COPD and heart failure comorbidity from patients to mechanisms

Mira Abou-Rjeili

Division of Experimental Medicine, Faculty of Medicine, McGill University, Montreal

March 2022

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Table of Contents

Abstract

Introduction: Chronic Obstructive Pulmonary Disease (COPD) and Chronic Heart failure (CHF) are two highly prevalent conditions that significantly impact patients, families and the health care system. They are often concomitant and the presence of both diseases negatively affects patient outcomes especially if the comorbidity is overlooked. A larger proportion of patients with COPD with comorbid CHF have preserved ejection fraction (HFpEF) compared to CHF with reduced ejection fraction (HFrEF), however, the abnormalities have not been well characterized in these patients. Chronic cigarette smoking is the most important risk factor for the development of COPD. Cigarette smoking induces pulmonary inflammation and activation of lung epithelial cells that release pro-inflammatory cytokines. The inflammatory state associated with COPD is not confined to the lungs and occurs systemically and leads to endothelial dysfunction which is a key mediator in the development of atherosclerosis and cardiovascular disease.

We hypothesized that patients with COPD and comorbidity of CHF will differ in lung structure, severity of airflow obstruction and blood biomarkers and that cigarette smoke affects the cross-talk and activation of different lung cells. The main objectives of the thesis were 1) to determine the prevalence of co-morbid CHF in COPD individuals in two separate studies, from a population-based and a clinical samples, and determine patients' characteristics which could be used to in clinical practice for active screening; and 2) to characterize the expression of inflammatory biomarkers in an *in vitro* model of cigarette smoke exposure. Three research projects were carried out to address these objectives (two projects for objective 1 and one project for objective 2).

Methods: In study 1, we characterized the prevalence of co-morbid COPD-early CHF in a population-based sample Multi-Ethnic Study of Atherosclerosis (MESA) of older adults, and associated imaging, neurohormonal, and inflammatory features. For study 2, we conducted a prospective observational study to determine the prevalence of comorbid COPD and CHF in stable patients with advance COPD in a specialized COPD clinic. Each patient underwent a detailed cardiopulmonary evaluation to establish diagnosis and was followed up for 12 months to collect data on exacerbation-like events and on hospitalizations for respiratory and cardiovascular adverse events. For study 3, we established an *in vitro* coculture model of cigarette smoke exposure. Normal human bronchial airway epithelial (NHBE) cells were treated with cigarette smoke extract (CSE) and cocultured with human lung microvascular endothelial cells (HMVEC-L) cells. We then assess levels of inflammatory biomarkers from these cells.

Results: Study 1- In the population-based sample (MESA), our study showed a prevalence of 12.7% of CHF in participants with COPD. Participants with COPD and early CHF were older and had a significantly higher BMI and significantly more comorbidities when compared to those with COPD only. They also had significantly worse lung function, however, no association was observed between the pulmonary structure, levels of serum biomarker and severity of airflow obstruction in COPD with CHF when participants with COPD without CHF were used as a reference group.

Study 2- In the clinical convenient sample of COPD patients, our study showed that unrecognized CHF in COPD is very common (prevalence 29.6%). Patients with both COPD and CHF were older, heavier smokers and a higher percentage of these patients have had an exacerbation in the past year. They also have higher rates of cardiac and diabetes comorbidities. Pulmonary function test variables and emphysema levels were similar between groups. Troponin

levels and eosinophils levels were also higher in patients with both diseases. Out of the COPD patients with CHF, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). A higher percentage in the HFrEF group had more than 1 exacerbation per year and more than 2 exacerbations per year. In the HFrEF group, more patients required a doctor's visit because of the exacerbation and required hospitalization. In the one-year follow-up, CAT score >10 was associated with an increased odds of having an exacerbation and an increase in fibrinogen levels was associated with a reduced odds of having an exacerbation. There was also an increased odds of having one exacerbation when the levels of eosinophils > 150 cells/μL.

Study 3- In the *in vitro* model of cigarette smoke exposure, we observed a significant time-dependent increases in IL-8, S100A8 and S100A9 expression in response to CSE in NHBE cells. NHBE cells exposed to CSE and cocultured with human lung microvascular endothelial cells (HMVEC-L) cells did not lead to the activation of HMVEC-L cells, as there was no significant increase in the levels of IL-6, VCAM-1 or E-selectin in these cells.

Conclusion: Our observational studies (population and clinical samples) provide evidence of a significant prevalence of undiagnosed CHF in COPD patients. Some clinical characteristics and more specifically age, smoking status, BMI, comorbidities and history of exacerbation could help targeting patients more likely to have CHF. However, we could not demonstrate distinct features with respect to the biomarkers, e.g., blood biomarkers, severity of airflow obstruction or emphysema levels. Our *in vitro* study demonstrated that cigarette smoke significantly increases the expression of inflammatory biomarkers including alarmins in lung epithelial cells, however, factors released from smoke-exposed lung epithelial cells do not activate lung endothelial cells. To improve patient care in people with COPD, the extent to which CHF co-morbidity co-exist with COPD needs to be recognized. We still don't have prognostic biomarkers that could be used

to distinguish COPD patient with CHF from those without CHF. However, targeted evaluation and treatment of early cardiac dysfunction in group of COPD individuals with clinical phenotypes should be done in an attempt to reduce the likelihood of cardiac adverse events and mortality.

Résumé

Introduction : La maladie pulmonaire obstructive chronique (MPOC) et l'insuffisance cardiaque (IC) sont deux pathologies très répandues qui ont un impact significatif sur les patients, les familles et le système de santé. Elles sont souvent concomitantes et la présence des deux maladies affecte négativement les résultats des patients, surtout si la comorbidité est négligée. Une plus grande proportion de patients atteints de MPOC avec une IC comorbide ont une fraction d'éjection préservée (HFpEF) par rapport à l'IC avec fraction d'éjection réduite (HFrEF), cependant, les anomalies n'ont pas été bien caractérisées chez ces patients. Le tabagisme chronique est le facteur de risque le plus important pour le développement de la MPOC. Le tabagisme induit une inflammation pulmonaire et une activation des cellules épithéliales pulmonaires qui libèrent des cytokines pro-inflammatoires. L'état inflammatoire associé à la MPOC n'est pas confiné aux poumons et se manifeste de manière systémique, entraînant un dysfonctionnement endothélial qui est un médiateur important dans le développement de l'athérosclérose et des maladies cardiovasculaires.

Notre hypothèse est que les patients atteints de MPOC et de comorbidité d'IC diffèrent en termes de structure pulmonaire, de sévérité de l'obstruction des voies respiratoires et de biomarqueurs sanguins, et que la fumée de cigarette affecte la communication et l'activation des différentes cellules pulmonaires. Les principaux objectifs de la thèse étaient 1) de déterminer la prévalence de la comorbidité de l'IC chez les personnes atteintes de MPOC dans deux échantillons distincts, un échantillon de population et un échantillon clinique, et de déterminer les caractéristiques des patients qui pourraient être utilisées dans la pratique clinique pour un dépistage actif ; et 2) de caractériser l'expression des biomarqueurs inflammatoires dans un modèle *in vitro* d'exposition à

la fumée de cigarette. Trois projets de recherche ont été menés pour répondre à ces objectifs (deux projets pour l'objectif 1 et un projet pour l'objectif 2).

Méthodes : Dans l'étude 1, nous avons caractérisé la prévalence de la comorbidité MPOC - début IC dans un échantillon de population Multi-Ethnic Study of Atherosclerosis (MESA) d'adultes âgés, et les caractéristiques d'imagerie, neurohormonales et inflammatoires associées. Pour l'étude 2, nous avons mené une étude observationnelle prospective pour déterminer la prévalence de la comorbidité MPOC et IC chez des patients stables atteints de MPOC avancée dans une clinique spécialisée dans la MPOC. Chaque patient a subi une évaluation cardiopulmonaire détaillée pour établir le diagnostic et a été suivi pendant 12 mois pour recueillir des données sur les événements de type exacerbation et sur les hospitalisations pour des événements indésirables respiratoires et cardiovasculaires. Pour l'étude 3, nous avons établi un modèle de coculture *in vitro* d'exposition à la fumée de cigarette. Des cellules épithéliales normales des voies respiratoires bronchiques humaines (NHBE) ont été traitées avec un extrait de fumée de cigarette (CSE) et mises en coculture avec des cellules endothéliales microvasculaires pulmonaires humaines (HMVEC-L). Nous évaluons ensuite les niveaux de biomarqueurs inflammatoires de ces cellules.

Résultats : Etude 1- Dans l'échantillon de population (MESA), notre étude a montré une prévalence de 12,7% d'IC chez les participants atteints de MPOC. Les participants atteints de MPOC et de début IC étaient plus âgés et avaient un BMI significativement plus élevé et significativement plus de comorbidités par rapport à ceux atteints de MPOC uniquement. Ils avaient également une fonction pulmonaire significativement plus mauvaise. Cependant, aucune association n'a été observée entre la structure pulmonaire, les niveaux de biomarqueur sérique et

la sévérité de l'obstruction des voies respiratoires chez les MPOC avec IC lorsque les participants atteints de MPOC sans ICC ont été utilisés comme groupe de référence.

Etude 2- Dans l'échantillon clinique commode de patients atteints de MPOC, notre étude a montré que l'IC non reconnue dans la MPOC est très fréquente (prévalence 29,6%). Les patients atteints à la fois de MPOC et d'IC sont plus âgés, plus gros fumeurs et un pourcentage plus élevé de ces patients a eu une exacerbation au cours de l'année passée. Ils ont également des taux plus élevés de comorbidités cardiaques et de diabète. Les variables des tests de fonction pulmonaire et les niveaux d'emphysème étaient similaires entre les groupes. Les taux de troponine et d'éosinophiles étaient également plus élevés chez les patients atteints des deux maladies. Parmi les patients MPOC atteints d'IC, 6 ont été classés comme ayant une HFrEF (37,5%) et 10 comme ayant une HFpEF (62,5%). Un pourcentage plus élevé dans le groupe HFrEF avait plus d'une exacerbation par an et plus de deux exacerbations par an. Dans le groupe HFrEF, un plus grand nombre de patients ont dû consulter un médecin en raison de l'exacerbation et ont dû être hospitalisés. Dans le suivi d'un an, le score CAT >10 était associé avec une probabilité plus élevée d'avoir une exacerbation et une augmentation des niveaux de fibrinogène était associée à une réduction des chances d'avoir une exacerbation. Il y avait également une augmentation des chances d'avoir une exacerbation lorsque les niveaux d'éosinophiles > 150 cellules/µL.

Étude 3- Dans le modèle *in vitro* d'exposition à la fumée de cigarette, nous avons observé une augmentation significative, en fonction du temps, de l'expression de IL-8, S100A8 et S100A9 en réponse au CSE dans les cellules NHBE. Les cellules NHBE exposées au CSE et cocultivées avec des cellules endothéliales microvasculaires pulmonaires humaines (HMVEC-L) n'ont pas conduit à l'activation des cellules HMVEC-L, car il n'y a pas eu d'augmentation significative des niveaux d'IL-6, de VCAM-1 ou de E-sélectine dans ces cellules.

Conclusion : Nos études observationnelles (population et échantillons cliniques) fournissent des preuves d'une prévalence significative d'IC non diagnostiquée chez les patients atteints de MPOC. Certaines caractéristiques cliniques et plus précisément l'âge, le tabagisme, BMI, les comorbidités et les antécédents d'exacerbation pourraient aider à cibler les patients les plus susceptibles d'avoir une IC, mais nous n'avons pas pu mettre en évidence de caractéristiques distinctes en ce qui concerne les biomarqueurs, par exemple les biomarqueurs sanguins, la sévérité de l'obstruction des voies respiratoires ou les niveaux d'emphysème. Notre étude *in vitro* a démontré que la fumée de cigarette augmente de manière significative l'expression des biomarqueurs inflammatoires, y compris les alarmines, dans les cellules épithéliales pulmonaires. Cependant, les facteurs libérés par les cellules épithéliales pulmonaires exposées à la fumée n'activent pas les cellules endothéliales pulmonaires.

Pour améliorer les soins aux patients atteints de MPOC, il convient de reconnaître dans quelle mesure la comorbidité de l'IC coexiste avec la MPOC. Nous n'avons toujours pas de biomarqueurs prognostiques qui pourraient être utilisés pour distinguer les patients MPOC atteints d'IC de ceux qui ne le sont pas. Cependant, l'évaluation et le traitement ciblés de la dysfonction cardiaque précoce dans le groupe de personnes atteintes de MPOC présentant des phénotypes cliniques devraient être effectués dans le but de réduire la probabilité d'événements cardiaques indésirables et de mortalité.

Acknowledgements

There have been numerous people who have helped and guided me throughout my PhD studies and all the work presented in this thesis would not have been possible without their support.

First and foremost, I am extremely grateful to my supervisor Dr. Jean Bourbeau for the opportunity to work with him on my PhD, for his invaluable advice, continuous support, and patience during my studies. His passion for research inspired mine and many of the accomplishments throughout my PhD would not have been possible without his constant encouragement. His immense knowledge and experience have encouraged me in my academic research; he helped guide my projects and provide critical and valuable feedback while allowing me to explore my own ideas and research questions. I can't thank him enough for always taking the time to meet with me and address any questions and concerns I had and provide feedback and careful editing on everything from scholarship applications, to manuscripts, to my thesis. This thesis would not be possible without his support, help, and guidance.

I would like to express my sincere gratitude to my committee members and academic advisor: Dr. Carolyn Baglole, Dr. Benjamin Smith, Dr. Sabah Hussain, Dr. Simon Rousseau, Dr. Michel White as well as my previous academic advisor Dr. Siham Sabri. They provided critical and valuable feedback on my work and helped steer my projects in the right direction with their insightful comments and suggestions.

I would like to extend my sincere thanks to all previous and current members of our research team headed by Dr. Jean Bourbeau for all their help throughout these past years. They were always willing to help and provide feedback on presentations and protocols.

A special thanks to Dr. Raquel Farias who I've worked with since I started working in the lab of Dr. Bourbeau. Her mentorship and guidance in every aspect of my research was essential to get

my studies started, develop new objectives and steer the projects in the right direction. With her help, we wrote study protocols for the projects and obtained ethics approval for the clinical study. Under her guidance, I learned essential clinical research skills and she always took the time to provide any feedback necessary on my work.

I thank Dr. Yousof Mostafavi, Celeste Laporte and Forest Day de Larrañaga for all their help with the clinical project from patient recruitment to data collection. I thank Dr. Suurya Krishnan for all this help with the MESA study and editing of the protocol. I thank Dr. Nadia Naderi, Dr. Hussein Traboulsi and Noof Aloufi for all their help and guidance with the experimental study; they always helped with any issues the project ran into.

I thank Pei Z Li for her help with statistical analyses for my projects, her patience and knowledge helped me tremendously.

I owe a great deal of appreciation to all members of the Centre for Innovative Medicine (CIM) where the clinical study was conducted. A special thanks to Meena Patel for her help and guidance in getting the clinical study started. She helped me get the ethics approval for the study, recruit patients and learn all the necessary tests.

I am grateful to the Research Institute of the McGill University Health Centre, the Meakins-Christie Laboratories and Fonds de Recherche Santé Québec for the awards I received during my doctoral studies.

I owe many thanks to all my friends who have supported me throughout this journey.

And finally, I am deeply grateful to my parents, my siblings and my fiancé for their unwavering support and belief in me. This would not have been possible without them.

Preface

One of the major goals of the projects constituting this thesis was to characterize chronic heart failure (CHF) as a cardiovascular comorbidity in individuals with chronic obstructive pulmonary disease (COPD). This thesis was prepared according to the McGill University rules for a manuscript-based thesis. This thesis consists of three manuscripts that are currently under review and will be submitted for publication in 2022. All the projects address important research topics related to COPD and CHF.

This thesis contains ten chapters:

Chapter 1 provides a comprehensive literature review of the topic that will be discussed in the thesis, it discusses comorbidities in COPD and more specifically CHF and the potential links between the diseases

Chapter 2 summarizes the rationale, hypothesis, and objectives of the thesis

Chapter 3 to 7 include the three manuscripts of original research (chapters 3, 5 and 7) that constitute the thesis and 2 bridging chapters (chapters 4 and 6)

Each manuscript will be organized as follows: title, abstract, introduction, methods, results, discussion, and tables/ figures and legends.

Chapter 3 consists of the manuscript entitled “Recognizing early cardiac dysfunction in chronic obstructive pulmonary disease (COPD): the Multi-Ethnic Study of Atherosclerosis (MESA)” that examined the characteristics of comorbid COPD and CHF in a population sample.

Chapter 4 is the bridging chapter which highlights the importance of studying characteristics of individuals with these comorbidities in a clinical setting

Chapter 5 consists of the manuscript entitled "Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure" that examined the characteristics of comorbid COPD and CHF in a clinical sample

Chapter 6 is the bridging chapter which highlights the importance of developing a translational research project to study the effect of cigarette smoke; the main risk factor for COPD and CHF

Chapter 7 consists of the manuscript entitled "Cigarette smoking mediates the expression of alarmins and other inflammatory biomarkers in lung epithelial cells" that examined the interaction and response of different lung cells following exposure to cigarette smoke

Chapter 8 summarizes the findings of the manuscripts and includes a general discussion and overall conclusion

Chapter 9 provides a reference list not included in the manuscripts

Chapter 10 contains supplementary material such as study protocols, consent forms and case report forms

Contribution of Authors

My thesis supervisor, Dr. Jean Bourbeau, significantly contributed to this thesis; he supervised and oversaw all aspects of it and the projects that constitute it. Dr. Bourbeau was actively involved in protocol design, research questions and hypothesis discussion, implementation of the projects and manuscript review.

All chapters included in this thesis were written by Mira Abou-Rjeili and thoroughly reviewed by Dr. Jean Bourbeau.

Mira Abou-Rjeili is the first author of the three manuscripts included in this thesis. She was involved in designing research questions, writing protocols, designing case reports forms and consent forms, implementing the study protocol, recruiting patients, performing baseline study visits with patients and performing necessary experiments.

All authors made substantial contributions to the development and implementation of the projects.

Dr. Raquel Farias, a former research associate in Dr. Bourbeau's team significantly contributed to protocol design of the MESA study, contributed to protocol design, case report form design, writing of consent forms and implementation of the clinical study as well contributed to all stages of the *in vitro* study from research question discussion to discussion and interpretation of the results.

Dr. Carolyn Baglole, a member of my doctoral thesis committee, significantly contributed to all stages of the *in vitro* project: from designing experiments and research questions to manuscript review.

Dr. Sabah Hussain, a member of my doctoral thesis committee, also significantly contributed to all stages of the *in vitro* project: from designing experiments and research questions to manuscript review.

Dr. Benjamin Smith, a member of my doctoral thesis committee, significantly contributed to all stages of the MESA study: from designing the research questions and writing of the protocol to statistical analysis and manuscript review.

Dr. Michel White, a cardiologist at the Montreal Heart Institute, contributed to the protocol design and research question design of the clinical project.

Pei Zhi Li significantly contributed to the statistical analyses of studies of manuscripts 1 and 2 in this thesis.

Seyed-Mohammad-Yousof Mostafavi- Pour-Manshadi assisted with patient recruitment and performing patients visits for the clinical study.

Suurya Krishnan assisted in editing the protocol that was submitted to MESA

All patient recruitments and visit tests were performed at the Centre for Innovative Medicine (CIM) at the Research Institute of the McGill University Health Centre (RI-MUHC). All *in vitro* experiments were performed at the Meakins-Christie laboratories at the RI-MUHC.

Contribution to original knowledge

The manuscripts in this thesis represent my original work.

Manuscript 1 entitled "Recognizing early cardiac dysfunction in chronic obstructive pulmonary disease (COPD): the Multi-Ethnic Study of Atherosclerosis (MESA)" examined the characteristics of comorbid COPD and CHF in a population sample. This study captures early disease by utilizing a validated score that relies on clinical characteristics and echocardiography to define early CHF. This study shows that despite the fact that we were looking at early undiagnosed disease, there's still a significant proportion of individuals in the population that qualify as COPD and a significant proportion of these individuals qualify as having early CHF. Characteristics such as older age, higher BMI, presence of atrial fibrillation and obesity as comorbidities and lower lung function more specifically lower pre and post-bronchodilator FEV1 and FVC were significantly different between individuals who have COPD alone and those who have COPD and CHF. We were not able to show a relationship between pulmonary structure as assessed by levels of emphysema, serum biomarker NT-proBNP, severity of airflow obstruction and having COPD with CHF compared to having COPD only. However, the data still provided evidence of the importance of evaluation and likely providing treatment of early cardiac dysfunction in susceptible individuals with COPD.

Manuscript 2 entitled "Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure" examined the characteristics of comorbid COPD and CHF in a clinical sample. This study for the first time performed a very extensive cardiopulmonary evaluation including biomarkers in every patient to diagnose CHF in COPD. Work-up bias was eliminated from our study by having all subjects undergo all the diagnostic tests necessary to classify CHF and COPD respectively. Even though we recruited

patients from a specialized COPD clinic, it was surprising to find that a significant number of COPD patients had unrecognized CHF. Some clinical characteristics and more specifically age, smoking status, heart disease, hypertension and diabetes as comorbidities and history of exacerbation were identified that could help clinicians targeting stable COPD patients who are more likely to have concomitant CHF, particularly those with HFrEF. We showed for the first time the differences in exacerbations between CHF phenotypes in COPD individuals. Blood biomarkers, lung function or CT scan abnormalities were not able to discriminate between stable COPD without or with CHF.

The study provides some evidence in favor of actively screening at minimum a subgroup of COPD patients for CHF comorbidities and modify treatment if necessary.

Manuscript 3 "Cigarette smoking mediates the expression of alarmins and other inflammatory biomarkers in lung epithelial cells" examined the interaction and response of different lung cells following exposure to cigarette smoke; the main risk factor for COPD and CHF. We showed for the first time an increased expression of alarmins S100A8 and S100A9 in lung epithelial cells in response to cigarette smoke stimulus. Another novel aspect of the study was mimicking the lung–blood barrier by using a bi-culture model of pulmonary epithelial and endothelial cells to understand how cigarette smoke affects this interaction.

