis, peripheral edema, detoriation in the neurological status, arrhythmias etc.
2. Having severe or very severe COPD and being under long term oxygen therapy at home.

3. No response to initial drug therapy.
4. Pulmonary (pneumonia), or non-pulmonary (cardiac disease, diabetes mellitus) comorbidities with high risk.
5. Having exacerbations often.
6. Newly diagnosed arrhythmias.
7. Diagnostic uncertainty.
8. Advanced age.
9. Detoriation in the arterial blood gas analysis results (pH< 7.35 or PaO_2< 60mmHg or SaO_2< 90%)
10. Insufficient home support

4. Treatment of COPD exacerbations

All moderate and severe exacerbations must be evaluated in the hospital and in all severe exacerbations; arterial blood gas analysis must be performed. The patient must be evaluated immediately in terms of respiratory failure and oxygen therapy must be initiated if needed. Life threatening exacerbations should be followed up in the ICU. Respiratory failure with hypercapnia and respiratory acidosis is related with high mortality both at the time of admission and also during the 12 months follow up. While carbon dioxide retention is possible in moderate and severe exacerbations under oxygen therapy, arterial blood gas analysis must be performed every 30-60 minutes in order to detect the $PaCO_2$ and pH levels.

The inhaled oxygen concentration must be titrated to achieve a SaO_2> 90% or a PaO_2> 60 mmHg. High-flow oxygen devices deliver oxygen more effectively than nasal canulas but nasal canulas may be tolerated better. If adequate oxygenation cannot be achieved by high flow masks or if the acidosis begins worsening (pH<7.35 and/or $PaCO_2$> 50mmHg), noninvasive ventilation (NIV) is indicated. Success rates of NIV in COPD exacerbations are reported as 80-85% (Mehta&Hill 2001). The effect of NIV must be evaluated at the end of first and second hour by an arterial blood gas analysis. If there is worsening in the arterial blood gas result or if the patients cannot tolerate NIV, has worsening hypoxemia or has severe comorbidities such as myocardial infarction, hemodynamic instability, severe arrhythmias or sepsis, intubation and invasive mechanical ventilation must be initiated immediately.

Short acting inhaled β-2 agonists are the first line preferred drugs for COPD exacerbations. The dosage and frequency of these drugs must be increased. Another option is to add an inhaler short acting anticholinergic drug or increase the dosage if the patient is already taking the drug. Nevertheless, the effectiveness of this combination still remains controversial. If there is a long acting bronchodilator the patient is not using, it can also be added to the therapy even though there is no clinical evidence showing the benefit of these drugs during an exacerbation. In severe exacerbations if the patient cannot inhale effectively, nebulised forms must be used. The role of theophylline in COPD exacerbations is controversial. If there is not enough response to short acting inhaled β-2 agonists, it can be used as a second choice drug. Serum levels must be obtained and patients must be followed carefully because of its cardiovascular side effects.

Characteristics of the patient and exacerbation	Possible causes	Oral therapy choices	Parenteral therapy choices
Mild exacerbation (no signs of respiratory failure and severe obstruction, no comorbidities, 3 or less exacerbations in the last one year, no antibiotic use in the last 3 months)	• H.Influenza • S.Pneumoniae • M.Catarrhalis • C.Pneumoniae • Viruses	• β-lactam (Penicilin,Ampicillin/ Amoxicillin) • Tetracycline • Trimethoprim/ Sulfhamethoxazole • β-lactam/ β-lactamase inhibitors (Co-amoxilav) • Macrolides (Azithromycin, Clarithromycin, Roxithromycin) • 2nd or 3rd generation cephalosporins • Ketolides (Telithromycin)	
Moderate/ Severe exacerbation (complicated exacerbation, risk factors for treatment failure but not for P. Aeruginosa	Added to above; • β-lactamase producing enteric gram (-) bacteria (K.Pneumonia, E.Coli e.g)	• β-lactam/ β-lactamase inhibitors (Co-amoxilav) • 2nd or 3rd generation cephalosporins • Floroquinolones (Gemifloxacin, Levofloxacin, Moxifloxacin)	• β-lactam/ β-lactamase inhibitors (Co-amoxilav, ampicillin/ sulbactam) • 2nd or 3rd generation cephalosporins • Floroquinolones (Levofloxacin, Moxifloxacin)
Severe exacerbation with a high risk of P. Aeruginosa*	Added to above; • P. Aeruginosa	• Floroquinolones (Ciprofloxacin, Levofloxacin-high dose)	• Floroquinolones (Ciprofloxacin, Levofloxacin-high dose) • β-lactams with antipseudomonal activity

*Hospitalization in the last one month, frequent antibiotic use in the last one year, exacerbation causing severe respiratory failure, isolation of *P. Aeruginosa* in the sputum culture during stable state or prior exacerbations.

Table 3. Antibiotherapy options in infectious exacerbations of COPD

Adding systemic (oral or intravenous) glucocorticosteroids to other therapies in the hospital management of exacerbations of COPD is recommended (Niewoehner et al. 1999). Systemic use of corticosteroids may lead to fast recovery and improvement in hypoxemia and lung functions in COPD exacerbations. The recommended dosage of prednisolon is 30-40 mg/day for 7-10 days if the patient has an initial FEV1 value below 50%. Prolonged treatment does not have a positive affect, besides it may increase the risk of side effects (e.g. muscle atrophy, hyperglycemia).

Bronchoscopic studies showed that the amount of bacteria is increased nearly in 50% of COPD patients during an exacerbation when compared to the stable state(Sethi 2004). The decision to use antibiotics and the choice of antibiotic should be guided by the patient's symptoms (e.g., presence of purulent sputum), recent antibiotic use, and local microbial resistance patterns. Prophylactic or continuous use of antibiotics does not improve outcome in patients with COPD (Rabe, Hurd et al. 2007).

Even though the most common bacteria responsible for the exacerbations are H.influenzae, S. pneumoniae and M. Catarrhalis; some enteric Gram (-) bacteria and Pseudomonas aeruginosa are also isolated in most of the patiens with hypoxemia, severe airway obstruction, malnutrition, frequent hospitalization and antibiotic use history and comorbidity(Incalzi et al. 2006). There are some studies suggesting that atypical bacteria can also be an etiologic reason for an exacerbation but antibiotherapy targeting these bacteria showed no positive affect on clinical outcomes(Diederen et al. 2007; Tasbakan et al. 2007). Viruses can also be responsible in 15-40% of all exacerbations. Most of these are present with a bacterial infection.

Antibiotherapy reduces the mortality rates and treatment failure especially in severe exacerbations of COPD(Puhan et al. 2007). Antibiotics also decrease the relapse rates of exacerbationsin outpatients(Adams et al. 2000). Therefore, antibiotherapy is strongly indicated expecially if the patient has purulant sputum and increase in dyspnea. Treatment options according to clinical status is summarized in Table 3.

5. Preperation for hospital discharge

In order to qualify for a discharge, the patient must have stable clinical conditions and a stable or improving arterial PaO_2 of greater than 60mmHg. The patient should not require short acting β-agonist more often than every 4 hours. If the patient is stable and can use a metered dose inhaler, there is no extra benefit of using nebulised forms (Jenkins et al. 1987). Patient education including topics such as medical treatment, nutrition, rehabilitation and physiotherapy programs and when to seek for professional medical help may improve the response to future exacerbations. Home support such as home mechanical ventilation, long term oxygen therapy, nebulisers or similar equipments should be arranged before discharge.

6. Preventing future exacerbations

Pulmonary rehabilitation, smoking cessation and immunization against influenza and pneumonia have been shown to improve health quality and reduce exacerbations in COPD

patients. There are also some data showing that long term oxygen therapy reduces the risk of hospitalization and shortens hospital stays in severely ill COPD patients. Long-acting inhaled bronchodilators and inhaled corticosteroids to improve symptoms and reduce the risk of exacerbations in patients with stable COPD are reviewed elsewhere with promising results.

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16

Novel Concept in Pulmonary Delivery

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1. Introduction

This chapter deals with recent advances in the nanovector approach to the pulmonary delivery of therapeutic substances; it also describes briefly the physiology of the lungs and the main factors affecting pulmonary delivery (Figure 1).

The development of an innovative nanocarrier, able to deliver the drug to the desired site of action, is highly dependent on the nature of the active substance and on its desired mode of action.

A delivery technology can thus be used to:

- reduce systemic exposure and improve drug targeting;
- circumvent the parenteral route or reduce the number of injections;
- achieve sustained plasma levels of the drug;
- reduce side – effects;
- modulate the effect of the drug, specifically in the case of vaccines, where the delivery system can modify the ratio of humoral and cellular response;
- increase patient compliance;
- reduce the price of the therapy;
- increase patent life and/or circumvent patents of competitors.

Numerous approaches involving non - parenteral routes, such as intestinal, nasal, buccal, transdermal and rectal, have been examined, but most of them are inadequate for a satisfactory therapeutic response. On the other hand, the pulmonary route represents a great promise for the systemic delivery and bioavailability of peptides and proteins, since lungs are highly permeable and accessible by normal inhalation methods. Actually, the pulmonary

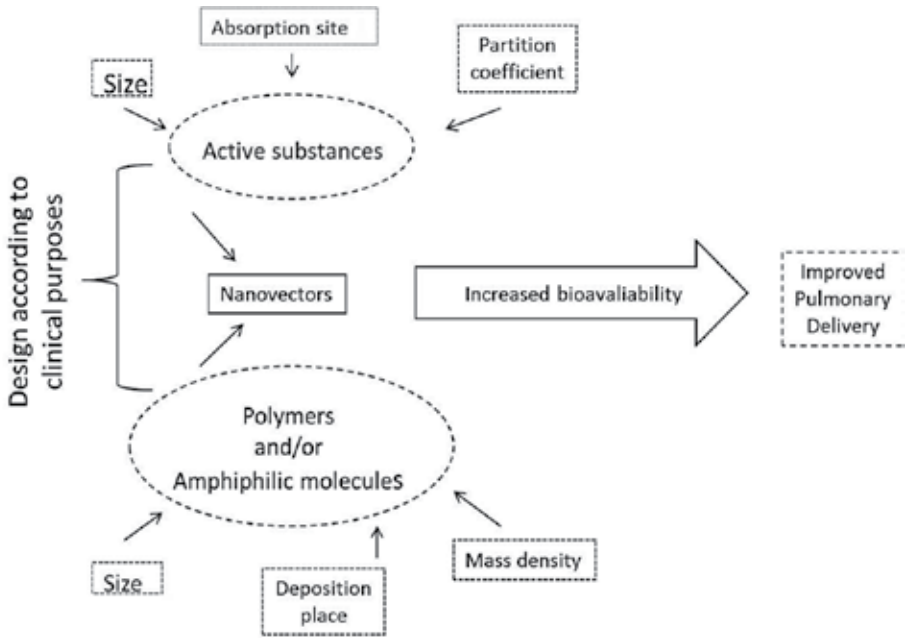

Fig. 1. Factors affecting pulmonary delivery

route typically shows from 10 to 200 times greater bioavailability with respect to other non - invasive routes (Patton & Byron, 2007). Thus, various pharmaceutical techniques have been introduced to take advantage of this route. These include formulations using specific and innovative excipients, chemical modifications of drugs, polymeric and/or amphiphilic Drug Delivery Systems (DDSs), particle engineering, inhalation devices.

In particular, over the past decade, the use of DDSs involving micro or nanocarriers and particle engineering techniques were remarkably developed and the resulting pharmaceutical techniques have been eagerly applied to the pulmonary delivery of drugs.

Inhalation is also a proven means of systemic delivery for drugs that have limited bioavailability by other routes or would benefit from rapid onset of action and a variety of products are being developed for this purpose (Gonda, 2006; Patton & Byron, 2007).

At the same time, in recent decades, advances in device design and formulation science have addressed the need for more efficient inhalers that are capable of delivering larger doses to the lung with low extrathoracic deposition. Once deposited in the lungs, drug disposition (dissolution, absorption, distribution, metabolism and elimination) and the influence of pulmonary pharmacokinetics (PK) are the critical determinants of clinical outcomes in terms of drug efficacy and safety. Pulmonary disposition remains poorly understood despite modern capabilities in imaging, analytical and biological science which make measurement of drug disposition and mode of action more accessible. For these reasons the development of new inhaled medicines capable to allow improvements in current therapy is a promising challenge in the development new DDSs.

A number of benefits result from inhalation of drugs and a continuously increasing number of inhaled drugs and nanomedicines are becoming available for the treatment various

diseases. Inhalation of drugs is convenient and extremely efficient to treat diseased airways. It allows a targeted therapy with high drug concentrations in the tissue of interest and low systemic drug exposure (and thereby reduced side effects). Inhalation aerosols have also been developed for systemic drug administration. The large absorptive surface area, the very thin diffusion path to the bloodstream and the elevated blood flow make the lung a port of entry to the systemic circulation and proteins are absorbed more efficiently from the lungs than from any other non-invasive route of drug administration.

Only one inhaled therapeutic protein is currently available on the market. It is recombinant human deoxyribonuclease I (Dornase alfa) indicated for the treatment of cystic fibrosis (CF) and marketed since 1994.

Recombinant human deoxyribonuclease I is a glycoprotein of 37 kDa, which selectively cleaves DNA. In CF patients, retention of viscous purulent secretions in the airways contributes both to reduced pulmonary function and to exacerbation of infection. Purulent pulmonary secretions contain very high concentrations of extracellular DNA released by degenerating leukocytes. Dornase alfa is delivered to CF patients by inhalation of an aerosol mist produced by a pneumatic nebulizer. It hydrolyses the DNA in airway secretions and reduces their viscoelasticity.

Inhalation can represent the most favourable non-invasive route of administration for insulin (5.8 kDa) because insulin bioavailability can reach 37% following inhalation while it reaches at most 1% following oral, sublingual, nasal or transdermal administration without chemical enhancers (Cefalu, 2004; Illum, 2002). The first inhaled insulin product, Exubera®, was approved in January 2006 but withdrawn from the market already in October 2007 due to disappointing sales.

Another inhaled insulin product, AFREZZA™, is currently under review by the FDA for the treatment of type 1 and type 2 diabetes (Neumiller & Campbell 2010; Rossiter et al., 2010).

AFREZZA™ is an ultra rapid acting insulin comprising Technosphere® insulin powder in unit dose cartridges for administration with the inhaler. The Technosphere® powder formulation is prepared by precipitating insulin from solution onto preformed diketopiperazine particles, which readily dissolve once in the lung environment. AFREZZA™ appears to overcome several limitations of Exubera®. Technosphere® insulin is both rapidly absorbed and eliminated and its pharmacokinetic profile mimics more closely normal physiologic insulin release than injection of regular human insulin as well as of rapid-acting analogues.

A few small-scale clinical trials have been conducted on inhalation of other systemically-acting therapeutic proteins, including interferon alpha-2b (19.3 kDa), human growth hormone (22 kDa) (Walvoord et al., 2009), an erythropoietin-Fc fusion protein (112 kDa) (Dumont et al., 2005). Inhalation of growth hormone is a potential alternative to growth hormone injection, which could offer improved patient adherence especially in pediatric patients. The bioavailability of inhaled growth hormone was 3.5% relative to subcutaneous injection in children, while it reached 7.6% in adults (Walvoord et al., 2009). The hypothesis behind this difference is that children have smaller oropharynx and larynx, which results in different deposition patterns as compared to adults. Although children preferred the inhalation route of therapy, ongoing development of growth hormone inhalation has been delayed due to its low bioavailability. An erythropoietin-Fc fusion protein was absorbed in

the bloodstream following delivery to the central lung regions in humans, with a dose-dependent concentrations in the serum, suggesting that large therapeutic molecules can be delivered to humans via the lungs using the FcRn-mediated transport pathway (Dumont et al., 2005).

2. Practical issues in the pulmonary delivery

2.1 Physiological features of the lungs

The lung resembles an inverted tree, where the trachea or trunk subdivides into two main bronchi and these latter successively branch into more and more narrow and short bronchioles. In total, the trachea undergoes 23 bifurcations before it reaches the alveolar sacs. The first 16 generations compose the conducting region where air is filtered, warmed, humidified and conducted to the respiratory region. Gas exchange between airspaces and blood capillaries occurs in the respiratory region, which includes the respiratory bronchioles, the alveolar ducts and the alveolar sacs.

Two different epithelia line the conducting and respiratory regions. A pseudo stratified columnar epithelium lines the proximal conducting airways and is composed of ciliated columnar cells, goblet or mucus secreting cells and basal or progenitor cells (Parkes, 1994). It is progressively replaced by a simple cuboidal cell layer in the more distal airways and by a very thin epithelial lining in the alveoli. Squamous type I pneumocytes cover 95% of the alveolar surface, owing to their large apical surface and thinness (0.05 µm). Cuboidal type II pneumocytes produce the lung surfactant and are progenitor for type I cells. Type II pneumocytes are located in the corners of the alveoli. The surface area of the alveolar epithelium reaches 100 m^2, which is enormous as compared to the 0.25m^2 surface area of the airways (Crapo et al.,1982; Mercer et al., 1994).

Mucociliary clearance is one of the most important defense mechanisms to eliminate dust and microorganisms in the lungs (Van der Schans, 2007).

The mucus is produced by goblet cells and sub-mucosa glands. It covers the entire airway surface and its thickness ranges from 5 µm to 55 µm (Clunes & Boucher, 2007; Lai et al., 2009). It consists of an upper gel phase made of 95% water, 2% mucin, a highly glycosylated and entangled polymer, as well as salts, proteins and lipids (Bansil & Turner, 2006). A periciliary liquid layer underlies the mucus gel and its low viscosity allows effective cilia beating. The mucus is transported by the coordinated beating of the cilia and by expiratory airflow towards the oropharynx at an average flow rate of 5 mm per minute. Mucus, cells and debris coming from the nasal cavities and from the lung meet in the pharynx, are mixed with saliva and are swallowed.

Pulmonary surfactant is responsible for biophysical stabilizing activities and innate defense mechanisms. It lines the alveolar epithelial surfaces and overflows into the conductive airways so that the surfactant film is continuous between alveoli and central airways (Bernhard et al., 2004). Pulmonary surfactant is composed of 80% phospholipids (half of which being dipalmitoylphosphatidylcholine), 5–10% neutral lipids (mainly cholesterol), 5–6% specific surfactant proteins and 3–4% non-specific proteins (Perez-Gil, 2008).

The phospholipids are mainly responsible for forming the surface active film at the respiratory air–liquid interface. In water, phospholipids self-organize in the form of bilayers.

Bilayers are also the structural form in which surfactant is assembled and stored by pneumocytes in lamellar bodies. At the air–liquid interface, phospholipids form oriented monolayers, with the hydrophilic headgroups oriented towards the aqueous phase and the hydrophobic acyl chains pointing towards the air. The higher the concentration of phopholipid molecules at the interface, the lower the surface tension, the lower the energy required to enlarge the alveolar surface during inspiration.

Specific surfactant proteins include SP-A, SP-B, SP-C and SP-D. SP-A and SP-D are hydrophilic while SP-B and SP-C are hydrophobic. SP-A is able to bind multiple ligands, including sugars, Ca^{2+} and phospholipids. This property allows SP-A to bind to the surface of pathogens, contributing to their elimination from the airways. Recognition of SP-A by specific receptors on alveolar macrophages stimulates phagocytosis of the pathogens. SP-B is strictly required for the biogenesis of pulmonary surfactant and its packing into lamellar bodies. Both, SP-B and SP-C promote rapid transfer of phospholipids from bilayers stores into air– liquid interfaces.

Luminal airway and alveolar macrophages are at the forefront of lung defence and their primary role is to participate in innate immune responses, that is, chemotaxis, phagocytosis, and microbial killing (Geiser, 2010). They also downregulate adaptive immune responses and protect the lung from T-cell-mediated inflammation (Holt et al., 2008). Macrophages are tightly applied on the surface of respiratory epithelia. They are immersed in the lung lining fluid beneath the surfactant film.

Although they occupy only 1% of the alveolar surface, they are capable to clean particles from the entire alveolar surface due to amoeboid movements (Geiser, 2010). In contrast to surface macrophages, interstitial macrophages are primarily involved in adaptive immunity by interfacing with lymphocytes via antigen presentation and production of cytokines (Geiser, 2010).

The lung presents a lower level of metabolism than the gastrointestinal tract and liver. Yet, various peptidases are distributed on the surface of different cell types in the lung, including bronchial and alveolar epithelial cells, submucosal glands, smooth muscles, endothelial cells, connective tissue. Proteases are largely present in lysosomes (Buhling et al., 2004). Proteases that degrade the extracellular matrix are secreted by different structural cells or are membrane bound (Stamenkovic, 2003).

Proteases play an essential role in cell and tissue growth, differentiation, repair, remodelling, cell migration and peptide-mediated inflammation (van der Velden & Hulsmann, 1999). Proteases can also be released in the airspaces by activated macrophages and neutrophils in case of inflammatory reactions in the respiratory tract (Buhling et al., 2006; Tetley, 2002). Blood supply to the lungs is divided among the pulmonary and systemic circulations (Altiere & Thompson, 1996). The pulmonary circulation consists of the pulmonary artery that leaves the right heart, branches into a dense pulmonary capillary bed that surrounds the alveoli and finally coalesces into the pulmonary vein that drains into the left heart. One hundred percent of the cardiac output flows through the pulmonary circulation. Its principal functions are gas exchange with air in the alveoli and nutrients supply to terminal respiratory units. The lungs receive a second blood supply via the systemic circulation, commonly referred to as the bronchial circulation. The bronchial circulation originates from the aorta and provides oxygenated blood and nutrients to all

structures of the tracheobronchial tree. Lymphatic vessels exist in close proximity of major blood vessels and of the airways (El-Chemaly et al., 2009). The lungs have unique physiological features and provide many conditions that favour the absorption of peptides and proteins.

2.2 Barriers to the pulmonary delivery of active substances

2.2.1 Deposition of nanocarriers through the respiratory tract

As pointed out in the section on lung physiology, the structure of the lung tissue largely varies according to airway generation and the fate of nanomedicines will similarly vary depending on the structures on which they deposit. The site of deposition of an inhaled formulation within the respiratory tract depends on the aerodynamic diameter of the aerosol particles. The aerodynamic diameter of a particle, d_{aer}, is equivalent to the diameter of a unit density (ρ_0) sphere that has the same terminal velocity in still air as the particle:

$$d_{aer} = d\sqrt{\frac{\rho}{\rho_0 X}} \tag{1}$$

where d is the geometric diameter of the particle, ρ is the particle density and χ is the particle dynamic shape factor denoting deviation of shape from sphericity (Hinds, 1999).

Filtering of large particles (d_{aer} >5 μm) occurs in upper airways (mouth, trachea and main bronchi) by inertial impaction. One to 5 μm d_{aer} particles deposit by gravitational settling in the central and distal tract. Particles with d_{aer} <1 μm remain suspended in the air and are mostly exhaled. Ultrafine particles (<100 nm) can largely deposit in the respiratory tract by random Brownian motion: particles <100 nm reach the alveolar region while particles <10 nm already deposit in the tracheo-bronchial region due to their high diffusion coefficients (Heyder at al., 1986).

Drug delivery inhalers, that include nebulizers, metered-dose inhalers and dry powder inhalers, generate particles with a d_{aer} in the micron-size range for deposition in the tracheo-bronchial tree (3–10 μm) in order to treat the airways (e.g., β2 mimetics) or in the alveolar region (1–3 μm) for systemic drug absorption (e.g., insulin) (Figure 2).

Therapeutic proteins can be delivered to the lungs by any medical inhaler. Yet, medical inhalers are not designed to produce ultrafine particles. Ultrafines require enormous energy for their creation, that is, for the atomization of the solution into nano-sized liquid droplets or for the complete de-agglomeration of nanosized dry powder particles. Therefore, drug formulations based on nanoparticles are most often delivered to the respiratory tract by nebulization of colloidal suspensions (Dailey et al., 2003). Developments of the preparation of dry powder microparticles as nanoparticles carriers for pulmonary drug delivery were also reported quite recently (Tsapis et al., 2002). It should be noted, ultrafines are generated in abundance in our environment by the most significant pollution sources, which are those related to combustion processes (Morawska et al., 2005). Epidemiologic studies have provided evidence that an increase in atmospheric ultrafines is associated with adverse pulmonary and cardiovascular effects in susceptible parts of the population. Therefore, significant research has focused on the fate of inhaled ultrafines in the body and ultrafines have been frequently produced in laboratories using spark generators (Geiser et al., 2008).

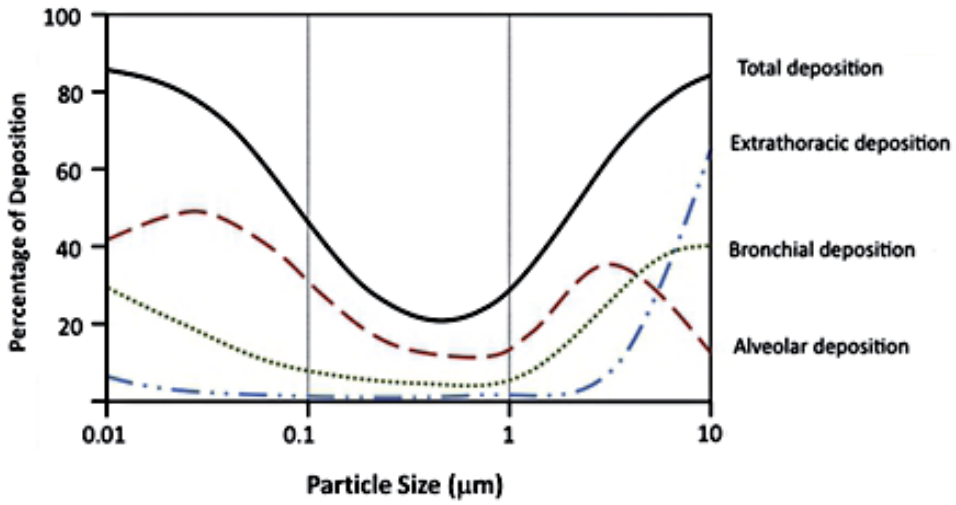

Fig. 2. Deposition of nanocarriers in human respiratory tract as a function of size

Data from several of these studies have been included in this review as the pulmonary fate of atmospheric ultrafines has likely similarities with the pulmonary fate of nanomedicines (Geiser et al., 2008; Furuyama et al., 2009).

2.2.2 Clearance mechanisms

Various elimination pathways for nanoparticles exist in the lungs, including coughing, dissolution, mucociliarly escalator, translocation from the airways to other sites, phagocytosis by macrophages and neuronal uptake (Figure 3); but the quantitative relationship among these pathways has not yet been established (Muhlfeld et al., 2008).

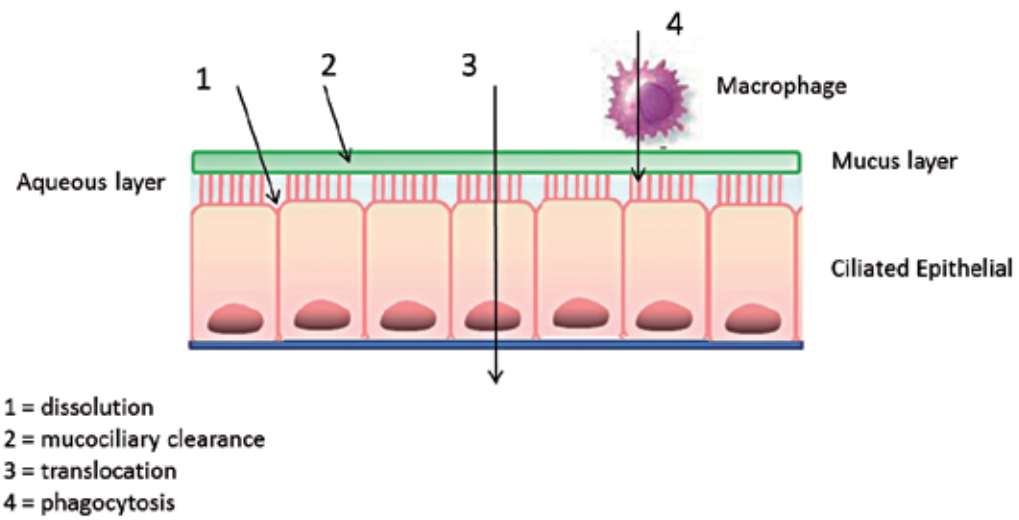

Fig. 3. Pathways involved in nanocarriers absorption

When a nanoparticle has landed on the airways, it first encounters the surfactant on the top of the airway lining fluid. The surfactant will enhance particle wetting thus helping it to sink into the fluid, passing first through the gel phase and then to the sol phase. Compared with sulphur colloidal particles (220 nm), human serum albumin molecules (HSA, 66 kDa) were cleared 3 times more slowly from the bronchi of dogs. This difference was attributed to the possibility that sulphur nanoparticles resided on the gel phase (i.e. the top layer of the periciliary fluid) whereas HSA dissolved and partitioned into the sol (i.e. bottom layer) which may be transported less effectively by mucociliary clearance (MCC). If extrapolated to inhaled drugs, the more soluble ones will behave like HSA and should be hence less susceptible to MCC (Edsbacker et al., 2008), but more likely absorbed through the epithelium. For nanoparticle agglomerates, it is likely that the particles will first reside on the gel phase. Depending on the aqueous dispersibility and solubility in the airway fluid, the agglomerates may remain in the gel phase behaving like the microsized particles, or they may then disperse into nanoparticles followed by dissolution and absorption. Nanoparticles delivered within a liquid droplet (e.g. from a nebulizer) might be different from dry particles, as the droplet liquid may interact with the gel layer making the nanoparticles easier to wet and partition into the sol layer, i.e. potentially more readily to escape the MCC (Zhang et al., 2011).

2.2.2.1 Dissolution

Dissolution depends on the site of deposition, which determines the volume of airway fluids available for dissolution and, hence, whether the dissolution occurs in sink or non-sink conditions, as well as on the solubility and dose of the drugs. Freely water soluble drugs include organic salts (e.g., salbutamol sulphate, terbutaline sulphate and disodium cromoglycate) and polar compounds (e.g. mannitol). These drugs will dissolve readily in the airway fluid followed by absorption or elimination by the mucociliarly escalator. Sparingly soluble drugs include the inhaled corticosteroids, which have aqueous solubility ranging from 140 to below 0.1 μg/mL (Edsbacker et al., 2008). Once dissolved, the drug molecules are diluted in the airway fluid where they can bind to proteins, opsonins, or other constituents and be metabolized and/or absorbed into the blood and lymph (Schmid et al., 2010).

Absorption of the drugs depends on the site such as alveolar or conducting airways (which affects the barrier thickness and surface area) and the drug molecule itself (which impacts on passive diffusion and active uptake by the epithelium. It must be pointed out that absorption of most drugs from the lungs is rapid: as an example, it has been reported that following inhalation of formoterol fumarate (4.5 nmol/L) and budesonide (136 pmol/L, the peak plasma concentrations occurred at 20 and 10 min, respectively.

2.2.2.2 Mucociliary clearance (MCC)

MCC operates in the ciliated airways where the movement of the cilia transports the mucus carrying the drug nanoparticles or dissolved drug (not yet absorbed) on the epithelial surface towards the pharynx/larynx. The drug-containing mucus will then be swallowed to the GI tract. The average transport velocity in the human trachea has been estimated at 3–10 mm/min, but the value varies widely among subjects. Using well-developed techniques of depositing radiolabelled sulfur colloids in the central airways, Daviskas and her colleagues reported a MCC rate remarkably reduced in patients with bronchiectasis, asthma, and CF,

with respect to healthy individuals. Actually, MCC and dissolution occur simultaneously and their relative importance should depend on the elimination rate from each of these contributions. While insoluble particles of 6 μm are practically all cleared from the bronchial airways by MCC in 24 h, smaller particles are retained for a longer period showing almost an inverse relationship between the 24 h airway retention and the geometric particle size. Nanoparticles with enhanced mobility may partition through the mucus into the periciliary spaces, where they can be taken up by the airway macrophages and bronchial epithelial cells, causing a reduction of MCC (Schmid et al., 2009).

2.2.2.3 Macrophage uptake

Alveolar macrophages are responsible for clearance of nanoparticles deposited in the alveolar region, in which MCC is absent. In response to the deposited nanoparticles, alveolar macrophages will migrate to the particles and phagocytize them via chemotaxis involving opsonisation. Macrophage uptake is believed to complete within 6–12 h after deposition of the particles in the alveoli (Oberdörster, 2007). nce internalized in the macrophages, the particles will be either disintegrated (e.g. by enzymes in lysosomes) or accumulated in the lymphatic system (Schmid et al., 2009) draining both airways and alveoli and finally terminating in the mediastinal and hilar lymph nodes (Geiser & Kreyling, 2010). A minor fraction of the particle-carrying macrophages will migrate to the ciliated airways where they are removed by MCC (Schmid et al., 2009). However, with a retention half-time of up to 700 days in humans (Oberdörster, 2007), clearance of solid particles by alveolar macrophages is a relatively slow mechanism. Phagocytosis of particles below 100 nm is not effective (Oberdörster, 2007), most probably because of a less effective recognition (~20%) of nanoparticles by the macrophages (Schmid et al., 2009). The reduced recognition is possibly due to more scattered and diluted chemotactic signals as a result of i) higher number concentration of nanoparticles (compared with micron-sized particles at the same dose) and ii) fewer opsonin molecules available per particle. Conversely, since nanoparticles are more readily taken up by epithelial cells, they become less available to be phagocytized by macrophages (Madl & Pinkerton, 2009). Macrophages are also present in the ciliated airway but their role in nanoparticle clearance is probably less important compared with MCC.

2.2.2.4 Translocation into cells, blood and lymph

This process involves transcytosis of the particles into the epithelial cells and/or across the epithelia of the respiratory tract into the interstitium and then to blood and lymph. As described earlier, translocation to the lymphatic system can be facilitated by macrophage uptake. The transport may be protein-mediated, requiring binding of certain proteins on the nanoparticle surface for recognition by the receptors (Schmid et al., 2009). The transport may also be receptor-mediated transcytosis via caveolae (Oberdörster, 2007), which have a diameter of 50–100 nm. Surface coating of the particles by albumin and lecithin may facilitate cellular uptake by pneumocytes and transcytosis across capillaries (Yang et al., 2008). Once internalized, nanoparticles can bind to mitochondria and even DNA in the nucleus (Muhlfeld et al., 2008; Oberdörster, 2007).

When translocated to the systemic circlulation, nanoparticles could cause unwanted effects on the blood (e.g. accumulation in platelets) and other organs in the body (Oberdörster, 2007). Some biological effects may include inflammation, oxidative stress, cytotoxicity, fibrosis, and immunologic responses (Madl & Pinkerton, 2009; Unfried et al., 2007).

Surface area has been proposed as the single most important particle dose parameter for the toxicity of nanoparticles (Schmid et al., 2009). This is particularly relevant for inflammatory and oxidative stress reactions, such as surface area of a catalyst (i.e. nanoparticles), that determines the oxidative reaction rate. However, oxidative stress involves the formation of reactive oxygen species (ROS) from the particles containing reactants such as transition metals or polyaromatic hydrocarbons (which induce the expression of the CYP1A1 protein). Drug nanoparticles, which do not contain such reactants, are therefore less likely to cause oxidative stress in the lungs. Biodegradabile nanoparticles indeed showed significantly lower inflammatory response in-vitro (Sung et al., 2007). Interestingly, translocation in the reverse direction with particles re-entrained from the lung capillaries or interstitium to the luminal side of the epithelium have been shown in animal models using rabbits and rats. Such back-translocation was suggested to be macrophage-mediated.

2.2.2.5 Neuronal uptake

Translocation into afferent vagal nerves in the tracheobronchial airways has been proposed but still not well studied (Oberdorster et al., 2005). Nanoparticles deposited in the nasal cavity have been reported to be taken up by the olfactory lobe and translocated to the central nervous system (Oberdorster et al., 2005). However, such a neuronal uptake pathway is relevant only if the drug nanoparticles are inhaled nasally. Existing data from epidemiologic and toxicological studies showed longer retention of inhaled nanoparticles in the lungs, but the applicability of these findings on nanoparticles is under investigation. In theory, inhaled nanodrug particles have the potential to be retained longer in the lungs followed by cellular uptake and translocation into the systemic circulation thus causing nanotoxicity. It can be speculated that the fate of the nanoparticles in the lungs, regarding the elimination pathways, will depend on the properties of both the particle and the drug molecule. Micron-sized aggregates of nanoparticles will deposit by sedimentation in the tracheobronchial (TB) region where MCC will operate to eliminate both the dissolved and undissolved drugs. Even discrete nanoparticles can deposit by diffusion in the TB region. Drug nanoparticles deposited in the alveolar region will dissolve in the airway fluid and be absorbed. This is likely to be the case even for hydrophobic drugs with low aqueous solubility like inhaled corticosteroids due to the relatively low doses that are used. As a result of the low persistence of drug nanoparticles, dissolution and mucociliary escalator will likely be the major clearance pathways responsible for these particles before macrophage phagocytosis and other translocation pathways would start to play a significant role.

3. Nanocarriers for lung delivery

Nanomedicine can be defined as the application of nanotechnology to medicine. Nanotechnology involves the understanding and control of matter at dimensions of 1 to 100-200 nm, where unique phenomena enable novel applications. Artificial nanostructures are of the same size as biological entities and can readily interact with biomolecules on both the cell surface and within the cell (Figure 4). Here our attention is focused on the fate of nanomedicines delivered to the lung, in particular an innovative glucocorticoid delivery system will be considered.

The understanding the fate of nanomedicines in the lungs is important because fate and therapeutic activity are closely related. Interaction of nanomedicines with cells of the

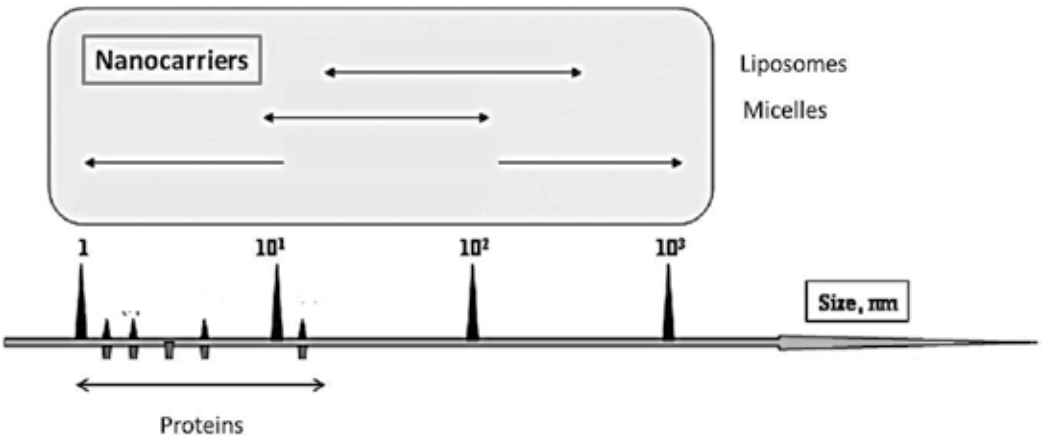

Fig. 4. Nanocarrier size

respiratory system will determine the pharmacodynamic response. For instance, the rapid uptake of particles by alveolar macrophages can be a way of targeting anti-tuberculosis drugs to this type of cells (Nimje et al., 2009). Conversely, macrophages uptake represents a clearance pathway for drugs acting on other cells within the lungs (e.g., β_2 mimetics).

Nowadays, biopharmaceuticals and conventional drugs are frequently engineered or incorporated in carriers in order to direct their fate in preferential pathways (Schmidt, 2009; Veronese & Pasut, 2005). Nano-size drug carriers can incorporate various therapeutics (e.g.,poorly water soluble drugs) and present several advantages for drug delivery to the lung including controlled release, protection from metabolism and degradation, decreased drug toxicity and targeting capabilities. Moreover, the successful integration of novel drugs with devices capable of delivering defined doses to the respiratory tract has resulted in a proven track record for inhalation as a route of administration that limits systemic exposure and provides localized topical delivery. Thus, a number of orally inhaled products have been successfully developed over the last 50 years, providing symptomatic relief to millions of patients with asthma and chronic obstructive pulmonary disease (COPD).

There are numerous types of nanoparticle systems now being explored for drug delivery to lungs, especially in cancer treatment (Haley & Frenkel, 2008).

The types of nanoparticle used at present in research for cancer therapeutic applications include polymeric nanoparticles, protein nanoparticles, ceramic nanoparticles, viral nanoparticles and metallic nanoparticles (Balak et al., 2010).

Liposomes are the most extensively investigated system for controlled drug delivery to the lungs (Mansour et al., 2009). A few liposome-encapsulated antibiotics have been delivered to the lungs in phase II clinical trials. These include amikacin (Weers et al., 2009) and ciprofloxacin (Bruinenberg et al., 2010) Multiple treatment cycles with ARIKACE™ (liposomal amikacin for inhalation) showed sustained improvement in lung function with significant reduction in bacterial density in CF patients who have chronic Pseudomonas lung infections (Okusanya et al., 2009).

A nanoscale liposomal formulation of amikacin has been shown to slowly release the drug in rat lungs and to penetrate Pseudomonas biofilms and CF sputum in vitro (Meers et al., 2008).

Mitsopoulos and Suntres reported that the delivery of N-acetylcysteine as a liposomal formulation improves its effectiveness in counteracting Paraquat-induced cytotoxicity (Mitsopoulos & Suntres, 2011).

Liposomal drug dry powder formulations, realized to obtain novel devices capable of delivering defined doses of drugs, represent promising tools for pulmonary drug administration, such as selective localization of drug, reduced local and systemic toxicities, increased patient compliance and high dose loading.

In liposomal dry powder formulations, drug encapsulating liposomes are homogenized, dispersed into the carrier and converted into dry powder by using freeze drying, spray drying or supercritical fluid technologies.

The most commonly used liposomes are composed of lung surfactants and synthetic lipids. Liposomal formulation have been proposed to delivery anticancer drugs, corticosteroids, immunosuppressants, antimicotic drugs, antibiotics for local pulmonary infections and CF and opioid analgesics for pain management using. Many of them have reached the stage of clinical trials for the treatment of several pulmonary diseases (Misra et al., 2009).

A promising application of nanocarriers to lung targeting is related to gene delivery. Gene therapy is currently being developed for a wide range of acute and chronic lung diseases, incuding CF, cancer and asthma (Griesenbach & Alton, 2009; Lam et al., 2011). Nguyen et al (2009) developed a highly efficient nanocomposite aerosol for pulmonary gene delivery, consisting of a biodegradable polymer core.

Respiratory diseases have attracted particular attention as targets of siRNA - mediated therapeutics, due to the lethality and prevalence of certain illnesses and the lung's accessibility to therapeutic agents via both local and systemic delivery routes. However, one of the major challenges to realize the RNAi therapeutic potential in lung diseases is to deliver the siRNAs to the lung tissue, in particular, to the target cells with high efficiency and high specificity (Yuan et al., 2011).

Several clinical trials have been conducted in order to assess the efficacy and safety of pulmonary DNA delivery using viral and non-viral vectors, especially in the case of CF. Yet, none of these formulations have been pursued due to low transfection efficiency, transient gene expression or immune elimination of the gene vector. Identifying the barriers to cell transfection might help to improve gene transfer efficiency of non-viral vectors (Griesenbach & Alton, 2009). An efficient and safe cationic lipid, 6-lauroxyhexyl lysinate (LHLN), was proposed to prepare cationic liposomes. *In vitro* tests showed that, compared with Lipofectamine2000, the new cationic liposome formulation using LHLN exhibited lower cytotoxicity and similar transfection efficiency in A549 and HepG2 lung cancer cells (Peng et al., 2011).

Ishitsuka et al. (2011) developed a multifunctional envelope-type nano device (MEND), in which plasmid DNA is condensed using a polycation to form a core particle that is encapsulated in a lipid envelope, modified with the IRQ peptide (IRQRRRR) to enhance transgene expression in lungs. (Ishitsuka et al., 2011).

Clinical applications of liposomes and nanoparticles for drug delivery to the respiratory tract are still in early stages. The key to future innovation may lie at the interface between biology and particle engineering. Improved understanding of biological processes

including particle clearance, cellular targeting, intracellular trafficking, and drug absorption are needed to better design formulations that deliver to the ''target'' with the optimal balance of pharmacodynamic, pharmacokinetic, and safety profiles. More specifically, continued advances are needed in the development of: (1) controlled release formulations; (2) formulations with improved regional targeting within the lungs (e.g., airway versus alveoli and vice versa); (3) formulations containing active targeting moieties; (4) formulation strategies for improving the systemic bioavailability of inhaled macromolecules; (5) formulation strategies for delivering macromolecules, including siRNA and DNA, into cells; and (6) formulations with improved dose consistency. It is likely that such innovation will require the development of novel excipients and particle engineering strategies. Future innovation must also take into account the changing marketplace and the diverse set of customers (patient, healthcare professional, heath authorities, payers, and politicians) who must be satisfied. The pharmacoeconomics of new delivery systems will be closely scrutinized, so it is imperative that cost factors should be taken into account. Otherwise, the new technology option may overshoot the evolving inhalation marketplace.

4. Toxicity of nanoparticles to the lung

Epidemiological studies have confirmed a positive correlation between levels of particulate pollution and increased morbidity and mortality rates among general populations (Gwinn & Vallyathan, 2006; Stone et al., 2007).

The adverse health effects seem to be dominated by pulmonary symptoms. For instance, many reports have addressed that occupational exposure of inhaled rigid nanoparticles (NPs) can lead to respiratory diseases such as pneumoconiosis (pulmonary fibrosis) and bronchitis (Byrne & Baugh, 2008; Lkhasuren et al., 2007).

Increasing inhalation of ambient ultrafine particles has been linked with exacerbation of respiratory symptoms and mortality among COPD sufferers (Xia et al., 2009). It has also been documented that NPs can instigate oxidative stress and cellular toxicity in various types of cells (Huang et al., 2009).

It was also reported that chronic exposure to NPs can potentially predispose humans to lung inflammation and increase the risk of COPD.

A concentration range of NPs within the level found in ambience and in nanotechnology industries (Klaine et al., 2008) can promote mucin aggregation.

The second safety aspect of deep lung deposition is the interaction of nanoparticles with the alveolar environment. The alveolar space is covered with a thin surfactant film. This film has important physiological functions e.g. to accelerate gas exchange and to lower the surface tension in the alveolar space. Compromising these functions by inhalable nanoparticles might cause life threatening consequences. Therefore, the compatibility of a delivery system with the alveolar environment must be considered (Azarmi et al., 2008).

For these reasons vesicular nanocarriers, composed of lung surfactants and/or synthetic amphiphiles, provide an efficient delivery system for the treatment of pulmonary disorders due to their biocompatibility, biodegradability and non-toxic nature (Taylor & Newton, 2004).

5. Chronic obstructive pulmonary disease (COPD)

COPD is the fourth leading cause of death in the United States and Europe, with COPD mortality more than doubling in the last two decades (Mannino et al., 2002). COPD can be defined as a preventable and treatable disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences (Celli & MacNee, 2004). It is interesting to point out how the definition of COPD has evolved including the systemic consequences of the disease. The natural history of the disease reveals numerous extrapulmonary manifestations and comorbidity factors that complicate the evolution of COPD, thereby altering the prognosis and quality of life of patients (Barnes & Celli, 2009; Agusti & Soriano, 2008). Many extrapulmonary effects of COPD have been described over the last two decades, including renal and hormonal abnormalities, muscle wasting (Remels et al., 2007), osteoporosis, anemia and reduction in circulating bone marrow progenitors (Palange et al., 2006). Although these systemic manifestations have been described for years in COPD patients, it is still unclear whether they represent consequences of the pulmonary disorder, or whether COPD should be considered as a systemic disease. The importance of establishing the distinction between a respiratory disease with extrapulmonary manifestations and a systemic inflammatory state with multiple compromised organs is justified by different therapeutic options: in the first definition, therapy is primarily centred on the lungs, whereas in the second, therapy could aim at the systemic inflammatory state. Both submucosal gland hypertrophy and airway surface goblet cell metaplasia are prominent features of the chronic bronchitis that occurs in most COPD patients. While cough and chronic expectoration helps to remove excess mucus from the large airways, impaired mucociliary function in COPD causes ineffective mucus clearance from small airways (≤2 mm diameter) which are not well cleared by cough. Like asthma and CF, COPD is strongly associated with the accumulation of inflammatory mucous exudates in the lumens of small airways (Hogg et al., 2004). Accordingly, declining lung function, respiratory infections, hospital admission, and mortality are significantly associated with chronic expectoration.

Among the numerous extrapulmonary effects of COPD, systemic inflammation has been widely studied and considered as an important key between the pulmonary disease and the related systemic manifestations. Many studies reported changes in various inflammatory cells and mediators, including neutrophils, lymphocytes, acute-phase reactants, and cytokines. Recently it was shown that systemic inflammation is present during COPD exacerbations and stable phases of the disease: increased numbers of leukocytes, levels of acute-phase response proteins (C-reactive protein and fibrinogen), cytokines such as interleukin (IL)-6, and tumor necrosis factor (TNF)-α are present in the peripheral blood of COPD patients (Gan et al., 2004). Systemic inflammation has been implicated in the pathogenesis of the majority of COPD systemic effects, including weight loss (Wouters, 2002), skeletal muscle dysfunction, cardiovascular diseases (Sin & Man, 2003), and osteoporosis, although it is still controversial whether this so called low-grade systemic inflammation represents the consequence of pulmonary inflammation into the systemic vascular bed (Agustı` et al. 2003), or whether it is a systemic inflammation. Although inflammation is certainly one of the major features of COPD, we still need to understand

whether the local inflammation is sufficient to induce systemic effects, or whether a second pathogenetic event is required (Evans & Koo, 2009; Huertas & Palange 2011).

Over the years it was evidenced that mucus hypersecretion is an important manifestation of COPD. In the classical phenotype of chronic bronchitis, mucus hypersecretion is the key presenting symptom that appears independent of airflow obstruction. A more recent work demonstrated that obstruction of the small airways by inflammatory exudates containing mucus is predictive of early death after volume reduction surgery in patients with advanced COPD (Hogg et al., 2007). It was suggested that such occlusion enhanced the probability of infection in the lower respiratory tract. In addition, several epidemiological studies showed an association between mucus hypersecretion and outcomes in patients with COPD. Mucus hypersecretion is not an innocent disorder. However, despite these observations, until now few studies have focused on the effects of mucolytic drugs in patients with COPD, even though some of these mucolytic drugs also appear to have antioxidant properties (Dekhuijzen, 2004; Rahman et al., 1997; Rahman & Kilty 2006; Decramer & Janssens, 2010).

Histopathological findings from surgical specimens clearly show that increased goblet cell numbers and increased MUC5AC and MUC5B production and secretion are found in the lumen of small airways in COPD patients (Caramori et al., 2004). These findings are inversely associated with pre-surgical Forced Expiratory Volume in the 1st second (FEV1). Thus, patients with higher FEV1 have less goblet cell metaplasia than patients with lower FEV1, suggesting that the presence of mucin-producing cells in the airways is related to increased airflow obstruction. The presence of a prominent goblet cell phenotype also negatively correlates with FEV1 improvement following lung volume reduction surgery (Kim et al., 2005). Collectively, these results show that mucus secretion may be significant enough to result in physiologically and clinically measurable mechanical obstruction of small airways, and it may significantly impact disease pathogenesis and prognosis. The main cause of COPD in humans is cigarette smoking. In mice, chronic cigarette smoke exposure causes strain dependent mucous metaplasia. Cigarette smoke itself has also been shown to promote mucin synthesis directly in vitro by activation of the EGFR cascade (Shao et al., 2004). Inhalation, of one of the many potential toxicants present in cigarette smoke, acrolein (acrylic aldehyde), induces mucous metaplasia and MUC5AC production in animals. Acrolein also induces MUC5AC production in human airway epithelial cell lines, and it is found at significantly elevated levels in the induced sputum and exhaled breath condensates of COPD patients, (Deshmukh et al., 2008).

It has been suggested that mucus can also serve as a suitable medium for adherence and growth of some bacterial pathogens, such as non-typeable H. influenzae. Gram positive and gram negative bacteria products up-regulate MUC5AC and MUC2 gene expression and mucin secretion in human respiratory epithelial cell lines in vitro, and the same effect can be seen in some animal models in vivo. Viral infections are also closely associated with COPD exacerbations in humans (Wedzicha & Donaldson, 2003; Beckham et al., 2005). Surgical specimens from smokers with COPD show increased goblet cell numbers in the epithelium of peripheral airways compared to non-smokers. This is accompanied by increased macrophages and CD8 positive T-lymphocytes, both of which are indicative of viral. Roles for IL-6 and virus-induced mucin overproduction have been suggested. In vivo, IL-6 production is enhanced during the early phase of bacterial or viral-induced inflammation.

Accordingly, IL-6 levels are increased in COPD patients (Bucchioni et al., 2003), and during experimental respiratory viral infections in humans and mice (Decramer & Janssens, 2010).

5.1 Mucus rheological properties

Mucus rheology plays a critical role in maintaining respiratory health. Mucins are large, highly glycosylated proteins. The polyanionic nature of mucin stems primarily from sialic acid, sulfate, and carboxyl groups present in these linked oligosaccharides. Beside physical entanglement, cationic calcium ions can act as crosslinkers that condense the mucin matrices inside mucin granules before exocytosis. Upon release, phase transition mainly driven by the Donnan effect triggers the massive decondensation of mucin networks. Hydrogen bonding, hydrophilic and hydrophobic interactions have also been proposed to contribute to the gel properties of mucin The gel characteristics and rheological properties of mucin are critical for the maintainance the integrity of epithelia by trapping bacteria and viruses and for mucociliary clearance (Bansil & Stanley, 1995; Verdugo, 1990).

Mucus is mainly composed of large and heavily glycosylated glycoproteins called mucins. The gel-forming mucins rapidly hydrate after exocytosis and, due to their tangle network properties, anneal with other mucins to form a protective barrier at the airway-surface liquid layer. The mucin gel layer lines the epithelial surface of various organs such as the vaginal tract, eyes, gastric wall and pulmonary lumen. Mucus in the airway of lungs serves as an innate immune defense against inhaled particulates, bacteria and viruses. Maintenance of the airway protection mechanism stems from the delicate balance between normal mucus production, transport and clearance. The mucin polymer network of mucus has a characteristic tangled topology. Since the rheological properties of mucus are governed mainly by the tangle density of mucin polymers, which decreases with the square of the volume of the mucin matrix, the mucin network hydration (degree of swelling) is the most critical factor in determining the rheological properties of mucus. The diffusivity of mucin matrices, which is closely related to mucin viscosity, can be calculated from polymer swelling kinetics. Based on the polymer network theory, polymer diffusivity is inversely proportional to its viscosity (Lodge, 1999). Thus, lower rate of mucin diffusivity is associated with higher viscosity, less dispersed and less transportable mucins that appear to characterize the clinical symptoms of thick mucus accumulation and obstruction commonly found in asthma, COPD and CF 44. (Rogers, 2007).

The clinical manifestation of major respiratory diseases (Rogers & Barnes, 2006; Quinton, 2008) are related to thick mucus.

The relationship between mucin dehydration and defective mucus clearance has been well established (Mall et al., 2004). As a result, the poorly hydrated, highly viscous and less transportable mucus appears to accumulate within airway passages (Randell et al., 2006). Obstruction of airway lumen with viscous mucus is usually accompanied by chronic bacterial infection, inflammation and impaired mucociliary transport.

6. Case studies

Although the use of liposomes for aerosol formulations is certainly encouraging, liposome nebulization still presents some problems, i.e. storage stability (mainly related to oxidation processes) and leakage of encapsulated drugs. In addition, it should be also considered that

synthetic phospholipids are usually expensive and, on the other side, natural phospholipids show a variable degree of purity (Desai & Finlay, 2002).

An alternative approach to the liposomal approach is the use of liposome-like vesicles made up of non-ionic surfactants, the so-called niosomes. These carriers were proposed for both topical (Carafa et al., 2000; Carafa et al., 2004; Paolino et al., 2007; Paolino et al., 2008) and systemic administration (Cosco et al., 2009).

Here we report the evaluation of the possible advantages of a new type of non-phospholipid vesicle system for pulmonary drug delivery that can lead to an improved mucus permeation. Vesicles consisting of one or more surfactant bilayers enclosing aqueous spaces (non ionic surfactant vesicles NSVs), are of particular interest because they offer several advantages with regard to chemical stability, lower cost and availability of materials compared to conventional liposomes

In the formulation of inhaled drugs for the treatment of asthma and COPD, considerable attention has been devoted to new aerosol morphologies which can either enhance the local effect and/or increase the penetration through the mucus, secreted in bronchial inflammatory diseases. In diseases characterized by bronchial hypersecretion, lipophilic substances, such as corticosteroids, can be remarkably impeded in reaching their receptors, which are localized within the cytoplasm of bronchial epithelial cells.

In particular, alveolar macrophages are important target cells for the therapeutic action of glucocorticoids, because these cells are the major source of both proinflammatory and antiinflammatory cytokines. The action of glucocorticoids is mediated by an intracellular receptor belonging to the steroid thyroid/retinoic acid receptor superfamily (Oakley et al., 1999).

With the purpose of carrying out research leading to an innovative formulation for lung delivery capable of permeating the mucous layer and of an efficient delivery to alveolar macrophages, beclomethasone dipropionate (BDP), clinically used for the treatment of asthma and COPD, was entrapped in non-phospholipid vesicles.

BDP, as a reference model drug, was encapsulated in vesicular structures obtained with polysorbate 20. The aim of the study was to evaluate *in vitro* the effectiveness of such delivery system that should enhance permeation through mucosal barriers because of the presence of vesicles formed with a remarkably hydrophilic non-ionic surfactant usually considered as unsuitable for the formation of vesicular structures because of its high HLB value (HLB 16.7) (Santucci et al., 1996).

The intracellular availability of BDP and the safety of the delivery system are the two main issues to be addressed to propose these innovative non-ionic surfactant vesicles as carriers for the pulmonary delivery of this drug to be effectively used for the treatment of pulmonary inflammatory diseases. Therefore, the aim of this investigation was the evaluation of the interaction between our innovative non-ionic surfactant vesicles and human lung fibroblast (HLF) cells, the carrier tolerability, the vesicle localization within the cells and the amount of BDP actually internalized by the cells.

Unilamellar vesicles were obtained from a non-ionic surfactant/BDP aqueous dispersion (Hepes pH 7.4) by means of the "film" method as previously reported (Santucci et al., 1996), according to the compositions reported in Table 1.

Sample	Polysorbate 20	Cholesterol	BDP
1	1.84	0.58	0.5
2	1.84	0.58	1.0
3	1.84	0.58	3.0
4	1.84	0.58	5.0
5	3.68	1.16	0.5
6	3.68	1.16	1.0
7	3.68	1.16	3.0
8	3.68	1.16	5.0

Table 1. Sample composition (expressed as % w/v)

All compositions are able to form nanovesicles with different size and zeta potential (ζ-potential) according to the various formulations (Table 1). The size and the polydispersity index (PDI) obtained by dynamic light scattering measurements indicated those formulations with the smallest size and the most homogeneous nanovesicles population that can be obtained: samples 2 and 8, chosen to perform further experiments.

Size measurement experiments indicate that BDP-loaded vesicles are slightly larger than empty ones as reported in Table 2 for samples 2 and 8 of Table 1, there is an increase in diameter between 10 and 20%, and this expected effect can be related to drug partition between the bilayer and the aqueous core of the vesicles. Accordingly, the presence of BDP in the formulation may affects the ζ-potential values; as it is possible to observe from Table 2, the corresponding samples 2 and 8 show a significant decrease in ζ-potential that approaches the value obtained with BDP alone. This effect can be related to the chemical steroidic structure of the drug that is somehow similar to that of cholesterol, thus allowing it to fit well within the vesicular structure.

Sample	Dimensions (nm)	ζ -potential (mV)
2	163±0.03	-32±0.2
8	174±0.02	-34±0.3
Empty vesicles	146±0.05	-40±0.2
BDP solution 0.05% w/v	=	-30±0.1

Table 2. Vesicle dimensions (nm) and ζ- potential (mV) of analyzed samples (n=3; ±SD)

Furthermore, it should be pointed out that electron microscopy carried out on numerous samples (10) indicated that nebulization does not influence drug-loaded vesicle dimensions (Figure 5A and B).

Analyzed samples showed a good stability in terms of possible changes in vesicle dimensions after aggregation.

Size measurement experiments indicated that after 1 month at 25 °C, no appreciable vesicle dimension variations could be detected.

The best entrapment efficiency (e.e.) was obtained for sample 8 and the calculated drug e.e. indicated that only about 20% of the overall amount of BDP is actually enclosed within the vesicles. This result is in agreement with the data reported by previous authors (Montenegro

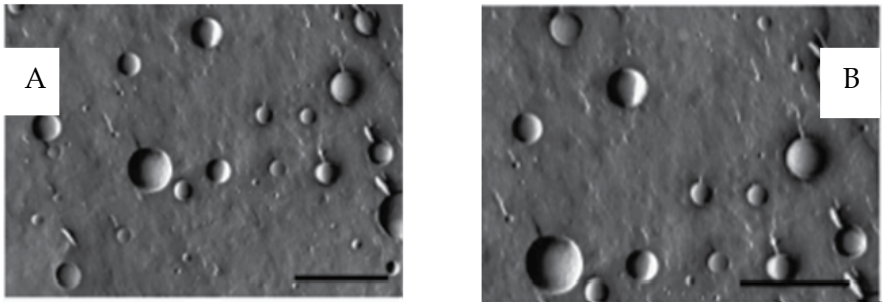

Fig. 5. Trasmission electron micrographs of BDP-loaded vesicles after freeze-fracture, before (A) and after (B) nebulization. The scale bar represents 0.5 μm.

et al., 1996; Darwis et al., 2001). For this reason, for permeation and nebulization experiments the formulation corresponding to sample 8 of Table 1 was used.

The possibility to use the novel vesicular dispersion in a conventional jet nebulizer, widely used in clinical applications, was also evaluated. For this purpose, samples were characterized also by means of rheological measurements and the aerodynamic diameter was determined (Table 3) as well as the nebulizer mass output (Figure 6) after completion of nebulization. Evaluation of Mass Median Aerodynamic Diameter (2.0±0.2 μm) and of geometric standard deviation (GSD) were also carried out; the GSD value (1.5) demonstrates the polydisperse nature of the distribution of the aerosolized droplets that, on the other side, contained a monodisperse vesicular system.

Aerodynamic diameter	Percentage
<10	100
<5	99.5
<2	65

Table 3. Percentage of particles with aerodynamic diameter <10, < 5, <2 μm, containing non-ionic surfactant vesicles, delivered by a jet nebulizer

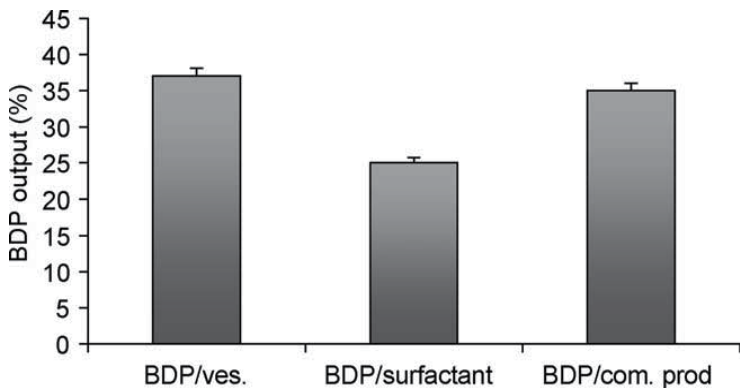

Fig. 6. Deposition of vesicle-encapsulated BDP (BDP/ves) on filters upon nebulization, compared to a BDP/surfactant solution and to a BDP commercial product (n=3, ±SD).

Furthermore, in all conditions of nebulization (TurboBoy nebulizer and Clenny nebulizer), the dispersion BDP/vesicles releases a greater amount of drug, dosed by HPLC, with respect to commercial formulations.

An important aspect to be taken into account for an actual application of these non-ionic surfactant vesicles as possible carriers to be aerosolized for the pulmonary delivery of drugs is their colloidal and storage stability.

In fact, the occurrence of aggregation phenomena can lead to a significant worsening of the biopharmaceutical features of nanosized colloidal suspensions, such as NSVs. Therefore, the colloidal stability of BDP-loaded NSVs was evaluated using the Turbiscan Lab® Expert (Celia et al., 2009) i.e. the optical transmission and the photon backscattering profiles of various samples were recorded. Any variation of the vesicle volume fraction (migration) or mean size (coalescence) triggers the variation of backscattering (BS) and transmission (T) signals, which are graphically reported as positive (backscattering/transmission increase) or negative (backscattering/transmission decrease) peaks. It can be assumed that no variation of particle size occurs when the ΔBS and ΔT profiles are within an interval of ±2% while variations greater than 10% either as a positive or a negative value are representative of an instable formulation.

Two different BDP concentrations, i.e. 50 mg/ml (sample BDP-50) and 0.4 mg/ml (BDP-0.4), were used in this stability investigations. The first concentration led to the maximum possible amount of drug entrapment within the NSVs, while the second concentration led to an amount of entrapped drug similar to that actually present in the most common commercial products.

The ΔBS and ΔT profiles of BDP -loaded and unloaded non-ionic surfactant vesicles are shown in Figure 7.

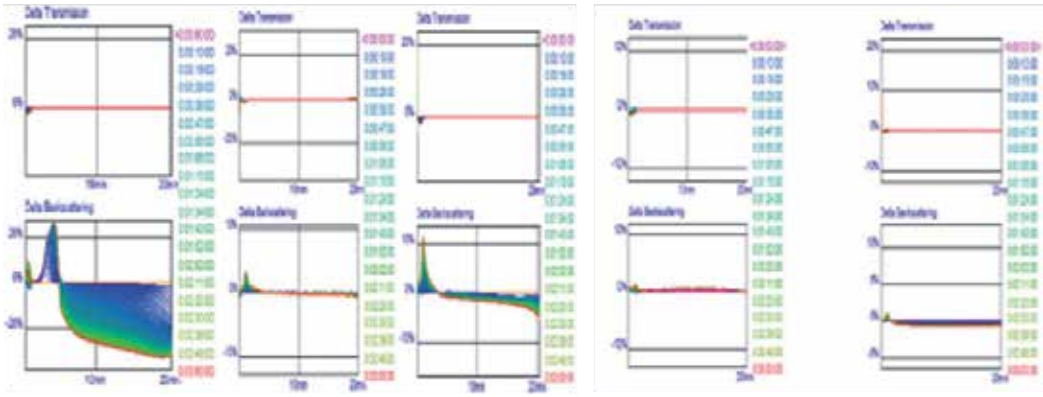

Fig. 7. Transmission and backscattering profiles of niosomes by using Turbiscan Lab® Expert. The image represents the analysis of different formulations: (1) unpurified BDP-50-niosomes; (2) purified BDP-50-niosomes; (3) unpurified BDP-0.4-niosomes; (4) purified BDP-0.4-niosomes; (5) unloaded niosomes. Data are reported as a function of time (0–3 h) and sample height (from 2 to 20 mm).

The transmission signal remained close to the base line value and ΔT profiles close to 0% were observed for all the investigated NSV formulations during the entire time of analysis.

Therefore, NSVs suspensions maintained a constant opalescent aspect along the height of various samples. At the same time, positive or negative variations of the backscattering profiles of the different formulations (Figure 7) were not correlated to destabilization processes under the sample height of 2 mm and over that of 20 mm, the values having been determined by enclosed air in the bottom and/or on the top of the cylindrical glass tube, respectively. Different NSVs formulations showed that backscattering emerged as the prevalent signal in the different measurements (Figure 7). ΔBS signals are close to ~1% during the time of analysis for the entire height of the samples of both purified BDP-loaded and unloaded non-ionic surfactant vesicles (Figure 7 panels B, D and F). It was interesting to observe that the different amounts of the entrapped drug do not influence the colloidal stability of non-ionic surfactant vesicles both in terms of vesicle migration and coalescence. Different stability behaviors, as shown by ΔBS profiles (Figure 7 panels A and C), were observed in the case of not purified BDP loaded non-ionic surfactant vesicles (i.e. before gel exclusion chromatography).

In particular, vesicles prepared in the presence of the highest drug concentration (50 mg/ml) showed a high colloidal instability just after the beginning of the analysis. A moderate stability (ΔBS profile within the 10% during the 3 h of analysis) was observed for unpurified non-ionic surfactant vesicles prepared in the presence of 0.4 mg/ml of the drug, while an elevated stability (equal to purified formulations) was observed for unpurified unloaded vesicles (Figure 7 panel E). These findings can be due to the presence of high amount of free drug, that with time leads to the formation of aggregates. Therefore, the purification procedure is essential to achieve a stable vesicular colloidal carrier for the delivery of BDP. The stability findings by Turbiscan Lab® Expert measurement were also supported by light scattering size analysis during a storage period of 3 months, which showed no appreciable vesicle size variation for purified BDP-loaded non-ionic surfactant vesicles.

According to the aim of this research, the capability of ensuring a better penetration through the mucus layer of vesicular formulation was tested.

In Figure 8, the permeation rate of BDP from the vesicular dispersion is reported and compared with that obtained using a BDP/polysorbate 20 (at the same concentration used for vesicle preparation) suspension as well as with that of the commercial preparation. The vesicular formulation (BDP-0.4) was used in its unpurified form, thus with the drug partitioned inside and outside the vesicular structure.

This situation allows an appropriate comparison with the other preparations used in permeation experiments: since both micellar surfactant solutions and the commercial one contained free BDP and BDP included within aggregated structures (micelles). As it can be observed, the presence of NSVs in the formulation remarkably increases the permeation rate through the model mucosal barrier with respect to the other tested preparation thus indicating that the novel BDP formulation can be proposed for a better targeting of corticosteroids in the treatment of COPD.

An important aspect to evaluate an innovative drug delivery system is its safety. This aspect is much more relevant in the case of pulmonary delivery, since several side effects may

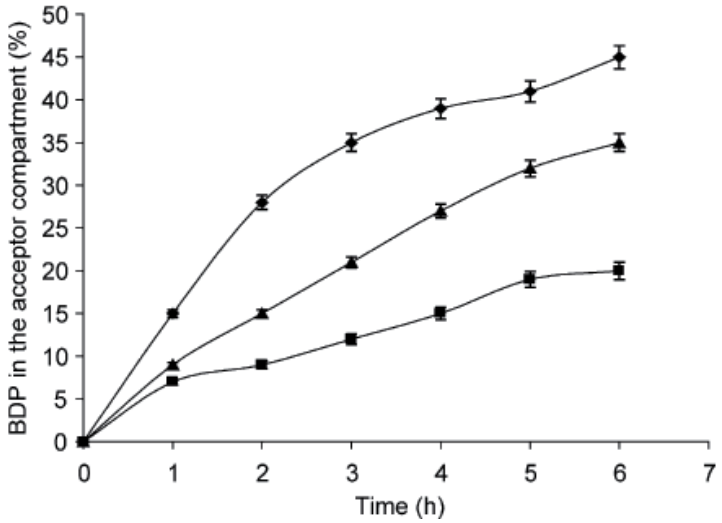

Fig. 8. Comparison of the permeation patterns through a gel-like mucin solution (0.1%w/v), expressed as percentage of permeated drug as a function of time (n=3, ±SD) (■BDP/surfactant ▲BDP/commercial product ◆BDP/vesicles).

result from an unsafe material, i.e. fibrosis, pulmonary oedema, inflammation, as reported in section 4.

Safety of empty non-ionic surfactant vesicles was evaluated in vitro on HLF cells by using the trypan blue dye exclusion assay (cell mortality) and MTT viability test. Purified and unpurified empty NSVs were assayed in vitro at different surfactant concentrations (from 0.01 to 10 μM) and incubation-times (24, 48 and 72 h).

Purified non-ionic surfactant vesicles did not show a significant cytotoxic activity on HLF cells at all incubation times for concentrations ranging from 0.01 to 1 μM, i.e. the mortality values ranged from 1.2 to 5.81%, respectively. Only at the highest investigated concentration (10 μM) and after 48 h of incubation a slight cytotoxic effect was observed (mortality value of 16%).

It is interesting to point out that unpurified vesicles showed a significant ($P<0.001$) increase of cytotoxicity with respect to purified vesicles at all the investigated conditions (exposition times and surfactant concentrations). In this sense it was also observed that the increase of the cytotoxic effect was dependent on surfactant concentration but not on the exposition time. This finding was due to the fact that the cytotoxic action of unpurified NSVs was determined by the presence of the free molecules of surfactants, which were able to exert immediately their cytotoxic action during the incubation period by noticeably perturbing the cellular membranes (Dimitrijevic et al., 2000; Lin et al., 2007) and hence causing the cellular death. This hypothesis was strongly supported by the evidence that HLF cells treated with the free surfactants showed a greater ($P<0.001$) cytotoxic insult than those treated with unpurified non-ionic surfactant vesicles. Therefore, the assembling of surfactant into non-ionic surfactant vesicles determined a drastic reduction of cytotoxicity, due to a concomitant reduction of the free surfactant molecules in solution and/or surfactant micelles, which are able to alter the cellular permeability and homeostasis.

Another important feature of an innovative drug delivery system is to increase the amount of active compound in the target district, thus improving the therapeutic effect of the drug. Therefore, to evaluate the delivery ability and the mechanisms of interaction of fluorescein-labelled non-ionic surfactant vesicles with HLF cells, CLSM experiments were carried out.

Figure 9 shows how the fluorescein-labelled vesicles interacted with HLF cells at different incubation times. A green fluorescence distribution was observed in the cells just after 1 h incubation. After 3 h incubation the fluorescence of the cellular membrane and the cytoplasm became more intense and increased slightly up to 24 h of incubation. These findings prompted us to suppose that the main mechanism involved in the NSVs/cell interaction was the endocytosis of the carrier (Di Marzio et al., 2008), which enabled a rapid internalization in the cytoplasm. It is worthy of note that at all incubation times the localization of fluorescence was in the cytoplasm compartment and these results represent an important aspect because the glucocorticoid receptor is localized in the cytoplasm.

No fluorescence was detected in the untreated HLF cell line (control) and hence there was no interfering auto-fluorescence phenomenon (Figure 9 panel 6).

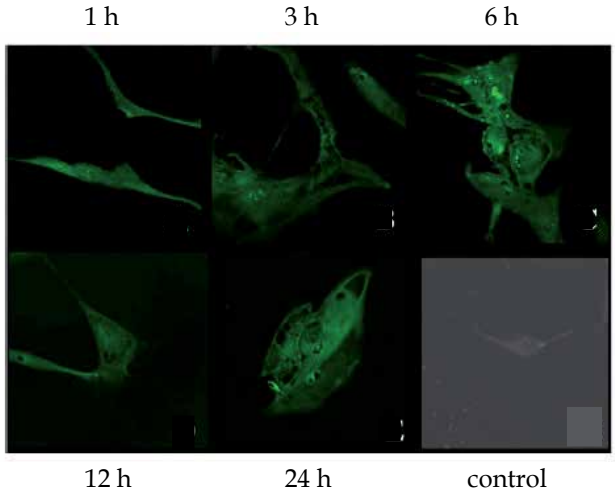

Fig. 9. CLSM micrographs of the interaction of fluoresceine-labelled NSVs with primary HLF cells as a function of the incubation times,. The reflectance CLSM micrographs of untreated cells were used as controls and no significant cellular fluorescence was observed

As a consequence, the improved interaction of NSVs with primary HLF cells should lead to a greater intracellular delivery of the entrapped drug.

For this reason the intracellular uptake of BDP prompted by the various formulations was investigated as a function of time (Figure 10).

Considering that surfactant molecules and unloaded non-ionic surfactant vesicles may act as drug cellular penetration enhancers and to evaluate the effective role of the vesicles in the promotion of the intracellular uptake of BDP, a mixture surfactant/BDP and a mixture empty vesicles/BDP was also assayed. As reported in Figure 10, BDP loaded non-ionic surfactant vesicles (BDP-0.4) showed a significant improvement of the intracellular uptake of the drug with respect to a mixture surfactant/BDP, a mixture empty non-ionic surfactant

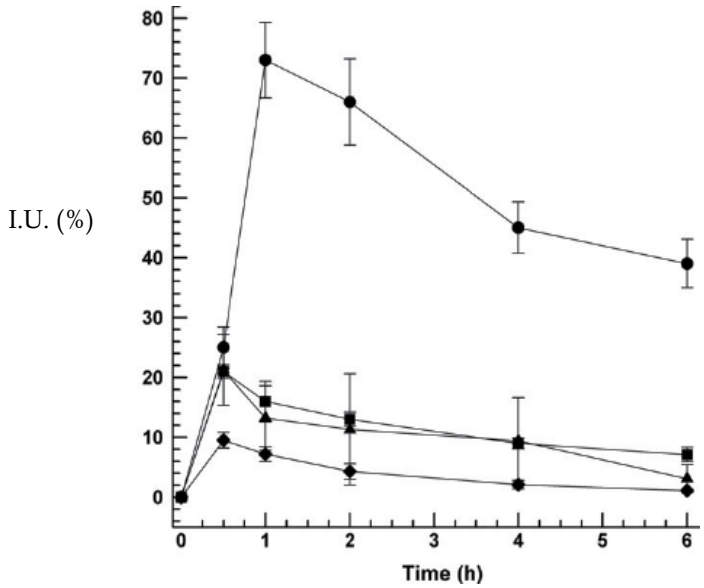

Fig. 10. HLF intracellular uptake (I.U. at 37 °C) of beclomethasone dipropionate (expressed as percentage of the applied dose) as a function of time by different formulations: BDP loaded NSVs (BDP-0.4), ●; surfactant/BDP mixture, ▲; empty non-ionic surfactant vesicle/BDP mixture, ■; free drug, ♦.

vesicle/BDP and a free drug solution. This result was correlated to the ability of NSVs to easily penetrate across cell membranes of primary HLF cells (in full agreement with CSLM experiments), thus achieving a noticeable cytoplasm accumulation of BDP. These results clearly evidenced that the improvement of the drug intracellular uptake was mainly mediated by the vesicular carrier and no positive influence was exerted by the surfactant molecules and/or empty vesicles, i.e. the drug has to be entrapped within the carrier. In fact, the mixtures surfactant/drug and empty vesicle/drug showed only a slight improvement of the intracellular uptake of BDP. The profiles of BDP intracellular uptake as a function of time showed (Figure 10) a Tmax (time at which the maximum drug concentration was reached) value of 1 h followed by a gradual reduction of the intracellular drug accumulation up to 6 h in the case of the drug-loaded non-ionic surfactant vesicles. On the other side, all other tested formulations showed Tmax values of 30 min.

The rapid internalization of BDP formulated in niosomes was not considered as a critical parameter.

The evidence of the improved intracellular entrance of NSVs and the noticeable increase of the intracellular uptake of BDP mediated by the vesicular carrier should match an improved pharmacological activity of the delivered drug. For this reason the anti-inflammatory activity of BDP-loaded non-ionic surfactant vesicles was evaluated in comparison with the free drug, as the capacity to inhibit the secretion of NGF.

NGF is an important inflammatory mediator, which contributes to the development of airway hyper-responsiveness (de Vries et al., 1999). The NGF production is stimulated by the presence of pro-inflammatory cytokines and asthma-associated cytokines, i.e. IL-1β, and

inhibited by anti-inflammatory glucocorticoids. Therefore, the in vitro determination of the levels of NGF secreted by HLF cells is a direct evidence of the pro- or anti-inflammatory effect of a substance.

As shown in Figure 11, the stimulation of HLF cells with IL-1β led to an over-secretion of NGF up to 210% of basal values (control). The treatment with the free BDP (1 μM) significantly reduced both the constitutive and the IL-1β-stimulated secretion of NGF by 18.7% and 61.23%, respectively. BDP-loaded non-ionic surfactant vesicles (BDP-0.4) were much more effective than the free drug, i.e. a reduction of 68% and 85% with respect to the constitutive and IL-1β-stimulated NGF secretion were respectively observed. These findings are in agreement with both CLSM and intracellular uptake experiments.

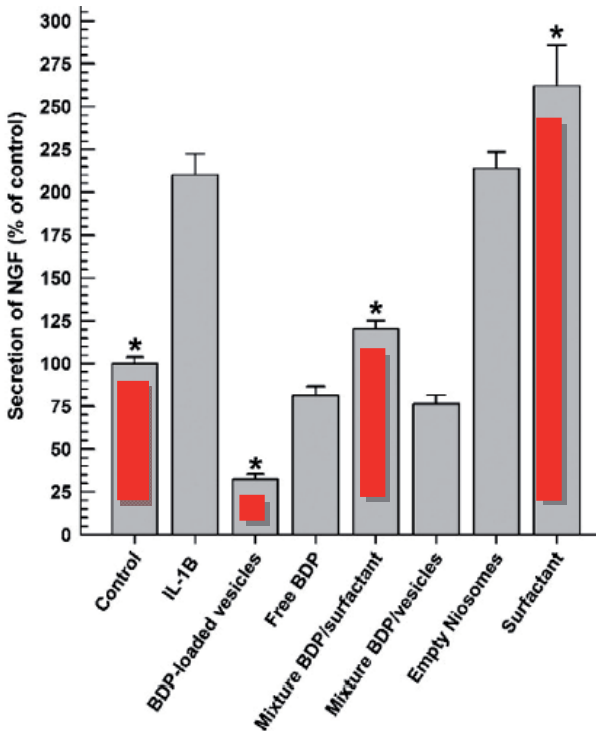

Fig. 11. Anti-inflammatory activity of various formulations containing BDP (1 μM) evaluated as inhibition of NGF secretion in primary HLF cells treated with IL-1β (as pro-inflammatory stimulating agent). Control was untreated HLF cells, which secrete the basal level of NGF.

The use of NSV formulations was investigated not only to increase intracellular uptake of BDP in HLF cells and to improve diffusion through mucus layer but also to design an innovative system able to increase therapeutic efficacy of BDP in pulmonary diseases thus reducing the dosage and potential side effects of this drug.

7. Conclusions

Despite the many promising proof - of - concepts of various delivery technologies, there is still a long way ahead that must be covered. This means there are still many challenges that

are being faced, which, in turn, mean there are still many chances for the academic and industrial scientist to make a decisive impact.

Further research efforts are needed to ensure the safety of long-term in vivo applications. There is an urgent requirement for cautiously designed toxicology and toxicokinetic studies for each nanocarrier type; the protocols should be customized for an appropriate clinical use. Furthermore, it should be pointed out that scale up from laboratory to industry is still poorly investigated in this specific area, despite its obvious importance in the ultimate goal of the development a product that can reach the market and actually give benefits to the patients.

In spite of the above reported difficulties and challenges; hopefully, within a few years, the safety and large - scale production at affordable costs of the delivery technologies described in this book will be a dream that will become true.

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Noninvasive Positive-Pressure Ventilation Therapy in Patients with COPD

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1. Introduction

Noninvasive positive pressure ventilation (NPPV) refers to the administration of ventilatory support without using an invasive artificial airway (endotracheal tube or tracheostomy tube). The use of NPPV has markedly increased over the past two decades. Rudimentary devices that provided continuous positive airway pressure were described in the 1930s, but the negative-pressure ventilators were the predominant method of ventilatory support until the polio epidemics overwhelmed their capacity in the 1950s. In the 1980s, increasing experience with positive-pressure ventilation delivered through a mask in patients with obstructive sleep apnea led to this type of ventilatory support, initially in patients with neuromuscular respiratory failure. Success led to its adoption in other conditions, and NPPV became especially promising in the treatment of patients with exacerbations of chronic obstructive pulmonary disease (COPD).

NPPV is defined as ventilatory support delivered by a non-invasive interface such as mask or similar device, acting as an alternative to intubation or tracheostomy. Consequently, by avoiding tracheal intubation, NPPV presents several potential advantages, such as reduction in pulmonary infections, barotrauma and need for sedation (British Thoracic Society Standards of Care Committee 2002). As a result, NPPV should be considered a standard of care to treat COPD exacerbation in selected patients, since it markedly reduces the need for intubation and improves outcome by lowering complication and mortality rates, and shortening hospital stay (Brochard et al. 1995; Kramer et al. 1995; Celikel et al. 1998; Martin et al. 2000; Conti et al. 2002; Squadrone et al. 2004; Lightowler et al. 2003; Nava, Navalesi, & Conti 2006). Weaker evidence indicates that NPPV could allow earlier extubation, avoid re-intubation in patients who fail extubation, and assist do-not-intubate patients, and thus could be beneficial for COPD patients who are suffering respiratory failure precipitated by superimposed pneumonia or postoperative complications, and COPD patients with severe stable disease who have substantial daytime hypercapnia and superimposed nocturnal hypoventilation.

This chapter will examine the evidence pertaining to the use of NPPV for various applications in COPD and make recommendation on patient, ventilation mode and interface selection as well as technical aspects of NPPV application in COPD. The literature review

and consensus processes used to reach the recommendations presented here are the American College of Chest Physicians [ACCP] consensus report on clinical indications for NPPV in CRF due to restrictive lung disease, COPD and nocturnal hypoventilation published in 1999, the British Thoracic Society guidelines published in 2002, the Indian Society of Critical Care Medicine guidelines published in 2006, the guidelines from 12 German Medical Societies published in 2008 and the most recent guideline published in 2011 from Canadian Critical Care Trials Group/Canadian Critical Care Society Noninvasive Ventilation Guidelines Group.

2. Physiologic mechanism of NPPV effect in patients with COPD

Severe COPD places the respiratory muscles at a mechanical disadvantage (Rochester, Braun, & Arora 1979). During COPD exacerbation, this situation becomes catastrophic. Exacerbations of COPD increase the respiratory load in these patients, exceeding their ability to adequately ventilate through a variety of mechanisms, including increasing hyperinflation with decreased diaphragmatic excursion and strength, increasing intrinsic positive end-expiratory pressure (PEEP), changes in respiratory patterns and increased respiratory frequency leading to ineffective or inadequate tidal volume generation. NPPV effectively unloads the respiratory muscles by increasing tidal volume, decreasing the respiratory rate, and decreasing the diaphragmatic work of breathing, which translates into an improvement in oxygenation, a reduction in hypercapnia, and an improvement in dyspnea. NPPV treatment counterbalances auto-PEEP, assists inspiration, reduces transdiaphragmatic pressure, lowers respiratory rate, rests the accessory muscles, increases functional residual capacity, decreases respiratory load and work of breathing and leads to favorable changes in the ventilation/perfusion ratio as well as the respiratory center and the sensivity of chemoreceptors (Mansfield & Naughton 1999; de Miguel et al. 2002). Expiration positive airway pressure (EPAP) counterbalances intrinsic PEEP. Inspiration positive airway pressure (IPAP) is capable of increasing tidal volume and subsequently decreasing the elevated levels of PC02.

3. Indications of NPPV in patients with COPD

3.1 Acute respiratory failure/Exacerbation of COPD

Based upon the overwhelming evidence that NPPV reduces the need for intubation, reduces mortality and complications rates, and shortens the length of stay in both the intensive care unit (ICU) and hospital (Kramer et al. 1995; Brochard et al. 1995; Celikel et al. 1998; Martin et al. 2000; Carlucci et al. 2001; Mehta & Hill 2001) , NPPV should be considered as a standard of care in acute respiratory failure (ARF) due to COPD exacerbations (Keenan et al. 2011). Brochard et al. were the first to show that pressure-support ventilation administered via face mask significantly reduced the need for intubation, duration of mechanical ventilation, and ICU stay in patients with COPD exacerbations (Brochard et al. 1990). The patients with relatively mild COPD exacerbations are not likely to benefit from NPPV, which suggests that NPPV should be applied to selected patients who have moderate-to-severe COPD exacerbations. Though, patients with milder exacerbations appear to demonstrate a more rapid improvement in their level of dyspnea with NPPV treatment, the addition of NPPV to standard therapy for patients with milder exacerbations of COPD is not well tolerated (Keenan, Powers, & McCormack 2005). NPPV should be the first option for ventilatory

support in patients with either a severe exacerbation of COPD or cardiogenic pulmonary edema(Keenan et al. 2011). Furthermore, consensus groups of experts advocate the routine use of NPPV for selected patients with COPD exacerbations (British Thoracic Society Standards of Care Committee 2002). High quality studies have shown that NPPV is an effective treatment for moderate to severe COPD exacerbation (Kramer et al. 1995; Celikel et al. 1998; Martin et al. 2000). In patients with mild to moderate ARF, characterized by pH levels between 7.25 and 7.35, the rate of NPPV failure was ranging from 15% to 20% (Elliott 2002; Lightowler et al. 2003). In more severely ill patients (pH<7.25), the rate of NPPV failure was inversely related to the severity of respiratory acidosis, rising up to 52%-62% (Conti et al. 2002; Squadrone et al. 2004). In patients with ''mild'' exacerbations, not complicated by respiratory acidosis, the use of NPPV was investigated in few studies, including patients in large majority with pH>7.35, which failed to demonstrate a better effectiveness of NPPV than standard medical therapy in preventing the occurrence of ARF (Bardi et al. 2000; Keenan, Powers, & McCormack 2005). Guidelines recommend the use of NPPV in addition to usual care in patients who have a severe exacerbation of COPD (pH<7.35 and relative hypercarbia) (grade 1A recommendation) (Keenan et al. 2011). Based on that evidence, the authors of the meta-analyses and the participants in the consensus groups recommended that NPPV should be used early in the course of a COPD exacerbation, in addition to the standard medical care (Lightowler et al. 2003; Keenan et al. 2003; British Thoracic Society Standards of Care Committee 2002) . NPPV is not appropriate for all COPD patients with ARF and the selection of candidates is important. Most of the indications and contraindications for NPPV in ARF are listed in Table 1 (Brochard et al. 1995). There are no absolute contraindications to NPPV although a number have been suggested (Ambrosino et al. 1995; Soo Hoo, Santiago, & Williams 1994). In part, these contraindications have been determined by the fact that they were exclusion criteria for the controlled trials. It is therefore accurate to state that NPPV is not proven in these circumstances rather than stating that it is contraindicated.

3.2 Severe community-acquired pneumonia in patients with COPD

The presence of pneumonia has been associated with poor outcome in patients treated with NPPV (Ambrosino et al. 1995). However COPD exacerbation is still an appropriate indication for NPPV even when complicated by community-acquired pneumonia (Confalonieri et al. 1999). In one randomized trial with patients suffering severe community-acquired pneumonia, NPPV reduced the need for intubation, and reduced mortality among the COPD subgroup of patients 2 months after hospital discharge (Confalonieri et al. 1999). But it is not clear whether NPPV should be used for severe community-acquired pneumonia in non-COPD patients.