List of Tables

List of figures

Manuscript 3:

Figure 7. mRNA levels of inflammatory biomarkers IL-6(A), IL-8(B), S100A8(C), S100A9(D) following exposure of NHBE cells to CSE for 24h and then coculturing them with HMVEC-L cells for 24h

Figure 8. Protein levels of inflammatory biomarkers IL-6 (A), and S100A8 (B) following exposure of NHBE cells to CSE for 24h and then coculturing them with HMVEC-L cells for 24h

Figure 9. mRNA levels of inflammatory biomarker IL-6 (A) and adhesion molecules VCAM-1 (B) and E-selectin(C)

List of abbreviations

Chronic obstructive pulmonary disease (COPD)

Cigarette smoking (CS)

Chronic bronchitis (CB)

Cigarette smoke extract (CSE)

Interleukin (IL)-1, IL-6, IL-8

Tumor necrosis factor-alpha (TNF-α)

Normal Human bronchial airway epithelial cells (NHBE)

Human lung microvascular endothelial cells (L-HMVEC)

Damage Associated Molecular Pattern Molecules" (DAMPs)

Pattern recognition receptors (PRRs)

Bronchoalveolar lavage (BAL)

Air-liquid interface (ALI)

Intercellular adhesion molecule-1 (ICAM-1)

Vascular cell adhesion molecule-1 (VCAM-1)

Forced vital capacity (FVC)

Forced expiratory volume in one second (FEV1)

Lower limit of normal (LLN)

Modified British Medical Research Council (mMRC)

COPD Assessment Test (CAT)

The Canadian Cohort of Obstructive Lung Disease (CanCOLD)

Asthma COPD overlap (ACO)

Cardiovascular disease (CVD)

Hazard ratio (HR)

Atrial fibrillation (AF)

Myocardial infarction (MI)

Acute exacerbation of COPD (AECOPD)

Heart failure (HF)

Left ventricular ejection fraction (LVEF)

The New York Heart Association (NYHA) functional classification

Natriuretic peptides (NPs)

B-type natriuretic peptide (BNP)

N-terminal pro-BNP (NT-proBNP)

Electrocardiogram (ECG)

HF with reduced EF (HFrEF)

HF with preserved EF (HFpEF)

HF with mid-range EF (HFmrEF)

C-reactive protein (CRP)

Surfactant protein D (SP-D)

Club cell secretory protein 16 (CCSP-16)

Global initiative for chronic obstructive lung disease stage(GOLD)

Total lung capacity (TLC)

Functional residual capacity (FRC)

Diffusing capacity for carbon monoxide (DLCO)

Left ventricle (LV)

Left atrium (LA)

Pulmonary artery systolic pressure (PASP)

Right atrial pressure (RAP)

Pulmonary Hypertension (PHTN)

Chronic lower respiratory disease (CLRD)

Ischemic heart disease (IHD)

Chapter 1: Literature Review

1.1 COPD definition, prevalence and burden

Chronic Obstructive Pulmonary Disease (COPD) is defined as a chronic, preventable and treatable disease caused by exposure to noxious particles or gases. It is characterized by progressive airflow limitation and chronic airway inflammation as well as chronic respiratory symptoms. COPD prevalence varies based on the definition and the method of diagnosis used, but most data shows that around 6% of the adult population have been told they have COPD. It is however likely that this number is underestimated[1].

The term COPD engulfs two types of chronic diseases: emphysema and chronic bronchitis. The two conditions usually occur together and can vary in severity among individuals with COPD. Emphysema is characterized by permanent enlargement and destruction of the alveoli. This destruction results in a reduction in the area available for gas exchange. The destruction of the lung parenchyma will lead to a decrease in the lung elastic recoil and gas trapping. The pathological patterns of emphysema can be divided into three subtypes : centrilobular, panacinar, and paraseptal. Centrilobular emphysema predominantly affects the upper portions of the lung and most commonly seen in smokers. Panacinar emphysema is seen predominantly in the lower lobes and has classically but not exclusively been described with α-1 antitrypsin deficiency[2].

Chronic bronchitis (CB) which is primarily clinically defined as a chronic cough and sputum production for at least 3 months per year for two consecutive years. The main pathological characteristic of CB is the presence of excessive mucus and this is due to overproduction and hypersecretion by goblet cells and decreased elimination. Mucus hypersecretion and overproduction is caused by exposure to cigarette smoke as well as bacterial or viral infections.

Mucus clearance is negatively affected by poor ciliary function, distal airway occlusion, and ineffective cough secondary to respiratory muscle weakness[3, 4].

COPD is a disease that is associated with a high social and economic burden as it is one of the causes of mortality and morbidity worldwide. Morbidity includes visits by the patient to their physician, visits to the emergency department and hospitalizations; all which are increased in patients with COPD. Studies have also shown that morbidity in COPD patients increases with age and when other concomitant conditions are present[5]. Beyond the clinical impact on patient, COPD is also associated with a significant economic burden that increases with disease severity with episodes of COPD exacerbations accounting for the greatest proportion of the burden[6].

1.2 COPD diagnosis and symptoms

The presence of airflow obstruction as well as symptoms are needed for the diagnosis of COPD. Symptoms include the presence of persistent dyspnea that is progressive over time and gets worse with exercise, the presence of chronic cough or sputum production. The symptoms can precede the development of airflow limitation by many years. Wheezing and chest tightness can also occur, as well as fatigue and weight loss. Symptoms are heterogenous with respect to the presence and severity of airflow obstruction and tend to vary between individuals and from day to day in the same individual[7, 8].

Other than symptoms, COPD should be considered in individuals with a history of exposure to risk factors including smoking as well as occupational or environmental exposures.

Spirometry is the standard test used to diagnose COPD; it offers reproducible and objective measurement of airflow limitation. Spirometry measures the volume of air forcibly exhaled from the point of maximal inspiration or forced vital capacity (FVC) and the volume of air exhaled

during the first second of the test or forced expiratory volume in one second (FEV1); the ratio of these two values is then calculated (FEV1/FVC). For a diagnosis of airflow limitation and confirm that a patient has COPD, the value used is a post-bronchodilator FEV1/FVC < 0.70. The values of FEV1 and FVC obtained for each patient are compared with reference values based on age, height, sex, and race[9].

The severity of airflow limitation in COPD patients is based on the global initiative for chronic obstructive lung disease stage (GOLD) criteria and is classified into 4 levels based on the values of FEV1 % predicted post-bronchodilator (Table 1).

Assessment of symptom burden in COPD patients can be done by using the Modified British Medical Research Council (mMRC) which is a simple measure of breathlessness (Table 2) or by using the COPD Assessment Test (CAT) (Appendix)[10]. The CAT is an 8-item questionnaire to measure health status impairment in COPD patients with the score ranging from 0-40. Higher scores on the CAT denote a more severe impact of COPD on a patient's life and a cut-point of 10 is used which means that scores <10 have a low impact[11, 12].

Table 1. Classification of airflow limitation severity in COPD based on post-bronchodilator spirometry in patients with FEV1/FVC < 0.70[13].

GOLD 1	Mild	FEV1 ≥ 80% predicted
GOLD 2	Moderate	50% ≤ FEV1 < 80% predicted
GOLD 3	Severe	30% ≤ FEV1 < 50% predicted
GOLD 4	Very severe	FEV1 < 30% of predicted

COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease stage, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity

Table 2. Modified Medical Research Council (mMRC) Dyspnea Scale[10].

mMRC Grade 0	Dyspnea only with strenuous exercise
mMRC Grade 1	Dyspnea when hurrying or walking up a slight hill
mMRC Grade 2	Walks slower than people of the same age because of dyspnea or has to stop for breath when walking at own pace
mMRC Grade 3	Stops for breath after walking 100 yards (91 m) or after a few minutes
mMRC Grade 4	Too dyspneic to leave house or breathless when dressing

mMRC: Modified Medical Research Council

1.3 COPD risk factors

Cigarette smoking is well established as the main risk factor for COPD, however, not all smokers develop COPD and some non-smokers can also develop chronic airflow limitation. Predictors of COPD in never smokers include age, lower education levels, occupational exposure and childhood respiratory diseases[14]. Never smokers with COPD have different clinical characteristics than current and former smokers with COPD, they tend to have fewer symptoms, milder disease and lower levels of inflammatory biomarkers[15].

The development of COPD is therefore the result of a complex interactions between genes, the environment and risk factors. Other than cigarette smoke, other types of tobacco like pipes and

cigars and marijuana are also considered risk factors for COPD[16]. Simultaneous use of marijuana and tobacco was shown to be associated with increased risk of respiratory symptoms and COPD if the lifetime dose of marijuana exceeded 50 marijuana cigarettes[17].

Age and sex are also considered risk factors for COPD. Aging is associated with changes in the airways similar to those in COPD, and a progressive decline in pulmonary function starts happening after the age of about 25 years[18]. Being male was previously associated with higher prevalence of COPD, however, with the changes in the pattern of tobacco smoking in recent years, recent data has showed that the prevalence of COPD is now almost equal in men and women[19].

A history of physician- diagnosed asthma and childhood hospitalization for respiratory illness were shown to be risk factors for developing COPD. Studies in the Canadian Cohort of Obstructive Lung Disease (CanCOLD) and by others found that a history of asthma was a factor for developing COPD regardless of smoking status. Individuals with chronic asthma have a greater than normal rate of decline in lung function with age, and this is further magnified by presence of smoking. A history of childhood hospitalisation for respiratory illness was also a significant predictor of COPD irrespective of smoking status; as a history of severe childhood respiratory infection is associated with reduced lung function and increased respiratory symptoms in adulthood[20-22].

Factors that affects lung growth and development during gestation and childhood have the potential to increase an individual's risk of developing COPD[23]. Indeed, a positive association between birthweight and lung function in adulthood[24].

One of the genetic factors that affects the development of COPD is alpha-1 antitrypsin deficiency. Alpha1-antitrypsin deficiency is a genetic disorder that is characterised by early-

onset emphysema. Alpha1 antitrypsin is protein produced in the liver, and whose function is to protect the lung against proteolytic damage from neutrophil elastase. A mutation in the gene coding for this protein leads to its deficiency. Individuals affected by this mutation start developing signs and symptoms of COPD at an early age (before 50 years old) [25].

Other risk factors that can lead to the development of COPD include: occupational exposures, like organic and inorganic dusts, chemical agents and fumes and lower socioeconomic status [26, 27]. A positive history of prolonged (>10 years) exposure to biomass fuels combustion for heating were factors independently associated with COPD in women. Indeed, exposure to biomass fuel combustion was related to moderate and severe COPD[21].

1.4 COPD and comorbidities

COPD can usually coexist with different comorbidities, this can be either due to shared risk factors or some disease can arise independently of the patient having COPD. The coexistence of COPD with other comorbidities negatively affects patients outcomes and can increase risk of hospitalization and mortality and worsening of one of the disease can also negatively affect the other [28, 29].

COPD is often associated with different comorbidities such as cardiovascular disease, cerebrovascular disease, osteoporosis, depression, lung cancer and diabetes[30, 31].

A meta-analysis studied data from 11 studies and looked at the prevalence of comorbidities COPD and non-COPD control patients. The study showed that the prevalence of cardiovascular comorbidities was significantly higher in the COPD patients (OR 1.90). The prevalence of cerebrovascular comorbidities was also significantly higher in COPD patients (OR 1.84). The prevalence of hypertension (OR 1.45) as well as diabetes mellitus (OR 1.22) was also

significantly higher in COPD than in the non- COPD patients. Also, the prevalence of neurological and psychiatric disorders (OR 1.78) and cancer (OR 1.67) was significantly higher in COPD[32].

The main respiratory comorbidities in COPD are asthma and lung cancer. COPD patients are at an increased risk of developing lung cancer and COPD is a factor that contributes to worse outcomes in lung cancer patients. The incidence of lung cancer is four times higher in COPD patients when compared to the general population. Three-year survival in patients with COPD and lung cancer was almost half that of the general population without COPD (15% vs 26%) and the highest mortality was observed in men aged 45-64[33]. The coexistence of both of these diseases could be due to a shared common risk factor which is smoking exposure, as smoking exposure is found in 85-90% of those diagnosed with either COPD or lung cancer.

Asthma and COPD are the two most common chronic pulmonary conditions; asthma is characterized by reversible airflow obstruction, whereas COPD is mainly characterized by irreversible airflow obstruction. Although asthma can be a risk factor to COPD, especially in people who have being smoking, the subgroup of patients with both diseases are often referred to as asthma COPD overlap or ACO[34]. There's a variety of definitions for ACO, all of them being clinical and combined persistent abnormality of the lung function. A common definition for ACO is a post-bronchodilator FEV1/FVC < 0.7 as well as a combination of clinical features including: smoke exposure, a diagnosis of asthma, post-bronchodilator response defined as an increase in FEV1 of > 12% and 200 ml after administrator of bronchodilator and a history of wheezing, and serum or sputum eosinophilia[35]. The presence of ACO is associated with higher healthcare utilization and associated cost compared to patients with one condition alone. Patients with ACO have an impaired quality of life, more frequent and severe exacerbations compared

with COPD only patients and tend to have more respiratory symptoms[36-38]. Patients with ACO tend to also have a higher levels of comorbidities[39].

An increasing number of elderly suffer from multi-morbidity also defined as the presence of two or more chronic conditions. As previously mentioned, comorbidities in COPD are increasingly recognized as important determinants of disease prognosis and management. There is currently no evidence that COPD should be treated differently when part of multi-morbidity and treatments should be kept simple to increase adherence to therapeutic interventions and reduce mortality.

1.5 COPD and cardiovascular comorbidities

Cardiovascular disease (CVD) is a frequent and important comorbidity in COPD, with the coexistence of both diseases being associated with worse outcomes than either condition alone. Cardiovascular comorbidities, together with lung cancer, are the leading causes of death in COPD patients with mild-to-moderate disease[29].

Cardiovascular comorbidities such as hypertension, coronary heart disease, heart failure, arrhythmias and heart attack were shown to be of a higher prevalence in COPD in comparison to smokers or non-smokers. Differences in treated hypertension, arrythmias, stroke and angina disappear after adjusting for age and sex[28].

CVD and COPD share similar risk factors including age, sex, smoking exposure and sedentary lifestyle which could in part explain the association between the diseases.

Patients with concomitant COPD and CVD experience worse quality of life, dyspnea and lower exercise capacity[28, 40]. Patients with both disease also experience higher risk of hospitalisation for either of the condition[41]. Having CVD with COPD can also increase the

frequency of exacerbation and mortality, more specifically, heart failure and coronary heart disease increase the risk of having frequent exacerbations compared to patients without these comorbidities[41, 42]. The ECLIPSE study investigated the effect of comorbidities on mortality in 2,164 COPD patients over a 1060-day follow-up period and identified heart failure (hazard ratio HR: 1.9), ischemic heart disease (HR: 1.5), heart disease general (HR: 1.5) as the cardiovascular comorbidities associated with increased mortality[28].

Hypertension is very common in COPD occurring in around 40% of the patients. It is not associated with increased mortality, it is however associated with increased mMRC dyspnea score and reduced exercise tolerance[41, 43].

COPD is also associated with a high frequency of cardiac arrhythmias. Atrial fibrillation (AF) is the most common arrhythmia in the elderly population and in COPD and a reduction in lung function is associated with new incidence of AF. The Copenhagen City Heart Study analyzed data from 13,430 individuals and showed that lower lung function is associated with a higher prevalence of AF. The risk of new AF at the 5 year follow-up was 1.8-times higher for FEV1 between 60-80% of predicted compared with FEV1 > or = 80%. The risk of AF hospitalisation was also 1.3 times higher for FEV1 between 60-80% and 1.8 times higher for FEV1 < 60% compared with FEV1 > or = 80%[44]. These observations are still valid when looking at a population of COPD individuals[45].

Ischemic heart disease (IHD) is another cardiovascular comorbidity that's frequent in COPD with the frequency ranging between 16.1% and 53%[43, 46]. There's a statistically significant increase of IHD (coronary artery disease, angina, and myocardial infarction) in COPD patients[47, 48].

The risk of myocardial infarction (MI) increases significantly during and following a COPD exacerbation[49, 50]. A 2.27 fold increase in the risk of MI was observed 1 to 5 days after a COPD exacerbation and this risk diminished over time[50].

An acute exacerbation of COPD (AECOPD) also increases the risk of other CVDs including coronary artery disease, peripheral arterial disease, stroke, myocardial infarction in the first 30 days and up to 1 year following the event[51].

To minimise the risk of poor outcomes, it is therefore important to ensure that patients with comorbid COPD and CVD are managed effectively. Treatment for the present CVD should be according to guidelines irrespective of the presence of COPD[52-54].

1.6 COPD and Heart Failure

Heart failure (HF) is another cardiovascular comorbidity that's frequent in COPD. The frequency of HF in COPD varies based on the type of population studies, but it usually ranges between 5.3% and 24.4%[46, 48, 55]. The frequency changes between national databases populations data, inpatient data and outpatients data, for example, stable COPD outpatients are less likely to have active investigation for comorbidities and less likely to be diagnosed. The prevalence of HF in COPD can reach up to 46% among those with an exacerbation[56].

A study by Finkelstein et al. using data from 18,342 individuals from the 2002 National Health Interview Survey (NHIS) investigated the association between COPD and CVD. The study showed that there is an increased prevalence of CVD in the COPD population (56.5% in COPD subjects vs 25.6% in non-COPD subjects) and the study also showed COPD patients were at highest risk of having HF (OR = 3.9) compared to other CVDs like coronary heart disease (OR = 2.0), angina (OR = 2.1) and myocardial infarction (OR = 2.2)[48].

The strong association between COPD and HF can be mostly explained by old age and smoking history, as well as the high prevalence of hypertension and IHD in patients with COPD.
Others processes that are thought to lead to the association between COPD and CVD including HF include: lung hyperinflation, hypoxaemia, systemic inflammation and exacerbations.
Patients with COPD are prone to exacerbations that are associated with airway inflammation and that lead to a decline in health status. It has been showed however that that patients who have frequent exacerbations have increased airway cytokine levels when stable which rise further during an exacerbation, specifically levels of IL-6[57, 58]. The acute exacerbations in COPD highly increase risk of having a major adverse cardiovascular event. It has been proposed that the inflammatory mediators from acute injury to the lung tissue can "spill out" into the systemic circulation and lead to atheromatous plaque initiation, progression and destabilization, and CVD. Acute lung injury could also lead to an increase in reactive oxidative species, and oxidized low-density lipoproteins and endothelial dysfunction, all factors that can affect the occurrence of a cardiovascular event[51, 59].
Hyperinflation, a driver of COPD burden, can compromise heart function. Abnormal lung function and hyperinflation in COPD negatively impact cardiac function as they can increase pressures in the cardiopulmonary system, cause right-ventricular dysfunction and impaired left-ventricular filling. Indeed, low lung function is an independent risk factor for incident HFrEF and cardiac events and gas trapping is associated with LV hypertrophy, a known risk factor for HFrEF [60, 61].
Airflow limitation and emphysema in COPD contribute to the development of hypoxaemia which can in turn lead to pulmonary vasoconstriction and vascular remodelling, resulting in right-ventricular diastolic dysfunction. In fact, pulmonary hypertension which is common in

patients with severe COPD, may progress to right HF, which is in turn associated with left HF[62, 63].

The presence of HF in COPD worsens the prognosis leading to increased risk of hospitalization and mortality[64]. Comorbid HF is significantly associated with worse self-rated health and decreases quality of life and health status[40].

Similarly, in HF, the presence of COPD leads to worse prognosis. An analysis on data from the Val-HeFT heart failure trial (an RCT designed to evaluate the efficacy and safety of valsartan: an angiotensin II receptor blocker) where patients were grouped according to the presence or absence of COPD, showed that those with COPD had higher mortality (27.4% vs. 18.4%). The study also showed that the presence of COPD is a strong predictor of non-cardiovascular mortality and increases all-cause and non-cardiovascular hospitalizations[65].

The presence of HF in COPD frequently goes undiagnosed because accurately diagnosing heart failure can be difficult. Around 20% of COPD patients can have undiagnosed HF, this can negatively impact patient outcomes and lead to more frequently hospitalizations and increased mortality rates[66, 67]. The diagnosis of HF in COPD can be difficult due to different factors: both conditions share similar symptoms and signs including exertion dyspnoea, functional disability, nocturnal cough, peripheral oedema etc. These clinical features can sometimes be attributed to other comorbidities that are already diagnosed or to the age of the patient[68]. Hyperinflation and gas trapping in COPD patients can also prevent accurate echocardiographic assessment of the left ventricular ejection fraction (LVEF), this usually occurs in 10–35% of patients especially in those with severe airflow obstruction. Hyperinflation can also mask increased cardiothoracic ratio and right ventricular enlargement can obscure left ventricular dilation[66, 69].

1.7 Heart Failure definition and prevalence

Heart failure (HF) is a clinical syndrome caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress[70, 71]. HF is a major public health problem and a heterogeneous syndrome; in 1997, HF was identified as an emerging epidemic due to its increase incidence and prevalence[72]. HF is defined as a syndrome and not as a disease because the diagnosis relies on clinical examination making it more challenging to diagnose.

It is essential to identify early stage patients to improve outcomes and reduce mortality. Before clinical symptoms are detected, patients can present with asymptomatic structural or functional cardiac abnormalities[73]. A study showed that among patients with asymptomatic left ventricular dysfunction, treatment with enalapril, an angiotensin-converting-enzyme inhibitor, reduced the incidence of heart failure and the rate of related hospitalizations at follow-up[74]. It is also important to identify the cardiac cause of HF to help guide treatment. A myocardial abnormality usually causes systolic and/or diastolic ventricular dysfunction, however, abnormalities of the valves, pericardium, endocardium, heart rhythm and conduction can also cause HF.

The number of patients living with HF has been increasing mostly due to an aging population, population growth and improved survival with an estimate of 64.3 million people worldwide living with HF[75]. In developed countries, 1-2% of the general adult population have known HF (in people who are >70 years of age, this number rises to ≥10%) and it's been estimated that over half of the all adults that have HF have HF with preserved ejection fraction [76-78]. A meta-

analysis taking into account undiagnosed cases of HF showed a prevalence of 4.2% which is twice as high as reported prevalence in registries containing only established cases. The difference between these numbers shows that HF can go undetected in about half the cases especially HF with preserved ejection fraction[79]. HF diagnosis is usually missed because it can get misclassified as COPD or obesity due to the similarity in symptoms, it can be assumed that the symptoms as just due to old age, or because echocardiograms are not usually available in primary care[80].

1.8 Heart Failure diagnosis and symptoms

Typical symptoms of HF include breathlessness, ankle swelling and fatigue and they may be accompanied by signs of HF including elevated jugular venous pressure, pulmonary crackles and peripheral oedema. These symptoms and signs can be hard to identify in some patients especially in those with comorbidities like obesity and chronic lung diseases[81]. The New York Heart Association (NYHA) functional classification has been used in HF to describe the severity of symptoms and exercise intolerance in patients (Table 3).

Table 3.The New York Heart Association (NYHA) functional classification of HF[71].

NYHA class I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
NYHA class II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
NYHA class III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.

NYHA class IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

NYHA: New York Heart Association

Other than symptoms, natriuretic peptides measurement, electrocardiogram and echocardiography are essential to establish a diagnosis.

The plasma levels of natriuretic peptides (NPs) are usually used to rule out HF or to identify individuals who need further cardiac evaluation but not to establish a diagnosis as they have high negative predictive values but low positive predictive values. Patients with normal NP values, below the cut-point for exclusion, are unlikely to have HF. The upper limit of normal in the non-acute setting for B-type natriuretic peptide (BNP) is 35 pg/mL and for N-terminal pro-BNP (NT-proBNP) it is 125 pg/mL; however, in the acute setting, higher values should be used: BNP <100 pg/mL and NT-proBNP < 300 pg/ mL[82, 83].

HF is unlikely in patients with a normal electrocardiogram (ECG) (sensitivity 89%), and an abnormal ECG increases the likelihood of a HF diagnosis but it has low specificity[84, 85].

Echocardiography is the most useful test in patients with suspected HF to establish the diagnosis. An echocardiogram provides data on ventricular systolic and diastolic function, ejection fraction, chamber volumes, wall thickness and pulmonary hypertension.

The main method to classify HF is by using the measurement of the LVEF. Patients with reduced LVEF (considered as <40%) are classified as HF with reduced EF (HFrEF) and those with normal LVEF (considered as ≥50%) are classified as HF with preserved EF (HFpEF).

Patients with an LVEF in the range of 40 – 49% are in a grey area defined as HF with mid-range EF (HFmrEF)[71, 86].

The diagnosis of HFrEF is done based on signs and symptoms of HF with an LVEF <40%. The diagnosis of HFpEF is however more challenging. To establish a diagnosis of HFpEF or HFmrEF, the following criteria should be met:

1) signs and symptoms of HF

2) a preserved LVEF (LVEF ≥50% or 40–49% for HFmrEF)

3) Elevated levels of NPs

4) Objective measures of cardiac dysfunction on the echocardiogram which includes: an increase in LV wall thickness and/or increased left atrial (LA) size as a sign of increased filling pressures.