3.3 Adjunct to early liberation

Patients with COPD can be considered for a trial of early extubation to NPPV in centres with extensive experience in the use of NPPV (Keenan et al. 2011). Guidelines suggest that NPPV be used to facilitate early liberation from mechanical ventilation in patients who have COPD, but only in centres that have expertise in this therapy (Grade 2B recommendation) (Keenan et al. 2011). Recent randomized controlled trials (RCTs) suggested benefit from NPPV after extubation in patients who had high risk of deterioration (Ferrer et al. 2006; Ferrer et al. 2009; Nava et al. 2005; Luo, Cheng, & Zhou 2001). The results of the RCTs of

Indications
• Increased dyspnea-moderate to severe • Tachypnea (>25 breaths per minute) • Signs of increased work of breathing, accessory muscle use, pursed lips breathing and abdominal paradox • Acute or chronic ventilatory failure (best indication), PaC02 >45 mmHg, pH <7.35 • Hypoxaemia (use caution), Pa02/Fi02 ratio < 200
Contraindications
Absolute • Cardiac or respiratory arrest • Severe encephalopathy • Unable to fit mask Relative • Severe haemodynamic instability with or without cardiac ischemia or arrhythmia • Severe gastrointestinal bleeding • Agitated, uncooperative state • Upper airway obstruction • Inability to protect the airway and/or high risk of aspiration • Inability to clear secretions • Multiple organ failure • Recent facial, upper airway or upper gastrointestinal surgery

[NPPV= non-invasive positive pressure ventilation; PaC02: arterial partial pressure of carbon dioxide; Pa02: arterial partial pressure of oxygen; Fi02: fraction of inspired oxygen]

Table 1. Indications and contraindications for NPPV in ARF

early extubation in COPD patients with NPPV are controversial, some showing significant benefit and the other showing no important benefit, but no attributable harm in either (Girault et al. 1999; Ferrer et al. 2003). Intubated COPD patients are appropriate candidates for early extubation by NPPV, but clinicians are advised to be cautious when selecting patients. The inability to sustain 5–10 min of unassisted breathing, a prior difficult intubation, multiple co-morbidities, copious secretions, a weakened cough, or the need for high levels of pressure support prior to extubation (>20 cm H2O) should exclude patients from consideration for early extubation (Hill 2004).

3.4 After planned extubation

Extubation failure occurs after 5-20% of planned (Epstein, Ciubotaru, and Wong 1997) and 40-50% of unplanned extubation (Chevron et al. 1998) NPPV may prevent the need for reintubation if applied immediately after planned extubation. NPPV is recommended to be used after planned extubation in patients who are considered to be at high risk of recurrent respiratory failure, but only in centres that have expertise in this type of therapy (Grade 2B recommendation) (Keenan et al. 2011). We should be careful to avoid delays in intubation in the face of deterioration and to select the patients for extubation.

3.5 Postoperative patients

It has been shown that NPPV in post-lung-resection patients with acute respiratory failure results in significantly less need for intubation, shorter ICU stay, and lower mortality rate than conventionally treated controls (Auriant et al. 2001) . The use of NPPV in selected postoperative patients (especially COPD patients) could maintain improved gas exchange and avoid reintubation and its complications.

3.6 Do-not-intubate patients

In the studies of patients in whom endotracheal intubation was contraindicated or postponed, COPD subgroup were supported with NPPV and weaned more successfully than the pneumonia or cancer subgroup of patients (Benhamou et al. 1992; Meduri et al. 1994). Thus, NPPV is indicated in do-not-intubate patients with acutely reversible processes that are known to respond well, including COPD exacerbations. However, if NPPV is to be used in a do-not-intubate patient, the patient and/or the family should be informed that NPPV is being used as a form of life support that may be uncomfortable and can be removed at any time (Hill 2004).

3.7 Overlap syndrome

The term ''overlap syndrome'' was introduced by Flenly to describe the association of obstructive sleep apnea syndrome (OSAS) and COPD (Flenley 1985). Even by chance alone, a patient with one of the disorders has a greater than 10% probability of also having the other disorder. Thus, when seeing a patient with either OSAS or COPD, it is reasonable to screen for the lower and longer nocturnal oxyhemoglobin desaturations, which produces more severe pulmonary hemodynamic complications (Chaouat et al. 1995; Bednarek et al. 2005). Concomitant COPD in patients with severe OSAS so called critical care syndrome is frequently associated with diurnal hypercapnia and acute ventilatory failure (Fletcher et al. 1991). There is an increase in the morbidity and mortality and risk of developing pulmonary hypertension and hypercapnic respiratory failure in patients with overlap syndrome than patients with OSAS alone and patients with usual COPD (Chaouat et al. 1995; Chaouat et al. 1999). NPPV with or without supplemental oxygen is now the treatment of choice for the patients with overlap syndrome (Mayos et al. 2001).

Improvement in daytime hypercapnia and gas exchange has been reported in overlap syndrome with continuous positive airway pressure (CPAP) treatment (Owens & Malhotra. 2010). Mild bronchodilatory effect due to amelioration of chronic irritation and responsiveness of the upper airway and reduction of the chronic airway has also been suggested as the possible mechanisms for the benefits of CPAP. Bilevel positive airway pressure (BPAP) may be preferred if the patient experiences difficulty in exhaling against a fixed pressure or has persistent intermittent hypoxemia despite adequate airflow (Kushida et al. 2006). Supplemental oxygen can be added to NPPV to eliminate persistent intermittent nocturnal hypoxemia (Kakkar & Berry 2007). In a cohort of overlap syndrome patients, CPAP added to long term oxygen treatment as compared to long term oxygen treatment resulted in a survival benefit with 5 years-survival rates of 71% and 26%, respectively (Machado et al. 2010). In another study including COPD and overlap syndrome patients, CPAP therapy eliminated the additional risk of mortality due to OSA in overlap syndrome

patients as compared to COPD- only patients (Marin et al. 2010) . One RCT and another study using a historical cohort showed reduction of mortality in overlap syndrome with NPPV (McEvoy et al. 2009; Windisch et al. 2009). In the study by Windisch et al., intensive pressure settings (average inspiratory pressure 28 cm H2O, average expiratory pressure 5 cm H2O and a high respiratory rate of about 21 breaths/min) were used with inhospital acclimatization and improvement in spirometry and arterial blood gas were reported (Windisch et al. 2009) . Finally, BPAP may be more comfortable and effective than CPAP in lowering CO2 and increasing tidal volume for patients with overlap syndrome, COPD component of which is much more related to moderate to severe hypercapnia and more prominent than the OSAS component.

3.8 Severe stable COPD/Chronic respiratory failure in patients with COPD

Despite the reported benefits of NPPV application in COPD patients with ARF, the role of NPPV in chronic respiratory failure (CRF) remains controversial. COPD patients with both increased hypercapnia and sleep-disordered breathing may be the ones, who are most likely to benefit from NPPV (Hill 2004). However the evidence to support the use of NPPV in CRF in the setting of severe stable COPD has been less consistent. COPD treatment guidelines does not recommend NPPV treatment routinely in end stage stable hypercapnic COPD in addition to conventional treatment (Global Initiative for Chronic Obstructive Lung Disease [GOLD] 2010).

Once hypercapnia develops, 2-year mortality is approximately 30-40% (Foucher et al. 1998). The reported studies show some physiological benefits for the use of NPPV in stable COPD, but clear survival benefit has not yet been demonstrated (Leger et al. 1994; Jones et al. 1998; Tuggey, Plant, & Elliott 2003). All of these and most other studies used a moderately aggressive ventilation to treat stable hypercapnic COPD patients and so an impressive reduction in hypercapnia was not achieved. In contrast, more aggressive form of ventilation with mean IPAP of up to 30 cmH20 or even higher was used in recent studies by Windish et al. and a remarkable reduction of PC02 was achieved (Windisch et al. 2002; Windisch et al. 2005; Windisch et al. 2006). Another RCT also has shown an improvement in survival with the application of nocturnal NPPV in end stage chronic hypercapnic COPD. The authors reported that the use of higher IPAP levels sufficient to be cardioprotective (but not to awake central respiratory drive) may result in greater treatment benefits (McEvoy et al. 2009). High intensity NPPV therefore offers a new and promising therapeutic option in the treatment of patients with CRF. High intensity NPPV is better tolerated in patients with severe chronic hypecapnic COPD and has been shown to be superior to the conventional and widely used form of low intensity NPPV in controlling nocturnal hypoventilation (Dreher et al. 2010). Nevertheless, higher leak volume, side effects and impairments in sleep quality are the main disadvantages of this modality.

NPPV might rest the chronically fatigued muscles and increase the muscle strength during daytime, could improve sleep time and efficiency, and sleep disordered breathing with episodes of hypoventilation. NPPV use in a select proportion of patients with severe stable COPD can improve gas exchange, exercise tolerance, dyspnea, work of breathing, frequency of hospitalisation, health-related quality of life and functional status (Kolodziej et al. 2007). Inconsistency in the effectiveness of all assessed outcomes may be due to the variability in degree of lung hyperinflation and NPPV levels and duration of use. As yet, no study has

provided convincing evidence that survival in COPD is prolonged by NPPV. Further work is also required to evaluate the effect of NPPV on reducing frequency and severity of COPD exacerbation. The general consensus, however, is that there is insufficient evidence to recommend NPPV for routine use in stable hypercapnic COPD (Kolodziej et al. 2007; Wijkstra et al. 2003). Despite the insufficient evidence, the ACCP consensus group opined that a trial of NPPV was justified with a symptomatic but stable and optimally treated patient who has daytime PaCO2 > 55 mm Hg, if OSA had been excluded. For PaCO2 between 50 and 54 mm Hg, , the ACCP consensus group suggested that there should be evidence of worsening hypoventilation during sleep, as suggested by a sustained (> 5 min) desaturation during use of the usual oxygen supplementation. In addition, the need for repeated hospitalizations was deemed a justification for a trial of NPPV (ACCP consensus conference 1999).

The other limitation of NPPV use in patients with stable hypercapnic COPD is poor compliance to NPPV in this group of patients. Criner et al., found that only 50% of COPD patients were still using NPPV after 6 months, compared to 80% for neuromuscular patients (Criner et al. 1999). Reasons for poor adherence are unclear, but probably include the advanced age of COPD patients, frequent occurrence of co morbidities and cognitive defects, lack of motivation and appropriate/inefficient setting of NPPV. Close follow-up is probably helpful to optimize compliance rates.

3.9 Sleep related hypoventilation/Hypoxemia due to COPD

The latest edition of The International Classification of Sleep Disorders: Diagnostic and Coding Manual (ICSD-2) subsumes a broad range of disorders under the heading ''Sleep Related Hypoventilation/hypoxemic Syndromes.'' (American Academy of Sleep Medicine. 2005). Some are quite common, such as COPD with worsening gas exchange during sleep; while some are exceedingly rare, such as congenital central hypoventilation syndrome. The ICSD-2 manual recommended the use of NPPV in addition to optimal treatment of the underlying disorder in selected subgroups of the patients (Casey, Cantillo, & Brown 2007).

In normal subjects, minute ventilation changes little, whereas minute ventilation in COPD patients falls approximately 16% from wakefulness to non REM sleep and almost 32% during REM sleep, compared to wakefulness, largely as a result of decreased tidal volumes. The greater drop in minute ventilation in subjects with COPD may reflect increased dependence on accessory muscles that become hypotonic during sleep, particularly in REM sleep leading to Sleep Related Hypoventilation/hypoxemic Syndrome due to COPD.

NPPV devices are used during sleep to treat patients with Sleep Related Hypoventilation/ hypoxemic syndromes. Compelling evidence exists to support the use of NPPV during sleep in the management of selected Sleep Related Hypoventilation/ hypoxemic syndromes. NPPV has been used in Sleep Related Hypoventilation/ hypoxemic due to central respiratory control disturbances, restrictive thoracic cage disorders, neuromuscular diseases and the obesity hypoventilation syndrome. A select subgroup of COPD patients also appears to have improved sleep after treatment with NPPV but specific characteristics that describe this subgroup well remain to be elucidated. It is unclear whether exclusively nocturnal hypoxemia in these patients will be deleterious and therefore whether isolated sleep-related hypoxemia should be treated. COPD patients with clear evidence of hypoventilation while awake as evidenced by daytime hypercapnia are a reasonable starting target group. Those COPD

patients who also show continued sleep disruption or worsening hypercapnia and nocturnal hypoventilation despite oxygen therapy should be further investigated probably with polysomnography to rule out other sleep related breathing disorders. Finally we need to define optimal NPPV and interface design and settings in hopes of improving compliance of long-term therapy for all types of appropriate patients, who are likely to benefit from NPPV.

3.10 Adjunct to exercise training in pulmonary rehabilitation programs

Another potential application of NPPV in patients with severe stable COPD is to enhance exercise training during rehabilitation. It has been shown that when delivered during cycle ergometry, CPAP, pressure-support ventilation, and proportional-assist ventilation all reduce inspiratory effort and dyspnea in hypercapnic COPD patients (Petrof, Calderini, & Gottfried 1990; Bianchi et al. 1998). Recent studies in patients with severe COPD in a 6-week exercise training program has reported that, NPPV alone was more effective than supplemental oxygen alone as adjunct to physical exercise in improving submaximal exercise tolerance and health related quality of life (HRQOL) (Borghi-Silva et al. 2010). These studies demonstrated that NPPV can be used to increase or prolong the intensity of exercise training sessions in patients with severe COPD.

4. Where to administer NPPV?

Any patient on NPPV is classified as receiving Critical Care Level 2 care, defined as ''Patients requiring more detailed observation or intervention including support for a single failed organ system''. This suggest NPPV should be administered in an intensive care unit (ICU) or high dependency unit (HDU) setting, but it has been widely recognised that NPPV can be successfully used outside the ICU and HDU with dedicated NPPV team able to provide 24/7 care. This is however only feasible in large units with many trained staff (Manuel, Russell, & Jones. 2010). NPPV is more frequently used outside the ICU, in HDU, respiratory wards and emergency departments (EDs) (Brochard, Mancebo, & Elliott 2002; Hill 2004). It has been suggested that each hospital should have a specific designed area with experienced staff, where patients requiring NPPV can be transferred with the minimum delay (British Thoracic Society Standards of Care Committee 2002).

5. Selection of optimal ventilator and mode of NPPV

NPPV is broadly classified into volume preset and pressure preset devices, early studies of long-term domiciliary NPPV mainly concern patients on volume preset ventilators, whereas in the last 5-10 years pressure preset machines, particularly bilevel pressure support equipment has become more prominent.

Volume preset machines gives the adjusted tidal volume regardless of mechanics of respiratory system (i.e. compliance, resistance and active inspiration) and if there is a leak from mask or mouth, patient cannot deliver the adjusted tidal volume.

On the contrary **pressure preset** machines gives the adjusted pressure according to respiratory system mechanics by changing the flow and compensates the mask leaks. However pressure preset machines may not to be sufficient in patients who need high inspiratory pressure. Pressure support ventilators on a first line basis, especially with pressure support mode, is easier to adjust and to synchronise with the patient. CPAP and BPAP are the pressure support

ventilators. CPAP as the name implies, requires the airway pressure not to change between inspiration and expiration. However BPAP therapy was originally conceived with the idea of varying the administered pressure between the inspiratory and expiratory cycles. BPAP is the commonly used pressure preset method. BPAP devices deliver separately adjustable inspiratory positive airway pressure (IPAP) and expiratory positive airway pressure (EPAP). The IPAP and EPAP levels are adjusted to maintain upper airway patency, and the pressure support (PS=IPAP-EPAP), which augments ventilation.

Three modes of NPPV were also defined according to principles of cycling of inspiration. NPPV devices can be used in the 1) **spontaneous mode** (the patient cycles the device from EPAP to IPAP), 2) the **spontaneous timed (ST)**/assisted-controlled (AC) mode (a backup rate is available to deliver IPAP for the set inspiratory time if the patient does not trigger an IPAP/EPAP cycle within a set time window otherwise patient the device from EPAP to IPAP), 3) the **timed (T)** /pressure controlled (PC) mode (patient cannot trigger and cycle the inspiration- inspiratory time and respiratory rate are fixed).

Volume assured pressure support / volume target BPAP (VT-BPAP) which is a hybrid mode of volume preset and pressure support ventilation was available by the end of the 1990s. Release of dual portable ventilators providing either pressure support ventilation or volume preset ventilation opened the way for new potent turbine pressure support ventilators able to deliver real volume ventilation with the average volume assured pressure support ventilation mode which represents a flexible way for managing the most difficult patients (Storre et al. 2006). Patient delivers the target tidal volume by the support of adjusted pressure support range. VT-BPAP has been developed in which the IPAP-EPAP difference is automatically adjusted to deliver a target tidal volume (Storre et al. 2006; Ambrogio et al. 2009; Janssens, Metzger, & Sforza 2009; Jaye et al. 2009)

Proportional Assist Ventilation is another mode still under investigation. It provides a level of ventilatory assistance which is proportional to the patient's respiratory effort throughout the respiratory cycle. Some studies reported better comfort and tolerance with proportional assist ventilation but found no differences in rates of mortality or intubation (Fernandez-Vivas et al. 2003; Gay, Hess, & Hill 2001). Guidelines make no recommendation about the use of proportional assist ventilation versus pressure support ventilation in patients who are receiving NPPV for ARF, due to lack of sufficient evidence.

6. Selection of interface

Interfaces connect the patient's airway to the NPPV tubing. The main six interfaces for NPPV are nasal mask, full face or oronasal mask, total face mask, helmet mask, nasal pillow or plugs and mouthpieces. Usually made of silicone, masks need to be carefully fitted to the individual to obtain optimum results. Variations include the bubble-type mask, and gel masks. Mask fit can be enhanced using mask cushions and seal/support rings which are supplied with the mask.

Nasal mask: Nasal mask covers nose and does not cover mouth so allows speaking, drinking and cough also reduces the risk of vomiting and asphyxia. Disadvantages of nasal masks are air leaks if mouth opens, possible nasal skin damage and the need for patent nasal passages.

Oronasal /Full face mask: Oronasal mask cover the nose and mouth and can prove valuable in patients with nasal airway blockage or acute confusional state. Oronasal mask is

recommended rather than nasal mask in patients who have ARF. Although there was no difference in endotracheal intubation or mortality rates, the oronasal mask was better tolerated (Keenan et al. 2011). The use of an oronasal mask seem a logical solution to maximize the NPPV efficacy, presumably due to lower leakage with oronasal mask compared to nasal mask in dyspneic patients who are mostly mouth-breathers (Carrey, Gottfried, & Levy 1990). However during long-term use the face mask can be poorly tolerated, thus causing a premature NPPV interruption (Carlucci et al. 2001).

Total face mask: Total face mask covers mouth, nose and eyes. Advantages of this type of masks are minor air leaks, little cooperation required and easy fitting application. Risks of asphyxia, claustrophobia, speaking difficulty are the main disadvantages.

Helmet: Helmet mask covers whole head and all or part of the neck without a contact with face. Advantages of this type of masks are minor air leaks, little cooperation required and absence of nasal or facial skin damage. The risk of vomiting, worsening of C02 clearance due to rebreathing, asynchrony with pressure support ventilation and discomfort of axillae are the disadvantages of the helmet.

Nasal pillow or plugs: These masks are inserted into the nostrils. This type of the mask may be suitable for claustrophobic patients with chronic stable COPD who do not need high pressures. Nasal irritation is the main disadvantage.

Mouthpieces: They are placed between lips and held in place by lip seal. Mouthpieces can be applied with other interfaces. The risk of vomiting and salivation, possible air leaks, gastric distension and speaking difficulty are the disadvantages of the mouthpieces. Mouthpiece ventilation is mainly used in patients with neuromuscular disease.

7. Application, setting and adjustments of NPPV

The first hours of NPPV are associated with an increased workload for health care personnel that requires a specific management protocol, including monitoring mask ventilation and monitoring the patient (Nava and Hill 2009). Recommended application, setting and adjustments of NPPV in the ICU, HDU, respiratory wards and emergency departments (EDs) are summarised as in the following:

1. Explain technique to patient (if competent).
2. Choose correct interfaces and size.
3. Set pressure starting from low levels (minimum starting IPAP and EPAP should be 8 cm H_20 and 4 cm H_20, respectively).
4. Place mask gently over face, holding it in place and start ventilation.
5. When patient is tolerant, tighten straps just enough to avoid major leaks, but not keep it too tight.
6. Set Fi02 on ventilator or add low-flow oxygen into the circuit, aiming for S02>90%.
7. Set alarms-low pressure alarm should be above PEEP level.
8. Be mindful of and try to optimise patient's comfort.
9. Reset pressures (pressure support increased to obtain inspired tidal volume 6mL/kg or higher, achieving a respiratory rate <25 breaths/min, PaC02 <45 mmHg and also raise EPAP to obtain S02 of 90% or higher). The recommended maximum IPAP should be 30 cm H_20 for patients ≥ 12 years. The recommended minimum and maximum levels of PS

are 4 cm H_20 and 20 cm H_20, respectively. PS should be increased in order to optimize CO2 removal and control of auto-positive end expiratory pressure (PEEP), according to the patient's tolerance. A backup rate (ST mode) should be used in all patients with low respiratory rate, in patients who unreliably trigger IPAP/EPAP cycle due to muscle weakness and in patients who do not achieve adequate ventilation or respiratory muscle rest with the maximum tolerated PS in the spontaneous mode. The inspiratory duration should be as short as possible.

10. Protect site of skin pressure from the interface.
11. Consider use of mild sedation if the patient is agitated.
12. Monitor comfort, respiratory rate, oxygen saturation and dyspnea every 30 minute for 6-12 hours and then hourly.
13. Measure arterial blood gases at baseline and within 1 hour from the start.
14. Humidification is advised for longer application.

Predictors of NPPV failure are no improvement or a fall in pH and PC02, no change or a rise in breathing frequency after 1-2 hours and lack of cooperation. Delays in intubation of these patients run the risk of unanticipated respiratory or cardiac arrest with attended morbidity and mortality. NPPV failure occurs more frequently in the first hours of ventilation, and was reported to be predicted by the following clinical factors: severe acidosis, high severity score, severe impairment of consciousness, presence of co-morbidities and lack of improvement of arterial blood gases after 1-2 hours of initial ventilation (Ambrosino et al. 1995; Elliott 2002; Nava & Ceriana 2004)

8. Complications of NPPV

Complications of NPPV therapy are minor and preventable. Major complications of NPPV such as pneumothorax and pneumocephalus are so rare (Grunstein 2005). The most common complications effecting almost half of the patients who are administered NPPV are due to mask leak and/or mask pressure injury (Pepin et al. 1999; Hoffstein et al. 1992; Abisheganaden et al. 1998; Lojander, Brander, & Ammala 1999; Sanders, Gruendl, & Rogers 1986). The main complications of NPPV therapy are listed in Table 2.

Due to Mask	**Due to Device**
Facial and nasal pressure injury/ ulcerations / pain	Rhinitis, Rhinorrhea
Mask allergy	Sinusitis
Conjunctivitis	Tinnitus
Dermatitis	Otitis /ear pain
Claustrophobia	Epistaxis
General	Gastric distension
Anxiety	Dry mucous membranes and thick secretions
Insomnia	Aspiration of gastric contents
Chest pain	Barotrauma (pneumothorax, pneumocephalus)
Headache	Central Sleep Apnea
Periodic Legs Movement Syndrome	Hypotension related to positive intrathoracic pressure

Table 2. Complications of NPPV Therapy

9. Conclusion

For COPD exacerbations NPPV should now be considered as a standard of care in properly selected patients, used in preference to invasive mechanical ventilation. Available evidence and experience have indicated that NPPV has an important role in managing COPD exacerbations, markedly by reducing the need for intubation and improving outcomes, including lowering complication and mortality rates, as well as shortening the hospital stay. NPPV can also be used in certain other situations in COPD patients: in respiratory failure precipitated by a superimposed pneumonia, in postoperative respiratory failure, in intubated patients to facilitate extubation with the aim of reducing the complications of prolonged intubation, in patients with postextubation failure to avoid reintubation, and in do-not-intubate patients; although the evidence to support these applications is not as strong as for NPPV in typical COPD exacerbations. For patients with severe stable COPD, currently available evidence suggests that NPPV can improve daytime and nocturnal gas exchange, prolong sleep duration, improve quality-of-life scores, and possibly reduce the need for hospitalization. However, the findings among studies have not been consistent on these benefits, partly related to numerous methodological shortcomings in most studies performed to date. Despite the weakness of the evidence base, however, some of the consensus and guidelines agree that COPD patients with substantial daytime carbon dioxide retention and evidence of superimposed nocturnal hypoventilation are the ones most likely to benefit (ACCP consensus conference 1999). Achieving desired NPPV adherence by COPD patients will remain still a challenge. Identification of eligible patients, establishment of the appropriate settings and close monitoring of the patients with trained staff are the key points of success of NPPV therapy. Technological improvement of NPPV devices and masks besides new guidelines on the selection of patient, ventilation mode and interface may achieve better NPPV adherence in patients with COPD in the future.

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18

Types of Physical Exercise Training for COPD Patients

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1. Introduction

Pulmonary diseases are increasingly important causes of morbidity and mortality in the modern world (Ries et al., 2007). Chronic obstructive pulmonary disease (COPD) is the most common chronic lung disease, and a major cause of lung-related death and disability (Fishman, 2008). COPD is characterized by chronic airflow limitation, progressive and largely irreversible, associated with an abnormal inflammatory reaction (Ancochea Bermúdez et al., 2009). COPD is very disabling and features extra-pulmonary manifestations, but it can be prevented and treated.

The disease is diagnosed by a clinical history based on the combination of history, physical examination and confirmation of the presence of airflow obstruction with the use of spirometry (Figure 1 Spirometry). Spirometric assessment is performed according to the guidelines of the American Thoracic Society (ATS) (Laszlo, 2006). The technician asks the subjects three exhaling exercises and the best is used for the analysis (Miller et al., 2005). If the Tiffenau rate (value of FEV_1/FVC) is less than seventy percent, COPD exists (Global initiative for chronic obstructive lung disease *[GOLD]*, 2010). Smoking is the major risk factor for the disease (Hilberink et al. 2011).

Fig. 1. Spirometry

The most common symptoms of COPD are breathlessness, chronic cough, wheezing, sputum production, recurrent respiratory infection may be associated with some of the following systematic effects such as undernourishment, weight loss, exercise limitation and muscle weakness (*GOLD,* 2010). Knowledge regarding the disturbance of muscle function that occurs in patients with COPD is continuously increasing. Initially muscular dysfunction was considered to be a self-limiting disease resulting from inactivity and lack of exercise. However, recent studies have shown that in addition to this factor, peripheral muscles such as the quadriceps seem to have some type of myopathy (Couillard & Prefaut, 2005). Although the presence of myopathy is still being debated, there is some evidence pointing to myopathy associated with oxidative stress (Rabinovich et al., 2001). Recent studies in COPD have highlighted the role of the ubiquitine proteasome system in the breakdown of skeletal muscle protein in COPD patients. Malfunction of the mitochondria has also recently been identified in these patients (Rabinovich & Vilaro, 2010).

COPD is a major cause of disability and mortality worldwide and the prevalence increases with age. COPD will increase by more than thirty percent in the next ten years, if the population does not cut down smoking (Ancochea Bermúdez et al., 2009). Actually, due to high prevalence, associated to high morbilidity, economic and social cost COPD is a major health problem (Ramsey & Sullivan, 2003; Sullivan, Ramsey, & Lee, 2000). COPD is not curable, but treatments can help to control symptoms and improve quality of life of patients. It is necessary to reduce risk factors such as smoking and physical inactivity *(GOLD,* 2010).

Many people suffer from COPD for years and die prematurely of it or its complications. The goals of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (Rabe et al., 2007) are to improve prevention and management of COPD through a concerted worldwide effort of people involved in all facets of health care and health care policy, and to encourage an expanded level of research interest in this highly prevalent disease. The GOLD report separates COPD patients into the four different stages (figure 2) *(GOLD,* 2010).

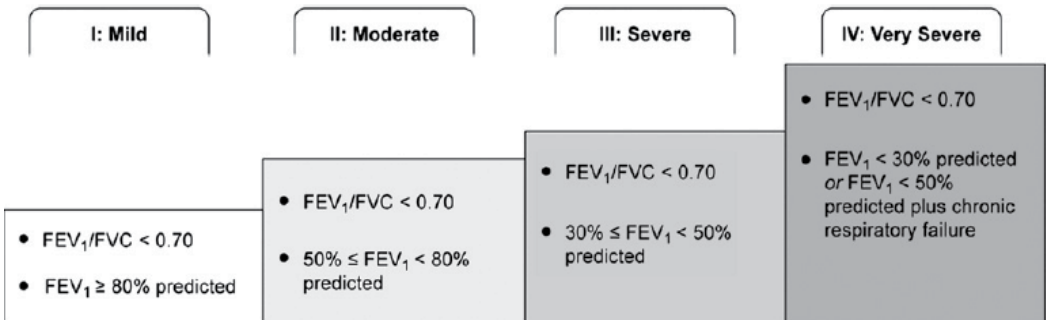

Fig. 2. Stages for Chronic Obstructive Pulmonary Disease

Pulmonary rehabilitation has emerged as a recommended standard of care for patients with chronic lung disease based on a growing body of scientific evidence. The American Thoracic Society and European Respiratory Society (ATS /ERS) published a document in 2006 defining respiratory rehabilitation as "a multidisciplinary and comprehensive intervention has proved effective from the perspective of evidence-based medicine for patients with chronic respiratory diseases who are symptomatic and often have decreased daily life activities. Integrated into individualized treatment of the patient, pulmonary rehabilitation is designed to reduce

symptoms, optimize functional status, increase participation, and reduce health-care costs by stabilizing or reversing systemic manifestations of the disease" (Nici et al., 2006). This definition focuses on three aspects of successful rehabilitation: a multidisciplinary approach; an individualized program; tailored to the patient´s needs; and attention to physical psychological and social functioning (Ries, 2008). Not forgetting a primary goal of rehabilitation interventions for people with COPD is to optimize function (Nici et al., 2006).

The components of multidisciplinary respiratory rehabilitation programs include education of patients and their families, chest physiotherapy, muscle training, the emotional support, nutritional support, occupational therapy (Ries et al., 2007). Physiotherapy consists of various phases of treatment (exercise training, peripheral and respiratory muscle training, and breathing exercises) that are considered cornerstones of the physiotherapeutic intervention (Langer et al., 2009). Also consider patients that are incorporated into a respiratory rehabilitation program must have an optimal pharmacological treatment, although not analyzed in this chapter.

There is no consensus of opinion regarding the optimal duration of the pulmonary rehabilitation intervention (Ries et al., 2007). The duration depends on changes in the patient´s lifestyle. A number of external factors also influence program duration including health-care systems and reimbursement policies, access to programs, level of functional disability, health-care provider referral patterns, and the ability of individual patients to make progress toward treatment goals.

Few clinical trials have focused on the impact of program duration on rehabilitation outcomes, but existing data suggest that gains in exercise tolerance may be greater following longer programs (Berry et al., 2003; Foy, Rejeski, Berry, Zaccaro, & Woodard, 2001; Green, Singh, Williams, & Morgan, 2001; Troosters, Gosselink, & Decramer, 2000). Besides Verrill et al. (2005) demonstrated that patients achieved significant gains in exercise tolerance in the six minute walk distance, after twelve weeks of pulmonary rehabilitation. However, in an older trial Wijkstra et al. (1995) showed that there was no difference noted between groups in the magnitude of gains in the six minute walk distance for patients who underwent 18 months and three months of home-based rehabilitation.

Moreover, although some studies suggest that the duration of the pulmonary rehabilitation program has an impact on exercise tolerance improvement, it is not clear that other outcomes such as health status or dyspnea are similarly affected by program duration (Ries et al., 2007). Thus, given the variations found in types of rehabilitation programs and content as on duration (Clini et al., 2001). Besides the differences found in clinical study design, patient populations, health systems in different countries, program location, and program content.

The purpose of this literature review is to compare the effectiveness of various exercises training programmes in the rehabilitation of COPD patients. This study analyzes the different types of aerobic exercises that are carried out with different intensities, doses and frequencies.

2. Exploratory testing

The chronic symptoms of COPD (cough, expectoration, wheezing, dyspnea and exercise tolerance) are the major factors responsible for altering the relationship between health and

quality of life. Studies of health-related quality of life (HRQoL) in patients with COPD with varying degrees of severity have consistently shown that patients have significant decrements in HRQoL (Okubadejo, Jones, & Wedzicha, 1996; Schrier, Dekker, Kaptein, & Dijkman, 1990). Therefore, HRQoL is an important clinical outcome in COPD. The Chronic Respiratory Disease Questionnaire (CRQ) (Guyatt, Berman, Townsend, Pugsley, & Chambers, 1987) and St George´s Respirtory Questionnaire (SGRQ) (Jones, Quirk, Baveystock, & Littlejohns, 1992; Jones, 2001) are the main questionnaires used to measure the quality of life in COPD patients.

The evidence-based clinical practice guidelines document concluded that there was a strong level of type A evidence, that Pulmonary Rehabilitation Programmes (PRP) improve the symptom of dyspnea in patients with COPD with a strong level of type A evidence (Jones, 2002). Dyspnea is a sensation of respiratory discomfort and the evaluation of the degree of dyspnea provides an independent dimension that is not provided by pulmonary function tests or by measuring dyspnea in an exercise laboratory. So, dyspnea is a main symptom associated with exercise performance and, therefore, quality of life. One of the major goals of COPD treatment is a reduction in dyspnea. The severity of the disease can be determined by the intensity of dyspnea (Camargo & Pereira, 2010). The severity of COPD is habitually classified by forced expiratory volume in the first second (FEV_1) after bronchodilator use (Rabe et al., 2007). Various instruments are available to measure the degree of dyspnea during exercise; the modified Medical Research Council (mMRC) dyspnea scale is the most used (Barbera et al., 2001). The mMRC has five levels that increase with the level of activity in which dyspnea appears. It assesses common tasks the patient can develop without displaying dyspnea. Levels of Dyspnea are graded as follows. Grade 0: "I only get breathless with strenuous exercise"; grade 1: "I get short of breath when hurrying or walking up a slight hill"; grade 2: "I walk slower than people of the same age because of breathlessness or have to stop for breath when walking at my own pace"; grade 3: "I stop for breath after walking 100 yards or after a few minutes"; grade 4: "I am too breathless to leave the house".

The mMRC was unidimensional, to overcome this limitation; Mahler (Mahler, Mejia-Alfaro, Ward, & Baird, 2001) designed the index known as the Baseline Dyspnea Index (BDT), which was later supplemented with the Transitional Dyspnea Index (TDI). BDT analyzes dyspnea from a triple perspective; the difficulty of the task, magnitude of effort and functional impairment, each of the sections will be assessed from 0 (severe) to 4 (none), so total amount can range between 0 and 12 (Mahler, 2006). TDI assessed changes over time compared to baseline (BDI), the changes in each of the three sections are measured between -3 and +3. Therefore, the total score can be between +9 and -9. A score of 0 indicates no changes have occurred, while -9 is very negative result (Sobradillo et al., 1999). Both multidimensional scales, BDT and TDI, are clinical instruments that can be used during cardiopulmonary exercise testing for clinical and research purposes. Besides, Borg et al., (Borg, Borg, Larsson, Letzter, & Sundblad, 2010) described the matching of the increase in dyspnea related to ventilation and oxygen consumption in exercise.

In a review of application of dyspnea and quality of life scales in COPD, it was concluded that a unidimensional scale can be used if applied in conjunction with specific quality of life scales. Alternatively, a multidimensional scale, which correlates better with quality of life, can be used (Bausewein, Farquhar, Booth, Gysels, & Higginson, 2007). Consequently, multidimensional clinical instruments were developed in order to provide a more

comprehensive assessment of the severity of dyspnea, combined with the Chronic Respiratory Disease Questionnaire (CRQ) incorporates five physical activities that are specific for individual patients (Guyatt et al., 1987). These instruments have been shown to be valid, reliable, and responsive (Reda, Kotz, Kocks, Wesseling, & van Schayck, 2010).

In 2004, Celli et al. created a mortality prediction index, known as the BODE index. It encompassed the body mass index (B), the degree of airflow obstruction as expressed by the FEV_1 (O), dyspnea with the modified medical research council (D), and exercise (E) measured with six-minute walk distance (Table 1 Variables and point value used for the computation of BODE index) data adaptated from Celli et al. (2004). The cut-off values for the assignment of points are shown for each variable. *The FEV_1 categories were identified by the American Thoracic Society (1995).† Scored on the modified Medical Research Council (mMRC) dyspnea scale can range from 0 to 4, with a score of 4 indicating that the patient is too breathless to leave the house or becomes breathless when dressing or undressing.

BODE index

Variables	Points on BODE index			
	0	1	2	3
FEV_1% of predicted *	≥ 65	50–64	36–49	≤ 35
Six-minute walk distance (m)	≥ 350	250–349	150–249	≤ 149
MRC dyspnea score †	0–1	2	3	4
Body mass index (kg/m^2)	>21	≤ 21		

Table 1. Variables and point value used for the computation of BODE index, adapted from Celli et al. (2004)

The BODE index is a multidimensional classification system that systemically determines the degree of mortality in individuals with COPD, that provides useful prognostic information in patients with COPD and might be able to measure health status. However, it is unknown whether the BODE index is a sensitive tool for predicting the impact of quality of life in such patients. Araujo (Araujo & Holanda, 2010) found correlations between the BODE index scores and all of the CRQ domains in COPD patients. Moreover, there are studies where patients who moved from moderate to high physical activity improved their SGRQ scores by 18.4 and their CRQ scores by 14.8 (Esteban et al., 2010).

Over recent decades, several organizations have championed pulmonary rehabilitation and developed comprehensive statements, practice guidelines, and evidence-based guidelines (Ries, 2008), however there are differences about how assessment of severity of disease. The 2010 NICE Guidelines defended that multidimensional assessment tool (BODE index) is a better predictor of mortality and exacerbation rate than FEV_1 alone (Gruffydd-Jones & Loveridge, 2011).

Exercise testing is frequently used in the clinical evaluation of patients with COPD to evaluate the functional impact of a treatment (American Thoracic Society & American College of Chest Physicians, 2003). Exercise testing is a useful evaluative tool, allowing standardized measurement of exertional dispnea and exercise tolerance *(GOLD,* 2010). There is, however, no consensus regarding which exercise testing protocol should be used for this application (Pepin, Saey, Whittom, LeBlanc, & Maltais, 2005). A research indicated that walking, as performed in the endurance shuttle walk, is sensitive to detect changes in exercise performance after bronchodilation (Pepin et al., 2005). Besides Pepin et al. (2007)

indicate that the response of the 6MWT test is not sensitive to change and may not be appropriate for an assessment tool. Another research also suggests that the endurance shuttle walk is more responsive to the effects of pulmonary rehabilitation than the 6MWT for detecting changes in exercise performance following broncholdilations (Eaton, Young, Nicol, & Kolbe, 2006). Together, these findings provide growing support for the use of the endurance shuttle walk as an evaluative tool to monitor response to treatment to COPD.

The six minute walk test (6MWT) is used in order to determine the six-minute walk distance (6MWD), which correlates with the performance of activities of daily living in patients with COPD (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002; Brooks, Solway, & Gibbons, 2003). The 6MWT measures the global and integrated responses of all organ systems involved during exercise, has been shown to be an important parameter related to morbility and mortality in COPD (Casanova et al., 2007), and is also part of the BODE index (Celli et al., 2004). Although rehabilitation improves both exercise tolerance and quality of life in COPD, it is not known whether these improvements are related to each other. Several trials show the weak correlation between quality of life and the six minute walking distance in patients with COPD suggests that these parameters measure different aspects of health (Wijkstra et al., 1995).

Recently, the use of accelerometer has been incorporated as an objective measure to assess physical activity level of the patient performs daily (Troosters et al., 2010). It is necessary to analyze physical activity in daily life in patients across different disease stages according to GOLD. Other studies have shown that grip strength in the wrist is a strong independent predictor of mortality in COPD (Cortopassi, Divo, Pinto-Plata, & Celli, 2011). A significant relationship was found between hand grip strength and peripheral muscle strength (flexion of elbow and knee) and strong relationship ($r = - 0.75$, $p < 0.0001$) with the force respiratory muscles (maximum inspiratory muscles, inspiratory capacity, forced vital capacity and maximum volume ventilation).

There is no clinical trial review that has found a connection between rehabilitation respiratory programs and an increase in exercise tolerance. It is necessary to clarify the change in quality of life was related with a change in exercise tolerance in COPD patients. The difference between current studies and previous controlled studies (Sinclair, 1980; Vale, Reardon, & ZuWallack, 1993) are the use of the 12 minute walking distance which is probably more sensitive to change than the six minute walking distance (Wijkstra et al., 1995).

COPD is often associated with exacerbation of symptoms. An exacerbation of COPD is defined as "an event in the natural course for the disease characterized by a change in the patient´s baseline dyspnea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD" (Burge & Wedzicha, 2003). The most common causes of an exacerbation are infection of the tracheobronchial tree and air pollution (White, Gompertz, & Stockley, 2003). Studies investigating effects on pulmonary function and oxigenation did not show benefits in either acute exacerbations of COPD (Newton & Bevans, 1978) or in chronic COPD (May & Munt, 1979). Assessment of the severity of an exacerbation is based on the patient´s medical history before the exacerbation, pre-existing comorbidities, symptoms, physical examination, arterial blood gas measurements, and other laboratory

test. Physicians should obtain the results of previous evaluations, where possible, to compare with the current clinical data. Specific information is required on the frequency and severity of attacks of breathlessness and cough, sputum volume and color, and limitation of daily activities (Vilaró et al., 2007).

Other targets of rehabilitation are anxiety control, dyspnea reduction and improvement of the health-related quality of life (Lacasse et al., 2006). The illness evolution can be associated with extra-pulmonary components, such as muscle loss is related with reduction of physical activity. After exacerbation, symptoms of depression have been identified as an independent factor of mortality risk (Yohannes, Baldwin, & Connolly, 2005), as well as risk a factor for rehabilitation program drop-outs (Garrod, Marshall, Barley, & Jones, 2006). The skeletal muscle dysfunction and depressive symptoms are potencially amenable to rehabilitation with exercise training (Rodrigues, 2010). We have made the following figure 3 in order to collect intra-pulmonary components (airways obstruction and dyspnea) with extra-pulmonary factors (muscle wasting, reduce mobility, exercise limitation, depression and sedentary lifestyle).

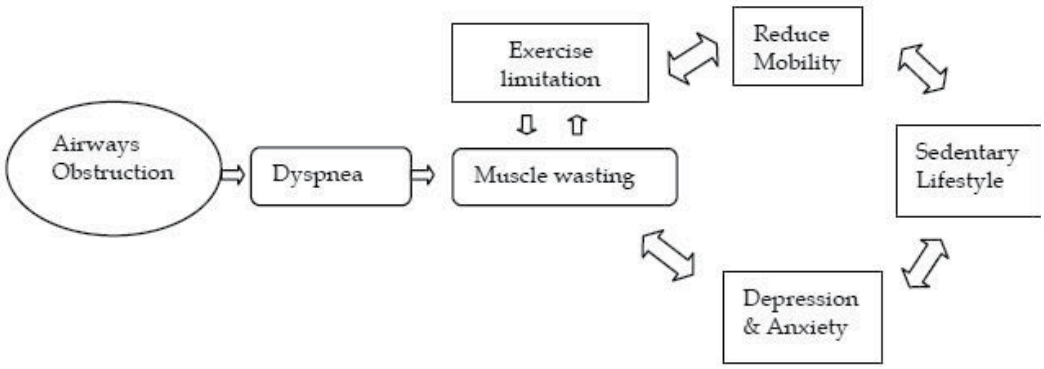

Fig. 3. Relation between intra-pulmonary components with extra-pulmonary factors.

3. Types of exercises

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure beyond resting energy expenditure (Thompson et al., 2003). Information on the importance of physical activity in COPD has grown, especially in the last few years, although major questions remain to be answered. The present chapter aims to provide an update on the most important studies of physical activity in COPD (Esteban, 2009).

Findings from meta-analysis of pulmonary rehabilitation strongly supports that exercise training as part of treatment of patients with COPD should last at least four weeks (Lacasse, Goldstein, Lasserson, & Martin, 2006). Exercise training should be available to people with COPD, because it improves breathlessness, quality of life, exercise tolerance and functional ability (Lacasse et al., 2006). Physical therapists are crucial to the delivery of rehabilitation because of their training in exercise and movement therapies (Garrod & Lasserson, 2007).

The primary goal of the rehabilitation programs is to restore the patient to the highest possible level of independent function (Ries et al., 2007). This goal is accomplished by

helping patients become more physically active, and to learn more about their disease, treatment options, and how to cope. Within the program of rehabilitation, the physiotherapeutic intervention is responsible for various treatment phases (specifically physical exercise training, peripheral and respiratory muscle training, and breathing exercises) (Langer et al., 2009).

Aerobic exercise is the main non-pharmacological treatment better tolerated by patients with COPD (Martín-Valero, Cuesta-Vargas, & Labajos-Manzanares, 2010). Exercise training is one of the key components of pulmonary rehabilitation. The exercise prescription for the training program is guided by the following three parameters: intensity; frequency; and duration.

The standarized criterions on intervention period, dose, intensity of physical exercises in COPD patients is needed. Seven (Coppoolse et al., 1999; Kurabayashi et al., 2000; O'Shea, Taylor, & Paratz, 2004; Puente-Maestu, Sanz, Sanz, Cubillo et al., 2000; Puente-Maestu, Sanz, Sanz, Ruiz de Ona et al., 2000; Wadell, Sundelin, Henriksson-Larsen, & Lundgren, 2004; Wijkstra et al., 1995) agreed with the criteria of the American College of Sports Medicine (ACSM) (Garber et al., 2011) for the intervention period and number of sessions varied from eight weeks in the majority on trials to twelve weeks in two trials and from two to four sessions a weeks. Therefore, the number sessions a week were at least between two or four sessions a week. Only one trial (Wijkstra et al., 1995) took into account, that patients had to practise twice a day for an individualised protocol, for 0 to 5 hours the first three months and then once a day only for 0-5. The time of sessions is variable in these seven articles with a minimum of 20 minutes up to 60 minutes because two articles do not talk about the time of sessions.

According to the recommendations of the American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR), *high-intensity training targets* have been operationally defined to be at least 60 to 80% of the peak work rate achieved in an incremental maximum exercise test. The intensity of the training sessions in five articles (Coppoolse et al., 1999; Puente-Maestu, Sanz, Sanz, Cubillo et al., 2000; Puente-Maestu, Sanz, Sanz, Ruiz de Ona et al., 2000; Wadell, Sundelin, Henriksson-Larsen, & Lundgren, 2004; Wijkstra et al., 1995) showed that the goal is 60-90% of heart rate maximum (HR_{max}) set by the ACSM for improving aerobic fitness ("American college of sports medicine position stand. exercise and physical activity for older adults,"1998a; "American college of sports medicine position stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults,"1998b)

Exercise training intervention can be adapted to the individual exercise limitations of the patient (Troosters, Gosselink, Langer, & Decramer, 2007). Troosters et al. review focused on different training types (endurance, interval and resistance training) (Troosters et al., 2007). In this chapter regarding types of exercise training intervention, it has been divided into aerobic and resistance training types. Aerobic exercise training for older people should have a target intensity of 50-85% of the oxygen uptake reserve – a range that includes both moderate exercise (minimum of 30 minute five days a week) or vigorous exercise (20 minutes three days each week)(Garber et al., 2011).

Resistance training is an ideal intervention for patients with peripheral muscle weakness and pronounced symptoms of dyspnea during exercise (O´Shea, Taylor, & Paratz, 2004).

There is not consensus on the optimal method of resistance training (callisthenics, resistance weight training, isometrics or isokinetic-type training) in patients with COPD. Each type produces strength gains highly specific to the type of training. There are no studies that compared different intensities of resistance training in patients with COPD. It is recommended to use (lower limb) resistance training according to ACSM (two or three times a week) ("ACSM", 1998a; "ACSM", 1998b; Garber et al., 2011). Exercises should be performed at 60-80% of the first repetition maximum (RM), resistance exercises should train 8-10 exercises involving the major muscle groups in bouts of 8-15 repetitions at least 30 minutes a day of moderate-intensity activity on two or three non-consecutive days each week (Nelson et al., 2007). Multiple sets of repetitions (2-5 sets) provide greater benefit (Langer et al., 2009). Resistance activities include a progressive-weight training program, done with therabands (wrist or ankle weights) or progressive weight.

Given that muscle weakness is a common problem in this population, progressive resistance exercise represents a beneficial treatment for improvements in muscle strength (O´Shea, Taylor, & Paratz, 2009). Moreover, improvements in muscle strength can be obtained when progressive resistance exercise is conducted alone or in combination with aerobic training, indicating that it can be successfully performed in conjunction with other training types during pulmonary rehabilitation (O´Shea, Taylor, & Paratz, 2009).

Careful consideration is also required when prescribing progressive resistance exercise programs for people with COPD who have comorbid health conditions (O´Shea, Taylor, & Paratz, 2004). Therefore, progresive resitance exercise may not be appropriate for all people with COPD attending pulmonary rehabilitation, and it is recommended that prescription be targeted to the individual (Storer, 2001).

It is essential to educate the patient about the importance of the training program begining with an initial phase: warming up and stretching (Table 2 Session outline). The central part consists in aerobic training (endurance or interval exercise), resistance training and breathing retraining. Finally, the sessions finish with stretching and relaxation exercises.

Initial phase	Central phase	Final phase
*Stretching *Warming up	*Aerobic training (Endurance or Interval) *Resistance training *Breathing retraining	*Stretching *Relaxation exercises

Table 2. Session outline

It is recommended to apply training strategies that enable patients to resume participation in a rehabilitation programme after an acute exacerbation as soon as possible (Puhan, Scharplatz, Troosters, Walters, & Steurer, 2009). Resistance training and interval training are best suited for early reactivation of patients. Moreover, arm exercises in patients with COPD were shown to increase arm muscle force (Epstein et al., 1997) and reduce symptoms of dyspnea and fatigue during arm activities (Bauldoff, Hoffman, Sciurba, & Zullo, 1996).

Patient education is included as an important recommendation in current clinical practice guidelines for COPD *(GOLD,* 2010; Celli, MacNee, & ATS/ERS Task Force, 2004) Education should be an integral component of pulmonary rehabilitation (Ries et al., 2007). Moreover,

education should include information on collaborative self-management and prevention and treatment of exacerbation. So, patient education interventions are necessary to ensure long-term maintenance of treatment effects. Studies with successful results in chronically ill adults both used physical activity self-monitoring (pedometers or diaries) and applied behavioural strategies to increase patient´s self efficacy and self-regulatory skills (Conn, Hafdahl, Brown, & Brown, 2008). It is necessary to initiate and maintain physical activity behaviour change during and after supervised physical exercise training programs. Rose et al., (Baraniak & Sheffield, 2011; Rose et al., 2002) evaluated psychosocial interventions to treat anxiety and panic in patients with COPD; however the data indicated that there were no changes in cognitive function. Overall, the educational intervention may have facilitated aspects of program adherence.

3.1 Continuous or incremental aerobic exercise

In this section different types of physical exercise training that can be applied to improve exercise performance in patients with COPD are presented. The authors have compared programmes with constant load training and incremental load training in COPD patients. There is high level evidence that aerobic training is effective for aerobic capacity and there is moderate evidence that interval training is effective for strength, endurance, functionality and psychosocial parameters (Normandin et al., 2002).

Endurance or continuous training

Supervised continuous training is recommended for patients in all stages of the disease who are able to perform continuous training of at least moderate intensity. Training frequency should be three times weekly in the first weeks of the exercise programme (Langer et al., 2009). Patients with severe symptoms of dyspnea during exercise are frequently not capable of performing high-intensity (70 to 80% of the peak work rate) continuous type training (Casaburi et al., 1997; Maltais et al., 1997). It seems that moderate intensity continuous training (50 to 60% of the peak work rate or 5-6 out of 10 according to the modified Borg Scale) is minimally required to achieve changes in physical fitness. Improvements in health-related quality of life after training at moderate intensities were comparable with those observed after high intensity training (Puente-Maestu, Sanz, Sanz, Cubillo et al., 2000).

Lower extremity exercise training at higher exercise intensity produces greater physiologic benefits than lower intensity training in patients with COPD. Moreover, both low-intensity and high-intensity exercise training produce clinical benefits for patients with COPD (Ries, 2008).

Two categories of tasks can be found during everyday activities, endurance and strength tasks. Endurance tasks require repetitive actions over an extended period of time (walking, cycling and swimming) as shown in figure 4. While strength tasks require explosive performance over short time periods (jumping, lifting weights, sprinting)(Ries et al., 2007). The addition of a strength-training component to a program of pulmonary rehabilitation increases muscle strength and muscle mass (Ries, 2008).

Interval training

Interval training is recommended as an alternative to continuous training in patients with severe symptoms of dyspnea due to the fact that they are unable to sustain continuous

Fig. 4. Endurance tasks taken from "Manual de Rehabilitación Respiratoria para personas con EPOC".

training at the recommended intensities. Short high intensity (at least 70-80% of peak work rate) exercise bouts of 30-180 seconds are necessary during interval training. Recommended frequency of training is the same as with continuous training (Langer et al., 2009).

Only one article (Puente-Maestu, Sanz, Sanz, Cubillo et al., 2000) showed that patients responded to supervised training with incremented loads also changed their ventilatory pattern to deeper, slower breathing. Therefore, improved ventilation this type of incremental training also tended to be more efficient with an average decrease in dead space. Perhaps, the quality of life questionnaires are not sensitive tools to detect changes in the functional variables of disease progression. The changes produced by aerobic physical training in COPD do not have clinical relevance, but they are a success because it slows down disease progression.

Most patients with severe COPD are not able to sustain a continuous exercise protocol. For these patients, interval exercise represents an alternative because it offers the same benefit as high-intensity exercise. Besides, incremental exercise is better tolerated, as expressed by fewer breaks during the rehabilitation program and better adherence to exercise protocols (Puhan MA et al., 2006). Therapeutic intervention can be done in or out of water; the next section explains the therapeutic aquatic exercise intervention.

3.2 Therapeutic aquatic exercise intervention

This intervention is known for its power of prevention and treatment in different conditions, although not considered part of standard pulmonary rehabilitation. Therapeutic aquatic exercise intervention is a discipline that includes hydrotherapy, spa therapy, balneotherapy and physiotherapy, and is used for the prevention and treatment of diseases through water (Geytenbeek, 2008). Hydrotherapy is defined as a complementary therapy that uses the temperature and pressure of water as a therapeutic agent at a given temperature (Geytenbeek, 2002).

There is controversy in the scientific literature regarding the beneficial and harmful effects of water exercise for the respiratory system in people with respiratory problems. Different types of exercises can be carried out: walking, cycling, lifting weights in a swimming pool (figure 5), and so on. Previous studies show that hydrostatic pressure exerts on inspiratory muscle strength and limited chest expansion; this effect is enhanced as the temperature of the pool water decreases (Frontera, Herring, Micheli, & Silver, 2008). In addition, the diaphragm moves during diving due to compression by the abdomen, thus decreasing respiratory vital capacity (Greenleaf, 1984). Patients with chronic obstructive pulmonary disease benefit from the hydrostatic pressure exerted during immersion, which facilitates expiration and reduces the residual volume, decreasing the air trapped in this pathology (Asanuma, 1999; Dahlback, 1975; Schoenhofer, Koehler, & Polkey, 2004). Previous studies show that water exerts hydrostatic pressure on inspiratory muscle strength and limited chest expansion, this effect is enhanced with decreasing the temperature of the pool water (Agostoni, Gurtner, Torri, & Rahn, 1966). Therapeutic aquatic exercise intervention is known for its ability to prevent and treat different conditions. This intervention is a specialized field of physical training and therapy, used to achieve certain physical and functional goals using the properties of water (Geytenbeek, 2008).

The reviewed articles covered incremental therapeutic aquatic exercise with an intensity ranging from 50% to 90% of maximal oxygen consumption (VO_{2max}) with sessions of 30 to 50 minutes 2 to 5 days a week, for a total of 8 to 24 weeks at a temperature of 29 ºC to 38 ºC (Kurabayashi et al., 2000; Wadell, Sundelin, Henriksson-Larsen, Lundgren, 2004). COPD patients walked in water to the level of their shoulders, and they breathed out slowly through their mouth into water after sinking their nose 3-5 cm below the water level. The patients` eyes were not under the water. After exercise, patients dressed and rested on a chair in a comfortable room (25ºC) for 30 minutes. Two studies showed clinical changes in the questionnaire of quality of life for respiratory patients. People who performed incremental exercise in the water showed functional changes in the distance walked in the walking test, in forced vital capacity and forced expiratory volume (Kurabayashi et al., 2000; Wadell, Sundelin, Henriksson-Larsen, & Lundgren, 2004). The aquatic intervention group that performed incremental exercise had improved health-related quality of life, compared to a control group without intervention (Wadell, Sundelin, Henriksson-Larsen, & Lundgren, 2004).

Fig. 5. Cycling and lifting weights

Physical therapy for COPD requires a certain duration and frequency in order to improve clinical parameters. Wadell et al. (2005a) indicated that training once a week (high intensity/low frequency) was not sufficient to sustain the improvements in physical capacity and quality of life achieved after a period of 3 months of high frequency aquatic exercise training with three sessions of 45 minutes each a week (high intensity/high frequency). However, high intensity physical training once a week for 6 months seemed to be enough to avoid deterioration compared to baseline. According to Kurabayashi's study, 6 consecutive days of exercise a week would be preferable to 3 alternative days of exercise a week, even if the cumulative exercise period was the same (Kurabayashi et al., 1998). The studies reviewed showed much heterogeneity with respect to the duration of treatment, ranging from 6 to 24 weeks. However, the typical duration of treatment was 8 to 12 weeks. Further studies should direct more attention to the specific duration, frequency and accuracy of aerobic intensity thresholds. Other authors found that exercise in water tends to provide even greater benefits than similar exercise training on land (Wadell, Sundelin, Henriksson-Larsen, & Lundgren, 2004).

Breathing exercises during immersion in water at 38 ^{o}C could be recommended as physical therapy after diagnosis of COPD. Elevation of the sub-peritoneal diaphragmatic pressure by the hydraulic pressure could help raise the diaphragm and assist in the evacuation of air during exhalation, resulting in a decrease in dead space. In addition, hydraulic pressure was reported to increase cardiac output, resulting in an improvement in blood gas exchange in lung capillaries. Besides these effects, inhalation of gas containing thermal hydrogen sulfate lowers the viscosity of sputum (Asanuma, Fujita, Ide, & Agishi, 1971). Only three studies (Kurabayashi et al., 2000; Kurabayashi et al., 1998; Perk, Perk, & Bodén, 1996) included breathing exercises during therapeutic aquatic exercise intervention.

3.3 Respiratory muscle training

In general, patients with COPD have weak inspiratory muscles (Polkey et al., 1996). This weakness may contribute to dyspnea and exercise limitation in patients with significant COPD. When evaluating the strength of respiratory muscles we should be aware that we are focusing primarily on the ability of these muscles to generate tension during a forced inspiratory or expiratory maneuver. The result of the maneuver can be measured with the mouth (Figure 6 Equipment to maneuver), and it is measured in centimeters H_2O. This primarily reflects a set of variables such as muscle mass (ability to generate force) and length-tension relationship.

The role of inspiratory muscle training (IMT) for individuals with stable COPD is unclear (Geddes, O'Brien, Reid, Brooks, & Crowe, 2008). The first systematic review on IMT found little evidence to support the use of IMT (Shoemaker, Donker, & Lapoe, 2009). The Amercian Thoracic Society/European Respiratory Society standards (Celli, MacNee et al., 2004) nor the Canadian Thoracic Society Recommendations for the Management of COPD (O'Donnell et al., 2008) recommend the incorporation of IMT into management plan. The Global Initiative for Chronic Obstructive Lung Disease *(GOLD,* 2010) states that "respiratory muscle training is beneficial, especially when combined with general exercise training" based on non-randomized trials and observational studies.

Fig. 6. Equipment to maneuver

In an attempt to reduce the severity of breathlessness and to improve exercise tolerance, IMT has been applied in many COPD patients (Weiner, Magadle, Beckerman, Weiner, & Berar-Yanay, 2003). Several different respiratory muscle training devices are available, ranging from sophisticated computerized systems to simple hand-held resistive devices. In addition, the relative benefits of strength versus endurance training, inspiratory versus expiratory training and effect in patients of differing severity are unknown (Garrod & Lasserson, 2007)

Types of intervention: Sham, low- and high-intensity IMT

There are studies comparing the effect of different types of intervention (Geddes, Reid, Crowe, O'Brien, & Brooks, 2005). In order to standardize studies that showed sham IMT and low intensity IMT at similar percentages of maximum inspiratory pression (PImax). Bégin et al., (Begin & Grassino, 1991) measured these loads using the tidal inspiratory pressure (PI) of individuals with COPD. Sham IMT was defined as that using the same type of device as the intervention group at an intensity less than or equal to the mean plus one standard deviation (SD). Since PI is directly proportional to the partial pressure of carbon dioxide in the arterial blood (PCO_2) of patients with COPD (Begin & Grassino, 1991), sham IMT for normocapneic individuals was defined as intensity p8.3 cm H_2O (mean PI +1 SD) and for individuals with moderate hypercapnia, as intensity p11.5 cm H_2O (Geddes et al., 2005).

Using IMT in combination with other interventions and using flow-dependent resistive training is important in the pulmonary rehabilitation program (Geddes et al., 2008). However, there are no established thresholds for what constitutes a clinically meaningful change in inspiratory muscle strength or endurance, other methods must be utilized to infer clinical benefit (Shoemaker et al., 2009). Geddes et al. (2005) recommended using IMT at least a total of 30 minutes daily but can be spread over more than one session a day. Training should occur at least 5 days a week. While gains may be measurable after as short

as 5 weeks, IMT should become part of the individual´s exercise program. The minimal training intensity necessary could start as low as 22% PImax and be progressed to as high as 60% PImax using a targeted inspiratory resistive or threshold trainer (Geddes et al., 2005). Therefore, IMT significantly increased inspiratory muscle strength and inspiratory muscle endurance (Lotters, van Tol, Kwakkel, & Gosselink, 2002). In addition, research review found a clinically significant decrease in dyspnea sensation at rest and during exercise is observed after IMT (Lotters et al., 2002).

In conclusion, IMT improves inspiratory muscle strength and endurance, functional exercise capacity, dyspnea and quality of life. Inspiratory muscle endurance training was shown to be less effective than respiratory muscle strength training. In patients with inspiratory muscle weakness, the addition of IMT to a general exercise training program improved PImax and tended to improve exercise performance (Gosselink et al., 2011).

Furthermore, maximal inspiratory pressure is a volitional test and therefore open to criticism (Polkey & Moxham, 2004). Futher research is needed to explore the impact that different training protocols (frequency, intensity and duration of IMT, supervision) may have on outcomes and to determine the extent to which changes in outcomes associated with IMT translate into clinically important improvement for adults with COPD (Geddes et al., 2008).

4. Implications

In the research reviewed, there are strong arguments that pulmonary rehabilitation is beneficial for improving the quality of life related to health at the beginning of the program. Furthermore pulmonary rehabilitation reduces symptoms and increases participation in everyday activities. However, it is necessary to do more randomized controlled trials to clarify which components of the lung rehabilitation are essential. Future studies to discover the ideal length of treatment, the necessary degree of supervision, training intensity and how long the treatment effect persists.

Without no doubt, it is necessary to individualize programs for this population taking into account their different levels of severity. The prescription should begin at low intensity and short duration, for both parameters gradually increasing to the threshold of fatigue.

In summary, incremental aerobic resistance physical exercises are better than constant load physical exercises at an intensity range from 90% to 50 % of $VO_{2\,max}$, with a frequency of two or four days a week, the session is from 30 to 60 minutes during a period of treatment from eight to twelve weeks. Exercise training induces several symptomatic and functional adaptations resulting in an increased aerobic capacity, although clinical relevance is not collected in the study population. Maybe, for further studies we should take intrinsic patient factor (severity of COPD) into account over a longer period of time and how extrinsic factors of the exercises affect disease progression. Moreover, it is important to determine whether these physiological benefits of COPD patients who have performed an incremental aerobic resistance physical exercises program supervised justify the increased costs. Therefore, a cost/effectiveness analysis is necessary to determining whether the type of intervention program is supervised or not.

It is essential to investigate physical activity in daily life in patients with COPD in accordance to the GOLD stages. Pulmonary rehabilitation programs should incorporate the

use of an accelerometer, the values of respiratory muscle strength and peripheral muscle strength (hand grip, knee- extension); also more sensitive tools for detecting changes in exercise tolerance should be included.

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Hospital at Home for Elderly Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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1. Introduction

Demographic, epidemiological, social, and cultural trends in European countries are changing the traditional patterns of care. The next decades will see increasing rates of care-dependent older people and non communicable diseases as the leading cause of chronic illness and disability. The break-up of the traditional large family group and urbanization will also lead to gaps in the care of older or disabled family members. These changes in needs and social structure require a different approach to health and social sector policy and services since a disease-oriented approach, alone, is no longer appropriate. An answer to these issues could be home care, a sustainable approach to prevent the need for unnecessary acute or long-term institutionalization and maintain individuals in their home and community as long as possible providing diagnostic, therapeutic and social support (Tarricone & Tsouros, 2008).

Home is a place of emotional and physical associations, memories and comfort. Although many people can be happy in assisted-living facilities, retirement communities or nursing homes – and for many people these are better options – leaving home can be disruptive and depressing for some people. Recent trends in health care favour alternatives to traditional hospital care for patients with acute or chronic diseases. Home care used appropriately decreases hospitalization and nursing home use without compromising medical outcomes. Moreover, patients generally prefer to remain in familiar surroundings. Physician support of home care services honors that preference (Levine et al., 2003).

Chronic Obstructive Pulmonary Disease (COPD) has been the focus of several hospital at home studies, however, most models studied have been early-discharge schemes that employed nursing care, without physician care in the home. There have been fewer studies of substitutive physician-led clinical unit model of hospital at home.

2. Ageing population: Demographics trends

Population ageing is progressing rapidly in many industrialized countries. For the world as a whole, the elderly will grow from 6.9% of the population in 2000 to a projected 19.3% in 2050 (Gavrilov & Heuveline, 2003).

Population ageing is a great challenge for the health care systems. As nations age, the prevalence of disability, frailty, and chronic diseases (Alzheimer's disease, cancer, cardiovascular and cerebrovascular diseases, COPD, etc.) is expected to increase dramatically.

Frailty is gaining attention in many fields because it increases the risk of hospitalization, falls, mortality and institutionalization. Geriatricians, gerontologists, and social scientists study frailty to better understand its impacts on health, individuals, and society. Frailty has been considered synonymous of disability or co-morbidity, but it is recognized that it is a biological syndrome identified by decreased reserves in multiple organ systems. The incidence of frailty increases with age, reaching more than 32% in those older than 90 years (Fried et al., 2001). Frailty can be a primary diagnosis, when the state is not associated directly with a specific disease, or a secondary diagnosis when the syndrome occur as a result of an acute event or the end stage of many chronic conditions, including severe congestive heart failure, stroke, chronic inflammatory diseases and dementia. The hospital, which is the "gold standard" for the delivery of acute medical care, is not an ideal environment for frail elderly patients. A new functional impairment and iatrogenic events such as nosocomial infections, pressure sores, falls and delirium are common during hospital stay.

Chronic obstructive pulmonary disease is a major cause of chronic morbidity and mortality. Patients with COPD usually have progressive airflow obstruction that is not fully reversible, which leads to a history of progressive, worsening breathlessness that can impact on daily activities and health-related quality of life. Winter outbreaks of COPD exacerbations, mostly occurring in elderly people with concurrent chronic co-morbidities, often generate dramatic increases in hospital emergency room admission. Such admissions have increased substantially over the past decade, comprising a significant proportion of all hospital admissions, and are associated with a high rate of readmission contributing to the high costs of care for COPD.

3. Chronic obstructive pulmonary disease: Epidemiological data

Chronic obstructive pulmonary disease is a leading cause of mortality and morbidity worldwide, affecting approximately 210 million people and leading to 3 million deaths annually (WHO, 2011).

The prevalence and morbidity data greatly understimate the total burden of COPD because the disease is usually not diagnosed until it is clinically apparent and moderately advanced. Furthermore, population-based estimates of COPD prevalence by region are problematic since the disease is progressive, measurement tools and definitions still vary among studies, and implementation of spirometry is often not feasible in developing regions (Lopez et al., 2006a).

A recent systematic review and meta-analysis on global burden of COPD reported a prevalence of physiologically defined COPD of 9-10% in adults (Halbert et al., 2006). These

data agree with results from the BOLD study, a population-based prevalence study including participants from 12 sites worldwide (n=9425), reporting a prevalence of COPD stage II or higher of 10.1% overall, 11.8% for men and 8.5% for women (Buist et al., 2007).

In England the rate of COPD in the population is estimated at between 2% and 4%, representing between 982.000 and 1.96 million people. The diagnosed prevalence of COPD was 1.5% of the population in 2007/08 according to the Quality Outcome Framework (QOF) statistical bulletin. Approximately 835.000 people in England have been diagnosed with COPD in 2008/09. However, it is currently estimated that over 3 million people have the disease and that an estimated 2 million have undiagnosed COPD, among whom it is considered that 5.5% will have COPD at the mild end of the spectrum (NICE guidelines, update 2010).

Recent available data suggest that a pooled prevalence on spirometric basis is about 9% in European adults, with 4-6% of them suffering from a relevant clinical form of the disease. In Italy, prevalence of COPD is 4.5%, on average.

The reported total prevalence of chronic bronchitis in U.S. adults ranged from a high of 55 (2001) cases per 1.000 to a low of 34 (2007). The prevalence of chronic bronchitis appears to have peaked in 2001, followed by a subsequent decline from 2001 to 2007. In 2008, however, there was an increase in the prevalence (44 case per 1.000) compared to the previous year, and this prevalence was the same in 2009 (data from the U.S. National Health Interview Survey-NHIS, 1999-2009).

The epidemiology of COPD in five major Latin American cities (São Paulo, Santiago, Mexico city, Montevideo and Caracas) has been provided by the PLATINO project , launched in 2002: rates of COPD range from 7.8% in Mexico city to 19.7% in Montevideo, suggesting that COPD is a greater health problem in Latin America than previously realized (Menezes et al., 2005). COPD is emerging as public health problem also in the Middle Est and North Africa countries. In 2001, the prevalence of COPD in Africa was estimated 179/100.000 and 301/100.000 in eastern Mediterranean countries (Lopez et al., 2006b).

Currently, in the European Union COPD and asthma, together with pneumonia, are the third most common cause of death, while in North America COPD represents the fourth leading cause of death. Five year survival from diagnosis is 78% in men and 72% in women with clinically mild disease, but falls to 30% in men and 24% in women with severe disease. (NICE guidelines, update 2010). Due to an aging population, increase in COPD prevalence and mortality are expected in the coming decades. The World Health Organization (WHO) has estimated that COPD will be the third leading cause of death for both males and females worldwide by the year 2030, surpassed only by heart disease and stroke (WHO, 2011).

Burden of COPD can also be measured in disability-adjusted life years (DALYs). Worldwide, COPD is expected to move up from the 12th leading cause of DALYs in 1990 to the 5th leading cause in 2020 (Lopez et al., 2006 b).

In the United States COPD accounts for 15.4 million physician visits, 1.5 million emergency department visits and 636.000 hospitalizations each year (Dalal et al., 2011). In Italy, COPD is the fourth highest cause of hospital admission (130.000 admissions every year). In the UK COPD is the second largest cause of emergency admission and the most common cause for emergency admission to hospital due to respiratory disease. One fifth (21%) of bed days

used for respiratory disease treatment are due to COPD, such that COPD accounts for more than one million "bed days" each year in hospitals in the UK (NICE guidelines, update 2010).

The impact of hospitalization for acute exacerbations is significant; mortality during admission is > 10% and mortality during the year after discharge following treatment for acute COPD exacerbation is 25-40% (Escarrabill, 2009).

An acute exacerbation of COPD is not an exceptional or unique event. The Risk Factors of COPD Exacerbation Study (EFRAM) found that 63% of patients were readmitted during the year following an exacerbation (Garcia-Aymerich et al., 2003). Patients with COPD experience exacerbations one to three times a year, with treatment often requiring emergency room care or hospitalization, which contributes substantially to the financial burden of the disease (Dalal et al., 2011).

Various observational studies have found that inpatient care accounts for 50-75% of the direct medical costs of COPD. This cost increases with disease severity: inpatient costs of patients with stage III (severe) disease are double those of patients with stage II (moderate) disease and 6.5 times greater than those of patients with stage I (mild) disease (Dalal et al., 2011).

The indirect cost of COPD are substantial with an impact on annual productivity amounting to an estimated 24 million lost working days per annum. There is little data available to quantify other indirect costs such as carer time and inability to carry out non-occupationally related activity (NICE guidelines, update 2010).

There continues to be high demand for acute care hospital beds for patients with an exacerbation of COPD. Recent reports highlight the fact that although the acute hospital is the standard venue for providing acute medical care, it may be hazardous for older persons, who commonly experience iatrogenic illness, functional decline, and other adverse events. One way to decrease or avoid admissions to hospital is to provide people with acute care treatment at home.

4. Current knowledge on home care for COPD exacerbations

COPD is often associated with exacerbations of symptoms. Exacerbations, particularly that result in admission to hospital, are significant events in the natural history of the disease. They are disruptive and distressing for patients, and account for a significant proportion of the total costs of caring for patients with COPD.

There is no generally agreed definition for an exacerbation of COPD. Definitions currently rely on clinical empiricism with little evidence-based scientific support (Caramori et al., 2009). Most common international guidelines and working groups provide very similar definition of a COPD exacerbation: "*an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD*" (ATS/ERS guidelines, Celli et al., 2004; GOLD, 2009; BTS guidelines, 2007; CTS guidelines, O'Donnel et al., 2008; SEPAR/ALAT joint guidelines, Peces-Barba et al., 2008; Rodriguez-Roisin, 2000).

When an exacerbation of COPD has been diagnosed, to define its severity is essential. Quantification of severity is important in medical management as well as in determing the setting of care (Celli et al., 2004). At present, there is not a validated method for quantifying the severity of exacerbation. Generally, the intensity of the underlying COPD must be considered, as well as comorbidity and a history of previous exacerbations. In addition to these factors, the progression of the symptoms, response to therapy, and availability of adequate home care must be considered in order to decide whether hospitalization is necessary. However, grading of the severity of mild to moderate exacerbations remains contentious since they can be categorised either on clinical presentation (essentially symptoms) or healthcare use resources (Rodriguez-Roisin, 2006).

The most recent position paper of the American Thoracic Society and the European Respiratory Society (ATS/ERS task force) provide a three levels operational classification of severity of COPD exacerbations which allows to identify the best setting of care according to specific elements of clinical evaluation and diagnostic procedures. Level I: patient can be treated at home, Level II: requires hospitalization, Level III: leads to respiratory failure (Celli et al., 2004).

In the National Institute for Clinical Excellence (NICE) guidelines (update 2010), hospital-at-home and assisted-discharge schemes are recommended as a safe and effective alternative to conventional hospitalization (Grade A), particularly for patients with less severe exacerbations. The same authors admit that, currently, there are insufficient data to make firm recommendations about which patients with an exacerbation are most suitable for hospital-at-home or early discharge, and patient selection should depend on the resources available, absence of factors associated with worse prognosis and patient's preference (NICE guidelines, update 2010).

The joint guidelines of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) and the Latin American Thoracic Society (ALAT) indicate home hospitalization only for patient without signs of severity such as diminished level of consciouness, abnormal chest radiograph, hypercapnia with acidosis, significant comorbidities, need of ventilatory support (Peces-Barba et al., 2008).

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (update 2009) state that "admission of patient with severe COPD exacerbations to intermediate or special respiratory care units may be appropriate if personnel, skills, and equipment exist to identify and manage acute respiratory failure successfully" (GOLD, 2009).

A first feasibility analysis of home-based services to prevent conventional hospitalization of COPD exacerbations was reported in 1999 by Gravil and collegues (Gravil et al., 1998). Subsequent controlled trials confirmed both safety and cost reduction when these types of services were applied to selected COPD patients (Cotton et al., 2000; Davies et al., 2000; Hernandez et al., 2003; Ojoo et al., 2002; Skwarska et al., 2000).

In a review and a meta-analysis including 7 robust RCTs (n=754 patients) Ram and collegues evaluated the overall efficacy of hospital at home schemes, showing that selected patients presenting to hospital emergency departments with acute exacerbation of COPD can be successfully treated at home when supported by visiting respiratory nurses at home. Authors suggested that approximately 25% of the patients with COPD who presented at the

emergency department with acute exacerbations would be suitable for home treatment (Ram et al., 2003, 2004).

In conclusion, there is an international consensus on home care for COPD exacerbations, especially for less severe episodes, although data on specific characteristics of patients suitable for this form of care are currently insufficient. In addition, the confusion on definition of "home hospitalization" and "hospital at home" can make difficult to clear up this problem.

Intermediate care is a treatment model which bridges the interface between hospital and community care. A specific subtype of intermediate care is Hospital-at-Home. There is a consensus on defining "Hospital-at-Home" a model of care where "active treatment is provided by healthcare professionals in the patient's home for a condition that otherwise would require hospital care, always for a limited period" (Cochrane Database of Systematic Review, Shepperd et al., 2001). Many disparate models exist with the general nomenclature of "Hospital at Home", "Home hospitalization". These include usual community-based care, outpatient infusion centre, nurse-only outpatient care and the direct clinical unit model of care. These models have distinct features.

Established models for delivering hospital-level care in the home setting exist internationally, including United States, Canada, Israel, Australia, New Zeland, Spain, United Kingdom, Italy, France (Leff et al., 2005; Lemelin et al., 2007; Stessman et al., 1996; Caplan et al., 1999; Montalto, 2002; Richards et al., 2005; Cerrillo –Rodriguez et al., 2009; Pérez-Lopez et al., 2008; Kalra et al., 2008; Wilson et al., 1999; Myles et al., 1996; Aimonino Ricauda et al., 2008).

For patients with exacerbations of COPD, over the last few years there has been considerable interest especially in hospital-based rapid assessment units and early discharge or admission avoidance hospital at home schemes.

Rapid assessment units aim to identify those patients that can be safely be managed at home. These units generally involve a full assessment of the patient in the hospital by a multidisciplinary team and discharge to the community with appropriate support (e.g. nebuliser and compressor or oxygen concentrator, nursing and medical supervision from respiratory specialists, increased social support). Patients remains under the care of the hospital but General Pratictioners are made aware of the fact that their patients are receiving home care.

Early or assisted or supported discharge schemes aim to identify patients in hospital who could be discharge before they have fully recovered by providing increased support in their homes. These schemes involve getting people out of hospital as quickly as possible. In a recent review Shepperd and collegues have demonstrated that mortality and disability for patients recovering from stroke, COPD or surgical interventions are similar in hospital and in early/supported discharge services. Patients may also be more satisfied with their care at home, and at the same time their cares, in most cases, do not report additional burden. However, authors concluded that there is little evidence of cost savings to the health care system (Shepperd et al., 2009a).

The **admission avoidance schemes** provide active treatment by hospital health care professionals (doctors, nurses and other professional figures) in the patient's home, always

for a limited time period. The key is that if the hospital at home service was not available, then the patient would need to be admitted to an acute hospital ward. In a systematic review of avoidance of admission through the provision of hospital care at home, 10 randomized trials involving elderly patients with medical condition were included (with a total of 1327 patients). For 5 of these trials individual patient data were obtained for meta-analysis, representing 87% of potentially eligible patients. Authors reported a significantly lower mortality at 6 months for patients who received hospital care at home, greater satisfaction and lower costs (if costs of informal care are excluded). (Shepperd et al., 2008; Shepperd et al., 2009b).

5. The Hospital at Home Service of Torino

In October 1984, with Resolution N. 1134/41/84, the Management Committee of the Local Health Unit 1/23 of Turin set up the 'Experimental Project of Home Hospitalisation'.

In October 1985 a team of doctors and nurses of the Turin Department of Geriatrics started an experiment that was unique in Italy at that time: medical treatment (including examinations and related medical and nursing services) at home rather than in hospital for patients with severe chronic or relapsing illnesses.

The Hospital at Home Service (HHS) is operating in Torino at S. Giovanni Battista Hospital, a large urban University teaching and tertiary-care hospital (Aimonino Ricauda et al., 2004, 2005, 2008; Tibaldi et al., 2004, 2009).

The HHS is a service that provides diagnostic and therapeutic treatments by health care professionals, in the patient's home, of a condition that otherwise would require acute hospital in-patient care. A quick admission to hospital is possible for examinations or interventions that cannot be carried out at home. Transport and acceptance are free for these patients, as part of the HHS service.

The HHS normally operates 12 hours a day (from 8 am to 8 pm), seven day a week. At night our Regional Emergency Unit ("118") can be contacted. For selected patients, medical staff is on-call 24 hours a day. Caregivers are instructed in the emergency plan and encouraged to telephone if problems arise.

The HHS team, equipped with 7 cars, is multidisciplinary and consists of 4 geriatricians, 13 nurses, 1 nurse coordinator, 2 physiotherapists, 1 social worker, 1 counsellor.

The main feature of HHS is that physicians and nurses work together as a team (Figure 1), with daily meeting to discuss the needs of each patient and to organize individualized medical care plans and day-to-day work. The three most important aspects of the nursing activity are:

- home visits to outpatients to give medical care as agreed with the doctors
- daily team meeting
- secretarial work, receiving applications for hospitalization, stocking pharmaceuticals and sanitary material, sending and collecting laboratory analysis, transporting patients for specialistic consultations or exams which can be done only in hospital

The team looks after 25 patients per day and 500 patients per year, on average. The most common diseases treated at home are cardiac, respiratory, cerebrovascular, metabolic and neoplastic diseases.

Fig. 1. Hospital at Home Service: doctor and nurse at patient's home

The HHS can be activated by a direct request of the general physician of the patient as an alternative to traditional hospital care, or by a request from hospital wards doctors to allow early and protected discharge from hospital.

Since 2001, a close collaboration has been started between the HHS and the Emergency Department (ED) of San Giovanni Battista Hospital, to propose, where possible, home care as an alternative to the traditional admission to hospital.

Now, approximately 60% of our patients are referred by the ED, 25% by hospital wards and 15% by specialist or general physicians in the community.

The relationship between the ED team and the "HHS mobile team" made up of 1 geriatrician and 1 nurse is very important. By using a multidimensional case sheet, the "HHS mobile team" carries out an assessment of the patient and his caregiver to evaluate the possibility of hospitalization at home and in order to give information on the service.

A "Module of interview with the family" was conceived and implemented to discover the willingness of the family to work together with HHS team, as a part of the patient's healthcare system.

When the availability is established, an "Informative Card" with information on the service has been given to the patient and his caregiver.

Then, the "HHS mobile team" together with the ED doctor writes a rough copy of patient's case sheet, which will be completed at home during the first HHS visit. In the ED all the

necessary diagnostic tests (e.g., blood tests, radiography, ECG) are provided and then the patient moves home by ambulance, usually within a few hours.

Entry criteria for home hospitalization are: informed consent of patient and caregiver; stable, diagnosed medical conditions needing hospitalization but not expected to require emergency intervention; appropriate care supervision; telephone connection; living in the hospital catchment area (all the southern part of the city).

Exclusion criteria are: need of intensive monitoring or mechanical ventilation, a monitoring more frequent than every 2 hours of blood pressure or haemogasanalysis, patients with an heart attack or with very low levels of oxygen in the blood or with a serious acidosis or alkalosis or with a suspect of pulmonary embolism.

Many services or treatments can be provided at home, as shown in Table 1.

Assessment in Emergency Department and transport home via ambulance
Services and treatment provided:
Physician and nursing visits
Standard blood tests
Pulse oximetry
Electrocardiogram
Spirometry
Echocardiogram
Internistic ecographies and Doppler ultrasonographies
Oral and intravenous medication administration, including antimicrobials and cytotoxic drugs
Oxygen therapy
Blood product transfusion
Central venous access (PICC, Midline)
Surgical treatment of pressure sores
X rays
Telemonitoring
Physical therapy
Occupational therapy
Counselling
Hospital-at-home patients are considered hospital patients, and all services are provided by the hospital, which retains legal and financial responsibility for care.

Table 1. Features of the Hospital at Home Service

A case history is made up for each patient and is always available at the patient's home, with an updated report available in the HHS office.

Medical consultation with other hospital specialists is possible in the hospital or at the home of the patient.

HHS has continued to increase its activity since its inception in 1985. Until now about 11000 admissions have been recorded. In 2010, 550 admissions were recorded, 9113 nursing visits

and 4317 medical visits were conducted. The mean age of our patients was 80 years (range 30-101). Mean length of stay was 14 days.

In 2010 the Piedmont Region issued a decree to regulate this HHS model and acknowledged a refund of 165 Euros/day for DRG included in MDC number 1, 4, 5, 16, 17 (neurological, respiratory, cardiovascular, haematologic and neoplastic diseases), and 145 Euros for the other diseases.

6. The Hospital at Home approach to elderly patients with COPD exacerbation: Principles for patient selection and management

About 20% of patients admitted to the Emergency Department and referred to the HHS of Torino are affected by an exacerbation of COPD.

From an operational point of view, an acute exacerbation of COPD is defined on the basis of Anthonisen criteria as an increase in breathlessness, sputum volume, or purulence for at least 24 hours requiring acute hospitalization (Anthonisen et al., 1987).

Patients that can't be safely managed at home by HHS are those without a family or social support, with severe hypoxemia (PO_2 < 50 mmHg), severe acidosis or alkalosis (pH < 7.35 or > 7.55), suspected pulmonary embolism, suspected myocardial infarction.

In the ED all COPD patients undergo baseline standard clinical evaluation; blood tests (blood cell count, routine biochemical tests and arterial blood gas tensions); pulse oximetry; 12-lead electrocardiography; chest radiographs and hand-held spirometry. Further investigations (including pneumologist's assessment) are performed when required, according to the clinical judgement of the ED physician. Patients eligible for HHS are immediately transferred home by ambulance.

HHS patients receive hospital-level treatments and services at home as dictated by their condition. Treatment of COPD exacerbations is based on the optimized use of bronchodilators as well as the administration of systemic corticosteroids and antibiotics, when requested, administered intravenously in about 90% of patients, and oxygen therapy by nasal cannula or Venturi mask. Non-invasive mechanical ventilation is administered at home in collaboration with pneumologists. Acute administration of nutritional support is possible at home, if requested.

The home care program emphasize patient and caregiver education on the knowledge of the disease giving advices about smoking cessation, nutrition, management of activities of daily living and energy conservation, understanding and use of drugs, health maintenance and early recognition of triggers of exacerbation that required medical intervention. Protocols for prevention of nosocomial infections, bed sores, immobilization, dysphagia are routinely adopted for frail patients. Moreover, a counselling service is offered to the most frail patients and caregivers . Aim of the counselling process is to offer to users the opportunity of exploring, discovering and clarify thought and action patterns, thus enabling them to make a better use of their resources in that specific situation of need. Within a situation of crisis and complexity, the counsellor aims at obtaining a safe, confident and cooperative environment capable of transmitting information, implementing support, modifying

attitudes, promoting health education to the patient and the family and finally enabling them to better cope with the situation. The counsellor do not provide standardized information to increase the caregiver's skill in caregiving; rather, counselors focused on helping caregivers understand and resolve their reactions to caregiving process.

In the first days after admission in HHS each patient is visited at home on a daily basis by physicians and nurses. In the following days the patients is seen every day by a nurse and at intervals of 2-3 days or less by the doctor, as required by the patient's clinical condition. Hospital at home staff is available at all times for urgent home visits, which occur within 20-30 minutes by the telephone call. Home visit include: physical examination, measurement of vital signs (pulse, blood pressure, respiratory rate, temperature, oxygen saturation), administration and revision of therapy, if necessary. Essential skills for members of the HHS team are the ability to take a comprehensive clinical history and assess clinical condition, familiarity with pharmacological and non-pharmacological approaches, good communication skills, understanding of airway clearance techniques.

Upon admission, for each patients are recorded: blood pressure, spirometric parameters (FEV_1, FVC, FEV_1/FVC%), hematocrit, blood glucose, serum creatinine concentration, serum hepatic enzymes, serum nutritional parameters (e.g, total proteins, albumin, transferrin, lymphocytes) and electrolytes, arterial blood gas levels (pH, partial pressure of oxygen, partial pressure of carbon dioxide, bicarbonate, pulse oximetry), sputum culture if possible. During the HHS admission clinical assessment and routine observations are useful in assessing the rate of recovery from an exacerbation. Blood tests, including arterial blood gases measurement and spirometry are repeated according to the clinical condition of the patient. A chest X ray at home is possible, if necessary.

At home, a multidimensional geriatric assessment is conducted using validated instruments. The multidimensional geriatric assessment include the evaluation of comorbidity using the Cumulative Illness Rating Scale (Conwell et al., 1993), severity of illness using the Acute Physiology And Chronic Health Evaluation (Knaus et al., 1985), depression status using the Geriatric Depression Scale (Yesavage et al., 1982), functional status using Katz Activities of Daily Living and Lawton Instrumental Activities of Daily Living (Katz et al., 1963; Lawton & Brody, 1969), cognitive status using the Mini-Mental State Examination (Folstein et al., 1975), quality of life using the Nottingham Health Profile (Hunt et al., 1985), nutritional status using the Mini Nutritional Assessment (Guigoz et al., 1997), characteristics of caregiver with special attention to the level of stress using the Relatives' Stress Scale (Greene et al., 1982), and satisfaction using an "ad hoc" questionnaire for customer satisfaction (Figure 2).

The HHS patients undergo acute rehabilitative care at home, including pulmonary rehabilitation when needed, and their caregivers are encouraged to actively participate in the rehabilitation process. Education and psychological support are important for the overall success of rehabilitation. Education improves knowledge, coping and self-management, actively engaging patients to maintain strategies that reduce dyspnoea, maintain good lifestyle habits and participate in decision-making when acute exacerbation occur.

When patients recover from an acute exacerbation of COPD the dimission is planned, making arrangements with General Pratictioner. District Health Services are activated if required.

Recently, two papers on hospital- at-home treatment of elderly patients with an acute exacerbation of COPD have been published by HHS of San Giovanni Battista Hospital of Torino (Aimonino Ricauda et al., 2007, 2008). Between April 2004 and April 2005 a prospective randomized controlled single-blind trial was conducted to evaluate hospital readmission rates and mortality at 6 month follow up in selected elderly patients with acute exacerbation of COPD. One hundred and four elderly patients admitted to hospital for acute exacerbation of COPD were randomly assigned to General Medical Ward (GMW, n=52) or to Hospital at Home Service (HHS, n=52). Baseline sociodemographic information, clinical data, functional, cognitive, nutritional status, depression and quality of life were obtained (Table 2). All patients were elderly, multimorbid, and functionally and cognitively impaired.