There's a current preference for stating preserved or reduced LVEF over preserved or reduced systolic function because most patients with HFrEF (previously referred to as systolic HF) can also have diastolic dysfunction; and even though most patients with HFpEF show evidence of impaired LV filling (or diastolic dysfunction), they can still have some subtle abnormalities in the systolic function. Individuals with HFmrEF usually have mild systolic dysfunction with features of diastolic dysfunction[71].

1.9 Inflammatory biomarkers in COPD

Lung inflammation is a known characteristic of patients with COPD. Continuous inhalation of cigarette smoke or other noxious particles over time leads to inflammation in the lungs. Airway inflammation starts at an early stage even many years prior to the onset of clinical symptoms[87]. Lung inflammation was observed in all cigarette smokers, however, in COPD, the inflammatory response appears to be abnormal and enhanced beyond the normal protective inflammatory response in the lungs and this can produce lung injury. The levels of inflammation in the lungs increase with disease severity and during an acute exacerbations and persist even

after smoking cessation[88]. Both innate and adaptive inflammatory and immune responses are involved in the lung inflammation in patients with COPD. Analysis of bronchial biopsies from COPD patients showed an increase in inflammatory cell infiltration to the central airways when compared with non-smokers or smokers who do not have COPD with T lymphocytes, mainly CD8+ cells, and macrophages being the most prevalent cells[89]. Macrophages from COPD secrete more inflammatory mediators when compared with macrophages from normal smokers including tumor necrosis factor α(TNF-α), interleukin (IL)-8 and proteases, and their numbers in the airways correlate with disease severity[90, 91]. Levels of activated neutrophils were shown to be increased in sputum from COPD patients, they also secrete mediators like neutrophil elastase and matrix metalloproteinases that contribute to alveolar destruction[92]. Numbers of neutrophils from sputum and bronchial biopsies of COPD patients were shown to be correlated with disease severity and the rate of lung function decline[91, 93].

The airway epithelium plays an important role in the process of inflammation, it is activated by cigarette smoke or other irritants and secretes inflammatory chemokines and cytokines, such as TNF-α, IL-1β, and IL-8, which recruit and activate inflammatory cells[94].

With increasing severity of COPD there is an increase in the inflammatory response, and it is now recognized that COPD is characterized by low-grade chronic systemic inflammation. A meta-analysis by Su et al. showed that COPD is associated with elevated serum CRP, leukocytes, interleukin (IL)-6, IL-8, and fibrinogen[95]. Systemic inflammatory markers can play a role in predicting clinical outcomes although their roles guiding treatment in COPD remains limited; more studies are needed to demonstrate their utility in clinical practice.

Fibrinogen is one of the most promising biomarkers in COPD, it was shown in multiple studies to be associated with the risk of COPD, disease progression, and mortality independent of other

risk factors like age, cigarette smoking and lung function[96]. A 1-g/L plasma increase in fibrinogen was shown to be associated with a 3.7-fold increase in the risk of COPD-specific mortality and elevated plasma fibrinogen levels were associated with an increased risk of exacerbations[97, 98]. The ECLIPSE study, a very large cohort of severe COPD patients, showed however that plasma fibrinogen was only weakly associated with total mortality and was outperformed by serum IL-6. In that study, IL-6 also outperformed C-reactive protein (CRP) and IL-8 in predicting total mortality over 3 years[99].

Surfactant protein D (SP-D) and club cell secretory protein 16 (CCSP-16) are pneumoproteins that are produced in the lungs so they are more specific to COPD. SP-D levels are increased in COPD patients but they are weakly associated with risk of exacerbation, and short-term use of either systemic or inhaled corticosteroids is associated with a fall in the levels of serum SP-D, which is then associated with improved health status[100].

CCSP-16 levels were not responsive to corticosteroids, however, it was the best biomarker for disease progression in the ECLIPSE study. A 1-SD increase in serum CCSP-16 levels was shown to associated with a 33-mL increase in baseline FEV1[101].

All these biomarkers have not yet impacted the clinical diagnosis and treatment of COPD patients. More studies are needed to allow their incorporation into clinical practice and guiding patient treatment.

1.10 Inflammatory biomarkers in HF

Biomarkers in HF have significantly impacted the way patients are evaluated and treated in the clinical setting. The use of NT-proBNP and BNP is the gold standard for the diagnosis and prognosis of HF. Patients diagnosed with HF have higher levels of BNP and these levels are

associated with increased severity of HF[102]. Similarly, patients with acutely decompensated HF had significantly higher levels of NT-proBNP compared with those without HF and symptom severity correlated with NT-proBNP concentrations[103]. Both BNP and NT-proBNP are used as predictors of death and hospitalization in both acute and chronic HF. A systematic review by Doust et al. showed that in HF patients each 100 pg/ml increase was associated with a 35% increase in the relative risk of death[104].

Cardiac troponins are also used for prognostic purposes in HF, levels of this biomarker are also elevated in acute and chronic HF. Results from the Val-HeFT heart failure trial showed that cardiac troponin T, a marker of cardiomyocyte injury, is associated with an increased risk of death (HR 2.08) and first hospitalization for HF (HR 1.55) at 2 years after adjusting for clinical risk factors. Some potential drivers that could contribute to elevated cardiac troponins include elevated filling pressures, increased wall stress and endothelial dysfunction. In addition, increased wall stress can lead to cardiomyocyte apoptosis, autophagy, and breakdown of the contractile apparatus releasing cardiac troponins [105].

Several inflammatory biomarkers have been shown to have prognostic value in HF. A measurement of plasma CRP in the Val-HeFT heart failure trial showed that CRP levels are elevated in patients with HF, patients with higher CRP show features of more severe HF, and there's a significant increase in the risk of mortality and first morbid event with increasing CRP quartile[106]. CRP has also been increasingly used to guide statin therapy for the primary prevention of cardiovascular events[107].

CRP production is influenced by levels of inflammatory marker IL-6 and left ventricular dysfunction even in the absence of clinical HF is associated with increased levels of IL-6 which

suggests that IL-6 could potentially be a marker of patients at risk for progression to clinical HF[108].

In addition to being elevated in COPD, a study assessing the predictive value of SP-D for cardiovascular mortality in patients who underwent coronary angiography, showed that SP-D is a good predictor of cardiovascular morbidity and mortality. In fact, patients who died during follow-up had significantly higher plasma SP-D levels than those who survived, and those that were in the highest quintile of SP-D had 4.4-fold higher risk of CVD mortality[109].

1.11 Alarmins in COPD and HF

Damage Associated Molecular Pattern Molecules (DAMPs) also known as alarmins are "danger" signals that are released from necrotic or injured cells to alert the immune system by binding to pattern recognition receptors (PRRs) found on the surface of neutrophils and monocytes. Under normal conditions, DAMPs are intracellular molecules that are involved in cellular function[110, 111]. S100A8 and S100A9 are alarmins that belong to the S100 family. They are of calcium-binding proteins that are responsible for successful cell migration, phagocytosis, and exocytosis under homeostatic conditions. S100A8 and S100A9 can form a stable heterodimer or homodimer both in vitro and in vivo [112].

S100A8 and S100A9 are constitutively expressed in neutrophils, monocytes but they can be induced in other cell types such as fibroblasts, mature macrophages and vascular endothelial cells once the cells are activated. Their release can induce the secretion of multiple cytokines in inflammatory cells to sustain and exacerbate inflammation [113-116].

In a mouse model of COPD, chronic exposure to cigarette smoking CS lead to an increase in the levels of S100A8 in the BAL fluid but not in the plasma of cigarette smoke-exposed younger

mice as compared to air-exposed controls[117]. Another study in COPD patients showed a significant increase in the serum levels of S100A9 during exacerbation compared with stable disease[118]. The airway epithelium forms the first barrier toward inhaled insults such as cigarette smoking separating lung tissue from the environment. Consequently, epithelial cells are one of the first cells to be exposed to inhaled noxious particles. An increase in apoptotic epithelial cells and decreased phagocytosis of apoptotic cells by airway macrophages in COPD might explain the increase in DAMPS in COPD. S100A8 and S100A9 play a role in leukocyte recruitment and their release can induce the secretion of multiple cytokines in inflammatory cells to sustain and exacerbate inflammation[119, 120].

S100A8 and S100A9 were also studied in the context of cardiovascular disease. A study by Morrow et al. investigated the risk of cardiovascular death or MI associated with S100A8/A9 levels measured at 30 days after an acute coronary syndrome. They found that S100A8/A9 levels were elevated in patients who suffered a recurrent event during the subsequent 30 days period. They also found that patients with the highest levels of S100A8/A9 had a 2 times higher risk to develop a recurrent event after adjusting for risk factors such as diabetes, hypertension, previous CV disease, heart failure, and CRP[121].

In severe HF patients, plasma levels of S100A8/A9 were found to be significantly increased when compared to patients with hypertension or healthy subjects. S100A8/A9 also predicted 1-year mortality and was positively correlated with IL-6 and IL- 8[122].

As cardiac myocytes subjected to ischemia do not upregulate S100A8 and S100A9 mRNA and protein levels, they are probably released from activated monocytes and neutrophils recruited to the site of the injury. In addition to their chemotactic function, S100A8 and S100A9 stimulate leukocyte migration by upregulating the expression of adhesion molecules and enhancing

leukocyte–endothelial cell interaction amplifying the inflammatory processes. S100A8/A9 are also thought to accelerate atherogenesis through increased recruitment and activation of neutrophils and monocytes in the arterial wall [119, 123].

1.12 Systemic inflammation as a link to COPD and HF

Several processes are thought to be important in the association between COPD and HF. COPD and HF share common risk factors including cigarette smoking, advanced age, and environmental pollution and even though these factors play a role in the association between the two diseases, studies have shown that this association persists independent of these shared risk factors[124]. Traditional cardiovascular risk factors such are hypertension are also common in COPD and could play a role in the association, however, it's been shown that the increased risk of CVD in COPD is not likely to be due to an atherogenic lipid pattern as lipid levels were comparable in the COPD and healthy group[125].

Low-grade systemic inflammation was extensively studied over the last few years as one of the pathophysiological pathways that explains the link between COPD and HF. It is well recognized that COPD is associated with systemic inflammation; as previously discussed, multiple inflammatory biomarkers are present in the serum of COPD patients and increased levels are associated with disease severity and mortality.

Different types of cells are involved in the systemic inflammation present in COPD. COPD is associated with not only pulmonary but also systemic inflammation. COPD is characterized by increased numbers of macrophages in peripheral airways, lung parenchyma and pulmonary vessels, together with increased activated neutrophils and increased lymphocytes . All of these

inflammatory cells, together with epithelial cells and other structural cells release multiple inflammatory mediators that can “spill out” into the systemic circulation[88, 126].

Chronic inflammation can lead to changes in the airways such as narrowing of the small airways and destruction of the lung parenchyma and can also affect endothelial function by reducing vasodilation and causing endothelial cell apoptosis, thereby acting a key mediator in the development of cardiovascular diseases such as atherosclerosis. Endothelial dysfunction is characterized by imbalanced vasodilation and vasoconstriction, elevated reactive oxygen species, and proinflammatory factors, as well as deficiency of nitric oxide bioavailability. COPD-induced endothelial dysfunction is associated with elevated cardiovascular risk[127, 128].

One of the proposed mechanisms by which systemic inflammation plays a role in the pathogenesis of cardiovascular disease is the amplification of the atherosclerotic process from plaque initiation, development, and rupture[129]. The process is amplified by the presence of pro-inflammatory cytokines eg. TNF-α and IL-8, which in turn increase the production of CRP in the liver. CRP can then upregulate the production of other pro-inflammatory cytokines which can increase the expression of cell adhesion molecules, such as ICAM-1 and VCAM-1, on the vascular endothelium, and allow the adhesion of circulation leukocytes to the damaged vascular endothelium. As a result, cardiovascular morbidity is common in patients with COPD[130, 131].

Other features of COPD such as exacerbations can further enhance the inflammatory profile and the negative effects on the cardiac system. Following an exacerbation, COPD patients are more susceptible to vascular events. Cardiac troponin T levels, a marker of cardiac injury, is elevated during a COPD exacerbation and this is associated with increased mortality after patient discharge[132].

Chapter 2: Rationale, hypothesis and objectives

Chronic Obstructive Pulmonary Disease (COPD) is a chronic lung disease characterized by progressive airflow limitation and chronic airway inflammation as well as chronic respiratory symptoms. The development of COPD is the result of a complex interactions between genes, the environment and risk factors with cigarette smoking being well established as the main risk factor for COPD. COPD is often associated with different comorbidities such as cardiovascular disease, cerebrovascular disease, osteoporosis, depression, lung cancer, diabetes and asthma which can negatively affect patient outcomes and can increase risk of hospitalizations and mortality. Cardiovascular diseases (CVD) are frequent and important comorbidities in COPD and include hypertension, coronary heart disease, heart failure, arrhythmias and heart attack. They have been shown to be of a higher prevalence in COPD in comparison to smokers or non-smokers. Heart failure (HF) is one frequent and important cardiovascular comorbidity in COPD. The presence of HF in COPD can frequently go undiagnosed due to both conditions sharing similar symptoms and signs. Unrecognizing and undertreating HF can be associated with poor quality of life, increased hospital admissions and mortality. The strong association between COPD and HF can be mostly explained by old age and smoking history, as well as the high prevalence of hypertension and IHD in patients with COPD. However, systemic inflammation associated with both diseases also plays a role. Multiple inflammatory biomarkers have been characterised in both COPD and HF.

We ***hypothesized*** that patients with COPD and comorbidity of HF will differ in lung structure, severity of airflow obstruction and blood biomarkers and that cigarette smoke will affect the cross-talk and activation of different lung cells.

The ***main objectives of the thesis*** were

1) to determine the prevalence of co-morbid HF in COPD individuals in two separate samples, a population-based and a clinical sample, and determine patients' characteristics which could be used to in clinical practice for active screening; and

2) to characterize the expression of inflammatory biomarkers in an *in vitro* model of cigarette smoke exposure.

Three research projects were carried out to address these objectives.

Chapter 3: Manuscript 1 "Recognizing early cardiac dysfunction in chronic obstructive pulmonary disease (COPD): the Multi-Ethnic Study of Atherosclerosis (MESA)"

3.1 Abstract

Chronic Obstructive Pulmonary Disease (COPD) and Heart failure (HF) are two highly prevalent conditions that significantly impact patients, families and the health care system. Although commonly studied independently, these diseases are often concomitant and the presence of comorbid HF and COPD affects patient outcomes. HF with preserved ejection fraction (HFpEF) is the predominant HF subtype among COPD patients with a higher prevalence compared to those with HF with reduced ejection fraction (HFrEF).

In a population-based study, the Multi-Ethnic Study of Atherosclerosis (MESA), we sought to characterize the prevalence of co-morbid chronic lower respiratory disease (CLRD)/COPD-early HF in a sample of older adults, and characterize the associated lung function, imaging, neurohormonal and inflammatory features.

The study showed a prevalence of 13.3% of HF in participants with CLRD and a prevalence of 12.7% of HF in those with COPD. In the main and subcohort, subjects in the CLRD/COPD with HF were significantly older and had a higher BMI and comorbidities including atrial fibrillation and obesity when compared to all the other groups including the CLRD/COPD without HF. Participants with CLRD/COPD with HF had lower lung function values when compared to those with only CLRD/COPD. No relationship was observed however between levels of emphysema, levels of NT-pro BNP, severity of airflow obstruction and disease phenotypes of CLRD/COPD with early HF.

Our study emphasizes the importance of recognizing early cardiac dysfunction in COPD and promoting treatment and prevention of disease progression to reduce the risk of adverse events and mortality.

3.2 Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation and chronic airway inflammation and has an estimated prevalence of 6%[1]. It is currently the fourth leading cause of death worldwide and is associated with a significant economic burden[2, 3]. In addition to affecting the respiratory system, COPD is associated with co-morbidities including heart failure (HF)[4, 5]. Although commonly studied as independent entities, both diseases are often concomitant with HF estimated to be present in 5 to 41% of patients with COPD [4, 6]. COPD and HF are two highly prevalent conditions, they both share similar risk factors, in particular smoking exposure, and they have significant impact on patient well-being and the health care system. The presence of comorbid COPD and HF increases the risk of hospitalizations and mortality[7]. Patients with comorbid HF and COPD had also higher in-hospital all-cause and non-cardiovascular (CV) mortality[8, 9].

COPD and HF have largely been studied separately, and given the similarities in the clinical signs and symptoms, the presence of concomitant COPD and HF is often overlooked in clinical practice: 20.5% of elderly COPD patients have unrecognized HF[10]. In a cohort study involving tertiary care centers, it was estimated that around 11% of COPD patients had echocardiographic evidence of left ventricular dysfunction[11]. In addition, HF with preserved ejection fraction (HFpEF) is the predominant HF subtype among COPD patients with a higher prevalence compared to those with HF with reduced ejection fraction (HFrEF)[12, 13]; which may contribute to the underestimation of co-existent COPD and HF. The functional pulmonary abnormalities have also not been well characterized in patients with HFpEF.

Both diseases are characterized by a chronic, sub-clinical pro-inflammatory state and several neuro-hormonal and thrombo-inflammatory biomarkers. In terms of neuro-hormonal activation, Brain Natriuretic Peptide (BNP), its pro-hormone N-terminal (NT) proBNP are elevated in both COPD and HF[7, 14, 15]. When added to clinical information, NTproBNP levels significantly improve the diagnostic accuracy of HF in patients with acute dyspnea[16]. NT-proBNP levels are also useful in detecting ventricular dysfunction in COPD patients and the measurements of BNP levels can help pick up unrecognized HF in COPD[11, 17]. In regards to markers of inflammation, levels of the cytokines interleukin (IL)-6 and IL-8, and levels of the pro-thrombotic mediators C-reactive protein (CRP), fibrinogen and troponin are also elevated in both COPD and HF[18-21]. Previous studies have shown that poor lung function in COPD negatively impacts the heart with gas-trapping being associated with left ventricular hypertrophy, a known risk factor for HF with reduced ejection fraction; as well as low lung function being associated with measures of impaired left ventricular filling in COPD[22]. Percent emphysema quantified by CT scan is associated with smaller right and left ventricular volumes[23].

Given the difference in participants characteristics of COPD and HF between a clinical sample where most of the prevalence studies have been conducted, we sought to characterize the prevalence of co-morbid COPD-early HF in a population-based sample of older adults, and associated imaging, neurohormonal, and inflammatory features.

Our central hypothesis is that individuals with the disease phenotype of chronic lower respiratory disease (CLRD) or COPD with concomitant early HF will differ in severity of airflow obstruction, lung structure (emphysema) and levels of biomarker NT-pro BNP when compared to those with CLRD or COPD alone and those at risk (i.e., no CLRD, no HF but ever smoker).

Our specific objectives are:

1) To determine the prevalence of individuals with the disease phenotype of CLRD or COPD with early HF in a community-based multi-ethnic sample;

2) To determine whether there are distinct characteristics in individuals with disease phenotype of CLRD or COPD-early HF compared to those with

i-CLRD or COPD without early HF;

ii-at risk (i.e., no CLRD, no HF but ever smoker); and

iii-healthy (i.e., no CLRD, no HF but never smoker);

3) To determine if there is a relationship between the pulmonary structure (emphysema), levels of serum biomarker NT-pro BNP, severity of airflow obstruction and disease phenotypes of CLRD (main cohort) and COPD (subcohort) with early HF

3.3 Methods

Multi-Ethnic Study of Atherosclerosis

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multi-center prospective community-based study designed to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease. In 2000 to 2002, MESA recruited 6,814 men and women aged 45- to 84-years old from the general population in six US communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; St Paul, Minnesota; and New York, New York). MESA participants are non-Hispanic white, African-American, Hispanic, or Asian. Exclusion criteria included individuals with clinical cardiovascular disease, pregnancy,

having weight > 300 lb, or a serious medical condition that prevented long-term participation[24].

MESA-Lung Study

The MESA-Lung Study enrolled 3,965 participants who were sampled randomly from MESA participants who consented to genetic analyses, underwent baseline measures of endothelial function, and attended MESA Exam 3 or 4 during the recruitment period from 2004 to 2006. The final cohort was 35% white, 26% African- American, 23% Hispanic, and 16% Asian-American. MESA Lung participants had performed full-lung CT and spirometry in years 2010 through 2012 (examination 5), with a follow-up assessment in years 2016 through 2018 (examination 6)[25].

Chronic lower respiratory disease

Spirometry was conducted in accordance with American Thoracic Society/European Respiratory Society guidelines[26]. Post-bronchodilator (BD) spirometry was performed after inhalation of two puffs of albuterol for 370 out of 2563 participants who completed spirometry. The term chronic lower respiratory disease (CLRD) was used in subjects of the main cohort and defined as a ratio of forced expiratory volume in one second (FEV1) / forced vital capacity (FVC) < 0.70, applying the COPD classification to pre-BD values without post-BD[27, 28]. COPD severity was classified as: mild, FEV1 ≥ 80% predicted; moderate, 50-79% predicted; and severe, FEV1 < 50% predicted[29]. To mitigate this potential limitation, a subcohort of COPD participants with post-BD spirometry was defined.

Definition of HF

The definition used in this study doesn't include the participants with "established or clinical heart failure" at Exam 6 which represented a relatively small number of subjects (around 70).

HF referred to HFpEF, early HF, was defined using an already developed and validated algorithm derived and tested in community-based patients and those with early-stage HFpEF[30]. H_2FPEF score (Heavy, 2 or more Hypertensive drugs, atrial Fibrillation, Pulmonary hypertension, Elder, Elevated filling pressures) was used in individuals who have symptoms of HF including dyspnea and/or exercise intolerance. The score relies on clinical characteristics and echocardiography that are available in clinical practice and allows the discrimination of HFpEF from noncardiac causes of dyspnea[30]. 6 variables that shown to be associated with HFpEF were included in this score: Obesity (body mass index >30 kg/m2), atrial fibrillation, age >60 years, treatment with ≥2 antihypertensive drugs, ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e') >9, and pulmonary artery systolic pressure >35 mmHg. A score was assigned to these 6 variables based on the strength of association with HFpEF (atrial fibrillation, 3 points; obesity, 2 points; others, 1 point each), creating an H_2FPEF score. The probability of HFpEF increased with increasing H_2FPEF score with a H_2FPEF score ≥ 5 associated probability of HFpEF > 80%[30]. Early HF was defined as H_2FPEF score ≥ 5.

CT scan and emphysema

Participants underwent full-lung CTs on 64-slice helical scanners following the MESA-Lung/SPIROMICS full-inspiration protocol[31]. Percent emphysema was defined using Apollo software (Vida Diagnostics, Coralville, IA) as the percentage of total voxels within the lung field that fell below -950 Hounsfield units [32]. The log transformed value was then obtained**.**

NT-proBNP Measurements

NT-proBNP was measured from serum collected during the baseline examination (Exam 1) that was stored at −70°C. NT-proBNP levels were measured using the Elecsys 2010 proBNP system (Roche Diagnostic, Indianapolis IN, USA) at a core laboratory (Veteran's Affairs San Diego Healthcare System, La Jolla, CA, USA)[33].

Covariates

Age, sex, race/ethnicity, pack-years of smoking, and medical history were self-reported. Height, weight were measured following MESA protocols. These data were collected from exam 6[24].

Statistical Analysis

The main cohort included individuals with CLRD without HF that was calculated using the H2FPEF score and those with the clinical phenotype of CLRD and HF. Participants with established or clinical HF were excluded from all the analysis. For the main cohorts, the prevalence of individuals with the clinical phenotype of CLRD and early HF was estimated using the H_2FPEF score. Participants were divided into 4 groups: healthy (no CLRD, no HF, and also never-smokers), at risk (no CLRD, no HF, but ever-smokers), CLRD alone, and CLRD and early HF. Sociodemographic, smoking history, clinical characteristics, lung function, emphysema and levels of NT-pro BNP were compared among the four groups, using ANOVA analysis for continuous variable, and Chi-squared test for category variables. Multiple comparisons (post-hoc testing) with Tukey adjustment were also performed for those variables with a significant overall p-value. To mitigate the potential limitation of using pre-BD spirometry to define CLRD, a

subcohort of COPD participants with post-BD spirometry was defined (post BD FEV1/FVC <0.70). Sensitivity analysis using this subcohort was performed to validate the results of the main cohort. The prevalence of individuals with the clinical phenotype of early HF was then calculated and participants were divided into 4 groups as previously defined but with COPD instead of CLRD. Similarly and using the same analysis, characteristics were compared among the four groups.

Multiple logistic models were performed to estimate the relationship between the severity of airflow obstruction, the pulmonary structure (emphysema) and the levels of serum biomarker NT-pro BNP and concomitant CLRD or COPD and early HF with CLRD or COPD used as reference populations. Model 1 was fully adjusted for age, sex, race or ethnic group and smoking status as well as HF risk factors (hypertension, heart attack, obesity and diabetes) and model 2 was minimally adjusted for age, sex, race or ethnic group, BMI and smoking status.

3.4 Results

Prevalence of early HF and subject characteristics in the main cohort and COPD subcohort

In the main cohort, there were 855 subjects classified as healthy, 503 as at risk, 462 as CLRD and 71 as CLRD + HF which represents a prevalence of 13.3% of HF. Subjects' characteristics are summarised in Table 1A. Race and sex distributions were similar between CLRD and CLRD + HF, however, subjects in the CLRD+HF group were significantly older and had higher BMI. Pack-years cigarette smoking were similar between the at risk, CLRD without and with HF groups; significant differences were observed when all 3 groups were compared to the healthy group. Subjects in the CLRD with HF group had significantly higher comorbidities including

atrial fibrillation and obesity when compared to all the other groups. Hypertension and diabetes were similar between groups.

Pre-bronchodilator FEV1 and FVC were significantly different between the CLRD and CLRD with HF groups with CLRD with HF having lowers FEV1 values. Pre-bronchodilator FEV1/FVC ratio was significantly different between CLRD and CLRD with HF with an average of 0.64 in the CLRD compared to 0.61 in CLRD with HF.

Percent emphysema was increased and significantly different between CLRD without HF and healthy or at risk as well as CLRD with HF and healthy, but no difference was shown between CLRD without and with HF. Levels of NT-proBNP measured at exam 1 were only significantly different between subjects in the at risk group and those in the CLRD with HF group.

In the subcohort, there were 906 healthy, 546 at risk, 158 COPD without and 23 COPD with early HF. A prevalence of 12.7% of COPD with early HF was observed in participants with COPD similar to that in the main cohort. Subjects' characteristics are summarised in Table 1B. Results show similar trends to the main cohort analysis. The male/female ratio was similar between COPD without and those with HF, however, subjects in the COPD with HF group had a significantly higher BMI. Even though participants in the COPD with HF group were older than those in the COPD group without HF, the difference did not reach statistical significance. Subjects in the COPD with HF group had significantly more comorbidities including atrial fibrillation and obesity when compared to COPD group. Hypertension and diabetes were similar between groups. Spirometry data showed similar COPD severity between the 2 groups. Pre and post bronchodilator FEV1 were significantly different between the 2 groups, but pre and post

bronchodilator FEV1/FVC ratio were not significantly different, neither were percent emphysema and levels of NT-proBNP.

Relationship between pulmonary structure, serum biomarker, severity of airflow obstruction and disease phenotype in the main cohort (CLRD) and the subcohort (COPD)

When the CLRD group was used as a reference, there was a significant decreased odds of percent emphysema in subjects with CLRD with HF (40% less likely) in fully adjusted model 1. No significant association was observed for NT-proBNP levels and pre-bronchodilator FEV1 % predicted (Table 2A). In the COPD subcohort, when the COPD group was used as a reference, no significant association was observed for percent emphysema, NT-proBNP levels and post-bronchodilator FEV1 % predicted (Table 2B).

3.5 Discussion

In a large population-based multi-ethnic cohort, this study showed a prevalence of 13.3% of HF in participants with CLRD, and a similar prevalence of 12.7% in participants with the COPD definition; those subjects were unrecognized as having HF. In the main cohort, subjects in the CLRD with HF were significantly older and had a higher BMI and comorbidities including atrial fibrillation and obesity when compared to all the other groups including the CLRD without HF. Participants with CLRD with HF had more severe chronic obstructive disease (lower pre-bronchodilator FEV1, lower pre-bronchodilator FVC values and lower pre-bronchodilator FEV1/FVC ratio) when compared to those with only CLRD. However, percent emphysema and levels of NT-proBNP were not significantly different. In the COPD subcohort where subjects

were defined based on the post bronchodilator FEV1 and FEV1/FVC ratio, similar results were observed.

In the main cohort and subcohort, no significant association was observed between percent emphysema, NT-proBNP levels and pre/post-bronchodilator FEV1 % predicted and disease phenotype.

The main aspect of this study was to estimate the prevalence of co-morbid COPD with early HF in a population-based sample as most of the prevalence studies have been conducted in a clinical setting which doesn't necessary mirror what is going on in the population. We observed a prevalence of 13.3% of HF in participants with CLRD, and a very similar prevalence was observed when we used the COPD definition in the sensitivity analysis (12.7%). In the general population, the prevalence of HF varies between 2-8% depending on the population studied and their age group, the proportion of HFpEF also varies between 40-70% [34, 35]. Our results show that the presence of COPD might increase the frequency of having HF which is consistent with the findings of previous studies that showed that cardiovascular disease including coronary artery disease, heart failure and cardiac arrhythmias is more frequent in COPD independent of shared risk factors[4, 36, 37]. It is unclear why the prevalence of HF is so much higher in COPD but it's been proposed that the increased prevalence of atherosclerosis in COPD and the increased smoking status could play a role[12]. We indeed observed in our study that the subjects with both CLRD/COPD + HF tend to be heavier smokers however the difference did not reach statistical significance. A higher proportion of subjects in the CLRD/COPD + HF had hypertension and treated diabetes and they were significantly more obese in comparison to the CLRD/COPD only group.

A rapid decline in both FEV1 and FVC was shown to be associated with a higher risk of incident heart failure in the Atherosclerosis Risk in Communities (ARIC) Study. The association pertained even after adjusting for potentially confounding cardiovascular risk factors, including smoking status and accounting for pack-years and NT-pro-BNP[38]. A population-based study of men without a history of MI or stroke showed similar results: low FEV1 and low FVC were significantly associated with the incidence of HF requiring admission to a hospital and this relationship was consistent in smokers and non-smokers even after adjusting for cardiovascular risk factors[39]. In our study, we used data from MESA exam 6 and therefore we do not have follow-up data on the participants, however, we showed significantly lower pre-bronchodilator FEV1, pre-bronchodilator FVC and pre-bronchodilator FEV1/FVC ratio in CLRD with HF as well as lower pre and post bronchodilator FEV1 in the COPD with HF group compared to CLRD or COPD alone. We also observed lower pre-bronchodilator FEV1 and pre-bronchodilator FVC as well as lower post-bronchodilator FEV1 and post-bronchodilator FVC in the COPD+HF group but this did not reach significance.

Participants in the CLRD and CLRD+ HF groups had significantly higher levels of emphysema compared to the healthy and at-risk groups with those in the CLRD having the highest percent emphysema. However, in the COPD subcohort, percent emphysema is very similar between the COPD and the COPD+HF group. This is expected as in the subcohort we used the COPD definition of post-bronchodilator FEV1/FVC < 0.70.

When considering the association of lung structure and disease phenotype, we did not observe a difference between having COPD alone and having both diseases. However, the presence of emphysema can negatively impact heart function; Barr et al have previously showed that a greater extent of emphysema on CT was associated with smaller left ventricular end-diastolic

volumes and reductions in stroke volume and cardiac output. In fact, the effect of emphysema on left ventricular end-diastolic volume and cardiac output was similar to that of traditional cardiac risk factors previously reported in MESA[22].

NT-proBNP levels were not significantly different between CLRD/COPD and CLRD/COPD+ HF groups in the main cohort and in the subcohort. Average NT-proBNP levels were 63 and 62 pg/ml in our main cohort and subcohort respectively; this is expected as measurements were taken at Exam 1 in a cohort that was free of clinical cardiovascular disease. NT-proBNP and BNP are gold standard biomarkers in determining the diagnosis and prognosis of HF, as very low concentrations of NT-proBNP were useful in excluding HF, with excellent negative predictive value. An age-independent cut-point of 300 pg/mL had 98% negative predictive value to exclude acute HF[40]. In COPD, higher NT-proBNP levels at baseline are associated with an increased risk of COPD exacerbations within one year of follow-up, regardless of the presence of underlying cardiovascular disease[41].

3.6 Strength and limitations

One of the main strength of the study is the use of MESA which is a large community-based multi-ethnic cohort with data collected on around 6,814 men and women aged 45- to 84-years old from the general population. MESA has data from diagnostic tests for CLRD as well as data on diagnostic tests for HF. We were able to use these diagnostics test to classify participants as CLRD/COPD and HF which eliminated misclassification due to recall bias.

However, the limitation of MESA is that this cohort is not a COPD cohort and only a small proportion of the participants had post-bronchodilator spirometry performed. However, by

performing a sensitivity analysis we were able to validate our results in the subcohort of participants with both pre and post-bronchodilator spirometry and mitigate this limitation.

Another strength in our study is we defined COPD in two different ways. We first defined CLRD based on an FEV1/FVC < 0.70 using pre-bronchodilator values. We then performed a sensitivity analysis on a subcohort of COPD participants defined based on an FEV1/FVC < 0.70 using post-bronchodilator values. The prevalence of CLRD in the population was 28% when we used pre-bronchodilator values and this changed to a prevalence of COPD of 11% when post-bronchodilator values were used. Our observed prevalence of COPD is consistent with the findings of previous studies that showed that COPD affects 5%–10% of the population in the United States[42]. Therefore, our study shows the importance of using the correct method of diagnosis to define COPD to avoid overestimation and overtreatment of individuals.

Another limitation of our study is that the participants were recruited from the general population and therefore have mild disease and may not represent the demographics of patients recruited by specialist from a specialty clinic (pulmonology and cardiology) or from a general practitioner cohort.

3.7 Conclusion

In conclusion, we showed in our study a prevalence of 13.3% of HF in participants with CLRD and a very similar prevalence of 12.7% of HF in participants with COPD. Participants with both diseases were older, had a higher BMI and more comorbidities. They also had worse lung functions with lower pre and post-bronchodilator FEV1 and FVC.

Even though we were not able to show a relationship between pulmonary structure, serum biomarker, severity of airflow obstruction and disease phenotype, this data still provides further evidence that, to improve patient care in people with COPD, the extent to which these conditions

co-exist needs to be recognized. Healthcare professionals must target risk factors and seek to promote evaluation and treatment of early cardiac dysfunction in susceptible individuals with COPD to reduce the risk of adverse events and mortality. Further studies are needed to follow up participants with early disease and track the development of disease.

3.8 References

1. Singh, D., et al., *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019.* Eur Respir J, 2019. **53**(5).
2. Quaderi, S.A. and J.R. Hurst, *The unmet global burden of COPD.* Glob Health Epidemiol Genom, 2018. **3**: p. e4.
3. Lozano, R., et al., *Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.* Lancet, 2012. **380**(9859): p. 2095-128.
4. Chen, W., et al., *Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis.* Lancet Respir Med, 2015. **3**(8): p. 631-9.
5. Koskela, J., et al., *Co-morbidities are the key nominators of the health related quality of life in mild and moderate COPD.* BMC Pulm Med, 2014. **14**: p. 102.
6. Hawkins, N.M., et al., *Heart failure and chronic obstructive pulmonary disease: diagnostic pitfalls and epidemiology.* Eur J Heart Fail, 2009. **11**(2): p. 130-9.
7. Tavazzi, L., et al., *Clinical profiles and outcomes in patients with chronic heart failure and chronic obstructive pulmonary disease: an efficacy and safety analysis of SHIFT study.* Int J Cardiol, 2013. **170**(2): p. 182-8.
8. Mentz, R.J., et al., *Clinical characteristics and outcomes of hospitalized heart failure patients with systolic dysfunction and chronic obstructive pulmonary disease: findings from OPTIMIZE-HF.* Eur J Heart Fail, 2012. **14**(4): p. 395-403.
9. Arnaudis, B., et al., *Impact of chronic obstructive pulmonary disease severity on symptoms and prognosis in patients with systolic heart failure.* Clin Res Cardiol, 2012. **101**(9): p. 717-26.
10. Macchia, A., et al., *Unrecognised ventricular dysfunction in COPD.* Eur Respir J, 2012. **39**(1): p. 51-8.
11. Griffo, R., et al., *Frequent coexistence of chronic heart failure and chronic obstructive pulmonary disease in respiratory and cardiac outpatients: Evidence from SUSPIRIUM, a multicentre Italian survey.* Eur J Prev Cardiol, 2017. **24**(6): p. 567-576.
12. Rutten, F.H., et al., *Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease.* Eur Heart J, 2005. **26**(18): p. 1887-94.
13. Kwon, B.J., et al., *Prognosis of heart failure patients with reduced and preserved ejection fraction and coexistent chronic obstructive pulmonary disease.* Eur J Heart Fail, 2010. **12**(12): p. 1339-44.
14. Calzetta, L., et al., *Brain natriuretic peptide: Much more than a biomarker.* Int J Cardiol, 2016. **221**: p. 1031-8.
15. Staszewsky, L., et al., *Clinical, neurohormonal, and inflammatory markers and overall prognostic role of chronic obstructive pulmonary disease in patients with heart failure: data from the Val-HeFT heart failure trial.* J Card Fail, 2007. **13**(10): p. 797-804.
16. Wright, S.P., et al., *Plasma amino-terminal pro-brain natriuretic peptide and accuracy of heart-failure diagnosis in primary care: a randomized, controlled trial.* J Am Coll Cardiol, 2003. **42**(10): p. 1793-800.
17. McCullough, P.A., et al., *Uncovering heart failure in patients with a history of pulmonary disease: rationale for the early use of B-type natriuretic peptide in the emergency department.* Acad Emerg Med, 2003. **10**(3): p. 198-204.
18. Celli, B.R., et al., *Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2012. **185**(10): p. 1065-72.
19. Bhatt, S.P. and M.T. Dransfield, *Chronic obstructive pulmonary disease and cardiovascular disease.* Transl Res, 2013. **162**(4): p. 237-51.

20. Tsutamoto, T., et al., *Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure.* J Am Coll Cardiol, 1998. **31**(2): p. 391-8.
21. Harvey, M.G. and R.J. Hancox, *Elevation of cardiac troponins in exacerbation of chronic obstructive pulmonary disease.* Emerg Med Australas, 2004. **16**(3): p. 212-5.
22. Barr, R.G., et al., *Percent emphysema, airflow obstruction, and impaired left ventricular filling.* N Engl J Med, 2010. **362**(3): p. 217-27.
23. Kawut, S.M., et al., *Cor pulmonale parvus in chronic obstructive pulmonary disease and emphysema: the MESA COPD study.* J Am Coll Cardiol, 2014. **64**(19): p. 2000-9.
24. Bild, D.E., et al., *Multi-Ethnic Study of Atherosclerosis: objectives and design.* Am J Epidemiol, 2002. **156**(9): p. 871-81.
25. Rodriguez, J., et al., *The association of pipe and cigar use with cotinine levels, lung function, and airflow obstruction: a cross-sectional study.* Ann Intern Med, 2010. **152**(4): p. 201-10.
26. Hankinson, J.L., et al., *Performance of American Thoracic Society-recommended spirometry reference values in a multiethnic sample of adults: the multi-ethnic study of atherosclerosis (MESA) lung study.* Chest, 2010. **137**(1): p. 138-45.
27. Bhatt, S.P., et al., *Discriminative Accuracy of FEV1:FVC Thresholds for COPD-Related Hospitalization and Mortality.* Jama, 2019. **321**(24): p. 2438-2447.
28. Vestbo, J., et al., *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary.* Am J Respir Crit Care Med, 2013. **187**(4): p. 347-65.
29. Celli, B.R. and W. MacNee, *Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper.* Eur Respir J, 2004. **23**(6): p. 932-46.
30. Reddy, Y.N.V., et al., *A Simple, Evidence-Based Approach to Help Guide Diagnosis of Heart Failure With Preserved Ejection Fraction.* Circulation, 2018. **138**(9): p. 861-870.
31. Sieren, J.P., et al., *SPIROMICS Protocol for Multicenter Quantitative Computed Tomography to Phenotype the Lungs.* Am J Respir Crit Care Med, 2016. **194**(7): p. 794-806.
32. Gevenois, P.A., et al., *Comparison of computed density and macroscopic morphometry in pulmonary emphysema.* Am J Respir Crit Care Med, 1995. **152**(2): p. 653-7.
33. Yang, S., et al., *NT-proBNP, race and endothelial function in the Multi-Ethnic Study of Atherosclerosis.* Heart, 2019. **105**(20): p. 1590-1596.
34. Roger, V.L., *Epidemiology of Heart Failure: A Contemporary Perspective.* Circ Res, 2021. **128**(10): p. 1421-1434.
35. Hogg, K., K. Swedberg, and J. McMurray, *Heart failure with preserved left ventricular systolic function; epidemiology, clinical characteristics, and prognosis.* J Am Coll Cardiol, 2004. **43**(3): p. 317-27.
36. Finkelstein, J., E. Cha, and S.M. Scharf, *Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity.* Int J Chron Obstruct Pulmon Dis, 2009. **4**: p. 337-49.
37. Feary, J.R., et al., *Prevalence of major comorbidities in subjects with COPD and incidence of myocardial infarction and stroke: a comprehensive analysis using data from primary care.* Thorax, 2010. **65**(11): p. 956-62.
38. Silvestre, O.M., et al., *Declining Lung Function and Cardiovascular Risk: The ARIC Study.* J Am Coll Cardiol, 2018. **72**(10): p. 1109-1122.
39. Engström, G., O. Melander, and B. Hedblad, *Population-based study of lung function and incidence of heart failure hospitalisations.* Thorax, 2010. **65**(7): p. 633-8.
40. Januzzi, J.L., et al., *NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study.* Eur Heart J, 2006. **27**(3): p. 330-7.

41. Labaki, W.W., et al., *NT-proBNP in stable COPD and future exacerbation risk: Analysis of the SPIROMICS cohort.* Respir Med, 2018. **140**: p. 87-93.
42. Mannino, D.M., et al., *Chronic obstructive pulmonary disease surveillance--United States, 1971-2000.* MMWR Surveill Summ, 2002. **51**(6): p. 1-16.

3.9 Tables

Table 1A. Characteristics of participants by study subset in the main cohort

	Total	Healthy	At Risk	CLRD alone	CLRD+HF	Over all p-value		p value for post hoc multiple comparisons with Tukey adjustment					
	n=1891	n=855	n=503	n=462	n=71			Healthy vs. At risk	Healthy vs. CLRD alone	Healthy vs. CLRD+HF	At Risk vs. CLRD alone	At Risk vs. CLRD+HF	CLRD alone vs. CLRD+HF
Age (year)	73.1 ± 8.2	72.1 ± 7.9	72.4 ± 7.8	76 ± 8.2	81.2 ± 8.1	<0.001		0.91	<0.001	<0.001	<0.001	<0.001	<0.001
Sex, n(%)													
Male	838 (44.3)	291 (34.0)	249 (49.5)	262 (56.7)	36 (50.7)	<0.001		<0.001	<0.001	0.028	0.12	0.998	0.779
Female	1053 (55.7)	564 (66.0)	254 (50.5)	200 (43.3)	35 (49.3)	<0.001		<0.001	<0.001	0.028	0.12	0.998	0.779
BMI (kg/m^2)	28.6 ± 5.8	28.7 ± 6	28.4 ± 5.5	26.4 ± 4.7	31.5 ± 6.7	<.0001		0.64	<.0001	0.0004	<.0001	<.0001	<.0001
Race n(%)													
White, Caucasian	740 (39.1)	289(33.8)	217 (43.1)	201 (43.5)	33 (46.5)	<0.001							
Chinese-American	279 (14.8)	169 (19.8)	41 (8.1)	65 (14)	4 (5.6)	<0.001		<.0001	0.0033	0.0182	0.0735	0.8557	0.2786
Black, African American	468 (24.8)	213 (24.9)	119 (23.7)	113 (24.5)	23 (32.4)	<0.001		0.1762	0.2608	0.9974	0.9988	0.848	0.8867
Hispanic	404 (21.4)	184 (21.5)	126 (25)	83 (18)	11 (15.5)	<0.001		0.9227	0.036	0.2761	0.1947	0.4269	0.9393
Pack-years of cigarette smoking	9.8 ± 18.8	3.3 ± 10.9	13.3 ± 17.9	13 ± 21.6	14.2± 19	<.0001		<0.001	<0.001	<0.001	0.9939	0.9729	0.9434
Cigarette smoking status n (%)													
Never	922 (48.8)	709 (83)	0 (0)	184 (39.8)	29 (40.8)	<0.001							
Former	888 (47)	94 (11)	503 (100)	249 (53.9)	42 (59.1)	<0.001		0.902	<0.001	<0.001	0.9394	0.9403	0.9938

Current	81 (4.3)	52 (6)	0 (0)	29 (6.3)	0 (0)	<0.001		1	0.0102	0.9999	1	0.9999	0.9998
pre-bronch: measured forced expiratory volume at 1 second (ml/sec)	2187.9± 686.8	2154.8± 654	2373.9± 658.9	2024.4± 686.1	1664.9± 592.8	<0.001		<.0001	0.004	<.0001	<.0001	<.0001	0.0001
pre-bronch: fev1 percent of predicted	96.5 ± 24	101.6 ± 28.2	102.4± 19	88.5± 21.2	83.64 ± 26.4	<0.001		0.9262	<.0001	<.0001	<.0001	<.0001	0.4007
pre-bronch: measured forced vital capacity (ml)	2985.6± 921.2	2775 ± 851.8	3078.7± 869.3	3163.8± 971.3	2791± 1041.7	<0.001		<.0001	<.0001	0.99	0.4523	0.055	0.006
pre-bronch: fev1 / fvc ratio	0.74± 0.09	0.78± 0.05	0.77± 0.05	0.64± 0.07	0.61± 0.09	<0.001		0.3	<.0001	<.0001	<.0001	<.0001	0.001
log950	-0.11±1.19	-0.47± 1.06	-0.16± 1.12	0.35± 1.25	0.15± 1.41	<.0001		0.0001	<.0001	0.0005	<.0001	0.2337	0.6171
Exam 1 NT-proBNP (pg/mL)	63.1± 87.1	67.2 ± 98.7	57.2± 56.9	72.3 ± 98.9	95.3± 77.2	0.0097		0.2657	0.8824	0.1129	0.1066	0.0161	0.2447
Hypertension n(%)	1222(64.7)	566 (66.2)	314 (62.6)	288 (62.3)	54 (76)	0.072		0.5254	0.4993	0.3338	0.9999	0.1254	0.1195
Heart attack n(%)	1 (0.05)	0 (0)	0 (0)	1 (0.22)	0 (0)	0.369		1	0.999	1	0.999	1	1
Atrial fibrillation n(%)	136 (7.2)	83 (9.7)	7 (1.4)	5 (1.08)	41 (57.8)	<.0001		<.0001	<.0001	<.0001	0.97	<.0001	<.0001
Obesity	583 (30.8)	308 (36)	152 (30.2)	82 (17.8)	41 (57.8)	<.0001		0.1287	<.0001	0.002	<.0001	<.0001	<.0001
Diabetes n(%)													
Untreated diabetes	65 (3.5)	25 (3)	15 (3)	22 (5)	3 (4.2)	0.0748		0.998	0.676	0.935	0.665	0.913	1.000
Treated diabetes	337 (18.2)	162 (19.5)	93 (18.8)	68 (15.1)	14 (19.7)	0.0748		0.914	0.068	0.999	0.339	0.972	0.557

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher Exact test (category variables) or ANOVA (normal distribution continuous variables) or Kruskal-Wallis test (not normal distribution continuous variables), with Tukey adjustment for post hoc multiple comparisons.