Characteristic	Geriatric Home Hospitalization Service (n=52)	General Medical Ward (n=52)	P-Value
Age, mean ± SD	80.1± 3.2	79.2± 3.1	.20
Male, n (%)	29 (56)	39 (75)	.06
Married, n (%)	27 (52)	29 (56)	.84
Family support at home, n (%)	52 (100)	52 (100)	.89
Smoking history, n (%)			
Current smoker, n (%)	7 (13)	6 (11)	.97
Ex-smoker, n (%)	34 (65)	35 (67)	.95
Nonsmoker, n (%)	11 (21)	11 (21)	.81
Number of cigarettes/d ± SD	20 ± 11	21 ± 15	.83
FEV1, mean ± SD	0.92 ± 0.4	1.04 ± 0.5	.18
Percentage of predicted FEV1	38	47	
Respiratory rate, mean ± SD	24 ± 5	25 ± 7	.32
Home oxygen use before admission, n (%)	18 (35)	12 (23)	.45
Arterial blood gas, mean ± SD			
pH	7.40 ± 0.04	7.41 ± 0.03	.19
Partial pressure of oxygen	69 ± 19	65 ± 14	.23
Partial pressure of carbon dioxide	44 ± 12	46 ± 12	.47
Activities of Daily Living score, mean ± SD*	2.3 ± 2.2	1.9 ± 2.2	.36
Instrumental Activities of Daily Living score, mean ± SD†	7.1 ± 4.9	8.1 ± 4.2	.27
Geriatric Depression Scale score, mean ± SD‡	16.1 ± 6.1	17.2 ± 6.8	.45
Mini Nutritional Assessment, mean ± SD§	17.1 ± 6.5	18.3 ± 6.2	.37
Mini-Mental State Examination score, mean ± SD‖	21.8 ± 6.9	21.8 ± 6.3	.89
Cumulative Illness Rating Scale score, mean ± SD			
Comorbidity index#	2.6 ± 1.5	3.0 ± 1.8	.24
Severity index**	2.5 ± 0.5	2.6 ± 0.5	.19
Acute Physiology and Chronic Health Examination II score, mean ± SD††	9.5 ± 4.0	10.3 ± 4.0	.29
Nottingham Health Profile score, mean ± SD‡‡	20.6± 9.6	19.3± 8.2	.46

Normal range * 0-6, † 0-14, ‡ 0-30, § 0-30, ‖0-30, # 0-14, **1-5, †† 0-100, ‡‡ 0-38.
SD = standard deviation; FEV1 = forced expiratory volume in 1 second.

Table 2. Baseline Characteristics of the Study Populations

QUESTIONNAIRE ON CUSTOMER'S SATISFACTION

Please, answer to the following questions.
Your answers will enable us to improve the quality of our care.
The questionnaire is anonimous and will be processed in a sealed envelope.
You may be helped by a family member or a friend.
Thank you for your comments on the back side of this sheet.

What I think about:

	Excellent	**Very good**	**Poor**	**Unsatisfactory**
1. Medical care				
2. Nursing care				
3. Medical explanations on diagnosis				
4. Medical explanations on disease course and treatment				
5. Nursing advice				
6. Medical and nurses attitudes				
7. Feeling of safety and protection about home hospital/inpatient treatment				
8. Satisfaction about your home hospital/inpatient treatment				

Detailed comments

Positive aspects

.....
.....

Issues to be improved

.....
.....

Date,...../...../.....

Fig. 2. Questionnaire on customer's satisfaction

Patients in both groups received COPD-related treatment at similar rates. The incidence of selected medical complications did not differ between the two setting of care, with the exception of urinary tract infections, which were observed in about 6% of GMW patients and only in 1% of HHS patients (p=.049). There was a lower incidence of hospital readmission for HHS patients compared with GMW patients at 6-month follow-up (42% versus 87%, p<0.001). Cumulative mortality at six months was 20.2% in the total sample, without significant differences between the two study groups. Patients managed in HHS had a longer mean length of stay than those cared for in GMW (15.5 $\pm$ 9.5 v 11.0 $\pm$ 7.9 days, p = 0.010). It is important to highlight that all patients discharged from HHS had completed the care program at home, whereas 11.5% of GMW patients continued their care in long-term facility after hospital discharge, with an average daily cost of $ 174.7 for a mean period

of 25 + 8.7 days. Only HHS patients experienced improvements in depression and quality of life scores. Satisfaction at discharge was very good or excellent for 94% of HHS patients and 88% of acute hospital patients (p=0.83). On a cost per patient per day basis, HHS costs were lower than costs in GMW ($ 101.4 ± 61.3 versus $ 151.7 ± 96.4, p=0.002). Analysis of costs for hospital-at-home patients revealed that 79% of costs were due to drugs, durable medical equipment, diagnostic procedures, medications, and other nonstaff costs.

7. New key aspects of COPD management at home: Telemonitoring and teleradiology

The challenges that are posed to the health care sector in terms of using innovative tools and methods are relevant. Issues like the growing of ageing population and of citizens in chronic conditions are the focus of the last medical progress, which offer new and better treatments.

Telecare and telemedicine are promising if considered as solutions for different particular conditions, such as rural regions and all the situations where the healthcare services could cope with a shortage of specialists or equipments.

Telemedicine, moreover, connecting hospital and homes could - in some cases - contribute to avoiding the traditional hospital admission, resulting less stressful for patients, and money saving as well. Technology can also improve the quality of life by supporting informal carers, making it more likely that people receiving care and their informal carers can continue to stay active at home and in the community instead of being institutionalized. With developments in medical and other technologies, people with very complex conditions may increasingly be treated at home rather than in hospital or institutional care. In San Diego, California, physicians arrive at patients' homes with a new version of the black bag that includes a mobile x-ray machine and a device that can perform more than 20 laboratory tests at the point of care. Landers recent opinion is that "the venue of care for the future is the patient's home, where clinicians can combine old-fashioned sensibilities and caring with the application of new technologies to respond to major demographic, epidemiologic, and health care trends. Five major forces are driving health care into the home: the aging population, epidemics of chronic diseases, technological advances, health care consumerism, and rapidly escalating health care costs" (Landers, 2010).

Telemonitoring devices have been tested on an elderly HHS population in Torino. In November 2008 Telecom Italia (TI), "San Giovanni Battista" Hospital and "Mario Boella" Institute (ISMB) of Torino started a project called *MyDoctor@Home*, for telemonitoring patients affected by an acute exacerbation of COPD or acute heart failure, managed at home by the HHS of "San Giovanni Battista" Hospital.

MyDoctor@Home (Figure 3) is an e-health service that enables the patient to measure at home, with portable and Bluetooth connected medical devices, his own physiological parameters and to transmit them in real time, through a mobile phone, to a platform operating in a TI data center, accessed by the sanitary structure. The patients use the mobile phone in order to transmit the measures, and they may also receive messages reminding them to take measurements and/or to follow their medication schedule.

Through the web platform "MyDoctor@Home", physicians or nurses can monitor in real time or from remote the received measures and can interact with the patient in different modalities (telephone, video-calling, visit at home) (Figure 4).

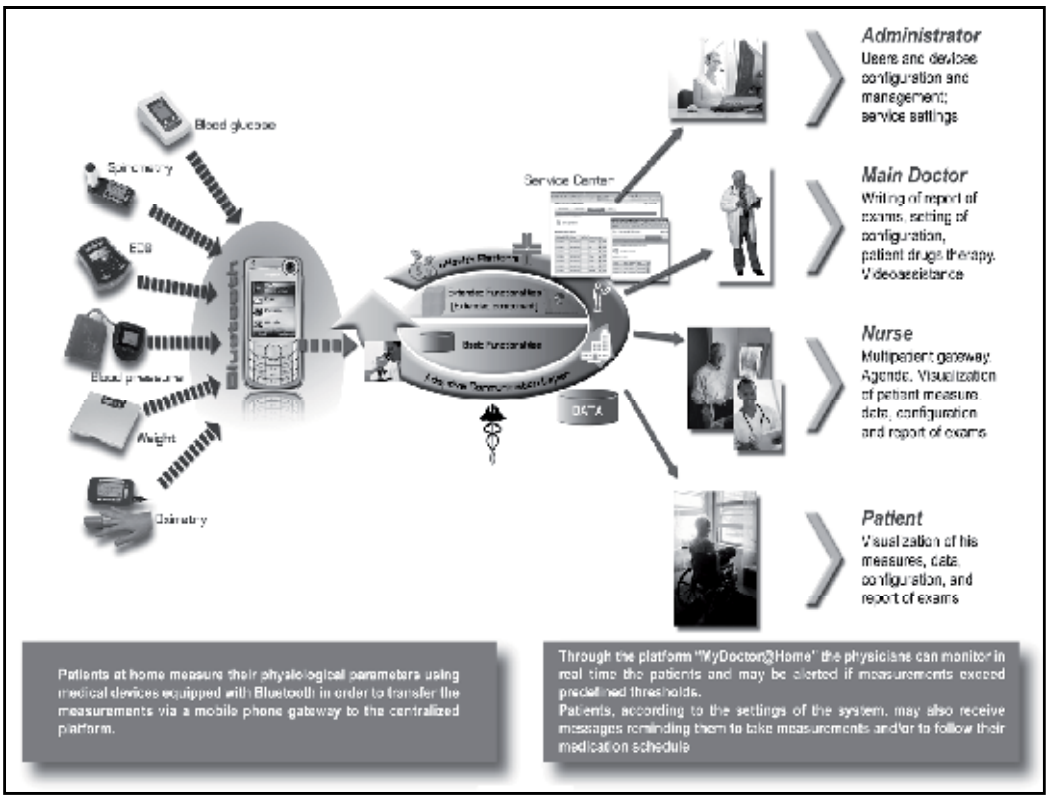

Fig. 3. The MyDoctor@Home Platform

Fig. 4. MyDoctor@Home: Computer work station at HHS office

The system enables the physician to the definition of value thresholds that can be personalized on the basis of single clinical situations. The platform informs the physician on recent measures by sending an SMS so that he can activate quickly the appropriate actions. There is a reduction of reaction times also when the nurse, during the visit at home, sends to the doctor measures performed with professional devices, like for example the ECG or the spyrometer, receiving, quickly, the feedback of the exams and instructions such as for example the variation of the therapy.

Eighteen patients have been involved in the study between June 2009 and June 2010 (27% with a COPD exacerbation), with a mean age of 86 years. All patients were functionally and cognitively impaired, with a poor quality of life. Instruments for telemonitoring resulted easy to use. The use of the equipment of telemonitoring had the benefit to avoid 24 visits by nurses and doctors on the sample in exam. Of them, 15 were substitued by phone contacts on therapy adjustments due to clinical parameter alterations registered by telemonitoring and 9 were substitued by phone counselling. Our preliminary data suggest that the use of our devises could have a reassuring role on the caregivers. Moreover, it has been demonstrated a significantly progressive reduction in the stress levels of caregivers from the baseline to the discharge (Aimonino Ricauda et al., 2011). Nevertheless, the sample size was small and the findings may not be generalizable, given that the study was conducted at only one centre and by an operationally mature hospital-at-home unit. There is the need for better quality studies in the future that can establish a clear role for telemonitoring as an adjunct to intermediate care.

Transporting radiology to the patient's home is challenging. Preliminary experiences indicate that the coupling of simple, light-weight X-ray equipment with an advanced CR-detector system proves effective for externalization of radiographic service. The image and examination quality has been proved to be the same or insignificantly lower than those performed with a stationary equipment and analysis on safety of radio-protection systems show a very low risk exposure for health staff as well as for the general population.

The study of Laerum and collegues showed that mobile, digital radiography service prove better for the nursing home patients at a compatible examination and imagine quality, and a substantially reduced cost for society (Laerum et al., 2005). The study of Sawyer concluded that domiciliary radiography services could be suitable for selected groups of patients (Sawyer et al., 1995).

A pilot study on domiciliary teleradiology service has been conducted at the HHS of Torino between June 2008 and June 2009. Acutely ill HHS patients in need of a radiological examination were randomly assigned to perform imaging at home (Intervention group, n=34) or in hospital (Control group, n=35). Inclusion criteria were: immobilization or chairbound, need for chest, pelvis/hips, joints, upper and lower limbs, hands and feet, abdomen X-rays, absence of definite delirium at enrollment according to the Confusion Assessment Method (CAM) (Inouye et al., 1990) and presence of intermediate or high risk of delirium according to the criteria of Inouye (Inouye et al., 1993), . The radiological examinations were performed at home by two qualified Radiology Technicians (RT) using a portable high frequency X-ray tube, improved cassettes (with imaging plate inside) and a mobile radiological station (Computed Radiography POC 260, Carestream) with visualization and real-time processing of acquired images (Figure 5, Figure 6). Using the Picture Archive and Communication System (PACS) of our hospital acquired images were

Fig. 5. Mobile tele-radiology station: equipment and Radiology Technicians at patient's home

Fig. 6. Mobile tele-radiology station: computed radiography system

transmitted directly via wireless broadband Internet to the radiologists in the hospital who were able to read a radiograph in real time. A firewall hardware has been used in order to protect the confidentiality of patient data. Only one radiography was performed at home in all patients, mainly a chest X-ray. All patients were very old (mean age 78 years in the entire sample), mostly multimorbid, functionally and cognitively impaired, at high risk of developing delirium in 62%. After radiological examinations an acute confusional status, according to the CAM criteria, requiring pharmacological treatment (antipsychotic drugs) appeared in 17% of patients in the Control group, whereas no one in the Intervention group developed delirium. Customer satisfaction for domiciliary X-rays was very good/excellent for 94%. This study demonstrates that a mobile, digital radiography service could be a good option for frail, vulnerable elderly and immobile patients at a compatible examination and image quality, and, due to our analysis, at a substantially reduced cost for the health care system (data in press in Arch Int J, August 2011).

8. Conclusion

Acute exacerbations of COPD are the most common cause of admission to hospital for respiratory illnesses. This causes an increased demand on hospital beds especially during the winter months. Increased provision of services in the community is one proposed method for reducing the pression on acute hospitals.

Intermediate care is a treatment model which bridges the interface between hospital and community care. It often involves cooperation between hospital doctors, general pratictioners, nurses, physioterapists and other healthcare professionals. A specific subtype of intermediate care is Hospital-at-home, were active treatment is provided by healthcare professionals in the patient's home for a condition that otherwise would require hospital care, always for a limited period. Providing acute hospital-level care in a patient's home can be a safe end efficacious alternative to hospital care, especially for frail elderly patients.

The physician-led substitutive "clinical unit" hospital-at home model of Torino provides care that substitutes entirely for an inpatient acute hospital admission; an intensity of care, including medical and nursing care, similar to that provided in the hospital, commensurate with the severity of illness treated; and care that usual community-based home care services cannot provide. Some prior studies of hospital at home for COPD have been of early discharge hospital at home models that treat patients at home with nursing care after they have been admitted to and stabilized in the acute hospital. Davies and collegues in their study of substitutive hospital at home care for COPD employed a nurse-based model that provided only twice daily nursing visits for a period of 3 days and although responsibility for patients rested with hospital physicians, patient's clinical condition did not necessarily require hospital physician's visits at home (Davies et al., 2000). Our intervention targeted very elderly patients with multiple comorbid illnesses, functional impairments and a fairly elevated degree of clinical severity, as shown by the APACHE mean score. These patients need frequent home visits by doctors, nurses and physiotherapists who work together as a team. In our experience HHS care was associated with a reduction in hospital readmission for COPD patients. In addition, HHS care was associated with improvements in quality of life and depression symptoms and a reduction in costs of care. HHS is appropriate for this target population that is especially susceptible to iatrogenic consequences of hospital care and to disruption in their common routines.

The importance of targeting appropriate interventions to appropriate patients has been seen in studies of home care services in which more intensive interventions that included multidimensional assessment were associated with positive outcomes.

Despite the evidence supporting hospital-at-home care, it has had relatively limited dissemination worldwide. Hospital-at-home care is a complex clinical model and, as such, faces substantial dissemination barriers (Leff, 2009).

Our experience suggests that a mature, physician-led, substitutive clinical unit model of hospital-at-home for elderly patients with acute exacerbation of COPD is feasible and is associated with reduction in hospital readmissions and better quality of life.

To date, the evidence base is focused nearly exclusively on patient-related outcomes, rather than on outcomes of interest to potential adopter organizations. There is a need for further studies that include a larger number of patients and an economic evaluation of direct and indirect costs. Moreover, the costs of implementation and the adoption process required within an health organization are to be well delineated.

Hospital at Home of Torino is a part of a comprehensive *continuum of services* at one end of which lies the hospital system and at the other end of which lie community services. Our model is well delineated from an organizational and administrative point of view, and may be considered an example for dissemination.

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20

Chest Mobilization Techniques for Improving Ventilation and Gas Exchange in Chronic Lung Disease

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1. Introduction

The clinical treatment and rehabilitation of chronic lung disease such as Chronic Obstructive Pulmonary Disease (COPD) is very challenging, as the chronic and irreversible condition of the lung, and poor quality of life, causes great difficulty to the protocol for intervention or rehabilitation. Most of the problems are, for example, air trapping and destroyed parenchymal lung, which cause chest wall abnormalities and respiratory muscle dysfunction that relate to dyspnea and decreased exercise tolerance (ATS/ERS 2006). Many intergrated problems such as increased airflow resistance, impaired central drive, hypoxemia, or hyperinflation result in respiratory muscle dysfunction, for instance, lack of strength, low endurance level, and early fatigue. Lung hyperinflation in COPD increases the volume of air remaining in the lung and reduces elastic recoil, thus giving rise to air trapping, which results in alveolar hypoventilation (Ferguson 2006). Thus, poor biomechanic chest movement and weak respiratory muscles affect respiratory ventilation (Jones & Moffatt, 2002). Furthermore, in COPD, the combination of V/Q mismatch, diffusion limitation, shunt and hypoventilation or hyperventilation is presented commonly, which leads to gas exchange impairment (West 2003). To solve inefficient ventilation from thoracic pump dysfunction, thoracic mobility exercise or mobilization techniques can be performed (Rodrigues & Watchie, 2010). Chest mobilization is one of many techniques and very important in conventional chest physical therapy for increasing chest wall mobility and improving ventilation (Jennifer & Prasad, 2008). Either passive or active chest mobilizations help to increase chest wall mobility, flexibility, and thoracic compliance. The mechanism of this technique increases the length of the intercostal muscles and therefore helps in performing effective muscle contraction. The techniques of chest mobilization are composed of rib torsion, lateral stretching, back extension, lateral bending, trunk rotation, etc. This improves the biomechanics of chest movement by enhancing direction of anterior-upward of upper costal and later outward of lower costal movement, including downward of diaphragm directions. Maximal relaxed recoiling of the chest wall helps in achieving effective contraction of each intercostal muscle. Thus, chest mobilization using breathing, respiratory muscle exercise or function training allows clinical benefit in chronic lung disease, especially COPD with lung hyperinflation or barrel-shaped chest (Jones & Moffat,

2002). Therefore, the technique of chest mobilization helps in chest wall flexibility, respiratory muscle function and ventilatory pumping, and results from this relieve both dyspnea symptoms and accessory muscle use. This technique is still controversial because it lacks clinical evidence, but it does show clinical benefit , especially in COPD by improving pulmonary function, breathing pattern and weaning from a ventilator.

2. Biomechanics of chest movement and thoracic spine

Movement of the thorax is like the pump-handle pattern (Hammon, 1978). Movement of the chest wall is a complex function within the rib cage, sternum, thoracic verterbra, and muscles. Basic observation reveals chest configuration for abnormality of the spine or chest shape, for example, scoliosis, kyphoscoliosis, barrel, or pectus excavatum (Bates, 1987). Normally, in all joint movement at the end of expiration, the intercostal muscles are at a suitable length before contraction during inspiration.

In assessment, chest stiffness may be caused by muscle structure being applied directly in the supine, side lying or sitting position. Stretching the rib cage, rotating the trunk or lateral flexion of the trunk can be evaluated. Furthermore, suitable lengthening of soft tissue around the chest wall and respiratory muscles is related to the efficency of contraction force and chest movement. In the case of emphysematus lung or air trapping in COPD, abnormal chest configurature and reduced chest movement with shortened muscle length and weakness are experienced (Malasanos et al., 1990).

Finally, increasing chest movement with stronger contraction of respiratory muscles can help in gaining lung volume, breathing control and coughing efficiency, and reducing symptoms by improving aerobic capacity, endurance, functional ability, and quality of life.

2.1 Functional movement

The thoracic cage is composed of three parts: thoracic spine, ribs, and sternum, which connect to costovertebral and condrosternal joints, and so movement occurs in three dimensions; transverse, antero-posterior and vertical directions (Landel et al., 2005). True ribs (2nd to 8th rib) move more flexibly because of no clavicle obstruction, whereas the 11th and 12th ribs connect to the cartilage, therefore causing less freedom to move.

1. **Flexion and extension**

The basic structure of the costovertebral joint comprises both the angle and neck articulation of the rib with the spine, and is attached to costotransverse and radiate ligaments. In the direction of thorax flexion (Grant, 2001), there is anterior sagittal rotation, when the costovertebral joint moves as anterior gliding that slightly rotates, whereas downward rotation and gliding occur during extension. The lower thoracic spine moves more freely than the upper one. The sternum is composed of the manubrium, body, and xiphoid process, and is anterior with upward expansion when breathing deeply. In fact, when it comes to movement, the manubrium is somewhat fixed to the first rib, whereas the body is more flexible around the 2nd to 7th rib. Thus, movement of the sternum looks like a hinge joint during deep inspiratory and relaxed expiratory phases. For extension, the extensor muscle group is the most active, with a motion range of

approximately 20-25 degrees. Thorax extension presents the opposite movement to flexion, with backward sagittal rotation by posterior translation and slight distraction of the spine (Neumann, 2002).

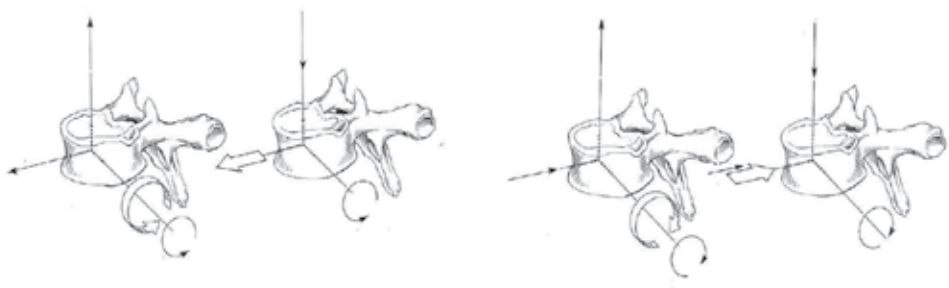

Fig. 1. Anterior rotation of the spine during flexion, and posterior rotation during extension. (Grant, 2001; Lee, 2002)

Fig. 2. Extension of the thorax; showing the movement in superior upward and posterior gliding of the costotransverse joint. (Grant, 2001; Lee, 2002)

2. **Lateral flexion**

In flexion direction, the thoracic body rotates slightly on the flexion side, while the posterior rotates in the opposite direction so that the costovertebral joint is opened and inferior

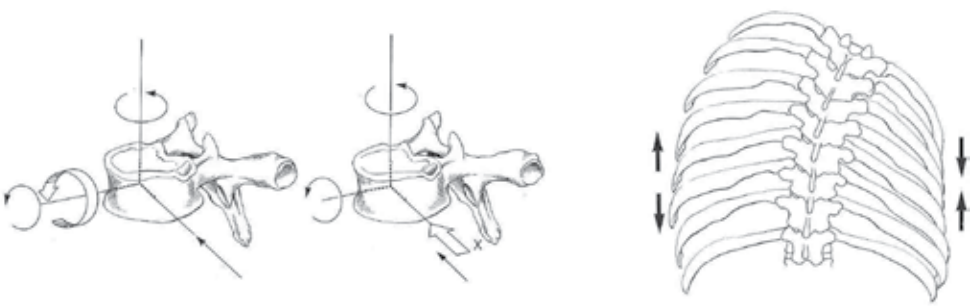

Fig. 3. Biomechanics of lateral flexion to the right; showing the movement of thoracic body and costovetebral joint on both sides. (Grant, 2001; Lee, 2002)

gliding occurs to increase rib space. Mobility of the thorax on flexion, either to the right or left, is found more in lower than upper thoracic parts. Thus, stretching of the lower thorax is rather more successful than that of the upper part. A normal range of motion is approximately 45 degrees: 25 degrees at the thorax and 20 degrees at the lumbar spines. During flexion to the left, the inferior facet of T6 on the left side moves above the superior facet of the T7 spine. In thorax movement, lateral flexion directly affects the rib space in both approximation and stretch away (Figure 3), which results in the transverse process, when the head of the rib glides in the opposite direction (Figure 4).

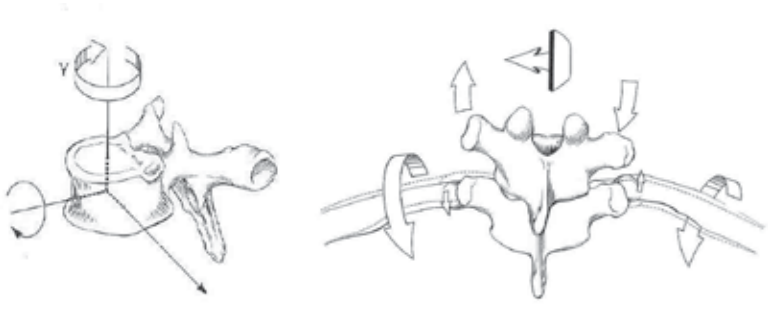

Fig. 4. Rotation of the trunk and thorax, with rib cage and costovertebral joint movement. (Grant, 2001; Lee, 2002)

3. Trunk rotation

Trunk rotation is a complex movement that involves many joints. For example, during rotation to the three left events are shown as; 1) rib rotation with costotransverse posterior gliding on the rotating side, whereas anterior rotation of the rib and gliding are on the opposite side, 2) thoracic body that is elevated and depressed in each segment, and 3) vertical asymmetrical torsion. Upper thoracic spine can move like pure axial rotation as well as thoracolumbar and cervicothoracic rotation. However, sometimes movement of the upper and lower thoracic spines also co-move with lateral flexion or rotation. Thus, articular facet between high and low spines is a sliding movement (Grant, 2001; Lee, 2002).

In conclusion, the chest wall, which is composed of spine, sternum, and ribs, moves in synchronization, no matter whether it is lateral flexion, flexion, extension, or rotation. However, the quality of movement affects individual direction because the costovertebral joint makes contact with the vertebral body, so that lateral expansion is affected more than anterior movement. Whereas, the 2nd to 8th ribs connect to the sternum anteriorly, thus expanding the chest in an anterior direction with pumping handle or anterior and superior motion, as well as bucket handle with lateral and superior motion (Norkin & Levangie, 1992) that occur in regular breathing (Greenman, 1996).

The chest mobilization technique is preferred in cases of COPD or chronic lung disease, with the basic theory of mainly improving ventilation. In addition, aging, prolonged use of a ventilator and chronic illness with neuromuscular dysfunction also concern chest wall mobility.

Rib torsion, passive stretching, trunk rotation, back extension, lateral flexion and thoracic mobilization are practiced to improve chest flexibility.

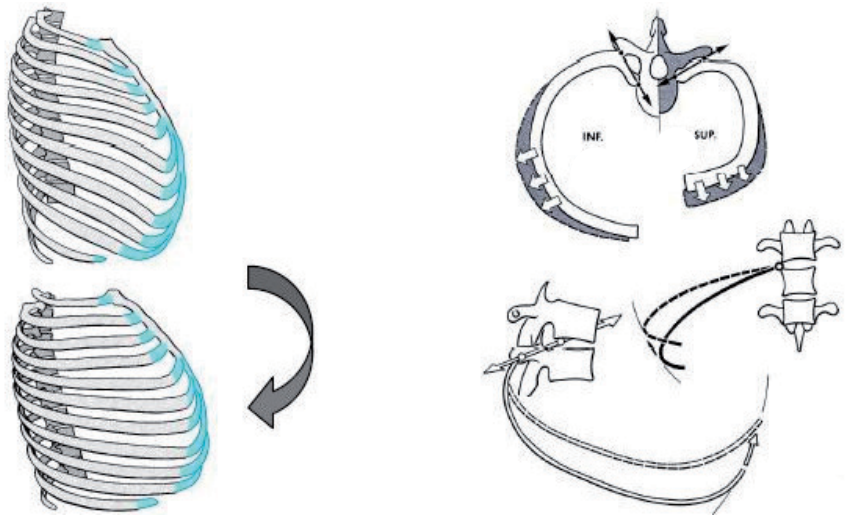

Fig. 5. Pump-Bucket pattern of chest movement. (Greenman, 1996)

3.1 Soft tissue flexibility

The theory of Laplace's law suggests that the length of muscle relates to the maximal force of either diaphragm or intercostal muscles, which affect ventilation in the lung (Kisner et al., 1996; Grossman et al., 1982). Previous evidence showed that stretching the anterior deltoid and pectoralis major muscles, including the sternocleidomastoid, scalenes, upper and middle fibers of trapezius, levaytor scapulae, etc., can increase vital capacity (Putt & Paratz, 1996). In the case of a patient with COPD, the lower diaphragm is depressed horizontally in a contracted length, thus, the resting length is insufficient for contraction. Tachypnea and dyspnea is then a common sign (Cane, 1992). This phenomenon still presents in patients who use a mechanical ventilator for a long period of time (Guerin, 1993). Muscle around the chest wall can be divided into two dimensions; anteriorly with pectoralis major and internal or external intercostal muscles; and posteriorly with erector spinae, latissumus dorsi, serratus posterior superior or serratus posterior inferior muscles, which are important for lung ventilation (Kacmarek et al., 2005). Thus, retraction or spasm of these soft tissues, or muscles, limits chest expansion.

Impairment or disease relates to ineffective chest wall movement

1. Scoliosis or kyphosis (Leong et al., 1999)
2. Osteoporosis or ankylosing spondylitis (Neill et al., 2005)
3. Nerve injury as spinal cord injury (Baydur et al., 2001)
4. Skin disease such as scleroderma, multiple sclerosis etc. (Woo et al., 2007)
5. Myofacial pain or chest pain (Wise et al., 1992)
6. Post thoracic surgery for lung or heart operation (Macciarini et al., 1999)
7. Prolonged use of a mechanical ventilator (Gillespine et al., 1985)
8. Chronic lung disease or pneumonia (Hoare & Lim, 2006)
9. Proloned bed rest (Suesada et al., 2007) or aging (Chaunchaiyakul et al., 2004)
10. Other factors; pain, posture, diaphragm dysfunction (Vibekk, 1991).

4. Physical examination and outcomes

Observation of respiratory symptoms and chest wall mobility

General screening of respiratory problems can be assessed from the signs or symptoms of respiratory depression such as tachypnea, use of accessory muscles, abnormal breathing pattern, cyanosis, nasal flaring etc. which refer to hard work in breathing (Irwin & Tecklin, 1995).

Normal shape of the chest can be observed by the diameter of anterior and lateral views, where the ratio of diameter between anterior and lateral measurement should be more than 1.0. However, in the case of COPD, this ratio may be less than 1.0 and the shape is called barrel chest (Jardins & Tietsort, 1997). In COPD, the barrel chest is shown simply from intrapulmonary air trapping or emphysema, which depresses the diaphragm downward and intercostal outward in a shortened position. The shortening of muscle length before inspiration causes insufficient contractile force. Shortness of breath and decreased chest expansion can be observed clinically. Finally, aggressive dyspnea and low ventilation induce physical deconditioning via low exercise performance (Celli, 2000).

Dyspnea intensity is quantified most easily by using the modified Brog (0-10) category ratio scale (Borg, 1982). This tool evaluates also within other protocols such as the Medical Research Council (MRC) scale, New York Heart Association (NYHA) scale, London Chest Activity of Daily Living scale and Pulmonary Functional Status and Dyspnea Questionnaire (Meek, 2004). Many reports and studies used a Brog scale for identification the dyspnea symptoms and interprets the effectiveness of program.

Palpation on chest expansion

Evaluation of chest expansion is very comfortable for the clinician. Various protocols such as the three levels of upper, middle, and lower lobes (Cherniack, 1983) can be performed manually. Circumferential change from full expiration to maximal inspiration at supine position can be applied with a tape at the axilla (upper lung) and xiphoid (lower lung) levels, as suggested by previous reports (Carlson, 1973), and this protocol has shown good reliability (Lapier et al., 2000). For example, 3 ¼ inches ± ¼ inch could be increased at the axillary level of 20-to 30-year old women (Carlson, 1973). Another level that can be measured to present chest expansion by tape is the 4th intercostal rib space (Fisher et al., 1990). Furthermore, the chest caliper is a new tool that can be used to evaluate chest expansion. Previous evidence has shown that application of the chest caliper enables measurement of thoracic diameters at rest and during activity, but it could not refer to the normal data for chest expansion (Davis & Troup, 1966).

Original palpable examination is of chest expansion in the respiratory system, and less expansion may reflect intrapulmonary lesion such as secretion obstruction or atelectasis. Sometimes, incomplete recoiling from expiration results in many issues such as mass, emphysema, or air trapping. Although, no scientific data have shown normal length of complete recoiling in chest expiration, clinical experience can adjust muscle tightness or shortening around the chest wall. Palpation of the chest wall for flexibility can be evaluated in sitting, side lying, supine, or prone position. Conventional chest movement can be performed with manual evaluation.

Upper costal chest expansion (Figure 6)

Position: Sitting.

Handling: All finger tips are placed at the upper trappezius with whole plamar on the upper chest above the 4th rib at the mid clavicle line, and the tips of both thumbs close to the midline at the mid-sternum line.

Command: Gentle compression and order the subject to breathe in deeply and release following chest expansion.

Results: Approximate calculation of different distances between the tips of thumbs in centimeters (cm) before an after full inspiration.

Direction: Upper costal expansion should be upward with anterior expansion.

Middle costal chest expansion (Figure 6)

Position: Sitting or lying supine.

Handling: All finger tips placed at the posterior axillary line with tips of both thumbs close to the horizontal mid line. The whole plamar should be placed on the middle chest area (4th to 6th rib anteriorly at the mid-clavicle line).

Command: Gentle compression and order the subject to breathe in deeply and release following chest expansion.

Results: Approximate calculation of different distances between the tips of thumbs in centimeters (cm) before an after full inspiration.

Direction: Middle chest expansion should be outward and slightly up ward.

Lower costal chest expansion (Figure 6)

Position: Sitting.

Handling: All finger tips placed at the anterior axillary line with tips of both thumbs close to the horizontal mid line. The whole plamar placed on the lower chest area (below the scapular line and not lower than the 10th rib posteriorly).

Command: Gentle compression and order the subject to breathe in deeply and release following chest expansion.

Results: Approximate calculation of different distance between the tips of thumbs in centimeters (cm) before an after full inspiration.

Direction: Lower costal expansion should be outward.

Sternocostal Movement Evaluation (Figure 6)

Position: Sitting

Handling: Palm placed to cover all sternum (head and body).

Command: Gentle compression and order the subject to breathe deeply.

Result: Anterior expansion during sternum expansion, then upward expansion during sternum (head part) movement.

(a) (b) (c) (d)

Fig. 6. Three levels of manual evaluation for upper (above the 4th rib anteriorly) (a), middle (between the 4th and 6th ribs anteriorly) (b), lower lung expansion (below the scapulae and above the 12th thoracic vertebrae, posteriorly) (c), and sternum flexibility (d).

Tape and Caliper Evaluation (Fisher et al., 1990; Carlson, 1973)(Figure 7)

Both of these methods can be applied in a sitting position, which is better than lying supine. From the author's experience, the three levels: upper, middle and lower, can be measured at the axillary, nipple line, and xiphoid process. The latest report on measuring the thoracic excursion or expansion was carried out by Bockenhauer and coworker (2007) (Bockenhauer et al., 2007). It suggests anatomic landmarks on the chest wall as follows;

Upper thoracic expansion is seen as the third intercostal space at the midclavicular line and the fifth thoracic spineous process.

Lower thoracic expansion is seen at the tip of the xiphoid process and the 10th thoracic spineous process.

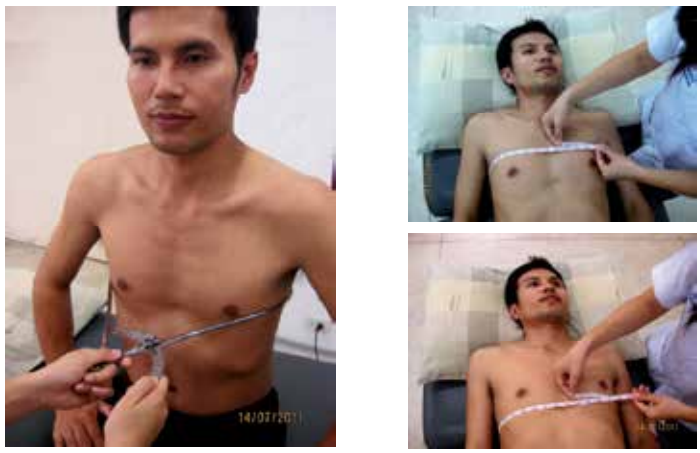

Fig. 7. Application of cloth tape for measuring the upper (right above), lower (right below) thoracic expansion and hand position, and use of the caliper to measure chest expansion (left).

The cloth tape method has been modified by placing the circumference on the specific landmarks transversly and measuring the different changes between full expiration and full

inspiration. Although results were studied in 9 healthy subjects, the mean of upper and lower expansion ranged from 1.0 to 7.0 cm, and 1.5 to 7.98 cm, respectively. For the chest caliper, there was no report or data for the range of normal chest expansion.

Thoracic Flexibility Evaluation (Figure 8)

The thoracic or chest wall flexibility is not determined or evaluated exactly for standard value or comparison between healthy and chronically ill subjects. Thus, many practitioners make decisions individually from clinical experience. Thoracic or chest wall flexibility can be evaluated by many procedures in different positions.

In supine or side lying positions, the examiner can evaluate in various directions, but the result is concerned with the lateral intercostal part.

A. Position:	Supine with head supported with or without a pillow at the mid-thorax (Figure 8)
Handling:	Two hands on the lateral lower chest (6^{th} to 8^{th} rib at the mid -axillary line).
Direction:	1. Hemi-cross counterpressure. 2. Hemi-caudal stretching force. 3. Bilater-caudal stretching force.
B. Position:	Side lying position with or without a pillow in the mid-thorax, combined with hand elevation (Figure 8)
Handling:	Two hands on the lateral lower chest (6^{th} to 8^{th} rib at the mid axillary line). One hand holding the subject's hand and the other on the lateral lower chest.
Direction:	Hemi-caudal stretching force with two hands, and opposite and cephalic stretching.
C. Position:	Sitting position without support (Figure 9) Sternum movement and upper chest expansion Trunk rotation test Lateral bending test or anterioposterial flexion test Trunk flexion and extension test.

Chest X-ray film: Evaluation of lung volume from a chest X-ray (CXR) film is measured possibly from previous evidence of using manual illustration for free hand tracing (May et al., 2009) or calculating total lung capacity from the thoracic roentgen image (Dieterich et al., 1990). In fact, improvement of air entry or volume can be observed from clinically increasing the dark field on the film. In COPD, silhouette sign and secretion retention are identified commonly, including atelectasis from a secretion block (Reid & Chung, 2004), which is the main problem in decreasing lung volume or resorptive atelectasis (Harden, 2009). Thus, the effectiveness of chest mobilization to improve lung ventilation can be reassessed by increasing the aeroted areas or resolving the lung collapse on the chest film.

Dynamic lung ventilation: In the case of lung volume evaluation, functional residual capacity (FRC), tidal volume (Vt) and forced vital capacity (FVC) from the pulmonary function test are challenging outcomes (Dexter, 2010). FRC decreases when there is an

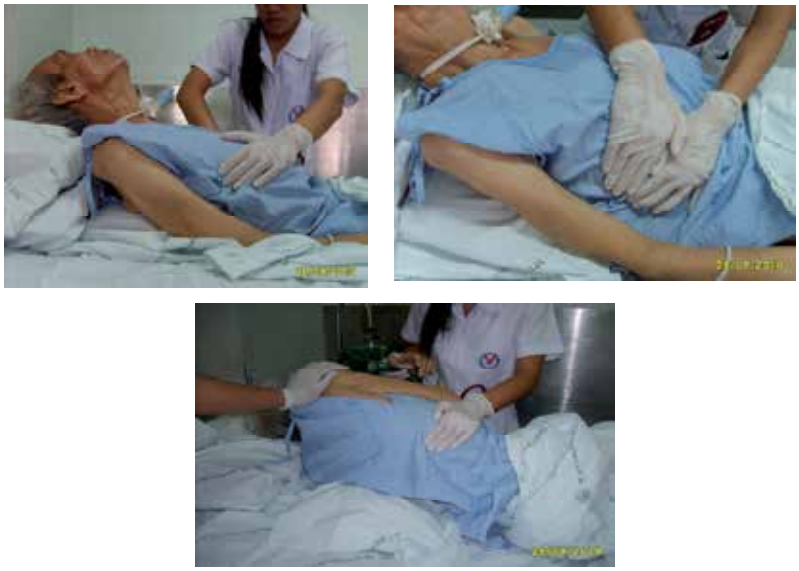

Fig. 8. Rib torsion (right above) and trunk extension (left above) and lateral stretching technique (below). (Leelarungrayub et al., 2009)

Fig. 9. Functional trunk test as flexion (right above), extension (middle above), rotation(left above), lateral flexion(right below), combined extension, and rotation tests (left below).

imbalance between the lungs and chest wall. Both atelectasis and kyphoscoliosis from abnormal posture affect the elastic recoil of the chest. A barrel chest affects the muscle length of the chest wall or diaphragm by either increasing or decreasing it , and a reduction in force results, which reduces vital capacity (VC) (Henderson & Clotworthy, 2009). In the case of patient who used a ventilator, improvement in lung volume or ventilation can be evaluated from tidal volume (Vt), expiratory tidal volume (ETV), or minute ventilation (VE). In the early exacerbation stage, evaluation of lung volume is difficult because of dynamic hyperinflation, but if the patient is on a ventilator with SMIV or CPAP modes, minute ventilation (VE) and FRC is very easy to measure (Vines, 2010). Finally, the weaning time from a ventilator is the final outcome that presents the improvement clinically.

Fig. 10. Passive stretching of the pectoralis major (above and middle) and active stretching of the pectoralis muscles with inspiration with exhalation during flexion and breathing in during extension (below).

From the overall outcomes, chest expansion, dyspnea, chest radiography, and dynamic lung ventilation are most important in representing the effectiveness of a technique. Other parameters can be evaluated such as breathing pattern, respiratory rate, oxygen saturation, etc., and respiratory muscle strength if protocol training is included.

5. Chest mobilization techniques

Chest mobilization techniques are the original protocol used in chronic lung disease, which has the tendency to cause poor posture, rigidity, or lack of thoracic spine and rib cage movement (Vibekk, 1991). These techniques are divided into passive and active chest mobilization, which depends on the patient's condition. In the case of an unconscious patient, as seen in an intensive care unit (ICU) where prolonged treatment is carried out with or without ventilator support, the" **Passive Chest Mobilization Technique**" can be performed on the chest wall by a therapist. Whereas, in the case of a patient in recovery or good condition, the "**Active Chest Mobilization Technique**" can be performed. In some general practices, patients who have just recovered can have modified Active-Passive Chest Mobilization to improve flexibility of the chest wall. The aim of these techniques is to improve thoracic mobility at the upper, middle or lower parts of the chest. Furthermore, these techniques need to be selected carefully to minimize dyspnea, and they should be applied in sitting, sitting leaning forward or high side lying positions (Lee, 2002; Rodrigues & Watchie, 2010).

Fig. 11. Chest Mobilization Techniques for improving thoracic mobility at the postero-lateral parts (trunk rotation) (Vibekk, 1991) by active and passive trunk rotation on both sides. Exhalation in a forward position is carried out at the beginning of flexion, and rotation of the left side is performed laterally with inspiration. However, an exhalation phase is carried out during passive trunk rotation.

5.1 Antero-posterior upper costal chest wall mobilization

The original technique is similar to the previously mentioned protocol (Frownfelter, 1987). This pattern is suitable for giving benefit in cases of shortening pectoralis muscles. Some evidence has shown that winging and trunk rotation can improve vital capacity (Pryor et al., 2000). The benefits of this pattern improve both ventilation in upper lobes of boths and also stretches the pectoralis muscle that may tight.

5.2 Postero-lateral chest wall mobilization

This technique has many procedures such as trunk torsion, rotation, and lateral bending (Frownfelter, 1987). It not only affects the ribs and tissue, but also moves the costovertebral and facet joints. This pattern is very useful in ordet to improve the ventilation around in the lower lobe of both lungs.

5.3 Lateral chest wall mobilization (Figure 12)

This technique can be applied in cases of unconsciousness and good consciousness. This part can be mobilized either by therapist likes lateral flexion on the bed, or rib torsion. Other procedures can be performed by passive stretching in sitting position. The last choice that is very strong and give the best result in order to stretching by side lying on the pillow and passive stretching. This pattern helps to improve the chest wall flexibility around the lower thoracic and improves the ventilation in both lower lungs. Sometime, lateral chesl wall stretching effects to the thoracic joints either sterocostal or costovertebral joints.