CLRD: chronic lower respiratory disease; HF: heart failure; BMI: body mass index; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; pre-bronch: pre-bronchodilator; log950: log value of percent emphysema; NT-proBNP: N-terminal pro B-type natriuretic peptide;

Table 1B. Characteristics of participants by study subset in the subcohort

	Total	Healthy	At Risk	COPD alone	COPD+HF	Overall p value	p value for post hoc multiple comparisons with Tukey adjustment					
	n=1633	n=906	n=546	n=158	n=23		Healthy vs. At risk	Healthy vs. COPD alone	Healthy vs. COPD+HF	At Risk vs. COPD alone	At Risk vs. COPD+HF	COPD alone vs. COPD+HF
Age (year)	72.6 ± 8	72.3± 8	72.5 ± 7.8	76 ± 8.2	79.9 ± 6.8	<0.001	0.96	<0.001	<0.001	<0.001	<0.001	0.12
Sex, n(%)												
Male	697 (42.7)	317 (35.0)	276 (50.6)	90 (57.0)	14 (60.9)	<0.001	<0.001	<0.001	0.0677	0.4874	0.7702	0.9848
Female	936 (57.3)	589 (65.0)	270 (49.5)	68 (43)	9 (39.1)	<0.001	<0.001	<0.001	0.0677	0.4874	0.7702	0.9848
BMI (kg/m^2)	28.8 ± 5.8	28.6 ± 5.9	28.3 ± 5.4	26.5 ± 4.8	33.3 ± 7.2	<.0001	0.7	0.0001	0.0007	0.0036	0.0002	<.0001
Race n(%)												
White, Caucasian	618 (37.8)	311 (34.3)	232 (42.5)	67 (42.4)	8 (34.8)	<0.001						
Chinese-American	231 (14.1)	173 (19.1)	42 (7.7)	14 (8.9)	2 (8.7)	<0.001	<.0001	0.0083	0.7468	0.9744	0.9784	0.9966
Black, African American	417 (25.5)	231 (25.5)	136 (24.9)	41 (25.9)	9 (39.1)	<0.001	0.3194	0.8074	0.8349	0.9976	0.5568	0.652
Hispanic	367 (22.5)	191 (21.1)	136 (24.9)	36 (22.8)	4 (17.4)	<0.001	0.9878	0.9349	0.9874	0.9822	0.9941	0.9995
Pack-years of cigarette smoking	9.1 ± 18.1	3.3 ± 10.9	13.7 ± 19.2	15.9 ± 22	20.9 ± 24	<.0001	<0.001	<0.001	<0.001	0.3832	0.1331	0.4889
COPD severity -2004 definition, n(%)												
Mild	113 (6.9)	0 (0)	0 (0)	104(65.8)	9 (39.1)	<0.001	1	0.9462	0.9987	0.9475	0.9987	1
Moderate	60 (3.7)	0 (0)	0 (0)	47 (29.8)	13 (56.5)	<0.001	1	0.9485	0.9985	0.9513	0.9985	1
Severe	8 (0.5)	0 (0)	0 (0)	7 (4.4)	1 (4.4)	<0.001	1	0.9655	0.9987	0.9731	0.9987	1

Cigarette smoking status n (%)													
Never	815 (49.9)	752 (83)	0 (0)	54 (34.2)	9 (39.1)	<0.001		0.6916	<0.001	<0.001	0.8108	0.8086	0.9993
Former	748 (45.8)	99 (10.9)	546 (100)	89 (56.3)	14 (60.9)	<0.001							
Current	70 (4.3)	55 (6)	0 (0)	15 (9.5)	0 (0)	<0.001		1	0.0002	0.9999	1	0.9999	0.9999
pre-bronch: measured forced expiratory volume at 1 second (ml/sec)	2223.8± 680.5	2154.4± 658.9	2367.6 ± 655	1942.9± 648.9	1564.5 ± 485.8	<0.001		<.0001	0.0011	0.0001	<.0001	<.0001	0.0478
pre-bronch: fev1 percent of predicted	97.9 ± 23.9	101.2 ± 27.8	101.7± 19	83.8± 21.3	79.1 ± 33.2	<0.001		0.9755	<.0001	0.0001	<.0001	0.0001	0.8287
pre-bronch: measured forced vital capacity (ml)	2969.1± 906.9	2797.7 ± 868.6	3102.9± 875.2	3145.9± 922.9	2810.4± 1064.7	<0.001		<.0001	<.0001	0.99	0.9489	0.4001	0.3188
pre-bronch: fev1 / fvc ratio	0.75± 0.08	0.77± 0.05	0.77± 0.05	0.61± 0.08	0.58± 0.11	<0.001		0.0799	<.0001	<.0001	<.0001	<.0001	0.0749
post-bronch: measured forced expiratory volume at 1 second (ml/sec)	2128.3 ± 692.2	2151.7± 722.5	2372 ± 624.7	2014.5 ± 652.2	1630.3 ± 509.4	<0.001		0.1229	0.396	0.0043	0.0004	<.0001	0.0447
post-bronch: fev1 percent of predicted	91.2± 22.3	97.1 ± 19.4	100± 18.3	87± 21.6	83 ± 37.8	<0.001		0.8081	0.003	0.0311	<.0001	0.0054	0.85
post-bronch: measured forced vital capacity (ml)	3111.1± 943.7	2848 ± 972.9	3119.9± 846.7	3207.7± 912	2870.3± 1073	0.0205		0.2148	0.019	0.9996	0.8944	0.6586	0.3591
post-bronch: fev1 / fvc ratio	0.69± 0.10	0.76± 0.05	0.76± 0.05	0.63± 0.08	0.59± 0.10	<.0001		0.9748	<.0001	<.0001	<.0001	<.0001	0.1209
	-0.20±1.2	-0.46± 1.07	-0.16± 1.12	0.43± 1.2	0.49± 1.31	<.0001		0.0001	<.0001	0.0025	<.0001	0.0774	0.9958

log950													
Exam 1 NT-proBNP (pg/mL)	61.7± 89.2	66.9 ± 96.8	55.92± 55.3	87 ± 144.5	71.7± 70.6	0.0062		0.1841	0.0945	0.9967	0.0036	0.9039	0.922
Hypertension n(%)	1062 (65.1)	596 (65.8)	338 (62.0)	113 (71.5)	15 (65.2)	0.15		0.4682	0.4944	0.9999	0.1279	0.9897	0.9261
Heart attack n(%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)								
Atrial fibrillation n(%)	113 (6.9)	88 (9.7)	7 (1.3)	4 (2.5)	14 (60.9)	<.0001		<.0001	0.0313	<.0001	0.6933	<.0001	<.0001
Obesity	527 (32.3)	320 (35.3)	163 (29.9)	29 (18.4)	15 (65.2)	<.0001		0.1407	0.0003	0.0276	0.0243	0.0051	<.0001
Diabetes n(%)													
Untreated diabetes	52 (3.3)	26 (3)	18 (3.3)	6 (3.8)	2 (8.7)	0.17		0.999	0.999	0.444	1.000	0.494	0.585
Treated diabetes	292 (18.2)	169 (19.1)	99 (18.4)	19 (12.1)	5 (21.7)	0.17		0.850	0.073	0.976	0.225	0.923	0.458

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher Exact test (category variables) or ANOVA (normal distribution continuous variables) or Kruskal-Wallis test (not normal distribution continuous variables), with Tukey adjustment for post hoc multiple comparisons.

COPD: chronic obstructive pulmonary disease; HF: heart failure; BMI: body mass index; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; pre-bronch: pre-bronchodilator; post-bronch: post-bronchodilator; log950: log value of percent emphysema; NT-proBNP: N-terminal pro B-type natriuretic peptide;

Table 2A. Association between pulmonary structure, serum biomarker, severity of airflow obstruction and disease phenotype in the main cohort

	CLRD alone	CLRD+HF	
		OR (95% CI)	P value
Model 1			
log950 - per increased 1 point	REF	0.6 (0.38,0.96)	0.04
ntprbnp1 - per increased 1 point	REF	1.00 (0.992,1.007)	0.93
pre-bronchodilator FEV1 % predicted-per increased 1%	REF	1.006(0.99,1.02)	0.51
Model 2			
log950 - per increased 1 point	REF	0.92 (0.7,1.22)	0.58
ntprbnp1 - per increased 1 point	REF	1.002(0.99,1.005)	0.14
pre-bronchodilator FEV1 % predicted-per increased 1%	REF	0.993(0.98,1.006)	0.3

Adjusted OR (95% CI) were obtained by performing multiple logistic regression models fully adjusted for age, bmi, gender, race, smoking status, hypertension, atrial fibrillation, diabetes in model 1 and minimally adjusted for age, bmi, gender, race, smoking status in model 2.

CLRD: chronic lower respiratory disease; HF: heart failure; BMI: body mass index; FEV1: Forced expiratory volume in one second; pre-bronch: pre-bronchodilator; log950: log value of percent emphysema; NT-proBNP: N-terminal pro B-type natriuretic peptide;

Table 2B. Association between pulmonary structure, serum biomarker, severity of airflow obstruction and disease phenotype in the subcohort

	COPD alone	COPD+HF	
		OR (95% CI)	P value
Model 1			
log950 - per increased 1 point	REF	0.85 (0.36,2.02)	0.71
ntprbnp1 - per increased 1 point	REF	0.999 (0.986,1.01)	0.83
post-bronchodilator FEV1 % predicted-per increased 1%	REF	1.007(0.97,1.04)	0.66
Model 2			
log950 - per increased 1 point	REF	1.27 (0.7,2.28)	0.42
ntprbnp1 - per increased 1 point	REF	0.999(0.992,1.007)	0.89
post-bronchodilator FEV1 % predicted-per increased 1%	REF	1.005(0.98,1.03)	0.63

Adjusted OR (95% CI) were obtained by performing multiple logistic regression models fully adjusted for age, bmi, gender, race, smoking status, hypertension, atrial fibrillation, diabetes in model 1 and minimally adjusted for age, bmi, gender, race, smoking status in model 2.

COPD: chronic obstructive pulmonary disease; HF: heart failure; BMI: body mass index; FEV1: Forced expiratory volume in one second; post-bronch: post-bronchodilator; log950: log value of percent emphysema; NT-proBNP: N-terminal pro B-type natriuretic peptide;

Chapter 4: Bridging chapter

In a large population-based multi-ethnic cohort MESA, we showed that in subjects sampled from the general population, there was a prevalence of 13.3% of HF in participants with CLRD, and a very similar prevalence of 12.7% in participants with the COPD. In the main cohort as well as the subcohort, subjects with CLRD/COPD and HF displayed distinct characteristics when compared to those with CLRD/COPD alone: subjects with both diseases were older, had higher BMI and more comorbidities. They also had worse lung function when compared to those with CLRD/COPD alone shown by lower FEV1 and FVC values. Percent emphysema and levels of NT-proBNP were not significantly different between subjects with CLRD/COPD with and those without HF. And no significant association was shown between percent emphysema, levels of NT-proBNP and severity of airflow obstruction and having CLRD/COPD with HF when CLRD/COPD without HF was used as the reference population.

As we discussed in manuscript 1, one of the limitations of MESA is that this cohort is not a COPD cohort and it's a population-based cohort which means that data from this population captures early disease and early biomarkers.

To address the differences between early undiagnosed disease and diagnosed more severe disease, we developed an observational clinical project with the goal of studying characteristics of patients with COPD and CHF in a specialized COPD clinic.

Chapter 5: Manuscript 2 "Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure"

5.1 Abstract

Chronic Obstructive Pulmonary Disease (COPD) and Chronic Heart failure (CHF) are two highly prevalent conditions that significantly impact patients, families and the health care system. Although commonly studied independently, these diseases are often concomitant and the presence of comorbid CHF and COPD is often overlooked. Therefore, a prompt diagnosis and treatment of comorbid COPD and CHF could improve patient outcomes and reduce healthcare use.

In this observational study, we determined the prevalence of comorbid COPD and CHF in stable patients in a specialized pulmonary clinic. Each patient underwent a detailed cardiopulmonary evaluation including: complete medical history and physical exam; clinical questionnaires; electrocardiogram(EKG); chest computerized tomography (CT) scan; echocardiogram; post-bronchodilator spirometry and blood samples for measurements of serum biomarkers.

Previously unrecognized CHF was present in 16 patients (prevalence 29.6%). Out of these, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). Patients with COPD and CHF tend to be older, heavier smokers and mostly males. They also have higher rates of comorbidities including heart disease, hypertension and diabetes. Troponin levels were significantly elevated in this group compared to those with COPD only. We did not observe any association between having a normal/abnormal echocardiography and adverse effect as a similar percentage of patients in both groups had an exacerbation in the 1-year follow-up and required a doctor's visit or hospitalization. However, patients with HFrEF had more frequent exacerbations

per year and more patients in this group required a doctor's visit and hospitalization for their exacerbation when compared to HFpEF. A significant association was observed between CAT score >10 and the occurrence of an exacerbation and we observed that higher fibrinogen levels are associated with lower likelihood of having 2 or more exacerbations.

Our study demonstrated that a high prevalence of undiagnosed CHF is present in COPD from a specialized COPD clinic which could potentially affect patient outcomes. Some clinical characteristics could help clinicians targeting stable COPD patients who are more likely to have concomitant CHF, particularly those with HFrEF.

5.2 Introduction

Chronic Obstructive Pulmonary Disease (COPD) and Chronic Heart Failure (CHF) are two highly prevalent conditions with a significant impact in the global burden of disease[1]. Although commonly studied as independent entities, both diseases are often concomitant; CHF is estimated to be present in 5 to 41% of patients with COPD [2, 3]. However, the majority of studies assessing the coexistence of COPD and CHF have been retrospective and lacked echocardiography to confirm co morbid CHF [4, 5]. Furthermore, the landscape of CHF and COPD has changed significantly over the last decades. Patient populations are older and chronic co morbidities are frequent[2]. In addition a larger proportion of patients with COPD with co morbid CHF now have preserved ejection fraction (HFpEF) compared to CHF with reduced ejection fraction (HFrEF)[6]. However, the functional pulmonary abnormalities have not been well characterized in patients with HFpEF. The presence of concomitant COPD and CHF is often overlooked. In primary care, two cross-sectional studies found that 20.5% of elderly patients with COPD have unrecognized CHF [7]. In a cohort study involving tertiary care centers, it was estimated that around 11% of COPD patients had echocardiographic evidence of left ventricular dysfunction[8]. Results from studies assessing the coexistence of COPD and CHF highlight the importance of performing a comprehensive cardiopulmonary evaluation in every patient with a COPD diagnosis. This includes chest imaging (x-ray, CT scan), electrocardiogram (EKG), echocardiogram and pulmonary function tests (PFTs)[3, 8].

The coexistence of COPD and CHF highly impacts patient outcomes and treatment. Studies have shown that patients with COPD that also have a diagnosis of CHF are less likely to receive β blockers, angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) even though these medications are safe and improve outcomes and are effective and safe

even in the presence of COPD [9-11]. Patients with comorbid CHF and COPD had also higher in-hospital all-cause and non-cardiovascular (CV) mortality[12, 13]. Therefore, a prompt diagnosis and treatment of comorbid COPD and CHF could increase the likelihood of improving patient outcomes and consequently reducing healthcare use. Unfortunately, this comprehensive evaluation is rarely performed in every day clinical practice as it is considered to add to the cost and it is time consuming. A good alternative would be to do active screening so that selected patients can be referred to a specialist for a more detailed assessment. In this regard, serum biomarkers could represent an attractive option for both, diagnostic and prognostic purposes[14]. Both, COPD and CHF are characterized by a chronic, sub-clinical pro-inflammatory state and several neuro-hormonal and thrombo-inflammatory biomarkers have been characterized in both conditions[15-18]. In terms of neuro-hormonal activation, brain natriuretic peptide (BNP), its pro-hormone N-terminal (NT) proBNP can be elevated in both COPD and CHF[19, 20]. When added to clinical information, NTproBNP levels improve diagnostic accuracy of CHF in patients with acute dyspnea[21]. Furthermore, NT-proBNP levels could be useful for the detection of ventricular dysfunction in COPD patients and unrecognized CHF in COPD[8, 22]. In regards to markers of inflammation, levels of the cytokines interleukin (IL)-6 and interleukin (IL)-8, and levels of the pro-thrombotic mediators C-reactive protein (CRP), fibrinogen and troponin are elevated in both, COPD and CHF[16, 23-25]. S100A8 and S100A9 are new markers of inflammation that have also recently been characterized in COPD and CHF. In CHF, plasma levels of S100A8/A9 were found to be significantly increased and serum levels of S100A9 in COPD exacerbation [26, 27].

Given the aging population and the change in landscape for COPD and CHF in the recent years, it is of high relevance to better characterize these patient populations and been able to actively

screen them for concomitant COPD or CHF. Therefore, a systematic approach for detecting CHF in patients with COPD could lead to early diagnosis and prompt treatment of the co morbid condition, with the subsequent improvement in long term outcomes.

Our central hypothesis is that concomitant COPD and CHF will be under diagnosed in a COPD specialized clinic, that HFpEF will be more frequent than HFrEF in COPD patients and that patients with concomitant COPD and CHF will exhibit higher levels of neuro-hormonal and trombo-inflammatory biomarkers than COPD without CHF. Our primary objective was to determine the prevalence of co-morbid CHF in patients with COPD sampled from a specialized COPD outpatient clinic and to assess the characteristics of these patients including blood biomarkers. Our secondary objectives were: i) to characterize the structural and pulmonary functional abnormalities and neuro-hormonal and inflammatory biomarker profiles in COPD patients with CHF associated with reduced versus preserved ejection fraction; ii) to compare adverse outcomes in COPD patients with and without CHF and determine if measurements of biomarkers can serve as predictors for adverse outcomes.

5.3 Methods

Participants

COPD patients were recruited from the COPD clinic at the Montreal Chest Institute. All COPD patients were older than 40 years of age, had COPD confirmed by post-bronchodilator FEV1/FVC <0.7, were classified as GOLD 1 to 4, and were either current or ex-smokers with a smoking history ≥ 10 pack-years. Exclusion criteria were: 1) New York Heart Association(NYHA) functional classification class 4; 2) unstable or advanced renal failure (GFR < 30ml/min); 3) heart failure caused by an active inflammatory condition such as sarcoidosis or any form of myocarditis; 4) history of thoracotomy with pulmonary resection; 5) unstable or life-

threatening cardiac arrhythmia; 6) respiratory failure that has required mechanical ventilation and/or admission to the ICU; 7) use of chronic home oxygen; 8) previous diagnosis of CHF.

Diagnostic procedures

At baseline, each patient underwent a detailed and standardized cardiopulmonary evaluation that included: complete medical history and physical exam with special attention to signs and symptoms of COPD and CHF, clinical questionnaires (CAT, SF-36, mMRC), electrocardiogram (ECG), chest CT scan, pulmonary function test, echocardiogram and blood samples for measurements of serum biomarkers. The Modified British Medical Research Council (mMRC) is a simple measure of breathlessness with an mMRC of ≥ 2 used as a threshold for separating "less breathlessness" from "more breathlessness"[28]. The COPD Assessment Test (CAT) provide measures of the symptomatic impact of COPD, it includes cough, phlegm, breathlessness, activity level, sleep, energy, chest tightness and confidence for a score out of 40, with CAT ≥ 10 indicating reduced quality of life from COPD symptoms[29].

Data was also collected on medication prescribed and comorbidities. Information regarding exacerbation-like events and on hospitalizations for cardiovascular adverse events and/or CHF decompensations was collected every 3 months during phone follow-ups over a 12 months period after the initial visit and verified with hospital databases. The study was conducted in accordance with legal and regulatory requirements and practice guidelines such as good clinical practice. The ethical committee of the MUHC approved the protocol, and informed consent was obtained from each patient.

Diagnostic criteria

The diagnosis of COPD was confirmed based on GOLD criteria: a post-bronchodilator Forced expiratory volume in one second/ Forced vital capacity (FEV1/FVC) <0.07. Disease severity was classified as mild (FEV1 >80% predicted; GOLD1), moderate (50%≤FEVI<80% predicted; GOLD2), severe (30%≤FEVI<50% predicted; GOLD3) and very severe (FEV1<30% predicted; GOLD4) in clinically stable patients[30]. The diagnosis of CHF was done based on the European Society of Cardiology (ESC) criteria; signs and symptoms of heart failure with objective evidence of structural or functional abnormality. Patients with reduced LVEF (considered as <40%) are classified as HF with reduced EF (HFrEF) and those with normal LVEF (considered as ≥50%) are classified as HF with preserved EF (HFpEF). Patients with an LVEF in the range of 40 – 49% are in a grey area defined as HF with mid-range EF (HFmrEF). The diagnosis of HFrEF was done based on signs and symptoms of HF with an LVEF <40%. To establish a diagnosis of HFpEF or HFmrEF, the following criteria had to be met: 1) signs and symptoms of HF, 2) a preserved LVEF (LVEF ≥50% or 40–49% for HFmrEF), 3) presence of diastolic dysfunction on the echocardiogram or objective measures of cardiac dysfunction on the echocardiogram which includes: an increase in LV wall thickness and/or increased left atrial (LA) size as a sign of increased filling pressures[31].

Blood sampling and biochemical analyses

All blood samples were non-fasting and were collected at the initial visit. Plasma and serum were both collected. Levels of troponin and CRP were measured in serum at the central laboratory. Eosinophils levels were also assessed at the central laboratory from complete blood count (CBC). The GOLD guidelines recommend the use of an absolute blood eosinophil count of

≥300 cells/μL to identify patients with COPD with the greatest likelihood of treatment benefit with ICS[32]. An association was also found between absolute blood eosinophil count of ≥150 cells/μL and an increased risk of severe exacerbations and response to treatment[33]. We defined elevated eosinophils in our study as ≥150 cells/μL and high eosinophils as ≥300 cells/μL. High CRP levels were defined as ≥ 2 mg/L based on established CVD guidelines[34].

Levels of NT-proBNP and fibrinogen were measured by Enzyme-linked immunosorbent assay (ELISA) (R&D systems, Ottawa, ON, Canada) according to the manufacturer's instructions. Levels of IL-6, IL-8, S100A8, S100A9 and SP-D were measured by Human Magnetic Luminex Assay 5 panel analytes kit (Bio Techne Canada Corporation R&D system) according to the manufacturer's instructions. Levels of all the biomarkers were compared among the following groups: outpatients with COPD only and outpatients with both COPD and CHF.

CT scan of the chest analysis

The presence of emphysema and gas trapping were determined in all subjects by computational analysis of chest CT scans using 3D SLICER software. Emphysema was measured as the percentage of lung with attenuation values less than or equal to -950 Hounsfield units (HU) on inspiratory images, and gas trapping was measured as the percentage of lung less than or equal to -856HU on expiratory images[35].

Cardiac and respiratory adverse events

Patients were followed up for 12 months by phone follow-ups every 3 months to collect data on COPD exacerbation-like events and cardiovascular adverse events. Exacerbations were defined as event-based, meaning an increase in respiratory symptoms from baseline that required a medication change (antibiotic and/or systemic corticosteroids) and contact with the health care provider. These contacts may have been a phone call to a COPD nurse case manager, a physician

visit, an unscheduled visit to the day hospital or the emergency department, or a hospital admission. Exacerbations were considered moderate if patients required a prescription of systemic corticosteroids, a course of antibiotics, or both. They were considered severe if patients required hospitalization.

Cardiovascular adverse events were defined as hospitalization or a visit to the emergency department for either of the following: CHF decompensation, angina, myocardial infarction and/or arrythmia.

Statistical analysis

The prevalence of CHF was reported in all patients enrolled with COPD. Patient characteristics and adverse events were reported as percentages and mean+/- SD, and were compared by performing Chi-square analysis/Fisher exact test (category variables) or T- test or Anova analysis (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables). The incidence of exacerbations was summarized as a per-person per-year rate, and differences in exacerbations between groups were analyzed with Poisson regression model.

To look at the association between each of the serum biomarkers and symptom biomarkers and the occurrence of exacerbations/hospitalization (a patient did have or did not have an exacerbation/hospitalization) during the 1-year follow-up, adjusted OR (95% CI) were obtained by performing multiple logistic regression models, adjusted for sex, age, and smoking pack-years. When looking at the association between each of the serum biomarkers and symptom biomarkers and increased exacerbation frequency, adjusted OR (95% CI) were obtained by performing multiple multinomial logistic regression models with the frequency of exacerbations during the 1-year follow-up classified as none, one, or two or more and adjusted for sex, age, and smoking pack-years.

5.4 Results

COPD participants and representativeness

Recruitment is still ongoing. The total number of patients to be recruited will be 100. Currently 83 COPD patients have completed the baseline visit, but 29 patients still have to complete an echocardiogram as of March 2022. Table 1 shows the representativeness of the sample of COPD patients recruited from the COPD clinic. Patients from the COPD clinic had more severe disease than the study sample as shown by more severe airflow obstruction with more patients being classified as GOLD3+, lower post-bronchodilator FEV1% predicted and higher symptom burden as assessed by the CAT. Although there was a higher percentage of patients that had more than 1 or 2 exacerbations per year, these differences were not statistically significant. When comparison was made with respect to echocardiogram variables, no statistically significant differences could be demonstrated on ejection fraction, abnormal left ventricle systolic and diastolic function.

Prevalence of co-morbid CHF and patient characteristics

Data are reported on the 54 patients who have completed an echocardiogram. Patients were first separated into 2 groups: COPD with normal echocardiogram and COPD with abnormal echocardiography that qualify as CHF, i.e. patients who had features of either HFrEF, HFmrEF or HFpEF. Previously unrecognized CHF was present in 16 patients (prevalence 29.6%).
Table 2 shows the characteristics of COPD patients in both groups of echocardiography. Patients with abnormal echocardiography (HFrEF, HFmrEF or HFpEF) were older with a higher percentage of males. Both groups had a heavy smoking history, however, those with abnormal echocardiography tend to be heavier smokers with a 63 packs-years on average. More patients in the abnormal echocardiography group have had an exacerbation in the past year, they also had

more comorbidities including heart disease, hypertension and diabetes, abnormal ECG, and significant increase in the use of cardioselective beta-blockers, statins and ACE-inhibitor. CAT score and MRC results were not different between groups. Spirometry results were not different between both groups with respect to post-bronchodilator FEV1 in % predicted and post-bronchodilator % predicted FEV1/FVC ratio, and around 80% of patients were classified as GOLD 2 and 3. As expected, the baseline echocardiography results show a significantly higher number of COPD patients in the abnormal group having abnormal left ventricle systolic function and abnormal left ventricle diastolic function as well as abnormal left atrial size.

Table 3 shows complete pulmonary function test, CT scan variables and blood biomarkers levels for all patients according to echocardiography abnormality. Lung volumes such as total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV), and diffusion capacity (DLCO) were not different between groups. On the CT scan analysis, whole lung % emphysema on inspiration and whole lung % gas trapping on expiration were not different between groups. Levels of blood biomarkers IL-6, IL-8, S100A8, S100A9, SP-D, CRP, troponin and fibrinogen were assessed in all patients. Troponin levels were significantly higher in the COPD group with abnormal echocardiography. Eosinophils levels were also assessed and there's a higher number of eosinophils in the group with the abnormal echocardiography however this did not reach significance.

COPD patients with CHF associated with reduced (HFrEF) versus preserved ejection fraction (HFpEF)

In the 16 patients recognized as having abnormal echocardiography that qualify as CHF, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). Table 4

summarizes patients characteristics, spirometry, CT scan, echocardiogram features and blood biomarker results between the following three groups: COPD with HFrEF, COPD with HFpEF and COPD without CHF. The results from this analysis were quite similar to the results obtained when patients were divided into 2 groups (COPD with normal and abnormal echocardiography). COPD patients with HFpEF were slightly older than those in the other groups. All three groups had a heavy smoking history, however, those with COPD with HFrEF tend to be heavier smokers with an average packs-years of 67.5. More COPD patients with HFrEF have had an exacerbation in the past year when compared to the other groups, had a significantly higher percentage of heart disease as a comorbidity, higher percentage of abnormal ECG and increase in the use of cardioselective beta-blockers, statins and ACE-inhibitor. CAT score was slightly higher in the COPD with HFrEF group when compared to the COPD with HFpEF and COPD without CHF and a more significant number of COPD patients in the HFpEF group have an mMRC score of 1 when compared to the COPD with HFrEF and COPD without CHF. Baseline spirometry results were not different between groups with respect to post-bronchodilator FEV1 in % predicted and post-bronchodilator % predicted FEV1/FVC ratio, and GOLD classification. Baseline pulmonary function test data show a significantly higher TLC in the COPD with HFrEF group, however, FRC, RV and DLCO were not different between the groups. Based on the entry criteria, the echocardiography abnormalities were different between groups.

On the CT scan analysis, we observed a statistically significant difference in the whole lung percent emphysema on inspiration with the HFpEF group having the lowest percent emphysema on CT scan and those with COPD without CHF and COPD with HFrEF not having different values. Troponin levels were higher in both COPD with HFrEF and those with HFpEF and

eosinophiles levels were higher in COPD with HFrEF with 50% of patients having >300 cells/μL.

Association between biomarkers and adverse events

We did not observe any association between having a normal/abnormal echocardiography and adverse events; a similar percentage of patients in both groups had an exacerbation in the 1-year follow-up and required a doctor's visit or hospitalization (Table 5A). However, according to HFrEF and HFpEF, we observed that a higher percentage in the HFrEF group had more than 1 exacerbation per year and more than 2 exacerbations per year, and more patients required a doctor's visit and hospitalizations. The incidence of exacerbations summarized as a per-person per-year rate was significantly higher in patients with HFrEF in comparison to the HFpEF group. (Table 5B).

Table 6A shows the association between clinical biomarkers, serum biomarkers and the occurrence of exacerbations/hospitalization during the 1-year follow-up. We observed a significant association between CAT score >10 and the occurrence of exacerbations (7 times more likely to have an exacerbation) and fibrinogen levels and the occurrence of exacerbations (85% less likely to have an exacerbation).

Table 6B shows the association between clinical biomarkers, serum biomarkers and increased exacerbation frequency during the 1-year follow-up. We observed that higher fibrinogen levels are associated with lower likelihood of having 2 or more exacerbations (84% less likely); similar results were observed for levels of IL-8 (36% less likely). When looking at the association between eosinophils levels and the occurrence of 1 exacerbation, we observed a 8 times increased odds for levels between 150 to 300 cells/μL and a 9 times increased odds for levels >300 cells/μL, however, this did not reach statistical significance.

Table 6C presents the association between each of the clinical and serum biomarkers and count of exacerbations in the 1-year follow-up. We observed a significant increase in exacerbation rate with mMRC ≥ 2 and CAT ≥10 (RR 2.2 and 3.5 respectively) and a significant decrease for levels of fibrinogen (RR 0.55).

5.5 Discussion

Our study confirmed that even in a specialized pulmonary COPD clinic, unrecognized CHF in COPD is very common (prevalence 29.6%). Patients with COPD and a co-morbidity of CHF were older, male and heavier smokers, most likely to have exacerbations in the past year and other cardiovascular comorbidities such as coronary artery disease and arrythmias, as well as hypertension and diabetes. However, patients were not different based on their pulmonary function tests and abnormalities on CT scan. Serum biomarkers including troponin levels and eosinophils levels were higher in the COPD group with abnormal echocardiography when compared to COPD with normal echocardiography. Finally, we did not observe any association between serum biomarkers and adverse cardiovascular or respiratory acute events in patients having a normal/abnormal echocardiography as a similar percentage of patients in both groups had an exacerbation in the 1-year follow-up and required a doctor's visit or hospitalization. Out of the COPD patients with CHF, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). The clinical features observed in COPD group with abnormal echocardiography were more obvious in the COPD with HFrEF group compared to those with HFpEF as COPD with HFrEF tend to have more exacerbation in the past year, tend to be heavier smokers, have more comorbidities including heart disease, and be more symptomatic with a higher MRC and CAT. The two groups could not be distinguished based on their

symptom burden and lung function but lung emphysema on CT scan was significantly lower in COPD with HFpEF. In the 1-year follow-up, COPD with co-morbidity of HFrEF compared to HFpEF or those without CHF had more exacerbation (1 or 2 per-person per-year) and more patients in this group required a doctor's visit because of the exacerbation and required hospitalizations.

In the one year follow-up, CAT score >10 was associated with the occurrence of exacerbations (7 times more likely to have an exacerbation) and an increase in fibrinogen levels was associated with a reduced odds of having an exacerbation. There is also an increased odds of having an exacerbation when the levels of eosinophils > 150 cells/μL. Finally, we observed a significant increase in exacerbation rate with mMRC ≥ 2 and CAT ≥10.

The landscape of COPD and CHF has been changing over the recent years and the diagnosis of CHF can be overlooked in COPD due to the similarities of signs and symptoms. The key aspect of this study is detecting COPD in patients with CHF using a systematic approach and diagnosing and treating the co morbid condition with the goal of improvement in long term outcomes. Studies have previously shown the coexistence of these 2 diseases, however, the majority of studies assessing the coexistence of COPD and CHF have been retrospective and lacked echocardiography and spirometry to confirm co morbid CHF or COPD, respectively[4, 5]. Our study showed that even in a specialized practice setting, unrecognized CHF in COPD is very common (prevalence 29.6%). Our results were consistent with previous findings of studies that estimated CHF to be present in 5 to 41% of patients with COPD. The reported prevalence in the literature varies according to multiple criteria including specialist care setting, cohort selection, diagnostic criteria and the measurement tools applied. The prevalence varied from 19% when administrative data was used, to 20.5% in a general practitioner cohort, to 17% in an

outpatient clinic [7, 36, 37]. We observed that the prevalence of CHF in stable COPD patients is about four times as high compared with subjects aged 65 or over in the general population[38]. It is unclear why the prevalence of CHF is so much higher in COPD but it's been proposed that the increased prevalence of atherosclerosis in COPD and the increased smoking status could play a role[6]. We indeed observed in our study that the patients with both COPD and CHF tend to be heavier smokers and heart disease as a comorbidity was significantly increased.

The presence of COPD is generally considered a complicating factor for the diagnostic of patients with suspected CHF and this is due to the similarities in the signs and symptoms. We indeed did not see significant differences in the clinical questionnaires such as CAT score, mMRC and NYHA grade in both our groups. The presence of COPD as well as old age can also lead to inadequate echocardiographic views due to thoracic cavities being filled with air, and changes in echo parameters such as reduced early diastolic filling and increased late diastolic filling[6, 39]. However, in our study, we studied patients that were in stable conditions which lead to less effect on the echocardiogram results.

In our study, we were also interested at looking at both subtypes of CHF specifically HFpEF as the functional pulmonary abnormalities have not been well characterized this group. There was a slightly higher prevalence of HFpEF in our cohort compared to having HFrEF (62.5% vs 37.5%). This was expected based on previous studies but we showed a much higher prevalence compared to other studies[6]. Patients with COPD and HFpEF tend to be older but seem to be less symptomatic based on CAT score and mMRC grade when compared to those with COPD and HFrEF. They also tend to have better lung volumes and are less likely to have an exacerbation in the 1-year follow up and less likely to require a doctor visit for their exacerbation. This comparison was done in another study by Gulea et al where they also showed

that the most common CHF phenotype in COPD was HFpEF, however, exacerbations were more frequent in COPD with HFpEF compared with the COPD HFrEF group. The study population and the way data on exacerbation was collected was different in that study, and they also mention that the COPD with HFpEF group had more severe COPD when compared with COPD and HFrEF[40].

Serum biomarkers can represent an attractive option for both, diagnostic and prognostic purposes when screening COPD patients for comorbidities. Troponin levels were significantly increased in the COPD group with abnormal echocardiography and levels were increased in both HFrEF and HFpEF. This is expected as troponin levels have been shown to be increased in CHF[41]. However there was no association between the levels of troponin and the occurrence of exacerbations. Previous studies have showed that serum troponin levels are commonly raised in acute exacerbations of COPD and they appear to reflect the severity of the exacerbation[25]. However, in our study, serum measurement were done on blood samples collected at baseline and not during the exacerbation experienced by the patients.

No significant differences were observed in levels of CRP, IL-6, IL-8, S100A8 and S100A9 and SP-D. These markers are markers of systemic inflammation and have been shown to be increased in COPD subjects. However the comparison group were control non-COPD subjects[42]. Serum levels of S100A9 were shown to be increased in COPD exacerbation and not in stable disease[26]. All the patients recruited in this study were stable at baseline and did not have an exacerbation at least a month prior to the baseline visit.

Fibrinogen levels were not different between groups and an increase in fibrinogen levels was associated with a lower likelihood of having an exacerbation. This was not expected as it was previously shown in the ECLIPSE study that elevated plasma fibrinogen levels were associated

with an increased risk of exacerbations in patients with moderate to severe COPD[43]. In the ECLIPSE study however, the mean baseline fibrinogen value was 397 mg/dL which translates to 3970 ug/mL which was the unit used in our cohort. Our mean baseline fibrinogen value was 2.4 ug/mL indicating that the COPD patients in our cohort had very low fibrinogen levels at baseline. Therefore, the association between fibrinogen levels and exacerbations may not be relevant at those levels. The difference is most likely due to the variation in the characteristics of the COPD populations studied and the technique used to assess fibrinogen levels.

We also considered symptom burden as a biomarker to predict exacerbations. A study by Lee et al., showed that higher CAT score categories (CAT score >10) were associated with significantly higher exacerbation risk[44]. Indeed a significant association was observed between CAT score >10 and the occurrence of exacerbations.

5.6 Strength and limitations

Our study is one of the first study to perform such an extensive comprehensive cardiopulmonary evaluation including biomarkers in every patient to diagnose CHF in COPD. We also eliminated work-up bias from our study by having all subjects undergo all the diagnostic tests necessary to classify CHF and COPD respectively. All patient went through the same baseline visits where they were asked clinical questionnaires, had an ECG, chest CT scan, pulmonary function test, echo and blood samples. Another strength is that the convenient sample of COPD patients that we recruited for the study was representative of the COPD clinic. We indeed showed that with respect to echocardiogram variables, no statistically significant differences could be demonstrated between our recruited sample and the COPD clinic that includes more severe cases.

One of the limitations of our study is the sample size. However the study is still not completed and the goal is to recruit 100 COPD patients and repeat the analysis. Another limitation is that the patients in our study were recruited from a pulmonology specialty clinic by specialists and may not represent the general population demographics. However, this limitation could be mitigated by the fact that the prevalence showed in our study was similar to the prevalence observed in primary clinics which was not expected as this is a specialized clinic so our sample could be representative.

5.7 Conclusion

In conclusion, our study demonstrated that a high prevalence of undiagnosed CHF is present in COPD from a specialized COPD clinic. This could potentially affect patient outcomes considering that the cardiac condition was often not recognized and optimal treatment was not implemented.

Some clinical characteristics could help clinicians targeting stable COPD patients who are more likely to have concomitant CHF, particularly those with HFrEF. These characteristics are older age, male, having high burden of symptoms and/or exacerbations complicated by hospital admissions, and other heart disease comorbidities. However, blood biomarkers, lung function or CT scan abnormalities cannot be used to discriminate stable COPD without or with CHF.

Our study provides some evidence in favor of actively screening at minimum a subgroup of COPD patients for CHF comorbidities and modify treatment if necessary. Because performing an extensive cardiopulmonary evaluation is time consuming and expensive in all COPD patients, using clinical traits are still the most valuable approach in clinical practice until more specific serum biomarkers are demonstrated to be predictive of CHF comorbidity.

5.8 References

1. Lozano, R., et al., *Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010.* Lancet, 2012. **380**(9859): p. 2095-128.
2. Chen, W., et al., *Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis.* Lancet Respir Med, 2015. **3**(8): p. 631-9.
3. Hawkins, N.M., et al., *Heart failure and chronic obstructive pulmonary disease: diagnostic pitfalls and epidemiology.* Eur J Heart Fail, 2009. **11**(2): p. 130-9.
4. Hawkins, N.M., S. Virani, and C. Ceconi, *Heart failure and chronic obstructive pulmonary disease: the challenges facing physicians and health services.* Eur Heart J, 2013. **34**(36): p. 2795-803.
5. Roversi, S., et al., *Chronic Obstructive Pulmonary Disease and Cardiac Diseases. An Urgent Need for Integrated Care.* Am J Respir Crit Care Med, 2016. **194**(11): p. 1319-1336.
6. Rutten, F.H., et al., *Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease.* Eur Heart J, 2005. **26**(18): p. 1887-94.
7. Macchia, A., et al., *Unrecognised ventricular dysfunction in COPD.* Eur Respir J, 2012. **39**(1): p. 51-8.
8. Griffo, R., et al., *Frequent coexistence of chronic heart failure and chronic obstructive pulmonary disease in respiratory and cardiac outpatients: Evidence from SUSPIRIUM, a multicentre Italian survey.* Eur J Prev Cardiol, 2017. **24**(6): p. 567-576.
9. Salpeter, S., T. Ormiston, and E. Salpeter, *Cardioselective beta-blockers for chronic obstructive pulmonary disease.* Cochrane Database Syst Rev, 2005(4): p. Cd003566.
10. Sin, D.D. and F.A. McAlister, *The effects of beta-blockers on morbidity and mortality in a population-based cohort of 11,942 elderly patients with heart failure.* Am J Med, 2002. **113**(8): p. 650-6.
11. Rutten, F.H., et al., *Beta-blockers may reduce mortality and risk of exacerbations in patients with chronic obstructive pulmonary disease.* Arch Intern Med, 2010. **170**(10): p. 880-7.
12. Mentz, R.J., et al., *Clinical characteristics and outcomes of hospitalized heart failure patients with systolic dysfunction and chronic obstructive pulmonary disease: findings from OPTIMIZE-HF.* Eur J Heart Fail, 2012. **14**(4): p. 395-403.
13. Arnaudis, B., et al., *Impact of chronic obstructive pulmonary disease severity on symptoms and prognosis in patients with systolic heart failure.* Clin Res Cardiol, 2012. **101**(9): p. 717-26.
14. Maclay, J.D. and W. MacNee, *Cardiovascular disease in COPD: mechanisms.* Chest, 2013. **143**(3): p. 798-807.
15. Su, B., et al., *Inflammatory Markers and the Risk of Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(4): p. e0150586.
16. Celli, B.R., et al., *Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2012. **185**(10): p. 1065-72.
17. Nymo, S.H., et al., *Inflammatory cytokines in chronic heart failure: interleukin-8 is associated with adverse outcome. Results from CORONA.* Eur J Heart Fail, 2014. **16**(1): p. 68-75.
18. Gaggin, H.K. and J.L. Januzzi, Jr., *Biomarkers and diagnostics in heart failure.* Biochim Biophys Acta, 2013. **1832**(12): p. 2442-50.

19. Tavazzi, L., et al., *Clinical profiles and outcomes in patients with chronic heart failure and chronic obstructive pulmonary disease: an efficacy and safety analysis of SHIFT study.* Int J Cardiol, 2013. **170**(2): p. 182-8.
20. Calzetta, L., et al., *Brain natriuretic peptide: Much more than a biomarker.* Int J Cardiol, 2016. **221**: p. 1031-8.
21. Wright, S.P., et al., *Plasma amino-terminal pro-brain natriuretic peptide and accuracy of heart-failure diagnosis in primary care: a randomized, controlled trial.* J Am Coll Cardiol, 2003. **42**(10): p. 1793-800.
22. McCullough, P.A., et al., *Uncovering heart failure in patients with a history of pulmonary disease: rationale for the early use of B-type natriuretic peptide in the emergency department.* Acad Emerg Med, 2003. **10**(3): p. 198-204.
23. Bhatt, S.P. and M.T. Dransfield, *Chronic obstructive pulmonary disease and cardiovascular disease.* Transl Res, 2013. **162**(4): p. 237-51.
24. Tsutamoto, T., et al., *Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure.* J Am Coll Cardiol, 1998. **31**(2): p. 391-8.
25. Harvey, M.G. and R.J. Hancox, *Elevation of cardiac troponins in exacerbation of chronic obstructive pulmonary disease.* Emerg Med Australas, 2004. **16**(3): p. 212-5.
26. Pouwels, S.D., et al., *Increased serum levels of LL37, HMGB1 and S100A9 during exacerbation in COPD patients.* Eur Respir J, 2015. **45**(5): p. 1482-5.
27. Ma, L.P., et al., *S100A8/A9 complex as a new biomarker in prediction of mortality in elderly patients with severe heart failure.* Int J Cardiol, 2012. **155**(1): p. 26-32.
28. Jones, P.W., et al., *Comparisons of health status scores with MRC grades in COPD: implications for the GOLD 2011 classification.* Eur Respir J, 2013. **42**(3): p. 647-54.
29. Jones, P.W., M. Tabberer, and W.H. Chen, *Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores.* BMC Pulm Med, 2011. **11**: p. 42.
30. Vogelmeier, C.F., et al., *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary.* Am J Respir Crit Care Med, 2017. **195**(5): p. 557-582.
31. Ponikowski, P., et al., *2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC.* Eur J Heart Fail, 2016. **18**(8): p. 891-975.
32. Singh, D., et al., *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019.* Eur Respir J, 2019. **53**(5).
33. Pascoe, S., et al., *Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials.* Lancet Respir Med, 2015. **3**(6): p. 435-42.
34. Arnett, D.K., et al., *2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.* Circulation, 2019. **140**(11): p. e596-e646.
35. Nakano, Y., et al., *Quantitative assessment of airway remodeling using high-resolution CT.* Chest, 2002. **122**(6 Suppl): p. 271s-275s.
36. Boudestein, L.C., et al., *The impact of concurrent heart failure on prognosis in patients with chronic obstructive pulmonary disease.* Eur J Heart Fail, 2009. **11**(12): p. 1182-8.
37. Curkendall, S.M., et al., *Chronic obstructive pulmonary disease severity and cardiovascular outcomes.* Eur J Epidemiol, 2006. **21**(11): p. 803-13.