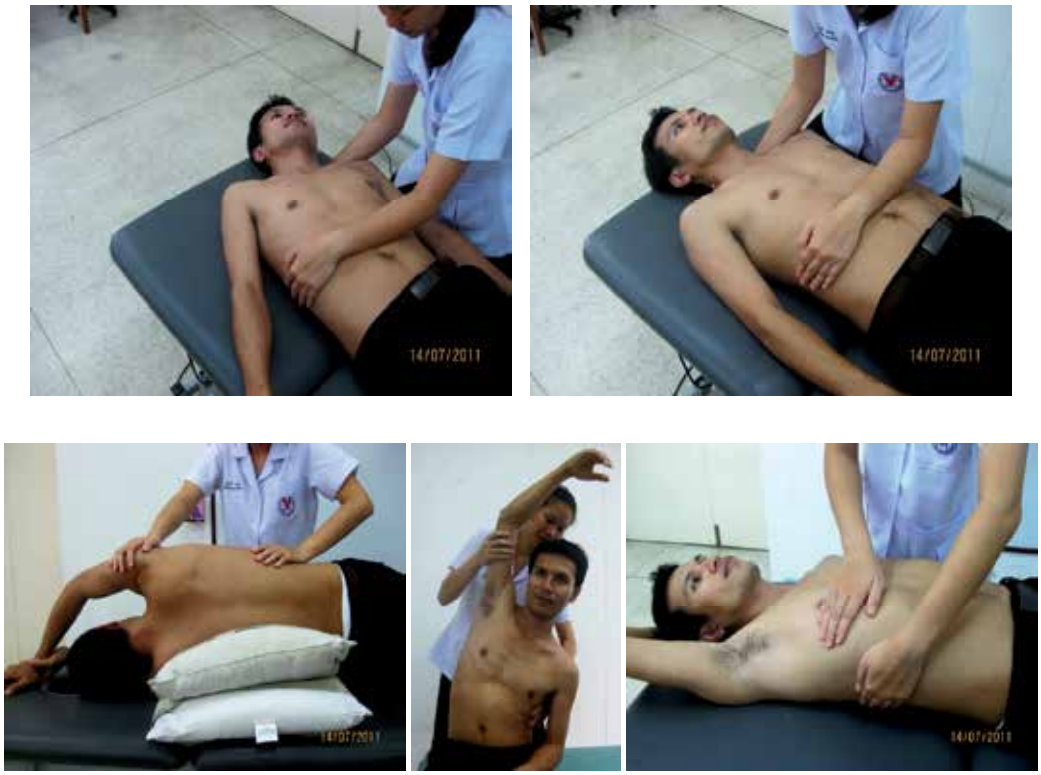

Fig. 12. Chest Mobilization Techniques for improving lateral thoracic mobility; Passive lateral flexion (above), passive rib torsion (right below) (Wetzel et al., 1995), and trunk flexion (middle below), including passive lateral flexion in side lying position on the pillows (left below)

5.4 Thoracic joint mobilization (Figure 13)

From the biomechanics of chest movement, vertebral joints connect to the ribs and sternum with a complex unit that promotes chest expansion. Although this movement is very hard to observe, it also is very effective for ventilation. Therefore, this joint movement is promoted for improving ventilation (Vibekk, 1991).

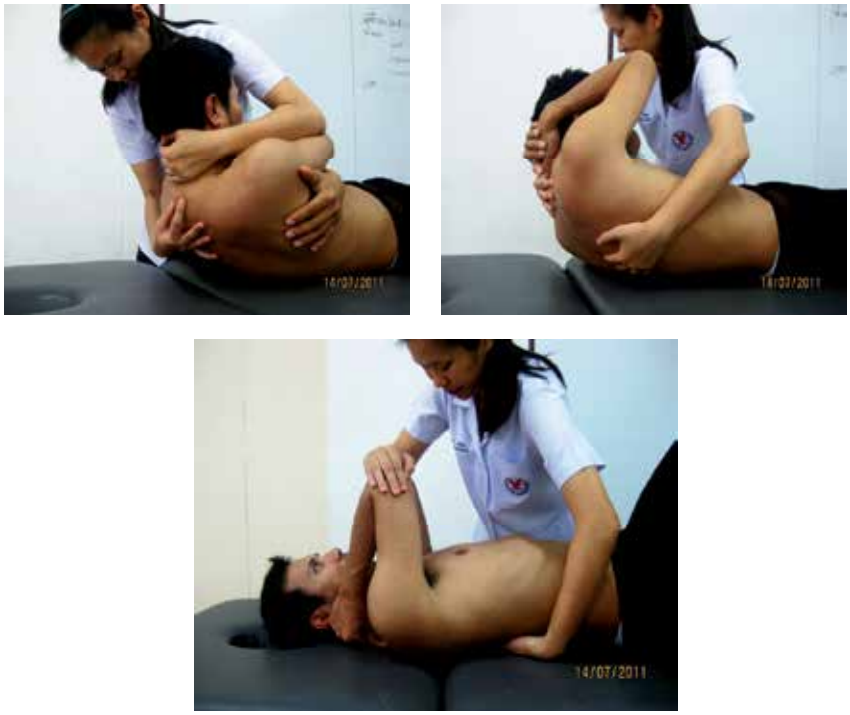

Fig. 13. Mobilization of the facet joint by flexion and extension (Vibekk, 1991), direct rib stretching at the supine lying (left above), facet joint (right above), and costovertebral joint (below).

6. Indication and contra-indication of chest mobilization techniques

There has been no information on the indication for chest mobilization before, which gives a tendency for limitation of chest movement; either structurally or physiologically. However, this technique can be used for various conditions such as COPD, prolonged bed rest, abnormal spine, deconditioning and aging.

The contra-indications for using this method are listed (Viekk, 1991) below:

- Severe and unstable rib fracture
- Metastasis bone cancer
- Tuberculosis spondylitis
- Severe osteoporesis
- Herination
- Severe pain
- Unstable vital signs

7. Clinical analysis on the effectiveness of programs

The clinical procedure for representing the efficiency of this treatment is very difficult because of the low number of cases. Representation of improvement using statistical analysis is limited by either parametric or non-parametric evaluation. In clinical rehabilitation, matching age and disease condition to set up a control or treated group is very difficult. Furthermore, presentation of a positive outcome in clinical improvement is very important.

Many reports of case studies from rehabilitation have shown results with explanations such as postural restoration from physical therapy (Spence, 2008). However, an interesting procedure for evaluating a single system was designed by Bloom and Fischer (1982). This system was designed basically to involve an individual or a single system by repeatedly taking recordings of dependent variables (Ottenbacher, 1986). The components of this design are composed of only sequential application and withdrawal or variation of intervention, with the use of frequent and repeated measures. Thus, this design is not a fixed procedure and can be applied in various study proposals.

The design of a case study has many models; A-B, A-B-A, A-B-A-B, and B-A-B, where A is the baseline period and B the treatment period. There is also an A-B-C model for use in different treatments. Various repeated data recordings are performed in each period, and more than 4 are enough for clinical analysis when a Bloom Table is used. Clinical explanation can be presented by visual inspection and raw data analysis. A simple line graph is an easy procedure for presenting the changes and tendency in each period. Improvement or deterious results in pre-treatment, during treatment or post-treatment can be explained from a changing or trend line. In addition, comparison of mean levels in each period is also a very important evaluation. Statistical analysis of this system can be performed using the Bloom Table (Bloom, 1975), which observes the proportion during baseline and number of treatments above or below the celeration line. Important analysis of data in each period involves changes in all parameters that must evaluate autocorrelation, which helps to separate changes between condition and treatment. Other procedures that present the statistical difference between baseline and treatment use the two standard deviation band method and C-statistic (Ottenbacher, 1986). Some researches have used this design such as the study of Cleland and Palmer (2004), who showed the effectiveness of manual physical therapy, therapeutic exercise, and patient education on bilateral disc displacement in a single-case A1 (control period) –B (intervention period) -A2 (withdrawal of the intervention) design, and also presented the results by visual analog scale and the two standard deviation band method (Cleland & Palmer, 2004). Overall, representation of effective rehabilitation or treatment in rare or few cases can be performed with a single case design.

8. Clinical implementation

Case 1: Chest mobilization treatment in the sub-acute stage

Illness history and medical treatment: A sixty years old man, diagnosed with aspirated pneumonia and underlying cysticercosis from obstructive hydrocephalus, was admitted to hospital with respiratory failure. A physician treated him with tracheostomy and on a ventilator (tidal volume = 450 mL, I:E = 2.1, and respiratory rate = 16 bpm). A hematology

test showed low haemoglobin (8.9 g/dL) and haematocrite (27.7%), and the chemistry lab test showed hyponatermia and hypoglycemia.

Chest X-ray: Interstitial infiltration of the left and right upper lobe (Figure 14).

Physical examination: A thin man, with general muscle atrophy, moderate dyspnea, use of accessory muscles during inspiration, decreased chest expansion on the left more than right side, dullness at the left lung, decrease of air entry with bronchovesicular breath sound and coarse crepitation in both lungs (Figure 14 right)

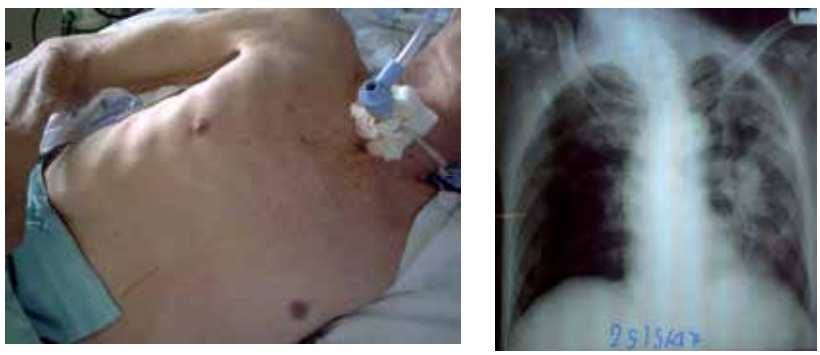

Fig. 14. Chest radiograph before treatment showing infiltration in the left lung and upper area of the right upper lobe (right), and general configurature of the chest wall showing very tight or stiff movement (left).

Treatment: Passive rib torsion at the left lung was added to the general chest physical therapy program; postural drainage, percussion, and breathing exercise (Figure 15) twice daily for 7 days.

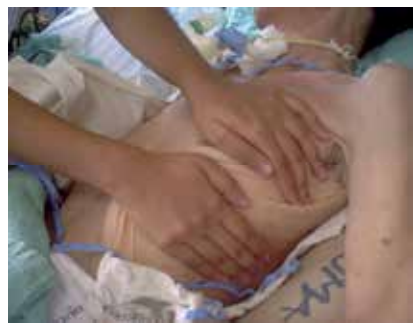

Fig. 15. Passive rib torsion at the left chest wall 10 times per session during ventilation.

Progession: After treatment, repeated chest radiography showed improvement of aerotion and less infiltration in the left lung (Figure 16). Medical treatment could stop using a ventilator to supplement oxygen at 10 Lpm, with a T-piece for 1 hr alternately in a 4 hr period, because hypoglycemia, hyponatermia and malnutrition, dyspnea and use of some accessory muscles were present.

Remaining problems: General weakness, ineffective breathing, shortness of breath, minimized chest expansion and stiffness, and air entry reduction without crepitation.

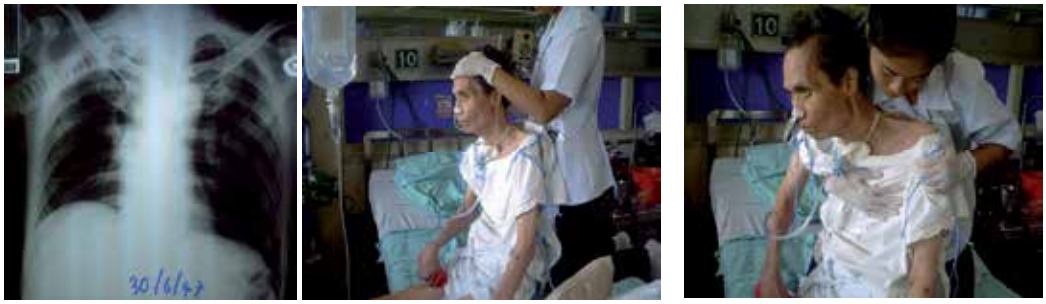

Fig. 16. Chest radiograph after 7 days of treatment (left) and Chest mobilization in sitting position with sternum compression, trunk extension and rotation (middle and right).

Progressive treatment: Passive chest mobilization in a sitting position by stimulating chest expansion in an anterio-posterior direction with sternum compression, back extension and trunk rotation.

Final outcomes: In this case, chest mobilization in anterio-posterior direction or stimulated sternum movement increased chest expansion by evaluating the expiratory tidal volume (TVE), tidal volume, and SpO_2. Patients who have stopped using a ventilator and are only on an O_2 with T-piece can be discharged from hospital after 2 weeks treatment with chest mobilization. However, there is more intensive treatment such as sitting, standing and walking training, and weight training to increase the upper and lower limbs' strength.

Case 2: Chest mobilization treatment in the acute stage

History of illness and medical treatment: A sixty-three years old man was diagnosed with chronic lung disease, pneumonia and sepsis. A physician treated him with an orotracheal tube on a ventilator (Pressure support= 12 cmH_2O, O_2 = 35%, VTE = 150 mL). Blood gas results showed respiratory acidosis and moderate hypoxia with metabolic compensation. Medical problems after treatment were prolonged use of a ventilator (for one month), with recurrent infection and pneumothorax at the right lung, which was resolved by intercostal drainage (ICD). Then, the medical program for weaning off the ventilator was unsuccessful.

Chest X-ray: Left lung atectasis and pneumothorax at the right lung with ICD (Figure 17 left)

Physical examination: A thin man was using a ventilator and presenting general weakness, muscle atrophy and malnutrition. He produced very little chest expansion on either side. Dullness presented at the left lung and hyperresonance at the right one.

Treatment: Initially, an upright position was combined with a chest mobilization technique on the left chest wall, and percussion to remove secretion was performed 3 times daily.

Progession: After 3 days of treatment, chest radiography was evaluated repeatedly (Figure 17), showing improvement of aerotion in the left lung, but atelectasis at the lower lung. The physician could not reduce pressure support while the patient was on the ventilator, but the expiratory tidal volume improved from 155 to 366 mL and an ICD was removed successfully. Unfortunately, remaining problems presented because of respiratory muscle weakness, and malnutrition, and the final goal of stopping the ventilator still had to be reached.

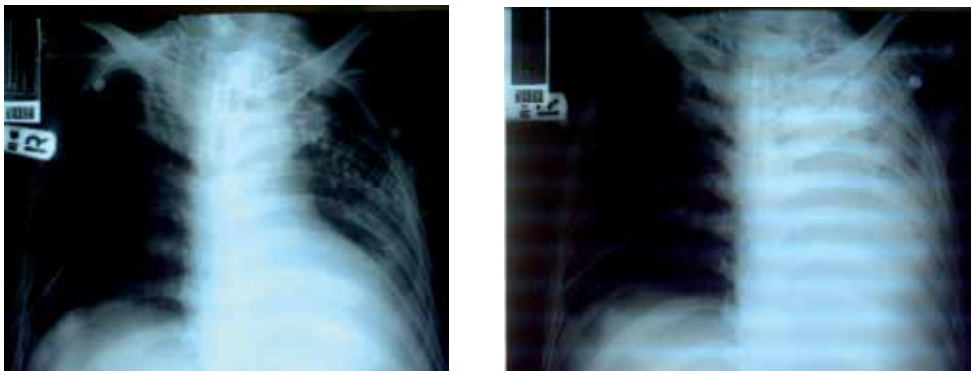

Fig. 17. Chest radiograph showing atelectasis of the left lung, and pneumothorax at the right lung with ICD before treatment (right) and after 3 days of treatment showing improvement of aerotion in the left lung with atelectasis of the left lower lobe (left).

Progressive treatment: An extensive program was carried out from previous treatment with passive chest mobilization in supine position because of weakness. Passive pectoralis muscle and breathing exercise were performed combined with diaphragmatic and intercostal muscle contraction by relearning.

Fig. 18. Passive chest mobilization being combined with breathing exercise of the intercostal and diaphragmatic muscles.

Final outcomes: For this case in the ICU stage, benefits of chest mobilization presented improvement of ventilation at the left lung, and more advantageous treatment was shown when combining other techniques such as breathing exercise with intercostal muscle and diaphragm relearning. However, treatment was unsuccessful in other factors such as pneumothorax, malnutrition and the patient's overall condition.

Case 3: Chest mobilization treatment in the chronic stage

Illness history and medical treatment: A sixty years old man was diagnosed with stable COPD and acute exacerbation because of prolonged use of a ventilator, no rehabilitation for 3 months, and unsuccessful weaning from the ventilator with recurrent infection and much secretion. Ventilator mode was maintained with pressure control (pressure support = 25 cmH_2O, rate = 15 bpm, I:E = 1:2, FiO_2 = 0.45, and PEEP =10 cmH_2O. Blood gas showed moderate hypoxemia (PaO_2 = 85 mmHg) with hypercapnea ($PaCO_2$ = 55 mmHg) and completed compensation. Berodual forth for preventing bronchospasm and Fluimucil A600 for diluting the secretion were administered routinely.

Chest X-ray: CXR shows specific atelectasis at the right lower lobe and hyperaerotion in the left lung before treatment (Figure 19 left).

Physical examination: A thin man using a ventilator presented with muscle weakness, atrophy and malnutrition. BMI was 13.5 kg/m^2. Chest expansion was very small on both sides. Dullness presented in the left lung and hyperresonance at the right lung.

Treatment: For general chest physical therapy with postural drainage, percussion and breathing exercises were carried out in the ward, such as upright position combined with a chest mobilization technique and compression on sternum, and trunk rotation was used to improve chest wall flexibility. An additional program of passive rib torsion, trunk extension and lateral stretching on a pillow was carried out as well. All programs were performed 3 times daily (Figure 19 middle and right).

Fig. 19. Chest radiograph showing atectasis of the right lower lobe and hyperaerotion in the left lung before treatment (right), Chest mobilization being performed with compression on sternum, trunk rotation of both sides, sterum and trunk rotation of both sides (middle and left) (Leelarungrayub et al., 2009).

Clinical evaluation: Efficiency of treatment was monitored using various parameters such as expiratory tidal volume, chest expansion at the mid axillary line with a tape and dyspnea, and followed up by CXR after treatment. This effective treatment used the single-case research design with the A (Pre-CPT), B (CPT treatment), and A (Post-CPT) model for 7 day periods.

Results (Figure 20)**:** A 7 day control period (Pre-CPT) showed low expiratory tidal volume (ETV) (mean = 195± 30 mL) and chest expansion (mean = 2.1± 0.54 cm), and during the 7 days of treatment (CPT) benefits were shown by increased mean of ETV (260±49 mL) and chest expansion (3.6±0.22 cm). The dyspnea score was reduced from 6.4±1.14 to 4.4± 0.54. Statistical comparison using the Bloom Table showed significant changes in ETV, with 5 points, 4 points, and all points above the trend line from a Pre-CPT period. However, the Post-CPT and ETV showed deterious effects when treatment was stopped and all points went below the trend line, except for the dyspnea score and chest expansion, which maintained the same level. In both the Pre-CPT and CPT period, all data showed non-significant results of autocorrelation, which meant that the changes in each period did not come from disease progression, especially during treatment (CPT).

Clinical implementation: The chest mobilization technique is very important for improving ventilation and gas exchange in cases that are measured by lung volume and chest expansion, including dyspnea. In figure 20 shows the significant changes of this technique by increasing in a mean of all paprameter when compared to the before treatment of

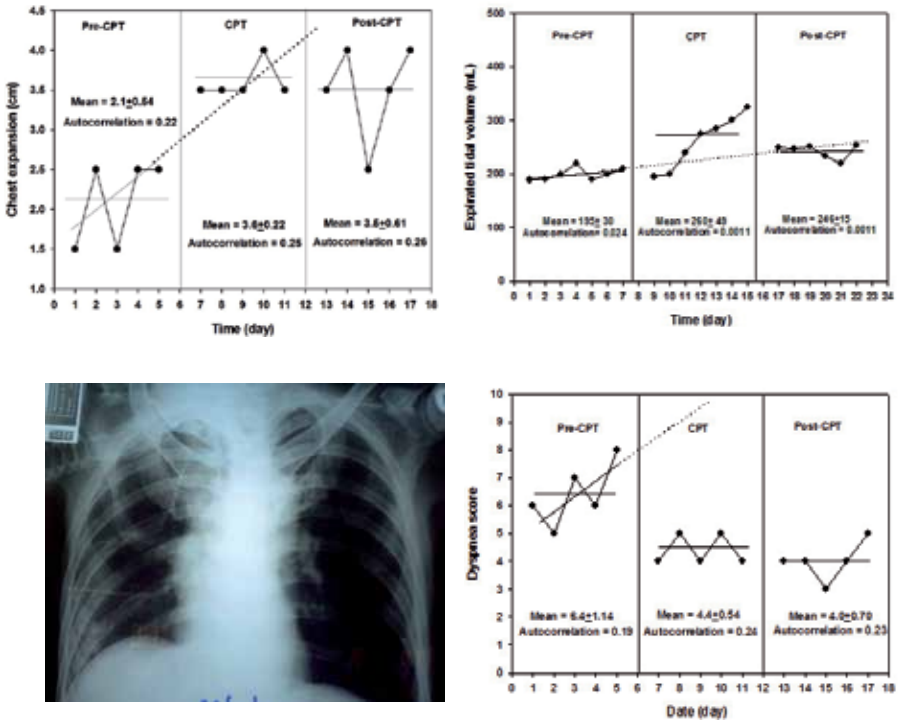

Fig. 20. Visual analog graphs of expired tidal volume (ETV) (right above), chest expansion (left above), dyspnea score (right below) with their autocorrelation with trend lines, and CXR showed an improvement of aerotion in the right lower lobe on the 7th day of treatment (left below). (Leelaraungrayub et al., 2009)

baseline. Expirated tidal volume and chest expansion were significant difference, and dyspnea score reduced. Moreover, chest radiography of post-treatment showed increasing in the lung volume and less infiltration.

9. Conclusion

Chest mobilization techqniues are very useful in clinical practice for improving lung ventilation and gas exchange. They also can be applied in various cases, for example, chronic obstructive pulmonary disease (COPD), pneumonia, chronic illness from stroke, spinal injury, prolonged use of a ventilator, etc. These techniques can be applied with others such as breathing exercise, cough training, or exercise in regular pulmonary rehabilitation. Before and after intervention, assessments of observations, palpation or chest expansion measurement,, including X-ray recheck and lung function test, are very important for confirmation of clinical improvement with a single case research design. Improvement of ventilation and gas exchange is very important in gaining health status or quality of life in ICU, or sub-acute or chronic stages. Efficiency of aerobic capacity directs the function and physical performance in daily life. However, this chapter is an example of interesting theory that needs more study to confirm its results. It is hoped that there will be more reports or wider application of chest mobilization in hospitals and communities for improving health status and pulmonary rehabilitation.

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21

Antipneumococcal Vaccination in COPD Patients

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1. Introduction

Streptococcus Pneumoniae, the most common cause of community-acquired pneumonia (CAP), remains a major cause of morbidity and mortality worldwide. Despite appropriate antibiotic therapy and intensive care treatment, mortality rates due to pneumococcal infections remain considerable, especially in elderly and high-risk individuals such as patients with chronic heart or pulmonary disease (Kyaw 2005).

The main reservoir of pneumococci is the nasopharynx, and the possible outcomes after colonisation are clearance by the organism, asymptomatic persistence of infection (carrier state), or progression to disease. Disease presentation depends on whether the bacteria spreads to adjacent mucosal tissues causing mucosal infections (otitis, sinusitis, bronchitis and nonbacteraemic pneumonias) or whether it invades the bloodstream, or other sterile sites, resulting in invasive pneumococcal disease (IPD), principally bacteraemic pneumonia, meningitis and sepsis. The outcome is a complex process that depends on interactions between factors related to the host, therapy and microorganism (Feikin 2000, Baddour 2004). Figure 1 illustrates the overlap between overall community-acquired pneumonia, pneumococcal pneumonia and IPD.

The reported incidences of IPD have widely varied in different studies. These differences probably reflect different rates of obtaining blood cultures from patients with pneumonia. The incidence of bacteremic pneumococcal pneumonia ranged from 9 to 18 cases per 100.000 adults-year in a multicentre study carried out in five countries (Kalin 2000). The true incidence of nonbacteremic pneumococcal pneumonia is unknown, but it is probably 3-4 fold higher considering that it has been estimated that 80% of all pneumococcal pneumonias happen without bacteremia (Orqvist 2005).

Chronic obstructive pulmonary disease (COPD) is a major risk factor for community-acquired pneumonia, and smoking (the most common cause of COPD) has been reported as an important risk factor for IPD (Torres 1996, Nuorti 2000).

Nowadays, COPD is a leading cause of morbidity and mortality worldwide. The prevalence of COPD increases with increasing age (approximately 1-3% in middle aged adults *vs* 6-10% in elderly people) and it is approximately three-fold higher in men than in women (Murtagh 2005). Likely, the prevalence of COPD is underestimated given the absence of systematic

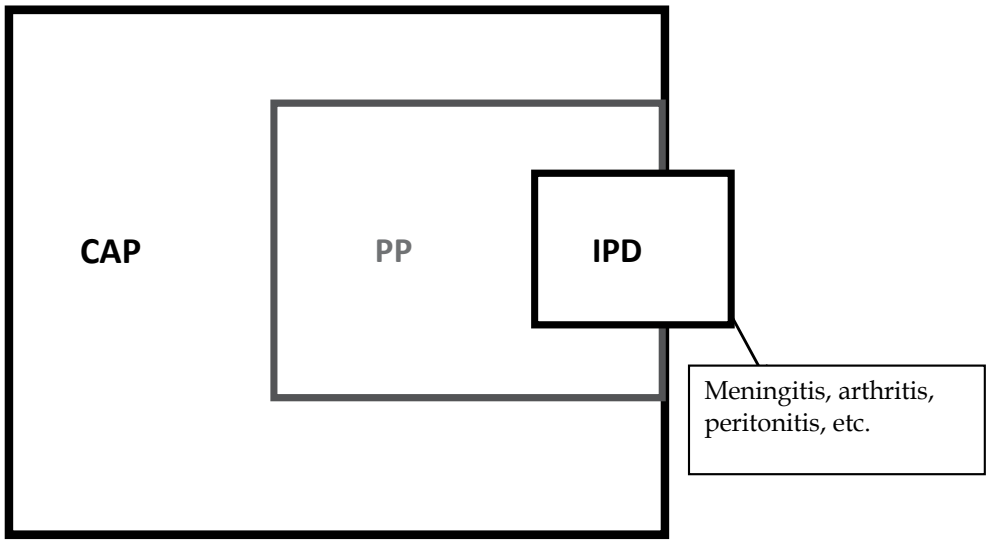

Fig. 1. Overlap between overall community-acquired pneumonia (CAP), pneumococcal pneumonia (PP) and Invasive Pneumococcal Disease (IPD).

investigations in clinical practice for those patients with apparently non-severe or trivial symptoms. It has been estimated that approximately 15-25% people over 45 years-old have a moderate obstructive ventilatory disorder (GOLD 2008). If we consider mortality, a according to World Health Organization estimates, COPD is the fourth leading cause of death worldwide, with more than 2.7 million deaths in 2000 (NHLBI 2001).

Incidence data of pneumococcal infections focused on COPD patients is scarce but, given these persons are considered to be at risk of pneumococcal infections, incidence is believed to be very large. Among patients with pneumonia, COPD is the most commonly reported comorbidity. Among COPD patients with pneumonia, hospital admission increases with the intensity of airflow obstruction. The incidence of all-cause pneumonia among people with COPD is around 40-50 cases per 1000 patients-year (approximately 3-4 fold greater than in the general population). In the United States, the reported annual incidence of hospitalisation for CAP was 11 cases per 1000 among the general population over 65 years-old and 41 cases per 1000 among those patients with chronic lung diseases (Jackson 2003). In Europe, incidences of 14 and 46 episodes per 1000 person-year have been reported among the general population and COPD patients, respectively (Vila-Corcoles 2006, Ochoa-Gondar 2008). Pneumococcus remains the most common microorganism identified among patients with chronic respiratory diseases with CAP (Liebermen 2002, Mandell 2007) although Gram-negative bacilli are increasing in patients with severe obstruction (Restrepo 2008, Ko 2008). Incidences of laboratory-confirmed pneumococcal CAP ranged from 0.5 to 2.1 per 1000 in the general population and 0.7 to 5.9 per 1000 among patients with chronic pulmonary disease (Jackson 2003, Vila-Corcoles 2006, Alfegeme 2006, Ochoa-Gondar 2008) of which approximately 25% were bacteremic and 75% non-bacteremic cases. These figures are likely to be an underestimation of the true incidence of pneumococcal bacteremia because they do not take into account persons from whom blood cultures were never obtained or those where the culture was performed after the start of antibiotic therapy. In

addition, those patients with COPD who develop pneumonia have more severe pneumonia and therefore are admitted to the intensive care unit more frequently and have significantly higher 30-day mortality than non-COPD patients (Restrepo 2008, Molinos 2009).

Acute exacerbations (although they represent a less serious illness than CAP) are also an important cause of morbidity and mortality in COPD patients (NICE 2004, Papi 2006, GOLD 2008). Approximately 50% of acute exacerbations in chronic bronchitis are triggered by bacterial infection (Sethi 2000) being pneumococcus responsible for almost a third of bacterial acute exacerbations (Saint 2001). There is an increased risk of exacerbations in COPD patients with persisting bacterial colonisation in the respiratory tract, especially in COPD patients with pneumococcal colonisation. It has been reported that pneumococcus was recovered from sputum in 33% of patients with COPD exacerbation (Bogaert 2004).

Immunizations with influenza and pneumococcal vaccines (together with smoking cessation, inhaled long-acting bronchodilators or inhaled corticosteroids) are a variety of strategies that may be effective in order to reduce incidence of pneumonia and acute exacerbations in COPD patients (CDC 1997, Black 2004, Poole 2009, Varkey 2009).

2. Types of antipneumococcal vaccines

The pneumococcus is surrounded by a polysaccharide capsule, and differences in this capsule permit serological differentiation into distinct serotypes (Hausdorff 2005). However, the existence of more than 90 distinct serotypes (differing in their chemical composition, potential immunogenicity and epidemiological impact on different population groups) has greatly complicated the development and evaluation of anti-pneumococcal vaccines.

At the moment, there are 3 established approaches to anti-pneumococcal vaccination: capsular polysaccharide pneumococcal vaccines (PPV), protein-polysaccharide conjugate pneumococcal vaccines (PCV) and protein-based pneumococcal vaccines (PBPV) (Fedson 2003, Abraham Van-Parijs 2004, Tai 2006). At present, only the "old" PPV-23 for use in adults and two "new" PCVs (PCV-10 and PCV-13), both licensed in 2010 for use in children, are available in clinical practice.

2.1 Pneumococcal polysaccharide vaccine

The currently available PPV-23 was licensed in 1983 and is usually recommended for all elderly people and some at-risk groups including those with chronic respiratory diseases. The vaccine contains capsular polysaccharide antigens from the 23 most dominant serotypes among clinical isolates of *S. pneumoniae*, accounting for approximately 80-90% of overall invasive infections in the adult population. These antigens induce type-specific antibodies (by a T cell-independent mechanism) that enhance opsonization, phagocytosis and killing of pneumococci by phagocytic cells (Fedson 2003).

Antibody response is generally satisfactory after vaccination, but children aged <2 years and immunodeficient persons do not consistently develop immunity, and certain high-risk individuals (including some people with medical co-morbidities and elderly individuals) may respond poorly (Sankilampi 1996, CDC 1997, Fedson 2003). Following vaccination there is a slow but steady decline in serotype-specific antibody titres, and pre-vaccination levels are generally reached within 5-10 years. An anamnestic response does not occur at

revaccination, although there is a significant increase in antibody levels (sometimes slightly lower than after the primary dose) (Sankilampi 1996, Artz 2003). Revaccination is only recommended for those persons who received PPV-23 before 65 years of age (CDC 1997) but its clinical effectiveness has not been clearly proved (Artz 2003).

Despite many studies of PPV efficacy in different populations, few randomized-controlled trials (RCTs) to date were focused on COPD patient (Leech 1987, Davis 1987, Alfageme 2006, Steentoft 2006, Ya Tseimakh 2006, Teramoto 2007, Furumoto 2008) and they have reported unconclusive results. Outcome measures in the different trials were very heterogeneous and included pneumonia, acute exacerbations, change in lung function, hospital admissions or visits to the emergency department and mortality (includes mortality from respiratory disease, causes other than respiratory disease and all-cause mortality). The heterogenity of outcomes reported in the distinct trials, together with the low accuracy of the criteria diagnosis for COPD (not verified by spirometric data in some trials), largely limits the comparison of the different results and their interpretation.

In two earlier RCTs published in 1987 evaluating a 14-valent PPV, Davis et al and Leech et al did not observe any efficacy of pneumococcal vaccination, but these negative results were attributed to the small number of patients included in the series and the low rate of pneumococcal bacteremia. Importantly, before vaccination, antibody titers were higher among the COPD patients than among the healthy control subjects in both trial, which suggests previous pneumococcus exposure and largely limits possible conclusions on vaccine efficacy in this population (Leech 1987, Davis 1987).

In the largest RCT on PPV efficacy in COPD patients published to date, Alfageme et al analysed the efficacy of PPV in a RCT including 596 Spanish patients with spirometric diagnosis of COPD (298 receiving PPV-23 and 298 receiving placebo), concluding that the efficacy of vaccination depends on the age and the severity of airflow obstruction. Considering overall study population, in Alfageme's trial, no differences in the risk of all-cause pneumonia was observed in vaccinated as compared with control subjects (OR: 1.03; 95% CI: 0.64-1.67). In subgroup analyses including only cases due to pneumococcus (5 cases) or unknown etiology (53 cases) pneumococcal vaccination appeared effective among subjects under 65 years (OR:0.24; 95% CI: 0.07-0.80), but it did not appear efficacious among COPD patients 65 years or older (OR: 1.14; 95% CI: 0.62-2.07). Among those patients with severe functional obstruction (forced expiratory volume in 1 second <40%) vaccination appeared to be more efficacious (OR: 0.52; 95% CI: 0.20-1.07), with greatest efficacy in younger patients with severe airflow obstruction (OR: 0.09; 95% CI: 0.01-0.65) (Alfageme 2006).

In a short trial including 49 COPD patients, Steentoft et al observed that a rise in antibody levels after PPV-23 occurred among patients with COPD despite the use of systemic steroid treatment, but a statistically significant clinical effect of vaccination was not demonstrated. In fact, no differences between vaccinated and control subjects were observed for the risk of pneumonia (OR: 0.59; 95% CI: 0.15-2.32), acute exacerbations (OR: 1.44; 95% CI: 0.29-7.14) or hospital admission (OR: 0.95; 95% CI: 0.26-3.48) (Steentoft 2006).

In 2006, Granger et al published the first Cochrane systematic review and meta-analysis on PPV efficacy focused on COPD patients, concluding that PPV was not effective in this population to reduce all-cause pneumonia (OR: 0.89; 95% CI: 0.58-1.37) or all-cause mortality (OR: 0.94; 95% CI: 0.67-1.33) (Granger 2006).

In 2010, Walters et al uptated the Cochrane review including a total of 7 RCTs in their meta-analysis specifically focused on COPD patients. According this meta-analysis, in six studies involving 1372 people, the reduction in the risk of developing pneumonia among vaccinated compared to control did not achieve statistical significance (OR: 0.72; 95% CI: 0.51-1.01). The reduction in likelihood of acute exacerbations of COPD from two studies involving 216 people neither reached statistical significance (OR: 0.58; 95% CI: 0.30-1.13). Of the secondary outcomes for which data were available there was no statistically significant effect for reduction in hospital admissions (two studies) or emergency department visits (one study). Considering mortality, according to three studies involving 888 people followed during periods up to 48 months post-vaccination, there was no significant reductions in the risk of all-cause death (OR: 0.94; 95% CI: 0.67-1.33), or death from cardiorespiratory causes (OR: 1.07; 95% CI: 0.69-1.66). The authors concluded that, while it is posible that PPV may provide some protection against morbidity in persons with COPD, no significant effect on any of the outcomes was shown in the meta-analysis, recomending that further large RCTs in this population would be needed to confirm the effectiveness of the vaccine suggested by results from some individual studies (Walters 2010).

In the present authors opinion, all RCTs on PPV efficacy focused in COPD patients has been largely underpowered considering that the most large RCT (Alfageme 2006) included less than six hundred patients (with only five definitive pneumococcal pneumonias observed during 3-year follow-up). Furthermore, given the effectiveness of the vaccine in protecting individuals against IPD, commencing new RCTs in populations at risk where vaccine effectiveness and disease burden is known would create ethical difficulties. Thus, although nonRCTs have inherent limitations (especially the possibility of selection bias), they can provide interesting data on the effectiveness and impact of the vaccination. In this way, several observational studies have reported benefits using the PPV-23 in patients with chronic respiratory diseases (Nichol 1999, Ochoa-Gondar 2008, Watanuki 2008, Sumitani 2008).

On other hand, given COPD is not a cause of immunodepression (apart from the impairment of local defences) and the reported antibody response is compatible with a vaccine efficacy despite its relatively rapid decline, data on efficacy in the general population can also be used to establish vaccine recommendations for these persons. Figure 2 shows point estimates of PPV efficacy against IPD, pneumonia and death according to the two last published meta-analyses (Moberley 2008, Huss 2009).

The last Cochrane review on PPV efficacy/effectiveness among the general population recommends the use of PPV to prevent IPD in adults (particularly otherwise healthy adults), but it also concluded that the meta-analysis did not provide compelling evidence to support the routine use of PPV to prevent pneumonia or death. This meta-analysis demonstrates strong evidence of protection against IPD, with an efficacy of 74% (95% CI 56% to 85%) in RCTs and an effectiveness of 52% (95% CI 37% to 61%) in observational studies (case-controlled and cohort studies). Vaccine efficacy appears poor amongst the subgroup of adults with chronic diseases, where vaccination efficacy did not reach statistical significance. In relation to all-cause pneumonia (the most reported outcome in the Cochrane review, the meta-analysis showed that the PPV provides an apparent protective efficacy of 29%, although substantial statistical heterogeneity was observed (OR: 0.71; 95% CI: 0.52-0.97) (Moberley 2008).

We note the limited amount of data regarding persons with chronic pulmonary diseases. Considering RCT's data, vaccination of younger patients with COPD appears best supported, while the evidence of a benefit to older patients is weaker. However, given

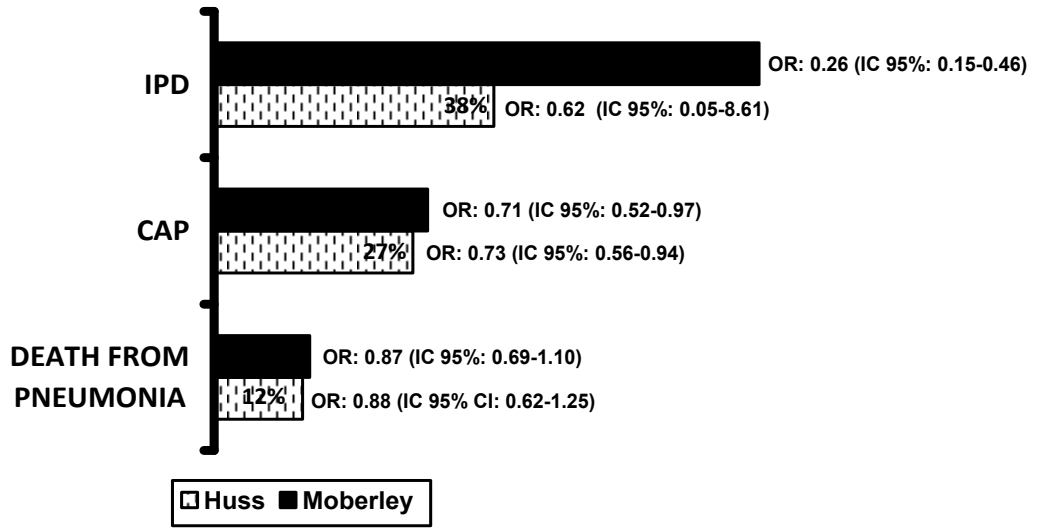

Fig. 2. Estimation of PPV efficacy against IPD, pneumonia and death according to the two last published meta-analyses (Moberley 2008, Huss 2009).

observational studies, PPV also appears effective in older patients with COPD. Because the risks of immunization are believed to be very small, public policy at this time continues to support immunization of all patients with chronic lung diseases regardless of age (CDC 1997, CDC 2010). New CDC's recommendations for using the PPV in adults have been publihsed in 2010. The CDC's new recommendations include some changes from 1997 recommendations.the indications for which PPV-23 vaccination is recommended now include smoking and asthma(CDC 2010).

2.2 Pneumococcal conjugate vaccines

Given the poor immunogenicity of PPV in children, extensive efforts have been made to develop a new generation of pneumococcal vaccines with good immunogenicity in infants. The result was a protein-polysaccharide combination, known as pneumococcal conjugate vaccine (PCV), which contains selected polysaccharides bound to a protein carrier. This renders the vaccine T-cell-dependent, and thus capable of stimulating antibody responses and priming for a memory response on rechallenge. The firstly available PCV contained specific antigen for the 7 most common pneumococcal serotypes in children, and was licensed for paediatric use in 2000 (Black 2000).

In contrast to the PPV-23, which only had a limited impact on the overall disease burden, the introduction of the PCV-7 as routine vaccination for infants has provided very encouraging results, even reducing incidences of pneumococcal disease in unvaccinated people (by herd immunity reducing the transmission of PCV-7 strains in the population) (Whitney 2003, Hicks 2007). In addition, an important reduction in drug-resistant Streptococcus pneumoniae isolates has been observed in all-age groups after the introduction of PCV-7 for children (Kyaw 2005).

Among people over 50 years in the United States, IPD declined by 28% (from 40.8 to 29.4 per 100,000 person-year between 1998-2003) (CDC 2005) with further reductions in recent years

(Pilishvili 2010). Nevertheless, it must be noted that for some groups of older adults the reduction was somewhat lower. There was only a very modest reduction in the number of cases in subjects with comorbid conditions, such as chronic renal disease, heart disease and chronic pulmonary disease (Lexau 2005, Lockhart 2006).

Considering the good immune response and efficacy shown in children, it has been proposed that the use of the conjugate vaccine could improve antibody responses and clinical efficacy in high-risk adults with poor response to PPV (Fry 2002, Lockhart 2006, Jackson 2008). An important immunological consequence of conjugation of polysaccharide antigen with a carrier protein is that the CD4+ helper T-cell fraction contributes to the immunological response. Thus a T-cell-dependent response is generated, with predominant IgG1 and IgG3 antibodies, instead of the T-cell-independent antibody response that occurs with simple polysaccharide antigens (Wuorimaa 2001). This is an important advantage for the conjugated vaccine, given that the response to polysaccharide antigens is much more varying and age-dependent, and antibody levels therefore more uncertain than with conjugated antigens. Thus, as in young children, adult population groups could obtain benefit from using a conjugate vaccine in the future.

Until now, the low serotype coverage has been a very important shortcoming for the "old" PCV-7, bbut he new PCVs including more serotypes (especially the PCV-13, which has broad serotype coverage for both children and adults) could be a good future alternative for all age groups (Scott 2008).

However, at the moment, there are important factors to consider before PCV could ever be used in adult populations. There are only limited immunogenicity data and no data on clinical efficacy in adults. Furthermore, it is not known how many doses of conjugate vaccine adults would require, what age groups should receive the vaccine, and what would be the optimal timing for pneumococcal conjugate vaccination (Abraham Van-Parijs).

2.3 Protein-based pneumococcal vaccines

Although the virulence of Streptococcus pneumoniae is largely dependent on its polysaccharide capsule, it has been demonstrated that numerous protein virulence factors are involved in the pathogenesis of pneumococcal disease (Orihuela 2004), and currently extensive efforts are being made to develop a new generation of pneumococcal vaccines. These vaccines, known as protein-based pneumococcal vaccines (PBPV), are composed of pneumococcal proteins or virulence factors, together with antibodies to them to neutralize their function and reduce the virulence of the infecting bacteria (Tai 2006).

Several formulations of experimental PBPV candidates containing different pneumococcal proteins (eg, PspA, PspC, Ply, or PsaA) have shown protective effects against invasive infections and nasopharyngeal carriage in animal models, and some studies assessing the development of natural antibodies after carriage and invasive disease in humans have reported development of an immune response against some of them (Tai 2006). It has been reported that the combination of various proteins with different protective functions may provide a broader protection (Ogunniyi 2007). Furthermore, other pneumococcal proteins identified very recently by exploiting molecular immunological techniques suggest interesting new vaccine directions (Giefing 2008).

Theoretical major advantages for a future PbPV could be the serotype-independent protection, the possibility of oral or intranasal administration, and probably a less complex production process and a lower cost than conjugate vaccines. However, at the moment, information on humans is scarce, and many studies and several years will be needed to elucidate the true potential of PbPV in human prevention. If finally these proteins can not provide sufficient protection as a sole component of the vaccine, it is posssible that they could be used either as a carrier protein for a conjugate vaccine or as a supplement component for the current vaccines to provide additional protection against pneumococcal infections (Wright 2008).

3. Conclusions

S. pneumoniae remains a major cause of morbidity and mortality worldwide. There are different preventive options but, at the moment, none is optimal. Among patients with chronic respiratory diseases, pending other more effective antipneumococcal vaccines, the PPV-23 (together with influenza vaccine) is currently the only preventive approach that has demonstrated an effect, even if it does not match up to expectations (Gaillat 2009).

COPD patients are commonly described as an at-risk population for pneumococcal infections, but RCTs on PPV efficacy in such patients are very limited and largely underpowered to obtain a reliable conclusion about the efficacy of the vaccine. Among the general population, most meta-analyses have concluded that the PPV is effective against IPD among immunocompetent persons. Recommendations for vaccinating COPD patients are based on this data, although the evidence for vaccine efficacy is less clear among persons with comorbidities.