38. Hogg, K., K. Swedberg, and J. McMurray, *Heart failure with preserved left ventricular systolic function; epidemiology, clinical characteristics, and prognosis.* J Am Coll Cardiol, 2004. **43**(3): p. 317-27.
39. Tighe, D.A., et al., *Influence of age on assessment of diastolic function by Doppler tissue imaging.* Am J Cardiol, 2003. **91**(2): p. 254-7.
40. Gulea, C., R. Zakeri, and J.K. Quint, *Differences in Outcomes between Heart Failure Phenotypes in Patients with Coexistent COPD: A Cohort Study.* Ann Am Thorac Soc, 2021.
41. Myhre, P.L., et al., *Cardiac Troponin I and Risk of Cardiac Events in Patients With Heart Failure and Preserved Ejection Fraction.* Circ Heart Fail, 2018. **11**(11): p. e005312.
42. Gan, W.Q., et al., *Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis.* Thorax, 2004. **59**(7): p. 574-80.
43. Hurst, J.R., et al., *Susceptibility to exacerbation in chronic obstructive pulmonary disease.* N Engl J Med, 2010. **363**(12): p. 1128-38.
44. Lee, S.D., et al., *The COPD assessment test (CAT) assists prediction of COPD exacerbations in high-risk patients.* Respir Med, 2014. **108**(4): p. 600-8.

5.9 Tables

Table 1. Baseline characteristics of the study COPD patients compared to the patients of the whole COPD clinic

	Sample n=52	**COPD clinic n=167**	**P values**
participant's sex, n (%)			
Male	27 (51.9)	71 (42.5)	0.233
Female	25 (48.1)	95 (56.9)	0.265
age	68.9 ± 8.8	66.8 ± 9.3	0.145
BMI	26.5 ± 5.2	26.6 ± 8.6	0.304
Pack years	55.1 ± 32.8	41.8 ± 18.8	0.004*
comorbidity_heartdisease: Yes, n (%)	11 (21.2)	26 (15.6)	0.348
comorbidity_hypertension: Yes, n (%)	27 (51.9)	66 (39.5)	0.114
comorbidity_diabetes: Yes, n (%)	8 (15.4)	23 (13.8)	0.771
comorbidity_lungcancer: Yes, n (%)	0 (0.0)	22 (13.2)	0.003*
Post-bronchodilator FEV1 in L	1.3 ± 0.6	1.1 ± 0.6	0.003*
Post-bronchodilator FEV1 in % predicted	50.3 ± 17.5	42.7 ± 19.3	0.003*
Post-bronchodilator FEV1/FVC, %	44.4 ± 11.0	48.5 ± 14.4	0.079
GOLD grade, n (%)			
GOLD1	2 (3.8)	10 (6.1)	0.735
GOLD2	22 (42.3)	41 (24.8)	0.022*
GOLD3+	28 (53.8)	114 (69.1)	0.047*
TLC in % reference	107.2 ± 16.9	116.6 ± 19.1	0.018*
DLCO in % reference	44.8 ± 16.1	54.4 ± 17.2	0.012*
CAT total score	17.8 ± 6.9	21.9 ± 7.1	0.001*
Ejection Fraction (EF)	60.6 ± 7.3	61.2 ± 8.1	0.493
Left ventricle systolic function: Abnormal, n (%)	6 (11.5)	8 (10.1)	0.798
Left ventricle diastolic function: Abnormal, n (%)	10 (19.2)	15 (19.2)	1
Right ventricle systolic function: Abnormal, n (%)	5 (9.8)	4 (5.0)	0.31
Right ventricle diastolic function: Abnormal, n (%)	0 (0.0)	4 (5.0)	0.302
PASP number (mmHg)	35.0 ± 9.7	38.3 ± 12.2	0.13
RAP number (mmHg)	3.9 ± 2.4	3.9 ± 2.2	0.989
PHTN: Yes, n (%)	1 (1.9)	5 (6.5)	0.4
eosinophils abs(cells/ul)	182.5 ± 121.5	176.4 ± 183.1	0.266
Exacerbation (completed 1-year FU)	N=39	N=166	
Exacerbation>=1 in 1-year FU, n (%)	24 (61.5)	135 (76.3)	0.071
Exacerbation>=2 in 1-year FU, n (%)	17 (43.6)	101 (57.1)	0.156
Exacerbation rate in 1-year FU (no./patient)[#]	1.5	2.5	0.001*

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher exacet test (category variables) or T- test (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables).

COPD: chronic obstructive pulmonary disease; CAT: COPD Assessment Test; GOLD: global initiative for chronic obstructive lung disease stage; TLC: total lung capacity; DLCO: Diffusing capacity for carbon monoxide; LV: left ventricle; LA: Left atrium; EF: Ejection Fraction (EF); PASP: Pulmonary artery systolic pressure; RAP: Right atrial pressure; PHTN: Pulmonary Hypertension

Table 2. Baseline characteristics of COPD participants according to CHF based on echocardiogram

	Total (n=54)	COPD without CHF based on echocardiogram (n=38)	COPD with CHF based on echocardiogram (n=16)	P value
participant's sex, n (%)				
Male	28 (51.9)	16 (42.1)	12 (75.0)	0.038*
Female	26 (48.1)	22 (57.9)	4 (25.0)	0.038*
age	69.0 ± 8.6	68.0 ± 8.6	71.5 ± 8.4	0.177
BMI	26.7 ± 5.5	26.6 ± 6.0	26.8 ± 4.3	0.891
Pack years	55.6 ± 32.6	52.9 ± 29.9	62.6 ± 38.8	0.608
Systolic blood pressure	132.1 ± 18.4	134.3 ± 18.9	124.5 ± 14.7	0.118
Diastolic blood pressure	70.4 ± 12.0	70.2 ± 11.1	71.1 ± 15.2	0.844
pulse_rate	71.3 ± 11.9	71.8 ± 11.0	69.9 ± 13.9	0.239
Post-bronchodilator FEV1 in L	1.3 ± 0.6	1.3 ± 0.6	1.4 ± 0.5	0.576
Post-bronchodilator FEV1 in % predicted	49.8 ± 17.4	49.4 ± 17.2	50.9 ± 18.4	0.765
FEV1 reversibility (or % change)	7.1 ± 7.3	7.9 ± 7.4	5.1 ± 7.0	0.343
Post-bronchodilator FEV1/FVC (% predicted)	44.3 ± 10.8	43.4 ± 11.2	46.4 ± 9.9	0.355
GOLD grade, n (%)				
GOLD1	2 (3.7)	2 (5.3)	0 (0.0)	1
GOLD2	22 (40.7)	15 (39.5)	7 (43.8)	0.77
GOLD3	23 (42.6)	17 (44.7)	6 (37.5)	0.623
GOLD4	7 (13.0)	4 (10.5)	3 (18.8)	0.41
mMRC grade, n (%)				
0 and 1	17 (31.5)	10 (26.3)	7 (43.8)	0.208
2	11 (20.4)	7 (18.4)	4 (25.0)	0.714
3	19 (35.2)	15 (39.5)	4 (25.0)	0.365
4	7 (13.0)	6 (15.8)	1 (6.3)	0.66
mMRC>=2, n (%)	37 (68.5)	28 (73.7)	9 (56.3)	0.208
CAT total score	18.1 ± 6.9	17.9 ± 6.2	18.4 ± 8.5	0.827
CAT>=10	46 (85.2)	33 (86.8)	13 (81.3)	0.682
exacerbation_history: Yes, n (%)	41 (75.9)	29 (76.3)	12 (75.0)	1
exacerbation_history_1year: Yes, n (%)	14 (26.9)	8 (21.6)	6 (40.0)	0.74
comorbidity_heartdisease: Yes, n (%)	11 (20.4)	4 (10.5)	7 (43.8)	0.010*
comorbidity_hypertension: Yes, n (%)	27 (50.0)	17 (44.7)	10 (62.5)	0.233
comorbidity_diabetes: Yes, n (%)	8 (14.8)	5 (13.2)	3 (18.8)	0.682
comorbidity_lungcancer: Yes, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
comorbidity_stroke: Yes, n (%)	5 (9.3)	4 (10.5)	1 (6.3)	1
comorbidity_tuberculosis: Yes, n (%)	1 (1.9)	1 (2.6)	0 (0.0)	1
Left ventricle systolic function: Abnormal, n (%)	6 (11.1)	0 (0.0)	6 (37.5)	<0.001 *
Left ventricle diastolic function: Abnormal, n (%)	11 (20.4)	0 (0.0)	11 (68.8)	<0.001 *
Ejection Fraction (EF)	60.9 ± 7.3	62.5 ± 3.9	56.8 ± 11.3	0.172

Right ventricle systolic function: Abnormal, n (%)	5 (9.4)	2 (5.4)	3 (18.8)	0.155
Right ventricle diastolic function: Abnormal, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
LV size: Abnormal, n (%)	4 (7.8)	2 (5.6)	2 (13.3)	0.571
LA size: Abnormal, n (%)	4 (7.5)	0 (0.0)	4 (25.0)	0.006*
LV mass: Abnormal, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
Hypokinesia, n (%)	5 (9.3)	2 (5.3)	3 (18.8)	0.148
PASP number (mmHg)	35.1 ± 9.5	35.1 ± 9.5	35.1 ± 9.9	0.95
RAP number (mmHg)	3.9 ± 2.4	4.0 ± 2.6	3.6 ± 1.5	0.745
PHTN: Yes, n (%)	2 (3.7)	1 (2.6)	1 (6.3)	0.509
Hyperdynamic state: Yes, n (%)	1 (1.9)	1 (2.7)	0 (0.0)	1
NYHA Grade, n (%)				
1	6 (11.1)	3 (7.9)	3 (18.8)	0.346
2	23 (42.6)	16 (42.1)	7 (43.8)	0.911
3 or 4	25 (46.3)	19 (50.0)	6 (37.5)	0.4
Angina Grade, n (%)				
0	39 (72.2)	29 (76.3)	10 (62.5)	0.301
1	9 (16.7)	4 (10.5)	5 (31.3)	0.106
2	6 (11.1)	5 (13.2)	1 (6.3)	0.657
Abnormal ECG: Yes, n (%)	31 (57.4)	21 (55.3)	10 (62.5)	0.623
orthopnea_pillow, n (%)				
1	38 (70.4)	26 (68.4)	12 (75.0)	0.751
2	10 (18.5)	7 (18.4)	3 (18.8)	1
3	3 (5.6)	3 (7.9)	0 (0.0)	0.547
nocturnal_dyspnea, n (%)	9 (16.7)	5 (13.2)	4 (25.0)	0.425
swelling_ankleabdomen, n (%)	17 (31.5)	11 (28.9)	6 (37.5)	0.537
palpitations_heart, n (%)	19 (35.8)	14 (37.8)	5 (31.3)	0.76
syncope_fainting, n (%)	3 (5.7)	2 (5.4)	1 (6.3)	1
medication_breathing, n (%)	54 (100.0)	38 (100.0)	16 (100.0)	
saba_med, n (%)	44 (81.5)	31 (81.6)	13 (81.3)	1
laba_med, n (%)	51 (94.4)	36 (94.7)	15 (93.8)	1
sama_med, n (%)	5 (9.3)	3 (7.9)	2 (12.5)	0.627
lama_med, n (%)	51 (94.4)	35 (92.1)	16 (100.0)	0.547
ics_med, n (%)	38 (70.4)	26 (68.4)	12 (75.0)	0.751
antibiotics_med, n (%)	16 (29.6)	12 (31.6)	4 (25.0)	0.751
ltra_med, n (%)	5 (11.4)	3 (10.3)	2 (13.3)	1
medication_heart, n (%)	38 (70.4)	24 (63.2)	14 (87.5)	0.106
cardio_bb_med, n (%)	3 (7.9)	0 (0.0)	3 (21.4)	0.043*
non_cardio_bb_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
statins_med, n (%)	25 (65.8)	12 (50.0)	13 (92.9)	0.012*
aceinhibitor_med, n (%)	9 (23.7)	3 (12.5)	6 (42.9)	0.052
arb_med, n (%)	10 (26.3)	7 (29.2)	3 (21.4)	0.715
diuretic_med, n (%)	10 (26.3)	7 (29.2)	3 (21.4)	0.715

calcium_chan_med, n (%)	11 (28.9)	7 (29.2)	4 (28.6)	1
vasodilator_med, n (%)	2 (5.3)	1 (4.2)	1 (7.1)	1
arni_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
mra_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	-
anticoagulants_med, n (%)	8 (24.2)	3 (15.8)	5 (35.7)	0.238
antiarrhythmic_med, n (%)	1 (3.0)	1 (5.3)	0 (0.0)	1
antiplatelet_med, n (%)	13 (39.4)	9 (47.4)	4 (28.6)	0.31

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher exacet test (category variables) or T- test (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables).

COPD: chronic obstructive pulmonary disease; BMI: body mass index; FEV1: Forced expiratory volume in one second; FVC: Forced vital capacity; GOLD: global initiative for chronic obstructive lung disease stage; mMRC: Modified British Medical Research Council; CAT: COPD Assessment Test; LV: left ventricle; LA: Left atrium; EF: Ejection Fraction (EF); PASP: Pulmonary artery systolic pressure; RAP: Right atrial pressure; PHTN: Pulmonary Hypertension; NYHA: New York Heart Association functional classification; ECG: electrocardiogram; SABA: Short-acting beta-agonist; LABA: Long-acting beta-agonist; SAMA: Short-acting muscarinic antagonist; LAMA: long-acting muscarinic antagonist; ICS: inhaled corticosteroid; LTRA: Leukotriene receptor antagonists; cardio bb:cardio selective beta blocker; noncardio bb: noncardio selective beta blocker; ACE-I/ARB: angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers; ARNI: angiotensin receptor neprilysin inhibitor; MRA: Mineralocorticoid Receptor Antagonists;

Table 3. Pulmonary complete function test, CT scan variables and blood biomarkers levels of COPD participants according to CHF based on echocardiogram

	Total (n=54)	COPD without CHF based on echocardiogram (n=38)	COPD with CHF based on echocardiogram (n=16)	P value
TLC in % reference	106.8 ± 16.8	108.4 ± 15.5	102.9 ± 19.6	0.27
FRC in % reference	143.6 ± 31.9	143.7 ± 31.7	143.5 ± 33.3	0.887
RV %reference	151.7±47.0	152.4±43.6	150.0±55.9	0.992
RV/TLC in %	55.8±11.2	55.4±11.4	56.7±11.1	0.704
DLCO in % reference	44.2 ± 16.1	44.8 ± 16.6	42.8 ± 15.3	0.687
Whole lung % emphysema on inspiration (LAA%-950)	15.5 ± 11.9	17.3 ± 12.3	10.7 ± 9.3	0.095
Whole lung % gas trapping on expiration (LAA% -856)	50.2 ± 18.8	50.7 ± 19.3	48.7 ± 18.0	0.564
CRP levels (in mg/L)	3.4 ± 2.6	3.4 ± 2.2	3.5 ± 3.8	0.509
CRP >2mg/L	24 (61.5)	19 (65.5)	5 (50.0)	0.384
Troponin I hs levels (ng/L)	4.8 ± 3.7	4.3 ± 3.8	6.3 ± 3.4	0.019*
Fibrinogen levels (in ug/mL)	2.4 ± 0.6	2.5 ± 0.6	2.2 ± 0.8	0.246
IL-6 levels (in pg/mL)	2.5 ± 1.2	2.3 ± 1.0	3.1 ± 1.7	0.294
IL-8 levels (in pg/mL)	2.7 ± 3.3	2.9 ± 3.6	1.9 ± 1.3	0.645
S100A8 levels (in pg/mL)	36.0 ± 36.6	34.3 ± 35.5	42.4 ± 42.3	0.829
S100A9 levels (in pg/mL)	284.6 ± 217.5	281.9 ± 228.6	293.2 ± 188.3	0.637
SP-D levels (in pg/mL)	13055.4 ± 8671.4	13654.3 ± 8832.7	11078.9 ± 8237.0	0.239
eosinophils abs(cells/ul)	177.2 ± 122.0	166.7 ± 116.0	204.3 ± 137.2	0.314
<=150 cells/µL	26 (52.0)	20 (55.6)	6 (42.9)	0.533
>150 to <=300 cells/µL	15 (30.0)	11 (30.6)	4 (28.6)	1
>300 cells/µL	9 (18.0)	5 (13.9)	4 (28.6)	0.245

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher exact test (category variables) or T- test (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables).

COPD: chronic obstructive pulmonary disease; TLC: total lung capacity; FRC: Functional residual capacity; DLCO: Diffusing capacity for carbon monoxide; CRP: C-reactive protein; IL-6, IL-8: Interleukin (IL) 6, 8; SP-D: Surfactant protein D

Table 4. Baseline characteristics of COPD participants having CHF with reduced or preserved ejection fraction (HFrEF or HFpEF) compared to those with COPD and no CHF

	Total (n=54)	COPD with HFrEF (n=6)	COPD with HFp EF (n=10)	COPD without HF (n=38)	Overall P value
participant's sex, n (%)					
Male	28 (51.9)	5 (83.3)	7 (70.0)	16 (42.1)	0.086
Female	26 (48.1)	1 (16.7)	3 (30.0)	22 (57.9)	0.086
age	69.0 ± 8.6	67.6 ± 10.1	73.8 ± 6.7	68.0 ± 8.6	0.151
BMI	26.7 ± 5.5	26.4 ± 3.1	27.1 ± 5.0	26.6 ± 6.0	0.962
Pack years	55.6 ± 32.6	67.5 ± 57.9	60.1 ± 28.8	52.9 ± 29.9	0.831
Systolic blood pressure	132.1 ± 18.4	121.2 ± 12.7	128.4 ± 17.5	134.3 ± 18.9	0.241
Diastolic blood pressure	70.4 ± 12.0	73.0 ± 15.7	68.8 ± 15.9	70.2 ± 11.1	0.808
pulse_rate	71.3 ± 11.9	62.8 ± 1.5	74.1 ± 16.3	71.8 ± 11.0	0.177
Post-bronchodilator FEV1 in L	1.3 ± 0.6	1.4 ± 0.6	1.3 ± 0.5	1.3 ± 0.6	0.853
Post-bronchodilator FEV1 in % predicted	49.8 ± 17.4	46.7 ± 17.0	53.5 ± 19.7	49.4 ± 17.2	0.723
FEV1 reversibility (or % change)	7.1 ± 7.3	9.3 ± 8.9	2.6 ± 4.4	7.9 ± 7.4	0.025*
Post-bronchodilator FEV1/FVC (% predicted)	44.3 ± 10.8	41.7 ± 9.6	49.3 ± 9.4	43.4 ± 11.2	0.259
GOLD grade, n (%)					
GOLD1	2 (3.7)	0 (0.0)	0 (0.0)	2 (5.3)	1
GOLD2	22 (40.7)	2 (33.3)	5 (50.0)	15 (39.5)	0.827
GOLD3	23 (42.6)	3 (50.0)	3 (30.0)	17 (44.7)	0.681
GOLD4	7 (13.0)	1 (16.7)	2 (20.0)	4 (10.5)	0.547
TLC in % reference	106.8 ± 16.8	115.2 ± 23.1	95.5 ± 13.4	108.4 ± 15.5	0.038*
FRC in % reference	143.6 ± 31.9	159.3 ± 46.0	134.0 ± 20.3	143.7 ± 31.7	0.694
RV %reference	151.7±47.0	182.0±64.7	130.8±42.4	152.4±43.6	0.21
RV/TLC in %	55.8±11.2	58.7±9.5	55.5±12.3	55.4±11.4	0.806
DLCO in % reference	44.2 ± 16.1	40.2 ± 11.6	44.1 ± 17.3	44.8 ± 16.6	0.84
mMRC grade, n (%)					
0 and 1	17 (31.5)	1 (16.7)	6 (60.0)	10 (26.3)	0.093
2	11 (20.4)	2 (33.3)	2 (20.0)	7 (18.4)	0.665
3	19 (35.2)	2 (33.3)	2 (20.0)	15 (39.5)	0.553
4	7 (13.0)	1 (16.7)	0 (0.0)	6 (15.8)	0.434
mMRC>=2, n (%)	37 (68.5)	5 (83.3)	4 (40.0)	28 (73.7)	0.093
CAT total score	18.1 ± 6.9	20.2 ± 7.1	17.3 ± 9.5	17.9 ± 6.2	0.713
CAT>=10	46 (85.2)	5 (83.3)	8 (80.0)	33 (86.8)	0.841
Whole lung % emphysema on inspiration (LAA%-950)	15.5 ± 11.9	17.8 ± 9.2	5.3 ± 4.8	17.3 ± 12.3	0.013*
Whole lung % gas trapping on expiration (LAA% -856)	50.2 ± 18.8	58.0 ± 12.0	41.7 ± 19.3	50.7 ± 19.3	0.322
exacerbation_history: Yes, n (%)	41 (75.9)	4 (66.7)	8 (80.0)	29 (76.3)	0.779

exacerbation_history_1year: Yes, n (%)	14 (26.9)	3 (60.0)	3 (30.0)	8 (21.6)	0.845
comorbidity_heartdisease: Yes, n (%)	11 (20.4)	4 (66.7)	3 (30.0)	4 (10.5)	0.005*
comorbidity_hypertension: Yes, n (%)	27 (50.0)	4 (66.7)	6 (60.0)	17 (44.7)	0.519
comorbidity_diabetes: Yes, n (%)	8 (14.8)	1 (16.7)	2 (20.0)	5 (13.2)	0.841
comorbidity_lungcancer: Yes, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
comorbidity_stroke: Yes, n (%)	5 (9.3)	0 (0.0)	1 (10.0)	4 (10.5)	1
comorbidity_tuberculosis: Yes, n (%)	1 (1.9)	0 (0.0)	0 (0.0)	1 (2.6)	1
Left ventricle systolic function: Abnormal, n (%)	6 (11.1)	6 (100.0)	0 (0.0)	0 (0.0)	<0.001*
Left ventricle diastolic function: Abnormal, n (%)	11 (20.4)	1 (16.7)	10 (100.0)	0 (0.0)	<0.001*
Ejection Fraction (EF)	60.9 ± 7.3	43.6 ± 8.3	63.4 ± 4.9	62.5 ± 3.9	<0.001*
Right ventricle systolic function: Abnormal, n (%)	5 (9.4)	3 (50.0)	0 (0.0)	2 (5.4)	0.012*
Right ventricle diastolic function: Abnormal, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
LV size: Abnormal, n (%)	4 (7.8)	2 (33.3)	0 (0.0)	2 (5.6)	0.109
LA size: Abnormal, n (%)	4 (7.5)	1 (16.7)	3 (30.0)	0 (0.0)	0.006*
LV mass: Abnormal, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
Hypokinesia, n (%)	5 (9.3)	3 (50.0)	0 (0.0)	2 (5.3)	0.011*
PASP number (mmHg)	35.1 ± 9.5	33.0 ± 3.4	35.9 ± 11.6	35.1 ± 9.5	0.943
RAP number (mmHg)	3.9 ± 2.4	3.0 ± 0.0	3.9 ± 1.8	4.0 ± 2.6	0.581
PHTN: Yes, n (%)	2 (3.7)	0 (0.0)	1 (10.0)	1 (2.6)	0.509
Hyperdynamic state: Yes, n (%)	1 (1.9)	0 (0.0)	0 (0.0)	1 (2.7)	1
NYHA Grade, n (%)					
1	6 (11.1)	1 (16.7)	2 (20.0)	3 (7.9)	0.282
2	23 (42.6)	3 (50.0)	4 (40.0)	16 (42.1)	1
3 or 4	25 (46.3)	2 (33.3)	4 (40.0)	19 (50.0)	0.755
Angina Grade, n (%)					
0	39 (72.2)	4 (66.7)	6 (60.0)	29 (76.3)	0.554
1	9 (16.7)	2 (33.3)	3 (30.0)	4 (10.5)	0.125
2	6 (11.1)	0 (0.0)	1 (10.0)	5 (13.2)	1
Abnormal ECG: Yes, n (%)	31 (57.4)	5 (83.3)	5 (50.0)	21 (55.3)	0.472
orthopnea_pillow, n (%)					
1	38 (70.4)	6 (100.0)	6 (60.0)	26 (68.4)	0.209
2	10 (18.5)	0 (0.0)	3 (30.0)	7 (18.4)	0.335
3	3 (5.6)	0 (0.0)	0 (0.0)	3 (7.9)	1
nocturnal_dyspnea, n (%)	9 (16.7)	2 (33.3)	2 (20.0)	5 (13.2)	0.373
swelling_ankleabdomen, n (%)	17 (31.5)	1 (16.7)	5 (50.0)	11 (28.9)	0.373
palpitations_heart, n (%)	19 (35.8)	2 (33.3)	3 (30.0)	14 (37.8)	0.906
syncope_fainting, n (%)	3 (5.7)	1 (16.7)	0 (0.0)	2 (5.4)	0.384
CRP levels (in mg/L)	3.4 ± 2.6	2.6 ± 2.8	4.9 ± 5.1	3.4 ± 2.2	0.582
CRP >2mg/L	24 (61.5)	2 (33.3)	3 (75.0)	19 (65.5)	0.408
Troponin I hs levels (ng/L)	4.8 ± 3.7	6.4 ± 4.3	6.2 ± 1.9	4.3 ± 3.8	0.05
Fibrinogen levels (in ug/mL)	2.4 ± 0.6	2.0 ± 0.4	2.6 ± 1.1	2.5 ± 0.6	0.182
IL-6 levels (in pg/mL)	2.5 ± 1.2	2.9 ± 1.9	3.3 ± 1.6	2.3 ± 1.0	0.421
IL-8 levels (in pg/mL)	2.7 ± 3.3	2.2 ± 1.2	1.6 ± 1.5	2.9 ± 3.6	0.612
S100A8 levels (in pg/mL)	36.0 ± 36.6	46.2 ± 48.6	34.7 ± 33.2	34.3 ± 35.5	0.969