Among COPD patients, the effectivenes of vaccination in preventing pneumonia and/or acute infective exacerbations is unclear. Two meta-analyses focused on COPD patients concluded that, although it is possible that PPV may provide some protection in persons with COPD, no significant protective effects were demonstrated in the meta-analysis. Considering nonRCTs, the clinical effectivenes of vaccination is also uncertain, but several studies have reported distinct benefits from pneumococcal vaccination in preventing distinct respiratory infections (using the PPV-23 alone and/or together with influenza vaccine).

Several studies have shown that the PPV-23 is cost-effective for preventing IPD among the general population over 65 years in developed counttries, but there is no data about cost-effectiveness of vaccination among COPD patients given the lack of efficacy data in these persons. Current CDC's recommendations for using PPV-23, besides COPD, include smoking and asthma. Revaccination (5-10 years after prime dose) is recommended for those persons who received PPV-23 before 65 years of age. It must not be forgoten, however, that the PPV-23 provides incomplete protection, it does not elicit long-lasting immunity, and no anamnestic effect occurs at revaccination. So, more effective vaccination strategies are needed.

In the next few years, the results of ongoing trials evaluating the efficacy of the PCVs in adults will be critical in determining the position of the conjugate vaccine in the prevention of pneumococcal diseases in patients with chronic respiratory diseases. In coming years, new PCVs including progressively more serotypes (most likely emerging types due to epidemiological changes) will probably be needed. However, the serotype replacement phenomenon can not be fully overcome by increasing the number of serotypes, so new

technologies, such as protein-based or genomic vaccines, will be greatly needed. Experimental protein-based pneumococcal vaccine candidates offer the potential advantage of serotype-independent protection and several are in various stages of development in animal models, but none can be expected to be available in clinical practice for several years at least.

Until better options are available, the PPV-23 should continue to be used in high-risk individuals, including younger and older adults with COPD. Although only moderately effective, the burden of pneumococcal disease is greatest in these persons and they can obtain benefit from vaccination.

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22

A Multi-Targeted Antisense Oligonucleotide-Based Therapy Directed at Phosphodiesterases 4 and 7 for COPD

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1. Introduction

Recent drug development for chronic obstructive pulmonary disease (COPD) has focused on strategies aimed at reducing the underlying inflammation by selective inhibition of phosphodiesterases (PDE), specifically the PDE4 isoforms. The anti-inflammatory and bronchodilator activities of PDE4 inhibitors have been well documented (Giembycz &Field 2010), however their clinical development has been hampered by their low therapeutic ratio and dose-dependent systemic side effects. PXS TPI1100 is an inhaled drug candidate consisting of two modified antisense oligonucleotides (AON) directed at PDE isoforms 4B, 4D and 7A. PXS TPI1100 has been designed to reduce the recruitment and persistence of inflammatory cells in COPD through an unique mechanism of action and has the potential to be a novel, highly effective approach for this respiratory disease.

In this chapter, we will present the rationale for the design of PXS TPI1100 including a summary of the PDE families and the proposed role they play in regulating inflammation in the lung. Next we will present an overview of the discovery and selection process for the drug candidate, including a summary of the key results from pre-clinical pharmacology, both *in vitro* models as well as two *in vivo* models of neutrophilic inflammation: cigarette smoke mouse model and LPS challenge model. These results will be compared to the first-in-class PDE4 inhibitor, roflumilast (Daxas/Daliresp). We shall conclude with the expected development plan for PXS TPI1100 including the design of upcoming clinical study trials.

2. Chronic obstructive pulmonary disease

COPD is a respiratory disease of airway obstruction and lung damage and is sometimes called chronic bronchitis and/or emphysema. COPD kills millions of people each year and it is currently the fourth leading cause of death worldwide, with forecasts to be the third leading cause by 2020 (ref www.goldcopd.com). COPD, as defined by the Global Initiative for Chronic Lung Disease (GOLD) is *"a preventable and treatable disease with some extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is*

usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" (Gold 2009). Symptoms of COPD include chronic cough, excessive sputum production, wheeze, shortness of breath and chest tightness. The 4 stages of COPD, designated as Mild, Moderate, Severe and Very Severe, are defined according to lung function as assessed by spirometry, usually the post-bronchodilator ratio of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC). The cellular and molecular mechanisms that contribute to COPD pathogenesis remain incompletely understood yet it is believed that COPD is caused by underlying inflammation characterized by increased presence of neutrophils, macrophages and CD8+ T cells (Gold 2009). Products of neutrophils induce mucus hypersecretion and are implicated both in the generation of mucus metaplasia in chronic bronchitis and the destruction of lung tissue in emphysema. Macrophages are also sources of proteinases and antiproteinases in the lung, oxidative stress and mucus hypersecretion (Ward 2010). Exacerbations play a large role in the disease progression of COPD, and exacerbations become more frequent and more severe as COPD progresses (Hurst et al. 2010).

2.1 Traditional management of COPD

Currently, the only intervention known to influence the loss of lung function is smoking cessation (Gold 2009). Besides treating symptoms and improving quality of life, the treatment focus includes prevention of future exacerbations, reduction of mortality and prevention of disease progression. Treatment for COPD falls into two categories: those medications which relieve symptoms of airflow limitations and those medications which control the underlying inflammation. As such, the current gold standard of treatment for COPD patients involves a step-up paradigm commencing with short-acting bronchodilators (either short-acting β2 agonists or antimuscarinic agents), then adding on long-acting bronchodilators again either long-acting β2 agonists (LABA) or long acting muscarinics, (LAMA) followed by inclusion of inhaled corticosteroids (ICS). Lastly, long term oxygen and possible surgical treatments are final treatment options. Typically, the most common treatment involves ICS/LABA class of drugs, but can also include methylxanthines (bronchodilator) and leukotriene antagonists (anti-inflammatory) (Hurst et al. 2010). The majority of novel treatments for COPD forecasted to launch prior to 2018, are in fact minimally differentiated from current options, with either being improved dosing or combining therapies such as combinations of LABA/LAMA.

Another dilemma is that although highly effective in asthma, ICS have provided little therapeutic benefit in COPD (Barnes 2006). In patients with severe COPD, histological analysis of their peripheral airways have shown an intense inflammatory response, despite treatment with high doses of ICS, suggesting steroid resistance (Hogg et al. 2004). Combinations of ICS and LABA have been shown to be more effective at reducing COPD exacerbations (Calverley et al. 2007) but have not been shown to statistically decrease mortality (Calverley et al. 2007) (Tashkin et al. 2008). ICS use has been associated with osteoporosis, glaucoma, cataracts and skin thinning (Giembycz &Field 2010) and increased risk of pneumonia in patients with COPD (Ernst et al. 2007). Even with the current and immediate future medications, there are clear unmet needs for more effective anti-inflammatories in COPD both for reducing progression of the disease and reducing mortality.

2.2 Phosphodiesterases as targets for COPD

PDE4 is a member of the PDE family of enzymes whose function is to selectively catalyze the hydrolysis of cycle adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP) (Bender &Beavo 2006). Second messengers perform intracellular signaling and cAMP is a key member. The level of cAMP can be regulated by its rate of degradation which is controlled by PDEs (Figure 1). As such, the regulation of PDEs is sophisticated and complex. This family currently includes 11 members (PDE1 to PDE11) of which there are multiple isoforms or splice variants. Several different PDEs can be expressed in a single cell type, and the localization of these PDEs within a cell regulates the local concentration of cAMP or cGMP. Besides being regulated through differential genetic expression, PDEs can be biochemically regulated by phosphorylation, binding of Ca2+/calmodulin and various protein-protein interactions (Bender &Beavo 2006). The PDEs with higher affinity for cAMP than cGMP include PDE3, PDE4, PDE7, PDE8 and PDE11 (similar affinities). These multiple isoforms and their differential expression across cell types

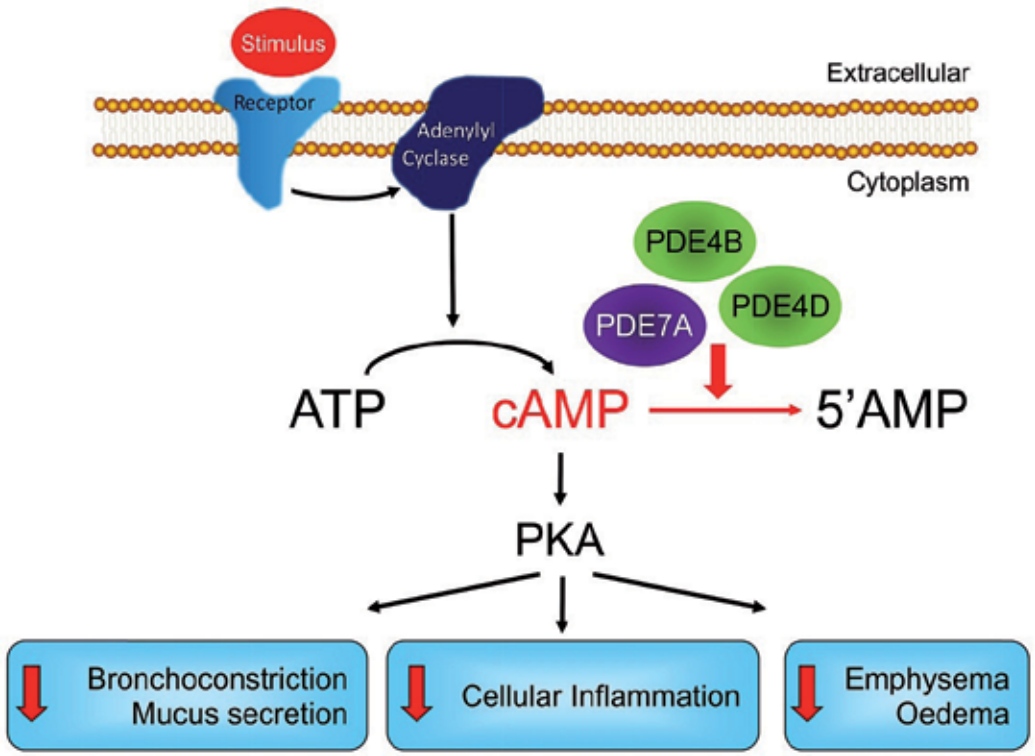

Fig. 1. Cartoon of the cAMP pathway, which is presumably activated upon binding of a stimuli to its receptor embedded in the cell membrane. Known components of this pathway include the calcium/calmodulin-activated adenylyl cyclase, the phosphodiesterase (PDE), and cAMP-dependent protein kinase (PKA) with its catalytic and regulatory subunits. Activation of PKA will lead to phosphorylation of cytoplasmic and nuclear targets. In the lung, inhibition of the PDE will lead to an elevation of the intracellular levels of cAMP resulting with a reduction of the bronchoconstriction, mucus secretion, cellular inflammation and in the long term decrease the emphysema/oedema.

are reasons PDEs are good drug targets as selective inhibition of a specific PDE isoform would limit nonspecific sides effects associated with broader PDE inhibition.

Another reason PDEs have been the focus of drug companies is based on the pharmacologic principle that a more rapid and larger percentage change in concentration is achieved through regulating the degradation of a second messenger than comparable regulation of the rates of synthesis (Bender &Beavo 2006). In most cells the levels of cAMP are between <1 to 10 μM which enables a competitive inhibitor to not need to compete with high levels of endogenous substrate to be effective, in contrast to many protein kinase inhibitors which need to have sufficient affinity to displace mM concentrations of ATP (Bender &Beavo 2006).

There are four PDE4 (A/B/C/D) genes which generate multiple variants as a result of splicing differences in their N termini (Bender &Beavo 2006). PDE4 isoforms, which are widely expressed in many tissues and cell types including the lung, have been shown to play a key role in macrophage and monocyte activation and functions, neutrophils infiltration and vasodilation (Table 1). There has been more information collated on PDE4 than other PDEs mostly from the work resulting from PDE4A, 4B and 4D knock out mice. In PDE4D knockout mice, their airways were shown to be refractory to cholinergic stimulation (Mehats et al. 2003) while PDE4B knockout mice were shown to have effects on immune cells (Jin &Conti 2002; Jin et al. 2005) and both genes were shown to be required for neutrophils recruitment in a model of lung injury in response to inhaled endotoxin (Ariga et al. 2004).

A new first-in class treatment, the PDE4 inhibitor Daxas/Daliresp (Nycomed), has recently been approved in Europe in 2010 and in the USA in 2011 for patients with severe COPD.

	Structural Lung Cells			Inflammatory Cells		
cAMP modulator	Lung Epithelium	Smooth Muscle	Epithelial Cells	Monocyte/ Macrophage	Neutrophils	T-Cells
PDE3	+	+	++	+++	+	+
PDE4	++	++	+++	++++	+++	++
PDE7	+++	+++	+++	+++	+	+
PXS TPI 1100	●	●	●	●	●	●

Table 1. Expression of different cAMP-modulating PDE isoforms in lung cells and inflammatory cells. PDE4 and PDE7 are highly expressed in lung structural cells as well as in inflammatory cells. Delivered to the lung, PXS TPI1100 can inhibit expression of PDE4 and PDE7 in both lung structural and inflammatory cells.

Daxas (3-cyclopropylmethoxy-4-difluoromethoxy-*N*-[3,5-di-chloropyrid-4-yl]-benzamide) is a once-a-day tablet, taken orally, whose principal action is to reduce inflammation. The clinical results from the six Phase III trials performed using Daxas will be reviewed below. Before, touting the benefits of PDE4 inhibitors in COPD, it is important to note that Daxas is not without its adverse events which include diarrhea, weight loss, nausea, headache and abdominal pain (Giembycz &Field 2010), which have been observed previously with other PDE4 inhibitor drugs (Down et al. 2006).

Like the PDE4 family, the PDE7 family, which consists of PDE7A and PDE7B, is highly selective for cAMP as a substrate (Bender &Beavo 2006). While the function of PDE7 has not been fully elucidated, PDE7 isoforms have been implicated in the activation of inflammatory cells (Li L et al. 1999), including T cells (Smith et al. 2003). PDE7A mRNA has been shown to be expressed in multiple tissues including the lung and inflammatory cells (Table 1) (Bloom &Beavo 1996) (Han et al. 1997) (Lugnier 2006). Inhibitors of PDE7 have shown to potentiate the effects of PDE4 inhibitors, suggesting that a combined PDE4:PDE7 inhibitor would be an effective drug.

3. PXS TPI1100: The drug

The relative lack of advancement and the slow pace of innovation to identify new drug products for COPD can be indicative of the complicated nature of this chronic diseases as well as a potential limited number of targets for conventional small molecule drugs and biologics. Moreover, the activity of cytokines, growth factors and chemokines depends on the interaction of these proteins with their cell surface receptors involving large protein-protein interactions or involving interactions between multiple sites on the protein, which could be particularly challenging to disrupt with small molecule inhibitors or biologics (Johnson et al. 2005). To side-step these complications, we have attempted to design an antisense oligonucleotide (AON) based therapy which functions by targeting RNA directly rather than the protein product.

3.1 Antisense oligonucleotides: An overview

Oligonucleotides (ODN) are short polymers of nucleotides that come in various forms, lengths and modifications which can be distinguished into two main groups based on two distinct mechanisms of action; ODN in the first group target RNA and those from the second group target proteins.

RNA-targeting ODN drugs are designed to bind to a specific sequence of a messenger RNA (mRNA) through Watson-Crick base-pairing interactions. Therefore, the site of action of this class of drug is not the protein itself, but rather "upstream" of it, the RNA coding for the protein. The principle of RNA-based therapy is the reduction in the level of a protein through hindrance of its translation. Archetypes of this class of ODN are AON and small interfering RNA (siRNA). AON drugs are single stranded, usually only approximately 20-bases long, which prevent translation of the target RNA via one of two mechanisms. The first mechanism involves the activation of the enzyme RNAse H, which cleaves the RNA moiety of the duplex formed by the binding of the AON drug to its target RNA leading to subsequent reduction in protein synthesis (Stein &Hausen 1969). The second mechanism involves a steric interaction of the AON with the target mRNA that prevents key maturation

steps processes such as splicing and thus preventing translation (Crooke 2008). siRNA drugs share the same mechanism of action to AON, degradation of the protein encoding RNA. However, these drugs are distinct from AON molecules, as they comprise double stranded RNA (varying from 19 to 27 base pair long) (Wu et al. 1998) and induces silencing via the RNA-induced silencing complex (RISC), which is composed of several proteins, including specific RNA-degrading enzymes (Holen et al. 2003).

Similar to conventional small molecules drugs or biologics, the second group of ODN comprises molecules that target proteins directly. Two examples of this group include aptamers and immunostimulatory sequences (ISS). Aptamers comprise either DNA or RNA and typically have a longer chain length (ie, approximately 40 nucleotides) than other ODN. These agents have a specific 3D structure (Ellington &Szostak 1992; Jayasena 1999) that determines their ability to bind specifically to their protein target acting in a similar manner to conventional antibody therapies (Lee et al. 2006). ISS molecules are single stranded, which sequence is enriched with unmethylated cysteine and guanine motifs (CpG) motifs (Vollmer et al. 2004). ISS can mediate immunostimulatory effects following binding to TLR9, a key member of the innate immune system (Agrawal &Kandimalla 2007).

ODN drugs share a relatively common chemical composition that is based on naturally occurring RNA and DNA, and comprises the three elements of nucleotide bases, pentose sugars and linking phosphate groups. In the past decade, medicinal chemistry has allowed significant improvements in the drug-like properties of ODN including the potential to optimize the stability as well as the pharmacologic, pharmacokinetic and toxicologic properties of these molecules. In general, three types of modifications of ODN can be distinguished. The first type of modification, and the one most commonly used, is the replacement of the oxygen atoms of the naturally occurring phosphodiester bond by sulfur groups (phosphorothioate (PS) linkages) to confer stability to the drug molecule. Nucleotide analogs have also been incorporated. For example, adenosine has been replaced with 2-amino-2'-deoxydenosine, which improves binding of the drug to the target and minimizes the potential for bronchospasm and inflammation induced by adenosine (Vollmer et al. 2004). Finally, the sugar moiety can be modified; for example, the addition of a 2'-*O*-methoxyethyl group to the pentose sugar confers stability to the ODN and enhances binding affinity to the target mRNA (Ward 2010).

The AON constituents comprising PXS TPI1100 incorporate two modifications: a modified phosphate backbone and the incorporation of 2-amino-2'-deozyadenosine. These modifications were aimed at improving the binding affinity of the drug to its mRNA target, reduce the immunostimulatory effect of this class of drug, and improve the lung tolerability after administration by the pulmonary route. *In vivo* testing of these molecules by multiple dosing via intratracheal (i.t.) administration in mice demonstrated that the modified chemistry contained in the PXS TPI1100 sequences was far less immunostimulatory than the typical PS-containing AON. Repeated daily i.t. delivery of PS-containing AON at a dose of 2.5 mg/kg induced a 4-fold increase in the recruitment of total cells in bronchoalveolar lavage (BAL) compared to control mice (treated with vehicle) and lung tissue changes as assessed by the presence of moderate (grade 3) perivascular mixed cell infiltrate and severe (grade 4) alveolar inflammation. In contrast, in mice treated with the same dose of AON bearing the modified chemistry no difference in BAL cells (total cells as well as differential cells) as compared to the vehicle group were observed, nor were there any histopathological

changes in the lung following administration of the modified AON demonstrating an overall improved lung tolerability.

3.2 Drug design

The drug candidate, PXS TPI1100, is a 1:1 mixture of two AON, one which targets two isoforms of PDE4 (4B and 4D) and the second AON targeting PDE7A (Fortin et al. 2008). The rationale for developing these new specific and multi-targeted AON is to provide a new class of anti-inflammatory agents that act more broadly on the underlying inflammatory-triad - recruitment, activation and potentiation of processes in chronic respiratory diseases and that is more potent than selective PDE4 inhibitors. Delivery directly to the site of action, the lung, will ensure local deposition of the drug and limited systemic exposure thus reducing potential side effects associated with the systemic delivery (e.g. oral delivery) of PDE4 inhibitors. Lastly, PXS TPI1100 consists of aerosolization of a simple aqueous solution, and does not require any specialized carriers.

AON drugs, while still early in development, possess properties that could be advantageous over classical small molecule drugs (Table 2). First, as a single mRNA strand can be translated into multiple copies of proteins (~5000 copies), there is a clear advantage of "upstream" targeting, that is targeting the mRNA rather than the protein (Popescu 2005). The "upstream" targeting approaching with AON can be achieved irrespectively of the location of the target protein, whether it is inside the cell or outside the cell. AON have the potential to amplify

Potential advantages	• High degree of specificity (primarily for RNA-targeted drugs) • Broad range of potential targets • Ability to modify the properties of the oligonucleotide through chemical modification • Ability to screen efficiently for off-target effects • Absence of hypersensitivity reactions • Relatively short development timelines • Relative ease of formulation for inhaled delivery • Relative ease of formulation of combination products • Relative stability of drug compound and product
Challenges	• Cellular uptake and intracellular release for larger oligonucleotides • Potential immunostimulatory effects • Oligonucleotide stability • Specific systemic toxicological findings
Potential advantages of application in lung disease	• Multi-targeting feasible • Direct delivery to the site of action in the lungs • Cellular uptake and release without additional carrier or formulation technologies • Low systemic exposure

Table 2. Advantages and challenges in the development of antisense oligonucleotides drug candidates.

potency as compared to small molecule drugs which target the protein directly. Furthermore, by targeting the mRNA this method avoids the complications of protein interactions and effects of phosphorylation which can be of concern for PDE inhibitors.

By its very nature, AON are designed to target a specific RNA sequence and this specificity lends an advantage over ICS. As comparison, corticosteroids which are believed to directly regulate between 10 to 100 genes per cell, with a further estimation of many other genes indirectly regulated through interaction of other transcription factors and coactivators by yet unclarified mechanisms (Barnes 2006). In contrast, the inherent specificity of AON for its target avoids the non-selective inhibition nature of steroids. However, AON, as all drugs, have the potential of causing unwanted toxicities or side effects, of which some of these unwanted toxicities can arise because of the inherent capacity of AON to hybridize to RNA. Such toxicities are termed hybridization-dependent and can be subdivided into effects caused by exaggerated pharmacology, i.e. inhibition of the intended target to a degree that produces deleterious effects, and hybridization-dependent effects on unintended RNA targets (off-targets) that happen to be completely or partially complementary to the AON sequence. For the former, with recent advances for the modifications of the AON chemistry to improve binding affinity, as well as improvements for more effective delivery systems, there could be potential risk in designing AON that are too effective. Correct dosing assessment would be imperative. With regard to off-target effects, the use of genomic information databases allows for identification of possible off targets early in the drug discovery process. Any potential off targets can then be monitored both during the preclinical development and safety assessment stage as well as in clinical studies if needed. Along with the hybridization-dependent toxicities, there are also hybridization-independent which are due to interactions between the AON and proteins. The majority of toxicities observed for AON tested to date are hybridization independent and result from AON chemistry or composition of the delivery system and such potential is assessed in animal toxicology studies (Levin et al. 2001).

A further advantage of AON is the common composition and chemical nature of AON allow for an ease in combining two or more AON for a multi-targeted drug, unlike typical combination therapies. Historically, combination therapies have resulted from combining two marketed drugs into a single drug product. In the respiratory space, the combination of a corticosteroid with a long acting β2-adrenergic receptor agonist has been effective at producing billion dollar drugs like Advair (fluticasone/salmeterol), and Symbicort (budesonide/formoterol). Each of the individual components of these drugs had undergone the development process as single entities which were then combined later for a final product. However, the current understanding of various disease systems would suggest the selection and development of drugs that contain at least 2 molecules directed against at least 2 targets from the beginning of the development process.

The rationale for developing these new specific and multi-targeted ODN inhibitors is to provide a new class of anti-inflammatory agents that act more broadly on the underlying inflammatory-triad - recruitment, activation and potentiation of processes in chronic respiratory diseases. Complex diseases require multiple approaches to circumvent the cellular signaling redundancy underlying inflammatory conditions. In an attempt to improve bronchoconstriction and airway hyperresponsiveness in respiratory diseases, drugs have been designed to modulate the immune response by targeting immune

mediators such as cytokines, chemokines or their receptors. It is believed that in order to treat chronic inflammation a single drug directed against multiple targets and pathways would be better at arresting the progression of these respiratory diseases. However, to date there has been limited success with therapies targeting either a single cytokine, chemokine or their receptor highlighting the challenge in treating these complex inflammatory diseases by focusing on a single component or aspect of the inflammation process. Drugs acting on individual molecular targets usually exert unsatisfying therapeutic effects or have severe toxicity or undesired side effects when used in diseases of complicated causes such as in oncology or in inflammatory diseases. One approach to address such limited efficacy and toxicity has been by the development of novel therapies using a mixture of molecules. In oncology for example, a prevailing idea is that inhibiting both cancer cells and cells of the stroma supporting the tumor or blood vessels would gain better results in fighting this disease.

There is a fine balance between specificity and reduced toxicity that can be obtained by targeting more than one cytokine or chemokine or receptor in the immune response without the overwhelming suppression observed with corticosteroids. The era of designing "one target for one disease" has evolved such that the single-target therapy is fading in favor of a multi-targeted approach and the new generation therapies are selected on the basis of their ability to simultaneously inhibit or affect several targets. Through combining two or more molecules which individually have their own target into a single therapeutic product, it may be possible to generate a drug that is potentially more effective, in particular in those patients non-responding to the conventional therapies. In addition, the lower doses could results in less side effects than with broader therapies like corticosteroids. This approach is especially important because of the redundancy of inflammatory pathways indicates the need for AON against multiple genes in one product.

Lastly, PXS TPI1100 consists of aerosolization of a simple aqueous solution, and does not require any specialized carriers unlike many other AON therapies. Indeed, direct administration of low doses to the site of action by inhalation permits AON to efficiently reach and enter the target cells (Figure 2).

3.3 Preclinical pharmacology

In vitro pharmacology studies of the AON candidates of PXS TPI1100 were conducted in both human and animal cell cultures. Results in normal human bronchoepithelial (NHBE) primary cells and a lung epithelial cell line (A549) confirmed the efficacy of PXS TPI1100 at reducing PDE mRNA target knockdown, which is the proposed mechanism of action of the drug. Moreover, in NHBE cells, inhibition of the PDE4B, PDE4D and PDE7A with PXS TPI 1100 resulted with a synergistic effect on the inhibition of IL-8 secretion in response to a stimulus (a mixture of cytokines TNF-α, IL-1β and IFN-γ) compared to when cells were treated with each AON alone (Figure 3). These results and the lack of efficacy of rolipram (small molecule PDE4 inhibitor) on IL-8 confirmed the benefit of PDE4 and PDE7 inhibition. Besides IL-8, cells treated with PXS TPI1100 had an inhibition of the expression and release of other inflammatory mediators (e.g. MCP-1, MMPs). A second model used the lung epithelial cell line, A549, stimulated with the cytokine IL-1β, and again the inhibitory effect of PXS TPI1100 upon the induction of key inflammatory mediators (IL-8, MCP-1) in response to IL-1β was observed.

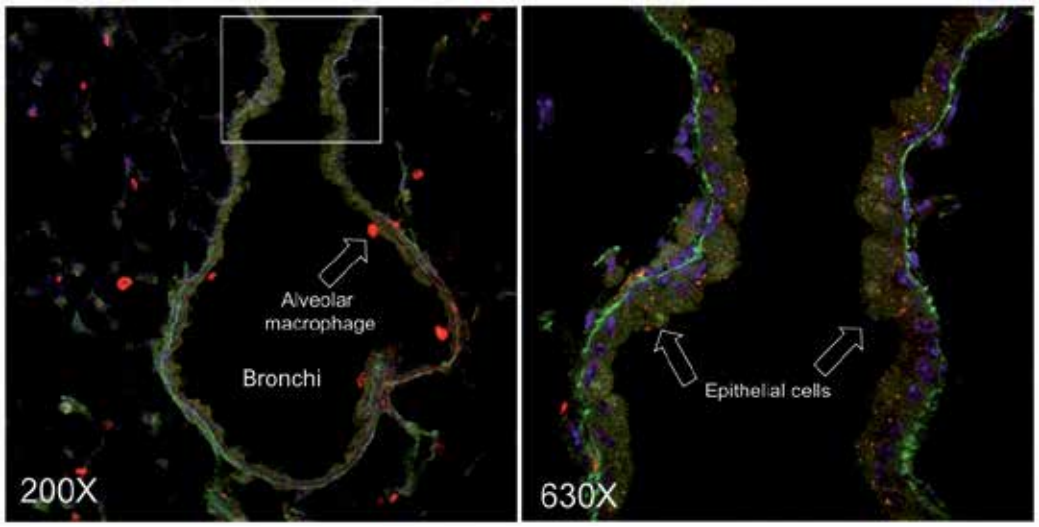

Fig. 2. Intracellular localization of PXS TPI1100 AON constituents in the lung of mice following cigarette smoke exposure. Mice exposed to cigarette smoke were treated intratracheally with a single dose of labelled PXS TPI1100 (a FITC-labeled AON against PDE4B/4D) and a Cy3-labeled AON against PDE7A. Images obtained using a confocal microscope (FITC in green, Cy3 in red and DAPI in blue). Magnification of 200X (left panel) and insert shown at 630X (right panel).

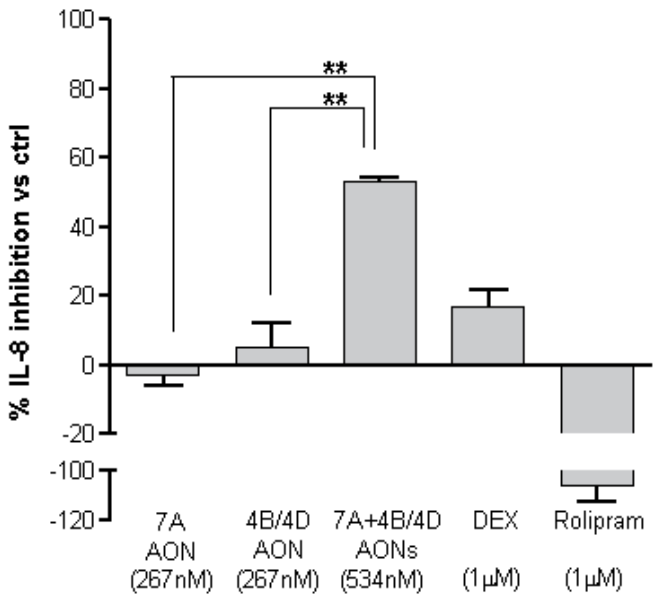

Fig. 3. Activity of PXS TPI1100 in NHBE cells. NHBE cells were treated with the PDE7A or the PDE4B/4D AON alone at indicated concentration or in combination prior to stimulation of the cells (mix of TNF-α, IL-1β and IFN-γ). Inhibition of the three PDE isoforms resulted with a synergistic effect (**$p<0.05$) on IL-8 secretion compared to each AON alone, and a more potent effect than rolipram or dexamethasone (DEX).

In two different *in vivo* models, PXS TPI1100 was shown to reduce the neutrophil influx in the BAL of mice either in response to cigarette smoke or to LPS challenge. Cigarette smoke exposure of laboratory animals reproduces many of the anatomic/physiologic lesions (neutrophilic inflammation, emphysema, small-airway remodeling and pulmonary hypertension) of human COPD (Wright et al. 2008) and has been used for the preclinical assessment of Daxas/Deliresp (Martorana et al. 2005). In this model, mice were exposed to cigarette smoke for 4 consecutive days and treated with PXS TPI1100 every other day (two treatments only) 3 h prior to cigarette smoke exposure. Following repeated smoke exposure, a significant increase (180-fold) recruitment of neutrophils in BAL collected the day after the last smoke exposure was observed compared to mice not exposed to smoke. The percentage of neutrophils in BAL also increased with smoke from 0.8% to 35%. When mice were treated with PXS TPI1100 at 0.1 or 0.4 mg/kg every other day, the smoke-induced neutrophil recruitment was significantly reduced (up to 52% inhibition $p<0.01$) when compared to mice treated with vehicle or a comparable dose of a control AON.

In the second model of acute lung inflammation, mice exposed to LPS (nasal instillation) had a strong inflammatory response with significant increase in neutrophils in BAL. PXS TPI1100 treatment at 1.2 mg/kg (1 h prior to LPS challenge) resulted in a 33% reduction of neutrophil recruitment induced by LPS ($p<0.05$) whereas treatment with the control AON had no effect.

The potency of PXS TPI1100 at reducing the smoke-induced or LPS-induced lung inflammation was compared to the PDE4 inhibitor roflumilast (Daxas). Roflumilast (5 mg/kg, p.o.) given daily 1 h prior to cigarette smoke exposure reduced neutrophil recruitment by only 25% (Fortin et al., 2009). In the LPS model, roflumilast, given once at a dose of 10 mg/kg (~10-fold more than PXS TPI1100) had no effect on the neutrophil influx, whereas at a higher dose of 100 mg/kg (~100-fold that of PXS TPI1100) it reduced neutrophil recruitment by 46% ($p<0.05$). This effective dose of roflumilast exceeds the current clinical dose for Daxas of 500 microgram per adult per day. PXS TPI1100 is continuing its pre-clinical development as a treatment for COPD.

PXS TPI1100 has not yet performed nonclinical drug depositions studies however, from tests with different AON that recognize the same PDE targets yet lacked the modified chemistry backbone we can extrapolate how PXS TPI1100 will behave following pulmonary delivery. In CD-1 mice, following 14 days of daily dosing with AON by inhalation, AON plasma concentrations were not detectable (< LLOQ of 5-10 ng/mL) at all time points for all dose levels. In the lungs, the AON concentrations were dose-related, and there was evidence of accumulation in lungs over the 14 days, based on the higher levels at 24 h after the last dose *vs.* 24 h after the first dose. The systemic exposure was extremely low with only small amounts of AON detectable in the kidneys and liver of high-dose mice (2.5 mg/kg/day), and the levels were similar following the first and last doses. In monkeys, following 14 days of inhalation of AON there were detectable levels of AON in plasma only in a few high-dose animals up to 1 h post dosing on Day 1 while samples from Day 14 were all <LLOQ (Guimond et al. 2008). In the lung of animals on the day after last drug exposure, the AON levels were approximately dose proportional. In kidney and liver, low levels of AON were quantified one day after the last dose and only in high dose animals, demonstrating that similar to mice, the systemic exposure was low. When AON were delivered by slow bolus intravenous (IV) administration in monkey, the highest plasma levels were measured immediately at the first time point after IV

injection (approximately 5 min) and these levels were greatly reduced by 4 h post-dose and near LLOQ by 24 h demonstrating the clearance of AON from the system.

The pharmacokinetics properties following pulmonary delivery has been well characterized (Templin et al. 2000; Ali et al. 2001; Guimond et al. 2008) and confers a significant advantage of AON over small molecule drugs. For example, orally-delivered Daxas/Daliresp has a bioavailabilty of 79%(David 2004) and with an elimination half-life of 14-18 h there is a greater opportunity for this drug to act upon PDE4 outside of the lung and for a long period of time. In comparison, PXS TPI1100 has reduced systemic bioavailability and based on results in mouse lung, the half-life of PXS TPI 1100 has been shown to be relatively short (<5h) suggesting a potentially safer drug that would work locally at the site of action in the lung.

3.4 Clinical experience

To date, PXS TPI1100 has not been dosed in human subjects, however, a review of the current literature on clinical study designs and using the Daxas/Daliresp background as guidance, the projected clinical path for PXS TPI1100 has been defined. Furthermore, reviewing its pharmacology profile, there are potential advantages for PXS TPI1100 which may be manifested in the clinic. In this section, we will first outline some general challenges facing COPD clinical study design, then capture some of the salient points from the Daxas/Daliresp experience in clinical study design.

3.4.1 Challenges in COPD clinical studies

Typically COPD clinical studies measure as a primary outcome lung function by spirometry, either through improvement in postbronchodilator FEV_1 or in cases where assessing the efficacy of nonbronchodilators is preferred, the measure is of the change from baseline in prebronchodilator FEV_1 (Giembycz &Field 2010). As a procedure for detection of airflow obstructions spirometry is a reliable, simple, non-invasive, safe, and non-expensive (Soriano et al. 2009). The test is relatively standardized with most COPD guidelines accepting the threshold to define a positive bronchodilation test as suggested by the Global Initiative for asthma (increase in FEV_1 larger than 12% and 200 mL from the prebronchodilator value) (Bateman et al. 2008) with the variation of suggesting minimum limits of 300 or 400 mL.

Besides the use of these spirometry measures, there have been attempts to determine a relevant easily accessible and rapid assessed biomarker as a measure of improvement. In preclinical pharmacology studies, animal models of neutrophil inflammation are routinely used for efficacy measures in an attempt to mimic the disease state in humans. It is known that in COPD patients the percentage of sputum neutrophils are increased with each GOLD stage, are also raised in COPD exacerbations (Caramori et al. 2003) (Papi et al. 2006), and that neutrophils are involved in the pathogenesis of emphysema through the secretion of proteases and elastases (Cowburn et al. 2008) (Sharafkhaneh et al. 2008). Taken together, these observations would suggest that sputum neutrophils have the potential to be a biomarker predictor of the degree of airflow obstruction, however the reality is far from clear. Reports with small cohorts of patients suggest a relationship between sputum neutrophils measures and FEV_1 (% predicted) (O'Donnell et al. 2004), however a larger cohort study by Singh et al. (Singh et al. 2010) demonstrated that this relationship is only weakly associated. A similar finding was shown with regard to sputum neutrophils

measures and the relationship to health status as defined by the use of the St. Georges Respiratory Questionnaire (SGRQ) (Singh et al. 2010). Furthermore sputum neutrophil measures in the stable state were shown not be predictive of the future rate of exacerbations (Singh et al. 2010). Lastly, no association between sputum neutrophils measures and emphysema or systemic inflammation as measured by serum levels of IL-6, IL-8, C-reactive protein (CRP) and surfactant protein D was observed (Singh et al. 2010). In short, although there is a plausible assumption for the use of sputum neutrophils as a biomarker, there is little validity in using them in face of the current evidence.

In lieu of the identification and validation of a biomarker that could predict the rate of lung function decline in COPD, most COPD clinical trials attempt to measure relevant changes in exacerbations. Exacerbation frequency has been considered to be an important outcome parameter in COPD as it is associated with increase in mortality (Patil et al. 2003) (Fuso et al. 1995). Measuring exacerbations is not without its challenges. It is difficult among studies to find consensus on what is defined as an exacerbation and to gauge the severity of the exacerbation. Symptom-based definitions include use of diaries, while event-based definitions may refer to hospitalizations or use of antibiotics and/or steroids (Miravitlles et al. 2004). Although a systematic literature review of studies reporting exacerbation frequency in COPD patients showed the relationship between increased exacerbation frequency with decreasing lung function to be borderline significance ($p=0.053$) (Spencer et al. 2004), exacerbations are still considered to be an important parameter in COPD. Exacerbations are more likely to occur in winter and according to current recommendations (Cazzola et al. 2008) studies need to have at least a 12 month follow up to give reliable estimate of exacerbation frequency, which requires the planning of lengthy clinical trials.

The clinical program of PXS TPI1100 has not been initiated yet we expect its design can follow that of other PDE4 inhibitors. An initial Phase 2 study design does not test in COPD patients but rather in allergic asthmatic patients following inhaled allergen challenge (2009). Another AON drug, ASM8 designed specifically for asthma and as such has targets different from PXS TPI1100, has demonstrated clinical efficacy in this allergen challenge model (Gauvreau et al. 2008; Gauvreau 2010) clearly showing the potential for the AON approach. An advantage of this allergen challenge model is that the studies are generally brief in duration and the fall in FEV_1 is a well-recognized response as well as the incorporation of monitoring induced sputum allows for other inflammatory indicators to be measured.

PXS TPI1100 has an advantage in that the clinical studies performed by Daxas/Daliresp can be used as a guide, as the two drugs share a common target. As Daxas/Daliresp was breaking new ground many studies had to be performed and it is plausible to conclude that for other drugs in the same class fewer studies may be required. In all, six phase 3 clinical trials were undertaken with Daxas/Daliresp which have been excellently reviewed by Giembycz and Field (Giembycz &Field 2010). Key aspects of these trials that can be used for PXS TPI1100's clinical development include criteria for patient selection and parameters selected for primary and secondary outcomes. In the phase 3 study named RECORD, patients with moderate-to-severe COPD (postbronchodilator FEV_1 of 30% to 80% predicted and a FEV_1/FVC ratio of less than 70%) were randomized to receive either Daxas/Daliresp at 250 µg or 500 µg or placebo (2:2:1 ratio) for 24 weeks (Rabe et al. 2005). Results showed treated patients experienced improvement in postbronchodilator FEV_1 (Rabe et al. 2005) and a change in SGRQ but this change did not reach clinical significant threshold. Although direct

comparisons between doses were not made, as it seemed that patients receiving the higher dose had better and earlier responses in most outcomes the daily dose of Daxas/Daliresp of 500 μg was then used in two subsequent identical trials (RATIO and OPUS). In these studies the patients had more severe COPD than in the RECORD study (postbronchodilator FEV_1 of 50% or less, FEV_1:FVC ratio of 0.7 or less, or FEV_1 reversibility of 5% or less). Although completed, the results from the OPUS trial have not been published, however results from the RATIO study showed an improvement for the change from baseline for post bronchodilator FEV_1, yet again no effect on the SGRQ (Calverley et al. 2007). A post-hoc analysis of a subgroup of patients with GOLD stage IV disease in the RECORD study showed a significant effect on reduction of exacerbation frequency (Calverley et al. 2007) which then led to the design of two identical studies AURA and HERMES where patients had a diagnosis of clinical COPD (confirmed by postbronchodilator FEV_1/FVC of at least 70%, and a FEV_1 at least 50% of predicted), had symptoms of chronic bronchitis and a history of exacerbations. Patients experienced an improvement in pre- and postbronchodilator FEV_1 and a reduction in exacerbation rate (Calverley et al. 2009) which were independent of LABA use, but no differences in mortality or C-reactive protein levels.

Taken together, the Daxas/Daliresp studies clearly show effects in patients with GOLD stage IV disease, with focus on measuring flow rates and exacerbation reduction as parameter outcomes. The clinical program for PXS TPI1100 can use this information in designing studies so as to sharply define the patient population at the onset and include the key primary outcomes as success measures.

As ICS and LABA have been shown to be more effective at improving lung function, health status and reducing COPD exacerbations when combined than when used individually (Calverley et al. 2007) the effect of combining Daxas/Daliresp with either the long-acting β2-agonist salmeterol (EOS study) or the long-acting inhaled antimuscarinic tiotroprium (HELIOS study) was studied in patients with less severely reduced lung function as compared to the previous studies. Results showed that the pre- and postbronchodilator FEV_1 improved in patients treated with Daxas/Daliresp versus placebo when combined with either LABA or LAMA (Fabbri et al. 2009). PXS TPI1100 can be expected to also function in combination with LABA, similar to that demonstrated by Daxas/Daliresp and could potentially replace ICS.

As with any drug, adverse events to Daxas/Daliresp were reported which included weight loss, diarrhea, nausea, headache, influenza and nasopharyngitis as well as certain cancers such as lung and prostate (Giembycz &Field 2010). There was a greater risk of discontinuation of therapy within the first 12 weeks of treatment for those patients taking Daxas/Daliresp than placebo although by the end of the studies, similar numbers of patients withdrew in both groups. In the Daxas/Daliresp treated groups, the most common reason for withdrawal were the gastrointestinal adverse events or headache (Giembycz &Field 2010).

There are aspects of PXS TPI1100 which may lend itself advantages over Daxas/Daliresp. Firstly, as PXS TPI1100 is administered via inhalation, it is delivered directly to the intended site of action of the lung (Ali et al. 2001; Duan et al. 2005; Gauvreau et al. 2008; Guimond et al. 2008) where the drug can enter target cells directly (Zhang et al. 2004; Griesenbach et al. 2006) thus potentially reducing total dose as compared to orally-available treatments. A further advantage of pulmonary administration of AON is that they are principally

metabolized in the lung with very limited systemic delivery after inhalation (Templin et al. 2000; Ali et al. 2001; Guimond et al. 2008) which leads to reduced systemic bioavailability of the drug. In comparison to Daxas/Daliresp, which is delivered orally and has a high level of bioavailability, the projected low systemic bioavailability of PXS TPI1100 may limit adverse events associated with PDE4 inhibitors, namely the gastrointestinal and neurological side effects. Another consideration is the projected brief half-life of PXS TPI1100. Based on the mouse lung, the half-life of PXS TPI1100 has been shown to be relatively short (<5h), although it is reassuring that this short tissue half-life does not appear to affect the efficacy of the drug as every-other day dosing of PXS TPI1100 in the smoking mouse model was highly effective. Reconciling the short half-life with longer term efficacy may be a reflection of the mechanism of action of the drug, suggesting that inhibition of PDE mRNA has longer term consequences on downstream effects including limitation of inflammatory responses.

Besides a projected favorable safety profile resulting from low systemic bioavailability, PXS TPI1100 can be expected to avoid the toxicity associated with the broader approach of anti-inflammatories such as ICS by specifically targeting PDE.

4. Conclusions

PXS TPI1100 faces challenges, in part of being the first respirable antisense drug product in COPD. As COPD is a chronic disease, it can be expected that patients will be dosed for years. The long-term effects of this drug class have never before been studied. In addition, in pulmonary/respiratory diseases, there is a risk that administration of therapeutic nucleic acids may lead to immune stimulation, inflammation and possibly hypersensitivity and bronchoconstriction of the airways. Except for the latter, these risks are not specific to the lung as they have been observed with other routes of administration. As with any novel inhaled medication, local tolerability and the absence of long-term effects following chronic dosing will require careful evaluation as drug candidate progresses through the later stages of development. The publicly available toxicological data on inhaled AON are not extensive (Guimond et al. 2008), and therefore deriving definitive conclusions on toxicology at this time is not possible. The phase 2a studies that have been performed until now have not shown any of this potential toxicity but longer term studies are needed to confirm these results. Furthermore, to date AON have been delivered via inhalation of a nebulisate to asthma patients (Gauvreau et al. 2008; Gauvreau 2010), but not to COPD patients who have severely decreased FEV_1. How well this patient cohort inhales the nebulisate would need to be determined. The range of delivery devices (including newer portable soft-mist inhalers) have increased and permit liquid aerosols to be targeted more effectively to the specific airways of interest (upper or lower airways), improve ease of use by patients and would be expected to improve compliance to therapy. In contrast, the particle processing and formulation of AON for delivery in dry powder inhalers or pressurized metered dose inhalers, which are most commonly used by COPD patients has, however, proven to be significantly more challenging than that of liquid aerosols.

Another challenge facing PXS TPI1100 is the selection of its targets PDE4B/D and PDE7A. While the success of Daxas/Daliresp demonstrates the effectiveness of targeting PDE4 in COPD, to date there is less corrobative clinical evidence for the efficacy of targeting PDE7A isoform. The success or failure of a specific drug development program is determined by a range of different factors, which includes the clinical relevance of the selected drug target.

One early pioneer in the respiratory field was the AON drug EPI 2010 (Epigenesis) targeting the promoter region of the adenosine A1 receptor. Although demonstrating efficacy *in vitro* and in animal models (Ball et al. 2004), EPI 2010 failed in later clinical studies to demonstrate efficacy to improve lung function in asthmatics. With the more recent understanding of the role the different adenosine receptors have in asthma (Brown et al. 2008), it could be argued that the absence of clinical efficacy for EPI 2010 could either be a result of targeting the wrong adenosine receptor or perhaps the need to combine it with other adenosine receptor inhibitors. Similarly, early preclinical efficacy and effect on biomarkers in a phase 1 study with AIR-645 (AON targeting IL-4/IL-13Rα Altair/Isis) met with apparent insufficient efficacy on lung function in phase 2 study (personal communication). This may perhaps be attributed to the target selection as other non-ODN drugs targeting these receptors have also had limited success in clinical trials. As mentioned, although few PDE7 inhibitors have been tested in clinical studies, our preclinical pharmacology results indicate a clear benefit in targeting this PDE isoform along with the PDE4. There is growing acceptance that multi-targeted approaches may provide significant therapeutic advantages, as demonstrated by the issuance of new guidance on drug combinations by the Food and Drug Administration.

There is a clear need for innovative products with novel mechanisms of action to complement today's inhaled products particularly for severe patients who seem resistant to current therapeutic interventions. In spite of many attempts, success in these respiratory indications has been modest, at most. This may reflect the challenge of delivering the therapies to the site of action (lung) or more importantly the complexity of these diseases. PXS TPI1100 belongs to a new class of therapeutics that is poised to expand in the upcoming decades because of its advantages, especially with lung administration. Outstanding challenges for PXS TPI1100 remain the need to establish long term safety and tolerability data as well as commence clinical efficacy. The future remains very promising for this novel drug.

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Cell Therapy in Chronic Obstructive Pulmonary Disease: State of the Art and Perspectives

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1. Introduction

The pulmonary diseases of obstructive character have high prevalence in the human population and has been subject of several clinical and experimental studies in order to seek a wider understanding of their pathogeny, physiopathology and, especially, the establishment of more rational ways for their treatment. Accordingly, this great effort has led to an extraordinary widening in the concepts of obstructive diseases in the last years, involving the integration of mechanical factors, inflammatory agents, autonomic regulation of airways and environmental aspects.

The COPD may be understood as a pathologic condition in which a non-reversible and limited gas exchange occurs. There are two clinical entities that constitute the COPD: chronic bronchitis and emphysema. Within the COPD spectrum, the main characteristic of pulmonary emphysema is air flow blockage and progressive dyspnea, arising out of the impairment of alveolar walls and increase of air spaces distal to terminal bronchiole, without significant pulmonary fibrosis (Barnes et al., 2003; GOLD, 2009; Oliveira et al., 2000).

The oxidative damage to which lungs are submitted to, as well as the inflammation occurring as a response to irritant agents, such as those coming from air pollution and cigarette smoke, contributes to the induction of the pulmonary degeneration (Lee et al., 2011). Therefore, it may be concluded that chief characteristic of COPD is the acceleration of functional and morphologic loss, with limitation of gas exchanges, resulting in progressive dyspnea, disability and premature death.

Therefore, the development and progression of the pathology are resulting from the interaction of genetic and environmental factors (Ribeiro-Paes et al., 2009). About 1-3% of cases of emphysema are generated by enzyme α1-antitrypsin deficiency, that characterizes a genetic abnormality as an inheritance of autossomal recessive pattern. The other risk factors include: age, infections, as well as social and economic factors (Mannino & Buist, 2007).

Smoking, however, has been established as the major cause related with COPD, resulting for active or passive exposure to the cigarette smoke, and corresponds to 15-20% of cases of

pulmonary emphysema (Ribeiro-Paes et al., 2009). The cigarette smoke in their gas and particle stages has a significant quantity of oxidant substances. A high number of particles and oxidant agents are contained in cigarette smoke. Oxidant agents are capable of reducing the effect of the anti-protease system through the oxidation of the active site of those enzymes and leading to a direct injury to the extracellular matrix (Barnes 2000; Barnes et al., 2003; Bast et al., 1991; Rufino & Lapa e Silva, 2006).

The Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2009) has pointed out COPD as a serious public health issue. The pathology is considered the fifth largest cause of death worldwide, and has 210 million patients, with 80 million already in the moderate and/or serious stage of the disease. Estimates put it at the third ranking of cause of death in 2020 (GOLD, 2009; WHO, 2008). Moreover, faced with the ageing of world population, the economic burden of COPD should represent a significant parcel of the future global investments in health (Mannino & Buist, 2007).

Several clinical strategies, associated with the pulmonary rehabilitation techniques have contributed to the extension and improvement of the quality of life of emphysema patients. Notwithstanding the significant advances resulting from the introduction of new therapeutic approaches and rehabilitation, there has not been any efficient form of treatment up to now, other than the one in the palliative scope. The surgery treatment entails highly complex procedures and, in the specific case of lung transplant, a shortage of donors. By taking these aspects into account, experimental models have been proposed, in order to advance the knowledge about the physiopathological processes and new therapeutic approaches to the pulmonary emphysema (Gross et al., 1965; Hele, 2002; Mahadeva & Shapiro, 2002; Martorana et al., 1989; Nikula et al., 2000; Ribeiro-Paes et al., 2009).

2. Experimental models in COPD

Experimental models represent an important tool, since they enable the broadening of knowledge about COPD physiopathology, besides allowing the application of new therapeutic approaches.

The methodology of papain intratracheal instillation, proposed by Gross and coworkers in 1965, represented an original model for pulmonary emphysema induction. Starting from the proposition of this pioneer methodology, a series of studies were conducted, which led to the development of the models of induced pulmonary emphysema by the instillation of other proteases. (Pushpakom *et al*., 1970; Fusco *et al.*, 2002).

The use of proteolytic enzymes, chiefly of porcine pancreatic elastase (PPE) for the generation of DPOC in an animal model is a widely employed methodology for the conduction of experimental studies, since it is mainly a simple and fast method and produces physiopathological effects similar to the human disease (March, 2000; Shapiro, 2000). The experimental models of pulmonary emphysema induced by proteases instillation have not reproduced precisely the mechanisms of alveolar destruction ensuing the inhaling of smoke and other toxic particles, and, therefore, they do not mimic exactly the sequence of pathological events that occur in the disease in humans (Cendron, 1007).

The use of animal models of cigarette-smoke-induced emphysema means seeking an accuracy in experimental models to match the human, chiefly with respect to the

physiopathological mechanisms involved in the formation of the emphysema. Up to the beginning of the 80's decade, the studies involving induced pulmonary emphysema in animals by exposure to cigarette smoke were scarce and their reliability questioned (March, 2000). In 1981, Huber and coworkers proposed a study based on the model of induced emphysema through exposure to cigarette smoke. Some achieved results in the study, with respect to morphometric and physiological aspects provided the basis for the ensuing research. According to the report from the First Siena International Conference on Animal Models of COPD held at the University of Siena in 2001, the induced lesions with the use of this model are similar to those observed in emphysematous humans, highlighting the importance of the stimulus through cigarette smoke in COPD experimental models. (Hele, 2001).

At our laboratory, a new apparatus (Figure 1) for induced emphysema through exposure to cigarette smoke is under test. The present device has a series of innovations when compared to the already existing inhaling models, such as the fact that the animals are contained inside acrylic containers making up the device, while in other cages the animals stay freed. Another important aspect worth highlighting relates to the smoke, which is pumped inside the box. In the device created by our team, the smoke pumped into the box interior comes from puffing on the cigarette; therefore, the situation of an active smoking human is mimicked. This apparatus is expected to lead to a model which mimics, as close as possible, the human pathology and, accordingly, which can be applied to research projects oriented to the analysis of physiopathological processes and to the development of new therapies in chronic degenerative pulmonary diseases.

Fig. 1. Apparatus created by the team of the Laboratory of Genetics and Cell Therapy – GenTe Cel to induce emphysema by cigarette smoke.

Notwithstanding the challenges involved in some parameters related to the cigarette-smoke-induced emphysema models, mainly with respect to the age of the animals, exposure time and reproducibility difficulty due to the required resources and time, this is a promising approach to turning animal models closer to the human, chiefly in relation to the physiopathological processes featured in the human pulmonary emphysema (March, 2000).

Currently, the creation and use of genetic models is a very important tool for DPOC study, since the strains mimic a series of aspects related to the human disease, mainly with respect to the α_1-antitrypsin deficiency (March, et al., 2000). At present, several mice strains are known to have natural or laboratory-induced mutations (gene targeting), which generate abnormal conditions in the animal development and are completed with the spontaneous arise of DPOC (March, 2000; Shapiro, 2000). Other methodological approaches to emphysema induction entail animal models with genetic modifications. Martorana *et al*. (1995), showed the installation and development of pulmonary emphysema in Tight skin transgenic mice, which show mutation in the fibrillin-1 gene, a protein related to the elastic fibers assembly making up the pulmonary tissue (Kietly, 1998).

The Table 1 shows some advantagens and disadvantages of the main animal models of induction of COPD.

Experimental model of COPD	**Advantages**	**Disadvantages**	**References**
Protease-induced Emphysema	- Simple method, easy to apply, quick results, high reproducibility - Morphological features similar to human disease	- Lack of inflammatory constituents - Physiological process is different from human	March *et al*. (2000)
Genetic models	- Reproduction of human pathology, mainly in relation to deficiency of α_1-antitripsin - Demonstrate the role of proteases in the development of the disease	- The aspects of pathology are reproduced just individually - Need for further studies with respect to inflammatory mechanisms	Shapiro (2000) Fujita and Nakanishi (2007)
Smoke cigarette	- Reproduction of aspects related to the inflammatory processes of COPD - Changes in the airways similar to the human disease	- Long time to onset of symptoms - Time of exposure highly variable	Zheng *et al.* (2009)

Table 1. Comparison of the main experimental models of COPD.

Taking into account these aspects, is evident the great importance of experimental models of COPD, even though none of them entirely mimic all the features making up the human

disease. The use of these models affords the broadening of knowledge, especially related to the physiopathology. The achieved results in animal models may be the grounds for the development of new therapeutic alternatives with the ensuing impact on the survival and improvement of the quality of life of COPD patients.

3. Stem cells and cell therapy: The rationale for use in the lung

The employment of cells for treating diseases is an ancient therapeutic practice, which dates back to the transfusion of whole blood or platelet concentrate in different acute or chronic clinical conditions. The first hematopoietic stem cells (HSC) transplantations were made according to the works of Till and Mculloch in 1961, on the response of mice with the bone marrow transplanted after lesion by ionizing radiation. Since then, new ranges and possibilities of use of other tissues according to the experimental model adopted by the authors have arisen.

The potential of differentiation of stem cells (SC), i.e., the wide range of options of commitments available for the cell (Smith, 2006), has aroused a growing and great interest, bearing in view the employment in the therapy of several types of degenerative diseases and in tissue bioengineering (Atala, 2008). According to The National Institutes of Health (NIH), SC can be defined as cells able to divide for indefinite time *in vitro* and to give origin to specialized cells. Melton and Cowan (2004) proposed a working definition of SC: "a clonal self-renewable entity which is multipotent and can generate several types of differentiated cells." Notwithstanding the concept variation, SC have two basic characteristic aspects: self-renewal, in order to maintain the pool of undifferentiated cells for tissue replacement, remodeling, and repair, as well as the differentiation into at least one mature cell type. These inherent properties for SC are afforded through particular asymmetric divisions, where undifferentiated cells are originated, or, alternatively, differentiation into specialized cells (Figure 2).

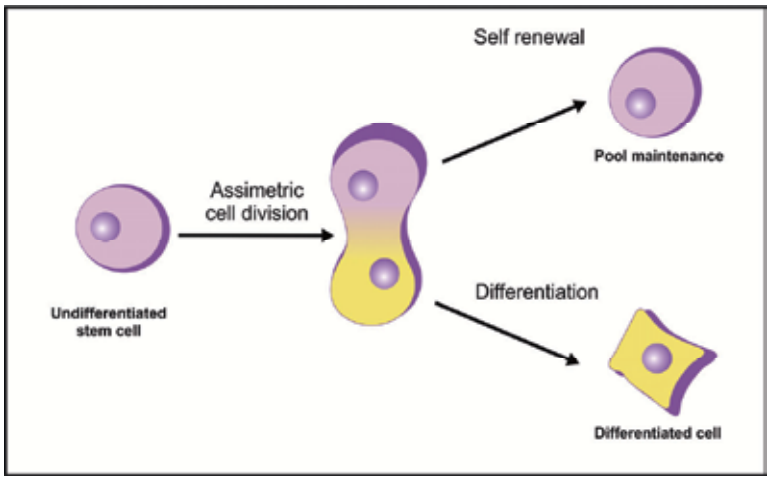

Fig. 2. Assimetric SC division. An undifferentiated SC under microenvironment stimulation start assimetric divisions producing two distinct daughter cells. One cell, undifferentiated, maintain the SC pool. In contrast, the differentiated cell acquires a new mature and specialized phenotype.

Considering their origin, SC are classified in three general types: embryonic stem cells (ESC), germinative stem cells (GSC), and adult or tissue-specific stem cells (ASC). The ESC are derived from the inner cell mass of the blastocyst, capable to generate any differentiated cellular type of the three primary germ layers (ectoderm, mesoderm and endoderm), as well as the GSC originated from the gonadal crest (Geijsen et al., 2004). On the other hand, ASC are undifferentiated cells, found in differentiated cell types in a tissue where they can renew themselves for long periods of time, and can differentiate to yield specialized cell types of the host tissue. By and large, ESC maintains the undifferentiated stage for a long period of time without losing their differentiation potential (Draper et al., 2004). Moreover, the ASC have a limited number of generations, and at each division there is loss of response to differentiation signals (Jiang et al., 2002).

The knowledge that undifferentiated cells exist in the bone marrow has been verified since the 40's decade; by the way, the blood progenitors are the first well characterized SC. Both in humans and in animal models, the literature reports consistent data with evidence for the existence of stained SC from the bone marrow in lungs after bone marrow transplant (Bittmann et al ., 2001; Kotton et al., 2001; Krause et al., 2001; Lama et al., 2007; Ribeiro_Paes et al., 2009; Schrepfer et al., 2007; Suratt et al., 2003; Yamada et al., 2004). At different experimental situations, these and others classical works have shown evidence for the migration of SC to the lung and have provided the theoretical reference which gives grounds for the idea of employing cell therapy in the regeneration of pulmonary tissue.

The experimental evidence of migration of SC from the bone marrow to the lungs was pioneering described in the work of Pereira et al. in 1995. Authors cultured murine cells expressing a collagen human gene and injected the expanded mesenchymal precursor cells into irradiated mice. The presence of transplanted cells in recipient animals for a period of up to 5 months was showed by PCR *in* situ assay. There was incorporation into the pulmonary tissue, where the cells disseminated through the mesenchymal parenchyma and could continue the replication process *in* vivo. Therefore, bone marrow cells can migrate and populate the pulmonary tissue and act as precursors of local cells.

Experimental animal models and clinical trials in regenerative tissue therapy by intravenous (IV) SC or BMMC infusion indicate a "pulmonary first-pass effect" as proposed by Fischer et al. 2009. The lungs act as a barrier, where administered cells are preferentially attracted and retained. Cell size and adhesion receptors of the stem and progenitors cells IV infused can determine this effect through pulmonary microvastulature (Fischer et al., 2009). Five minutes after labeled MSC IV infusion was verified, in animal model, a significant greater bioluminicensce signal in the lungs, in relation to several other organs, such as heart, spleen, liver and kidney. Therefore, the mean size of injected cells larger than the caliber of lung capillaries provides an efficient and fast cell trapping in lungs (Schrepfer et al., 2007).

Interesting works found on literature indicate the initial migration and chimerism in lungs after cell transplantation. Krause et al. (2001) transplanted male mice cells into females with bone marrow depleted by ionizing radiation and tracked the presence of Y chromosome in gastrointestinal tract, liver, lung and skin. It was verified co-staining of pneumocytes type II and Y chromosome in bronchi and alveoli showed by FISH assay (Y chromosome and surfactant B mRNA staining) and immunohistochemistry (anti-cytokeratin antibodies for the detection of epithelial cells). However, authors proposed that the significant damage to

the lungs, arising out of the radiation, provided high levels of incorporation in the alveolar tissue.

In the same year, Kotton *et al.* (2001) IV infused Lac-Z stained cells of transgenic mice into recipient wild animals, which underwent pulmonary lesion by intratracheal instillation of bleomycin. There was typical staining of lac-Z expression (Incubation in medium containing X-gal), with statistically significant increase in the animals sustaining lesion with bleomycin. The grafted cells showed evidence for morphologic and molecular phenotype of pneumocytes type I. So, cultured or fresh aspirates of bone marrow cells can express pulmonary markers. Thereby, these cells could represent a potential therapy in extensive alveolar degeneration.

An elegant experimental model of suppression of bone marrow and later lesion with bleomycin was elaborated by Rojas *et al.*, (2005). The authors obtained full survival index and protective effect in mice which underwent MSC transplantation. The immunohistochemistry analysis of the pulmonary tissue of the animals with suppression of bone marrow disclosed, when compared to group without suppression, that the transplanted cells (GFP^+) were present in the organ and in a large number, even 14 days after the administration of bleomycin.

As in the animal models, cell migration and chimerism were also observed in human patients who received, for different reasons, bone marrow allogeneic transplant, as in the models of animal studies. Suratt et al. (2003), in a pioneer work, showed pulmonary chimerism upon the incorporation of cells with Y chromosome in women receiving HSC allogeneic transplant from male donors. Another study, 7 patients who underwent pulmonary transplant between (donor and recipient) individuals of opposite sexes showed, by means of different assays of histochemistry staining and molecular analysis (RT-PCR), the presence of mesenchymal stem cells (MSC) in lungs of recipients with cytogenetic expression of the sex of donor. In a period of up to 11 and a half years after the transplant was verified donor cells in the recipient patients (Lama et al., 2007).

Nevertheless, the SC migration to the lungs can be overestimated and, therefore, they are allegedly present at a much lower rate with a questionable clinical meaning. So, the results obtained and reported have been evaluated more carefully by some authors, who challenge the accuracy of the employed detection techniques. For example, after transplanting MSC GFP^+ in mice which had previously received an LPS intraperitoneal injection, Xu et al. (2008) did not find, in the immunohistochemistry analysis of the pulmonary tissue conducted 14 days after the transplant, circumstantial evidence for a significant presence of cells with positive sign of GFP. However, although the authors did not find evidence for an actual integration of MSC to the pulmonary tissue and the presence of cells with the pulmonary phenotype, there was demonstration that the SC transplant afforded a decrease in the lungs inflammation and edema induced by the LPS. There are, accordingly these results, the indication that the action mechanism of cells would be mediated by paracrine factors that stimulate tissue regeneration rather than cell engraftment into lungs (Huh et al., 2011).

More recently, Katsha and collaborators (2011) reported a significant improvement resulting from the use of MSC from the murine bone marrow for the repair and regeneration of the pulmonary parenchyma, in an elastase-induced experimental model of emphysema. The

authors suggest in the same study the importance of paracrine factors derived from MSC as the regenerative mechanism operating in the pulmonary parenchyma.

Notwithstanding the diversity of used methodologies, in human patients and animal models, has been proposed that ASC from several tissue sources can migrate and populate injured areas in the lung. It is propounded that the regenerative property of SC involves cellular migration to the site of tissue damage and probable promotion of functional and structural organ repair. This mobilization process (homing) is related to liberation of chemotactic mediators by injured organ (Chen et al., 2011).

4. Use of stem cells in chronic obstructive pulmonary disease: Experimental basis

In lungs, affected by chronic inflammation, there is intense production of molecules that signal and can recruit SC (endogenous and transplanted) capable of tissue reconstruction (Rojas *et al*., 2005). In this context, the rationale for cell therapy in COPD comprehends the ability of SC homing toward injured pulmonary tissue, allowing repair of the lung parenchyma and probable clinical efficacy.

Two groups of Japanese researchers reported in 2004 the first consistent results of pulmonary regeneration in an experimental mouse model (C57BL/6 strain) of lesion and later infusion of SC from bone marrow. The mice were submitted to lipopolysaccharide (LPS) intranasal treatment after irradiation. An experimental group received bone marrow-derived progenitor cells transplant from transgenic mice donors expressing GFP. There was protection of the lungs against the lesion of the emphysematous type in the animals transplanted with BMMC. It was also detection of stained cells (endothelial and epithelial) only in the recipient animals in which the induced pulmonary lesion (Yamada et al, 2004).

In a model of elastase-induced pulmonary emphysema, Ishizawa et al. (2004) reported that the treatment with retinoic acid or granulocyte colony-stimulating factor (G-CSF) led to the alveolar regeneration and the treatment, concurrently with both factors, resulted in an additive effect. There was BMC mobilization to injured alveoli by retinoic acid and G-CSF besides regeneration process.

Several authors around the world reported experimental and interesting results with cell therapy in animal models of COPD. Some of these works are shortly described in the Table 2.

At our laboratory, several research projects have been directed for the study of morphologic and functional pulmonary recovery after the treatment with ASC in mice with experimentally-induced COPD. Our model basically consists of the induction of emphysema by intranasal instillation of papain or elastase and later treatment with BMMC or MSC pool originated from the bone marrow (Figure 3).

Female mice of the C57BL/6 act as recipients. Transgenic male mice (with C57BL/6 background), which express the green fluorescent protein (GFP) are used as donors of BMMC and MSC for the purpose of cellular tracking and validation of the post transplant chimerism.

The achieved results both in quality and in quantity have shown the regeneration of the pulmonary tissue in animals with emphysema and treated with BMMC pool or MSC (Figure 4).

Animal	COPD induction	Stem cell type / source	Therapeutic effects	Probable action mechanism	Reference
Rabbit	Elastase	BMMC	Improves pulmonary function, decreases airspace enlargement	-	Yuhgetsu et al., 2006
Rat	Papain Co-60	MSC / bone marrow	Improves alveolar parameters (mean alveoli area and linear interval)	Inhibition of the apoptosis of alveolar cell wall	Liu et al., 2008
Rat	Cigarette smoke for 6 months	BMC MSC Conditioned medium of MSC/bone marrow	Attenuates cigarette induced emphysema, restores the increased Lm, increase pulmonary microvastulature,	Paracrine effects	Huh et al., 2011
Sheep	Elastase	MSC/ lung	Increases tissue mass, lung perfusion, cellularity and ECM content.	Paracrine effects	Ingenito et al., 2011
Mice	Cigarette smoke for 6 months	Human or murine MSC / cell-free contidioned medium adipose tissue	Decreases inflammation and airspace enlargement, prevents cigarette-induced weight loss, restores cigarette-induced BM dysfunction	Paracrine effects	Schweitzer et al., 2011
Mice	Elastase	MSC / bone marrow	Ameliorates alveolar structure, restores increased Lm and destructive index	Paracrine factors	Katsha et al., 2011

Table 2. Experimental animal models of cell therapy for COPD.

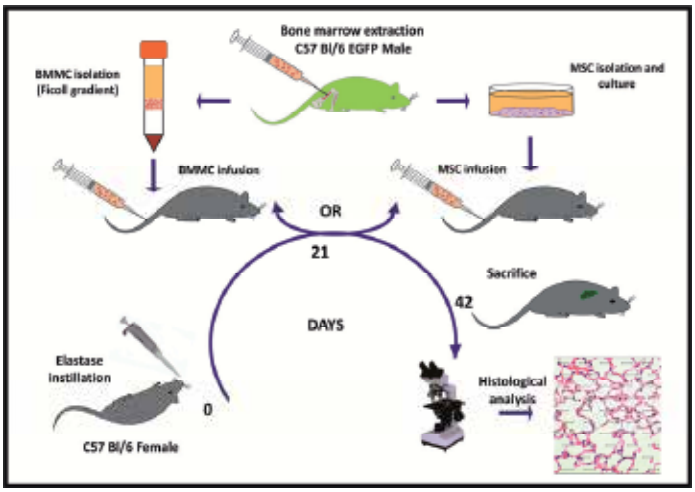

Fig. 3. Experimental design of protease-induced emphysema and ASC treatment.

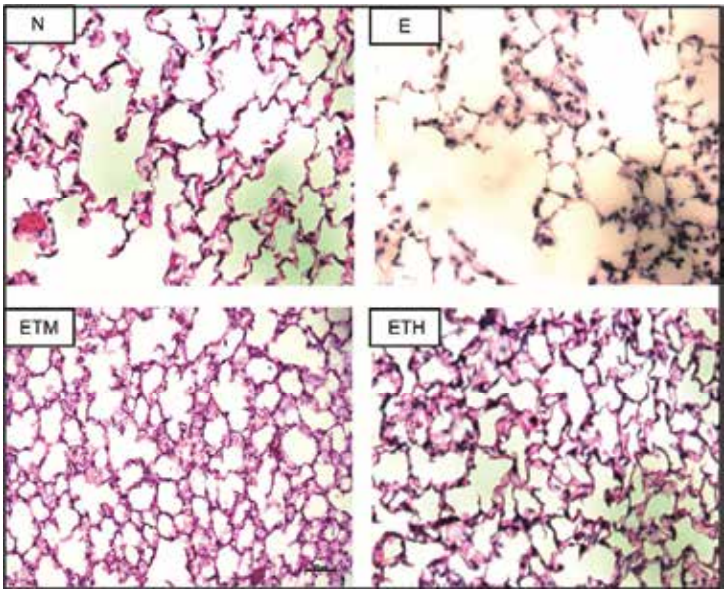

Fig. 4. Pulmonary tissue from female mice C57Bl/6 in representative histological cuts. Hematoxylin and eosin staining. Groups: N - no treatment, E - instillation of elastase only, ETM - instillation of elastase and MSC transplant and ETH - instillation of elastase and infusion of BMMC. Original magnification 200 x.

The regeneration of the pulmonary tissue, expressed in a quantitative way as the measurement of the mean linear intercept (Lm), had a significant statistical difference between animals treated with ASC and controls.

In accordance with the data showed in Figure 5, there is a statistically significant difference between E group, treated with elastase only, and N group, with no treatment, which shows evidence for the efficacy of elastase via intranasal administration in the induction of pulmonary emphysema. Between groups treated only with elastase (E) and treated with elastase and growth medium (EME) there is no statistically significant difference, which suggests the inability of the infusion vehicle in the regeneration of the pulmonary parenchyma. Furthermore, the experimental groups, treated with HSC or MSC have not shown statistically significant difference in comparison with N group, with no treatment. It is worth noting that groups treated with HSC and MSC have not turned out significant difference, which shows the therapeutic equivalence between the two stem strains originated from the bone marrow.

The comparison between the achieved values of Lm equivalent to the groups undergoing the elastase instillation (E) and treated with DMEM (EME), as well as the groups with experimentally-induced emphysema and treated with HSC or MSC has shown statistically significant difference, according to Figure 5 ($p>0.05$).

Accordingly, it is possible that MSC and BMMC hold a potential role to deliver the required cellular strain diversity during the tissue regeneration process, possibly by paracrine mechanisms (Katsha et al, 2011) and to check, in a significant and effective way, the repair of the lesion in the pulmonary tissue.

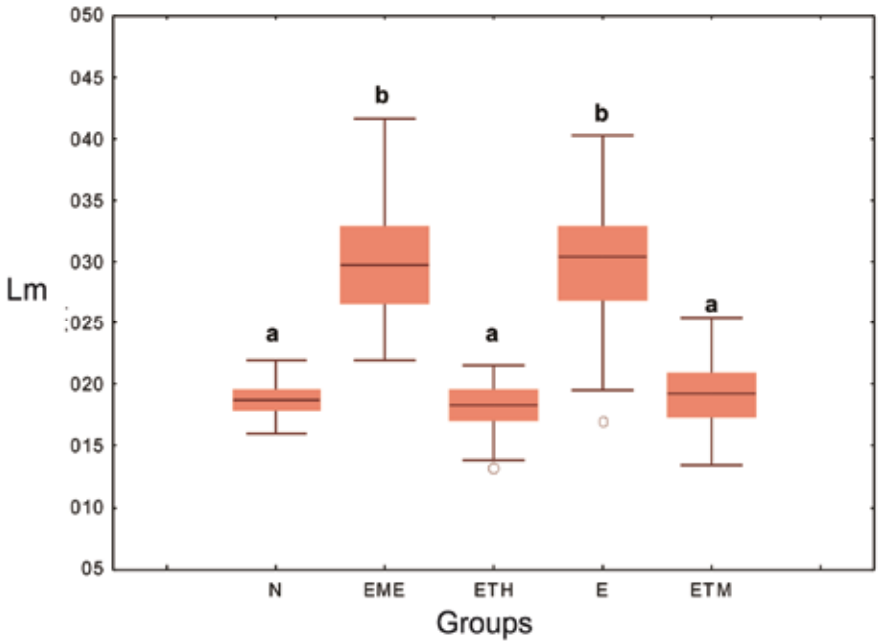

Fig. 5. Mean linear intercept (Lm) of the animals in the control and treated groups. N Group - no treatment, EME - instillation of elastase and infusion of DMEM growth medium, ETH - instillation of elastase and infusion of BMMC, E - instillation of elastase only, ETM - instillation of elastase and MSC transplant. Medians followed by the same letter indicate no significant difference (p>0,05).

As it can apprehended from the literature, there is a consistent set of results generated by several laboratories, including those achieved by our research group, which supplied the experimental basis and afforded the cell therapy application by our group in COPD patients.

5. Clinical application: Cell therapy as a new therapeutic approach for COPD

Due to the high prevalence and significant economic and social impact caused by COPD, there are, as already presented, several researches in cell therapy, described in animal models, which sustain the use of ASC in human patients with COPD.

The results arising out of the basic research in animal models of COPD cell therapy, at our laboratory, have shown regeneration of the pulmonary parenchyma both in the qualitative and in the quantitative forms, as demonstrated by the histological analyses and by the measurement of the Lm. These results were the grounds for the preparation of a research project submitted to the National Committee of Ethics in Research (CONEP-Brazil) in April 2008. The clinical protocol was approved in April 2009 (registration nº 14764, CONEP 233/2009) and, on May 11th, 2009, the first patient, with CPOD in advanced stage, was submitted to BMMC pool infusion (Ribeiro-Paes et al., 2011).

This first work corresponds to a phase 1 clinical screening for the evaluation of safety concerning SC infusion in COPD patients and it was registered with Clinical Trials – NIH – USA (NTC01110252). The experimental outlining consists, basically, of the autologous

transplant of Bone Marrow Mononuclear Cells (BMMC) pool in patients with COPD in advanced stage, higher than 3 according to the Modified Medical Research Council (MRC) Dyspnea Scale Score (Curley, 1997; Mahler & Wells, 1988). The study design is shown in Figure 6.

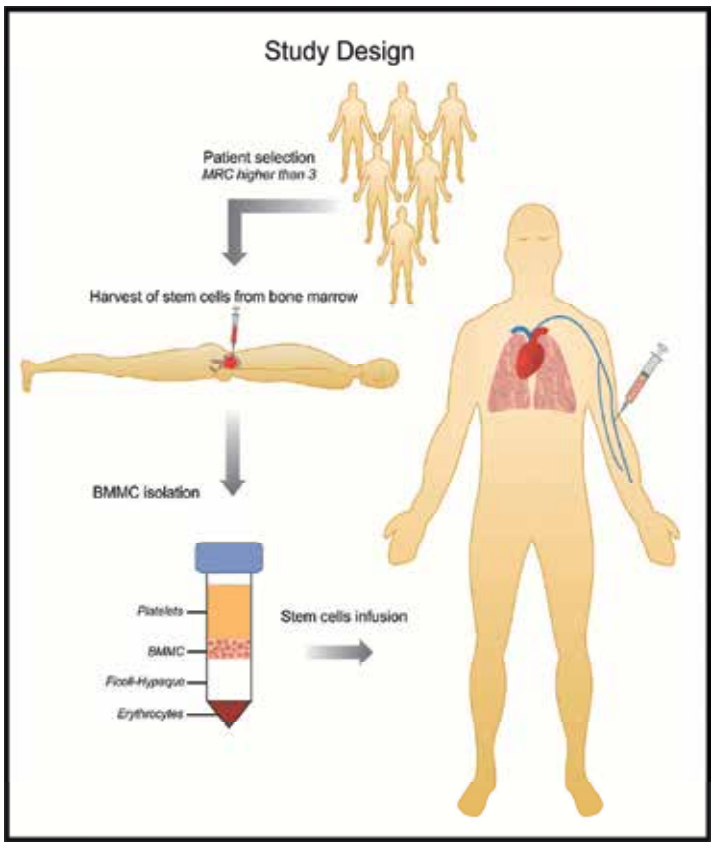

Fig. 6. Clinical protocol adopted for cell therapy in patients with advanced pulmonary emphysema (Ribeiro-Paes et al., 2011).

In the pre-procedure period, the selected patients were submitted to a full pulmonary and cardiac evaluation. Routine laboratory tests were also performed and the Dyspnea Scale Score test, modified according to the British MRC, was also conducted.The selection criteria is presented below.

Inclusion criteria: 1) age between 40 and 76 years; 2) severe obstructive pulmonary disease; 3) ineffective clinical treatment; 4) limited life expectancy; 4) limitation in daily physical activities; 5) possibility of pulmonary rehabilitation physiotherapy; 6) acceptable nutritional condition; 7) acceptable cardiac function; 8) no tobacco use for at least six months; 9) satisfactory psychosocial and emotional profile and family support and 10) Dyspnea Scale Score greater than 3.

Exclusion criteria: 1) active pulmonary or extra-pulmonary infection; 2) serious coronaropathy and/or ventricular dysfunction; 3) significant renal illness and/or hepatitis;

4) detected immunosuppressive illnesses, including HIV; 5) hepatitis B or C; 6) smoking habit; 7) carrier of known neoplasies; 8) pregnancy; 9) noncompliance with established medical protocol; 10) psychosocial problems, including drug or alcohol abuse; 11) lack of family support. After the selection, the participants received written and verbal information explaining the study and written consent was obtained from all participants before the beginning of the procedure.

After a thorough clinical evaluation, bone marrow of the voluntary patients was collected, processed and the BMMC pool achieved after isolation in Ficoll density gradient. The infusion of the achieved mononuclear fraction was made by peripheral IV (brachial medial) way and the clinical evolution of patients after the transplant has been monitored until the present date by the conduction of pulmonary function tests.

The use of BMMC pool for cell therapy in COPD patients has shown to be quite safe. No intercurrent disease occurred that could put the research's voluntary subjects in clinically serious situations or long lasting discomfort.

All the voluntary subjects of the research had some kind of clinical improvement. The spirometry tests showed a very slight improvement, as shown in Figure 7. The VEF 1 showed an improvement in all patients after thirty days.

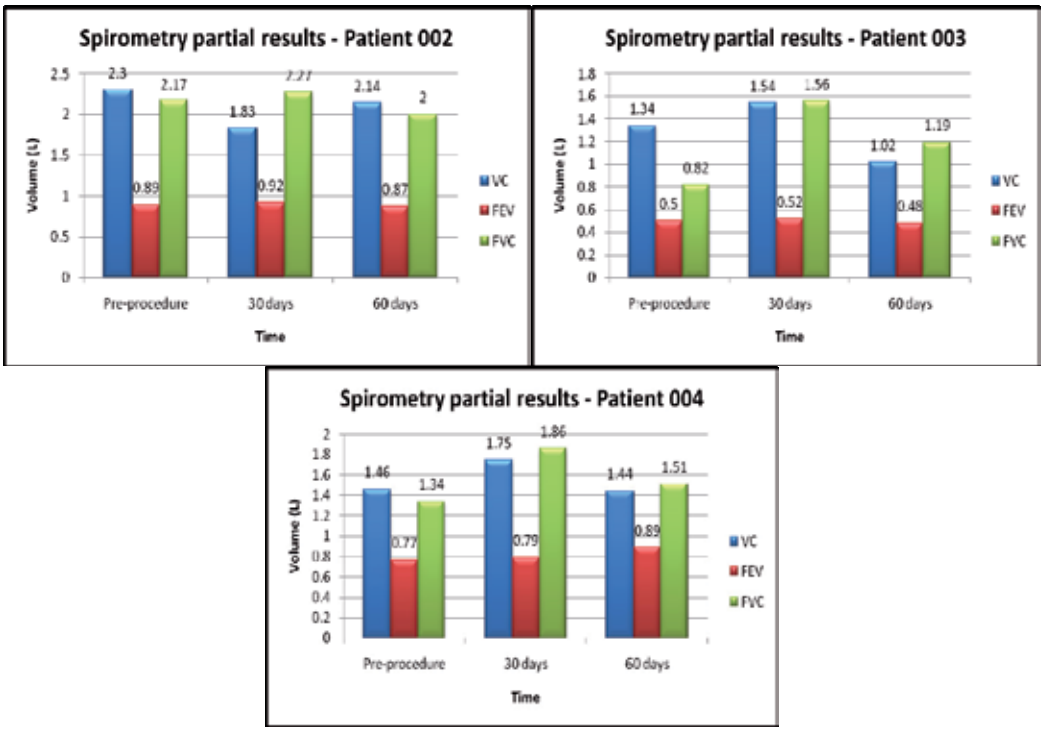

Fig. 7. Spirometry absolute values from 3 research patients included in clinical protocol and submitted to autologous BMMC transplantation.

Likewise, the increase in the CVF and CV parameters occurred in all patients after 30 days had lapsed from the procedure (Figure 7). However, after this period, there was a decrease

in CVF; in spite of this fact, an important aspect is that the functional parameters remained always higher than the ones found before the procedure.

An interesting information turned out in the long term results, approximately 2 years of clinical monitoring. The spirometry parameters along the post transplantation period, by and large, maintain a certain regularity and similarity to those found before the procedure. One of the research subject disclosed a significant increase in the forced vital capacity, after 1 year and 3 months of treatment (Figure 8); The analysis of this parameter suggests a proximity to normality and reduction of the severity of the disease.

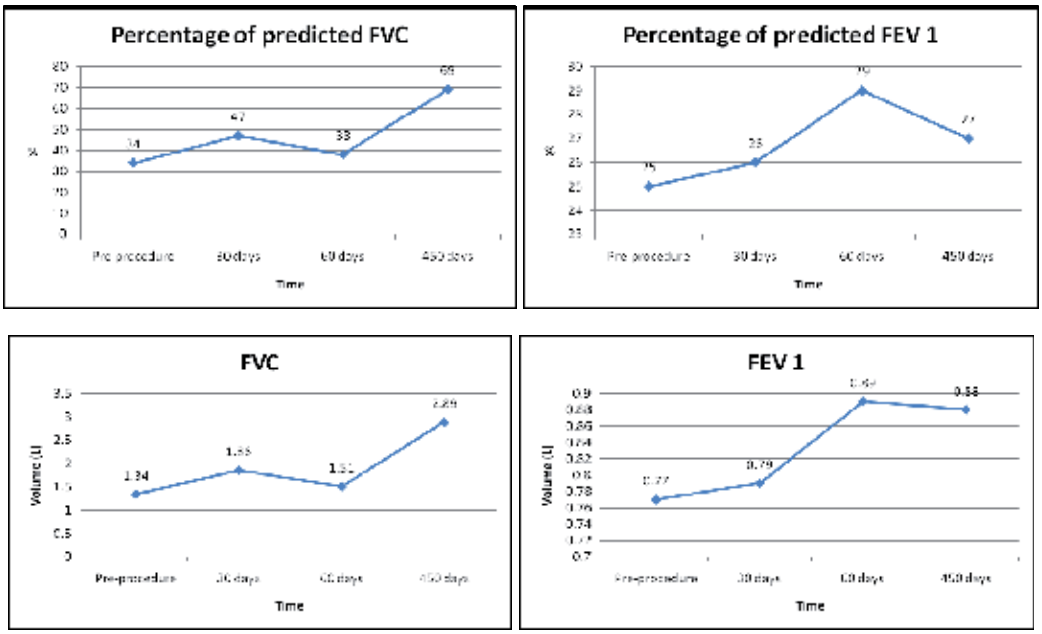

Fig. 8. Percentage of predicted and absolute values from a patient spirometry until 1 year and 3 months after BMMC autologous transplant.

The results from this clinical protocol show the procedure should be conducted at an earlier stage, that is, at a less advanced stage of the pathology. As mentioned, the laboratorial analysis, confirmed by clinical response, has reported a significant improvement in all patients, chiefly in the first 30 days after the procedure was carried out. After this period, laboratory tests displayed a tendency to decrease; however they did not drop to the base values obtained before the BMMC therapy treatment. These results advance the possibility that cell therapy may be applied in repeated doses from time to time for the purpose of stimulating pulmonary regeneration.

Another protocol under registration with Clinical Trials (NTC00683722) corresponds to a multicenter, double-blind, placebo controlled phase II study for patients with moderate to severe COPD. The clinical protocol, sponsored by Osiris Therapeutics Inc. (Columbia, MD), concerns the employment of *ex vivo* cultured adult human SC (PROCHYMAL) in the treatment of pulmonary emphysema. The purpose comprehends the evaluation of safety and efficacy of MSC multiple infusion.

As proposed by Osiris Therapeutics "Preclinical and clinical data suggest that Prochymal's unique mechanism of action may provide a first-in-class treatment option with the ability to reverse the underlying disease". However, there is no publication to date reporting the results arising out of the screening made in 62 patients. By virtue of the lack of results from the use of PROCHYMAL cell therapy in COPD, it is not possible to check and uphold the effect of regression of chronic inflammation in lungs as a response to the MSC treatment. Therefore, no critical evaluation may be made about the results of the protocol proposed by Osiris Therapeutics.

More recently, a phase 1 clinical study sponsored by Leiden University Medical Center (Leiden, Netherlands) was registered with Clinical Trials.gov (NCT01306513). The clinical protocol consists of the autologous transplant of bone-marrow-derived MSC in patients with COPD (MRC 3) before the surgery to reduce pulmonary volume. The purpose of the work, still in progress, is the evaluation of the cell therapy safety, as well as the feasibility of cultivating MSC.

The results achieved by our group, as well as the registration of clinical protocols concerning cellular therapy by other research centers, have led to the opening of new strategies of therapeutic investigation. Thus, it is possible to establish ew perspectives in regard to the formulation of cell therapy experimental designs which will be surely incorporated into future research projects for the purpose of optimizing the clinical effect and the quality of life of COPD patients.

6. Perspectives and challenges

COPD represents a serious public health problem, which, according to the latest projections of the World Health Organization, should gradually change for the worse in the coming years, with a great impact on the economy, on a global scale.

The incorporation of new drugs having more effectiveness and longer effect unquestionably has contributed to the improvement in quality of life of the patients; however, up to now, no significant change in the natural history of the disease has been achieved. In this context, cell therapy turns out as a potentially promising treatment option, which, perhaps, may represent a change of paradigm in therapeutics and in the natural course of the disease.

The results achieved at our laboratory and by several other coworkers, at different research centers, have shown a morphological recovery of the pulmonary parenchyma in animals with experimentally-induced emphysema by the employment of proteases and/or cigarette smoke. From said results, a pioneer treatment with BMMC pool was administered for patients with emphysema in advanced stage. It is a project in an initial phase and the sample of treated patients is still small, which limit the analyses from the statistical point of view. At our research center, a new project will soon start. It will comprehend a larger sample (about 40 patients) and the employment of a new methodology, which the use of MSC.

Notwithstanding the statistical limitations, the pioneer publication of the results by our research group (Ribeiro-Paes *et al.*, 2011), has afforded the preparation of some logical inferences and methodological suggestions which will be incorporated into future projects. The use of MSC obtained from adipose tissue has disclosed a highly promising future perspective. Furthermore, the feasibility of establishing a protocol with repeated SC infusions should also be taken into account, just like in chronic treatments with drugs.

There are, finally, a series of questions and possibilities that arise from this pioneering studies and results obtained in our laboratory. The sample of treated patients is still small. There is, indeed, in these beginnings of research, far more doubts than certainties. Also, extreme caution should be exercised so as not to arouse false expectations and unrealistic hopes in COPD patients. Only the first trials have been carried out. We do not know exactly how this story is going to unfold. However, the first steps of a long and challenging journey have been taken, but surely it looks potentially promising, in therapeutic terms. For our group, it means a very stimulating journey of research and work.

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A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.

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