S100A9 levels (in pg/mL)	284.6 ± 217.5	306.8 ± 182.0	272.7 ± 224.1	281.9 ± 228.6	0.747
SP-D levels (in pg/mL)	13055.4 ± 8671.4	13336.5 ± 9713.5	7692.5 ± 4561.7	13654.3 ± 8832.7	0.285
eosinophils abs(cells/ul)	177.2 ± 122.0	290.0 ± 141.2	170.0 ± 126.3	166.7 ± 116.0	0.27
<=150 cells/μL	26 (52.0)	1 (25.0)	5 (50.0)	20 (55.6)	0.648
>150 to <=300 cells/μL	15 (30.0)	1 (25.0)	3 (30.0)	11 (30.6)	1
>300 cells/μL	9 (18.0)	2 (50.0)	2 (20.0)	5 (13.9)	0.196
medication_breathing, n (%)	54 (100.0)	6 (100.0)	10 (100.0)	38 (100.0)	
saba_med, n (%)	44 (81.5)	5 (83.3)	8 (80.0)	31 (81.6)	1
laba_med, n (%)	51 (94.4)	6 (100.0)	9 (90.0)	36 (94.7)	0.66
sama_med, n (%)	5 (9.3)	0 (0.0)	2 (20.0)	3 (7.9)	0.308
lama_med, n (%)	51 (94.4)	6 (100.0)	10 (100.0)	35 (92.1)	1
ics_med, n (%)	38 (70.4)	3 (50.0)	9 (90.0)	26 (68.4)	0.182
antibiotics_med, n (%)	16 (29.6)	2 (33.3)	2 (20.0)	12 (31.6)	0.805
ltra_med, n (%)	5 (11.4)	0 (0.0)	2 (20.0)	3 (10.3)	0.613
medication_heart, n (%)	38 (70.4)	5 (83.3)	9 (90.0)	24 (63.2)	0.237
cardio_bb_med, n (%)	3 (7.9)	2 (40.0)	1 (11.1)	0 (0.0)	0.022*
non_cardio_bb_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
statins_med, n (%)	25 (65.8)	5 (100.0)	8 (88.9)	12 (50.0)	0.021*
aceinhibitor_med, n (%)	9 (23.7)	4 (80.0)	2 (22.2)	3 (12.5)	0.011*
arb_med, n (%)	10 (26.3)	0 (0.0)	3 (33.3)	7 (29.2)	0.435
diuretic_med, n (%)	10 (26.3)	0 (0.0)	3 (33.3)	7 (29.2)	0.435
calcium_chan_med, n (%)	11 (28.9)	1 (20.0)	3 (33.3)	7 (29.2)	1
vasodilator_med, n (%)	2 (5.3)	0 (0.0)	1 (11.1)	1 (4.2)	0.607
arni_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
mra_med, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-
anticoagulants_med, n (%)	8 (24.2)	1 (20.0)	4 (44.4)	3 (15.8)	0.269
antiarrhythmic_med, n (%)	1 (3.0)	0 (0.0)	0 (0.0)	1 (5.3)	1
antiplatelet_med, n (%)	13 (39.4)	2 (40.0)	2 (22.2)	9 (47.4)	0.435

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher exacet test (category variables) or Anova analysis (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables).

COPD: chronic obstructive pulmonary disease; CHF: chronic heart failure; HFrEF: HF with reduced EF; HFpEF: HF with preserved EF; BMI: body mass index; mMRC: Modified British Medical Research Council; CAT: COPD Assessment Test; NYHA: New York Heart Association functional classification; ECG: electrocardiogram; SABA: Short-acting beta-agonist; LABA: Long-acting beta-agonist; SAMA: Short-acting muscarinic antagonist; LAMA: long-acting muscarinic antagonist; ICS: inhaled corticosteroid; LTRA: Leukotriene receptor antagonists; cardio bb:cardio selective beta blocker; noncardio bb: noncardio selective beta blocker; ACE-I/ARB: angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers; ARNI: angiotensin receptor neprilysin inhibitor; MRA: Mineralocorticoid Receptor Antagonists;

Table 5A. One year follow up in COPD participants according to CHF based on echocardiogram

	Total (n=54)	**COPD without CHF based on echocardiogram** (n=38)	**COPD with CHF based on echocardiogram** (n=16)	P value
Exacerbation (completed 1-year FU)	N=53	N=37	N=16	
Exacerbation>=1 in 1-year FU, n (%)	32 (60.4)	23 (62.2)	9 (56.3)	0.686
Exacerbation>=2 in 1-year FU, n (%)	23 (43.4)	16 (43.2)	7 (43.8)	0.973
Requiring ER or doctor visit>=1 in 1-year FU, n (%)	19 (35.8)	13 (35.1)	6 (37.5)	0.869
Requiring hospitalization>=1 in 1-year FU, n (%)	5 (9.4)	3 (8.1)	2 (12.5)	0.632
Exacerbation rate in 1-year FU (no./patient)[#]	1.5	1.5	1.4	0.648
Hospitalized/ visited the ER for any heart problem in 1-year FU, n (%)	1 (1.9)	1 (2.7)	0 (0.0)	1

Table 5B. One year follow up in COPD participants having CHF with reduced or preserved ejection fraction (HFrEF or HFpEF) compared to those with COPD and no CHF

	Total (n=54)	COPD with HFrEF (n=6)	COPD with HFpEF (n=10)	COPD (n=38)	Overall P value
Exacerbation (completed 1-year FU)	N=53	N=6	N=10	N=37	
Exacerbation>=1 in 1-year FU, n (%)	32 (60.4)	5 (83.3)	4 (40.0)	23 (62.2)	0.223
Exacerbation>=2 in 1-year FU, n (%)	23 (43.4)	4 (66.7)	3 (30.0)	16 (43.2)	0.392
Requiring ER or doctor visit>=1 in 1-year FU, n (%)	19 (35.8)	4 (66.7)	2 (20.0)	13 (35.1)	0.213
Requiring hospitalization>=1 in 1-year FU, n (%)	5 (9.4)	1 (16.7)	1 (10.0)	3 (8.1)	0.77
Exacerbation rate in 1-year FU (no./patient)[#]	1.5	2.3	0.8	1.5	0.042*
Hospitalized/ visited the ER for any heart problem in 1-year FU, n (%)	1 (1.9)	0 (0.0)	0 (0.0)	1 (2.7)	1

Data are presented as mean ± sd unless otherwise specified; P-value was obtained by performing Chi-square analysis/Fisher exacet test (category variables) or Anova analysis (normal distribution continuous variables) or Wilcoxon test (not normal distribution continuous variables). #. P-value was obtained by performing Poisson regression model.

COPD: chronic obstructive pulmonary disease; CHF: chronic heart failure; HFrEF: HF with reduced EF; HFpEF: HF with preserved EF; FU: follow-up

Table 6A. Relationship of different blood biomarkers and the occurrence of exacerbations/hospitalization during the 1-year follow-up

	Exacerbation>=1		Hospitalization>=1	
	OR (95% CI)	P value	OR (95% CI)	P value
CRP levels (in mg/L)— per increase of 1 point	0.94 (0.71, 1.23)	0.636	1.16 (0.81, 1.67)	0.413
Troponin I hs levels (ng/L)— per increase of 1 point	0.98 (0.81, 1.19)	0.83	0.79 (0.47, 1.34)	0.385
Fibrinogen levels (in ug/mL)— per increase of 1 point	0.15 (0.03, 0.65)	0.011*	0.18 (0.02, 1.68)	0.131
IL-6 levels (in pg/mL)— per increase of 1 point	1.18 (0.65, 2.13)	0.585	1.90 (0.84, 4.29)	0.123
IL-8 levels (in pg/mL)— per increase of 1 point	0.94 (0.76, 1.16)	0.55	1.02 (0.69, 1.51)	0.931
S100A8 levels (in pg/mL)— per increase of 1 point	1.01 (0.99, 1.03)	0.553	1.01 (0.99, 1.04)	0.243
S100A9 levels (in pg/mL)— per increase of 1 point	1.00 (1.00, 1.01)	0.092	1.00 (0.99, 1.00)	0.588
SP-D levels (in pg/mL)— per increase of 1 point	1.00 (1.00, 1.01)	0.241	1.00 (0.99, 1.00)	0.053
eosinophils abs.— per increase of 1 point	1.00 (1.00, 1.01)	0.149	1.00 (0.99, 1.01)	0.759
mMRC>=2 vs. <2	2.44 (0.67, 8.82)	0.174	2.76 (0.26, 29.15)	0.398
CAT>=10 vs. <10	7.60 (1.06, 54.34)	0.043*	-	-
CRP >2 vs. <2 mg/L	1.24 (0.28, 5.46)	0.775	-	-
eosinophils abs(cells/ul)				
<=150 cells/μL	Ref	-	Ref	-
>150 to <=300 cells/μL	1.86 (0.46, 7.44)	0.948	2.25 (0.26, 19.51)	0.658
>300 cells/μL	3.81 (0.51, 28.59)	0.307	1.99 (0.14, 28.94)	0.823

Adjusted OR (95% CI) were obtained by performing multiple logistic regression models, adjusted for sex, age, and smoking pack-years.

CRP: C-reactive protein; IL-6, IL-8: Interleukin (IL) 6, 8; SP-D: Surfactant protein D

Table 6B. Relationship between different blood biomarkers and increased exacerbation frequency during the 1-year follow up

	Number of Exacerbations						
	1 vs. 0		≥2 vs. 0		≥2 vs. 1		P Value for Overall Model
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	
CRP levels (in mg/L)— per increase of 1 point	0.91 (0.55, 1.51)	0.725	0.93 (0.70, 1.23)	0.612	1.02 (0.62, 1.67)	0.944	0.861
Troponin I hs levels (ng/L)— per increase of 1 point	0.79 (0.48, 1.31)	0.365	0.99 (0.82, 1.21)	0.951	1.25 (0.76, 2.06)	0.373	0.657
Fibrinogen levels (in ug/mL)— per increase of 1 point	0.13 (0.02, 1.09)	0.06	0.16 (0.03, 0.73)	0.019 *	1.21 (0.17, 8.63)	0.846	0.042*
IL-6 levels (in pg/mL)— per increase of 1 point	0.68 (0.23, 1.96)	0.47	1.34 (0.73, 2.48)	0.343	1.99 (0.72, 5.54)	0.187	0.335
IL-8 levels (in pg/mL)— per increase of 1 point	1.19 (0.90, 1.58)	0.226	0.77 (0.53, 1.10)	0.152	0.64 (0.43, 0.98)	0.038 *	0.113
S100A8 levels (in pg/mL)— per increase of 1 point	1.01 (0.98, 1.04)	0.539	1.01 (0.98, 1.03)	0.562	1.00 (0.98, 1.02)	0.874	0.798
S100A9 levels (in pg/mL)— per increase of 1 point	1.00 (1.00, 1.01)	0.099	1.00 (1.00, 1.01)	0.115	1.00 (1.00, 1.01)	0.682	0.217
SP-D levels (in pg/mL)— per increase of 1 point	1.00 (1.00, 1.01)	0.18	1.00 (1.00, 1.01)	0.422	1.00 (1.00, 1.01)	0.318	0.364
eosinophils abs.— per increase of 1 point	1.01 (1.00, 1.01)	0.077	1.00 (1.00, 1.01)	0.358	1.00 (0.99, 1.00)	0.31	0.21
mMRC>=2 vs. <2	2.46 (0.35, 17.11)	0.363	2.45 (0.59, 10.16)	0.218	0.99 (0.13, 7.81)	0.996	0.394
CAT>=10 vs. <10	-	-	4.34 (0.57, 33.16)	0.158	-	-	0.368
CRP >2 vs. <2 mg/L	1.11 (0.13, 9.69)	0.928	1.23 (0.25, 5.97)	0.8	1.11 (0.14, 9.08)	0.923	0.968
eosinophils abs(cells/ul)							
<=150 cells/µL	Ref	-	Ref	-	Ref	-	
>150 to <=300 cells/µL	8.67 (0.83, 90.61)	0.071	1.20 (0.26, 5.61)	0.817	0.14 (0.01, 1.49)	0.102	0.347
>300 cells/µL	9.35 (0.65, 135.21)	0.101	2.70 (0.29, 25.52)	0.386	0.29 (0.02, 3.96)	0.353	

Adjusted OR (95% CI) were obtained by performing multiple multinomial logistic regression models, adjusted for sex, age, and smoking pack-years

CRP: C-reactive protein; IL-6, IL-8: Interleukin (IL) 6, 8; SP-D: Surfactant protein D

Table 6C. Relationship between different blood biomarkers and count of exacerbation in the 1-year follow-up

	RR (95% CI)	P value
CRP levels (in mg/L)— per increase of 1 point	0.959 (0.860, 1.069)	0.454
Troponin I hs levels (ng/L)— per increase of 1 point	0.998 (0.929, 1.071)	0.946
Fibrinogen levels (in ug/mL)— per increase of 1 point	0.545 (0.348, 0.854)	0.008*
IL-6 levels (in pg/mL)— per increase of 1 point	1.200 (0.996, 1.446)	0.055
IL-8 levels (in pg/mL)— per increase of 1 point	0.933 (0.849, 1.026)	0.155
S100A8 levels (in pg/mL)— per increase of 1 point	1.002 (0.995, 1.008)	0.636
S100A9 levels (in pg/mL)— per increase of 1 point	1.001 (1.000, 1.002)	0.055
SP-D levels (in pg/mL)— per increase of 1 point	1.000 (1.000, 1.000)	0.296
eosinophils abs.— per increase of 1 point	1.001 (0.999, 1.003)	0.376
mMRC>=2 vs. <2	2.182 (1.178, 4.041)	0.013*
CAT>=10 vs. <10	3.448 (1.191, 9.984)	0.022*
CRP >2 vs. <2 mg/L	1.156 (0.660, 2.025)	0.612
eosinophils abs(cells/ul)		
<=150 cells/µL	Ref	-
>150 to <=300 cells/µL	0.979 (0.569, 1.685)	0.939
>300 cells/µL	1.326 (0.721, 2.441)	0.364

COPD: chronic obstructive pulmonary disease; CHF: chronic heart failure; CRP: C-reactive protein; IL-6, IL-8: Interleukin (IL) 6, 8; SP-D: Surfactant protein D

Adjusted RR (95% CI) were obtained by performing multiple Poisson regression models, adjusted for sex, age, and smoking pack-years

RR: Exacerbation rate ratio from Poisson regression models; CRP: C-reactive protein; IL-6, IL-8: Interleukin (IL) 6, 8; SP-D: Surfactant protein D

Chapter 6: Bridging chapter

In a specialized pulmonary COPD clinic, unrecognized CHF in COPD was still very common (prevalence 29.6%). Patients with COPD and a co-morbidity of CHF exhibited distinct characteristics: they were older, male and heavier smokers, most likely to have exacerbations in the past year and other cardiovascular comorbidities such as heart disease including coronary artery disease and arrythmias, hypertension and diabetes. In the 16 patients recognized as having abnormal echocardiography that qualify as HF, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). These distinct features observed were more obvious in COPD with HFrEF compared to those with HFpEF as COPD patients with HFrEF tend to have more exacerbations that were complicated with a hospital admission and to be more symptomatic. Groups however could not be distinguished based on their lung function pulmonary function tests and abnormalities on CT scan. Finally, we did not observe any association between serum biomarkers and adverse cardiovascular or respiratory acute events in patients having a normal/abnormal echocardiography. In the one-year follow-up, certain markers were associated with having an exacerbation.

Cigarette smoking is the most important risk factor for the development of COPD as well as a major cause of cardiovascular disease. We show in our clinical study that patients in the COPD as well as the COPD and CHF groups were heavy smokers. The inflammatory state in COPD is not confined to the lungs and occurs systemically in other organs. Cigarette smoking induces pulmonary inflammation and is associated with prolonged epithelial cell activation. Once cigarette smoke gets into the lungs, it first interacts with the layer of epithelial cells. Some toxic particles can cross the alveolar barrier and interact with endothelial cells or immune cells to be

transferred into the system. When particles interact with cells, defense mechanisms are activated and cell damage can occur. The lung-blood barrier is an important entity for investigating pulmonary and cardiovascular toxicity, this barrier is composed by pneumocytes, alveolar macrophage and endothelial cells. Endothelial function can be affected by reduced vasodilation and causing endothelial cells apoptosis, thereby acting a key mediator in the development of atherosclerosis.

Therefore, a translational project was developed to assess the effect of cigarette smoke on the interaction of different lung cells and characterize the expression of several inflammatory biomarkers.

Chapter 7: Manuscript 3 "Cigarette smoking mediates the expression of alarmins and other inflammatory biomarkers in lung epithelial cells"

7.1 Abstract

Cigarette smoking is the most important risk factor for the development of chronic lung diseases, including chronic obstructive pulmonary disease (COPD). Cigarette smoke induces pulmonary inflammation by prolonged activation of lung epithelial cells and the consequent release of pro-inflammatory cytokines such as IL-6 and IL-8. S100A8 and S100A9 are alarmins that, when released from injured cells, can recruit immune cells to the lungs, which can exacerbate the inflammatory process. Epithelial cells do not act in isolation but can directly communicate with other lung structural cells, including endothelial cells. Moreover, the inflammatory state in COPD is not confined to the lungs, but also occurs systemically, leading to endothelial dysfunction. In this study, we considered the lung epithelium as the source of activation of lung endothelial cells and characterized the expression of alarmins and adhesion molecules using an *in vitro* coculture model. We hypothesized that acute exposure to cigarette smoke extract (CSE) increases alarmin and cytokine release from pulmonary epithelial cells, leading to subsequent activation of endothelial cells. First, normal human bronchial airway epithelial (NHBE) cells were treated with CSE and the levels of inflammatory cytokines IL-6 and IL-8 as well as alarmins S100A8 and S100A9 were assessed. We observed a significant time-dependent increases in IL-8, S100A8 and S100A9in response to CSE in NHBE cells. NHBE cells were then exposed to CSE and cocultured with human lung microvascular endothelial cells (HMVEC-L) cells; however, this did not lead to the activation of HMVEC-L cells, as there was no significant increase in the levels of IL-6, VCAM-1 or E-selectin. This study demonstrates that cigarette smoke promotes inflammation in lung epithelial cells, and characterizes the expression of alarmins in those cells under the experimental conditions tested.

7.2 Introduction

Cigarette smoking (CS) is the most important risk factor for the development of chronic lung diseases, including chronic obstructive pulmonary disease (COPD) [1]. COPD is characterized by an ongoing inflammatory response in the lungs that drives airway remodelling and/or emphysema, leading to accelerated lung function decline in susceptible individuals [2]. There is growing evidence that the inflammatory state in COPD is not only confined to the lungs but also occurs systemically in other organs [3, 4]. While systemic inflammation can lead to changes in the airways, it also affects endothelial function by reducing vasodilation and causing endothelial cell apoptosis, thereby acting a key mediator in the development of cardiovascular diseases such as atherosclerosis [5, 6].

Lung epithelial cells form the first barrier against environmental insults and against inhaled toxicants such as cigarette smoke as well as microbial pathogens; lung epithelial cells are also important regulators of the innate and adaptive immunity. CS induction of pulmonary inflammation is associated with prolonged epithelial cell activation through the release of pro-inflammatory cytokines, growth factors, and chemokines that recruit and activate immune cells into the airways [7, 8]. *In vitro* studies have shown that CS causes increased levels of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-8 from lung epithelial cells [9]. *In vivo* studies with mice exposed to CS, as well as studies with human cigarette smokers, demonstrate higher levels of pulmonary neutrophils, macrophages and CD8+ T lymphocytes [10-12]. Furthermore, COPD subjects have increased levels of these immune cells in the lungs when compared to smokers without COPD [13, 14].This inflammatory response is thought to drive the

development of COPD, as continued recruitment of these immune cells can exacerbate the inflammatory process via secretion of cytokines, impairment of phagocytosis, production of reactive oxygen species (ROS) and expression of surface antigens [15, 16]. In addition, there is cellular crosstalk between lung epithelial and endothelial, an interaction that facilitates the recruitment of immune cells from the circulation [17]. Thus, the inflammatory response in the lungs caused by smoking affects other organ systems, including the cardiovascular system. Indeed, CS alters vascular function, activates the lung endothelial cells which affects inflammatory cell accumulation within the endothelium. *In vitro* studies using cigarette smoke extract (CSE) as an *in vivo* surrogate for smoke exposure have also shown an increase in the surface expression of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, as well as cytokines including TNF-α, IL-6 and IL-1α in endothelial cells [18]. CSE also increases adherence of monocytes to the endothelium and trans-endothelial migration, in addition to neutrophil transmigration across endothelial cells [19, 20].

Additional factors that contribute to the ongoing pulmonary and systemic inflammatory response include damage associated molecular pattern molecules (DAMPs), also known as alarmins. DAMPs are intracellular molecules that are involved in the regulation of cell proliferation and differentiation under normal conditions but act as "danger" signals when released from necrotic or injured cells by binding to pattern recognition receptors (PRRs) found on the surface of neutrophils and monocytes [21, 22]. S100A8 and S100A9, two alarmins that belong to the S100 family, are calcium-binding proteins that are responsible for successful cell migration, phagocytosis, and exocytosis under homeostatic conditions [23]. These alarmins are constitutively expressed in neutrophils and monocytes and can be induced in other cell types such as fibroblasts upon

activation [24, 25]. However, their expression in lung epithelial cells have not been well-characterized. Therefore, the goal of the present study was to characterize the expression of inflammatory biomarkers, including alarmins (S100A8 and S100A9), in lung epithelial cells exposed to CSE.

We further sought to characterize the expression of adhesion molecules in endothelial cells exposed to lung epithelial cells in an *in vitro* coculture model of CS exposure to better understand the dynamics of interaction between these two types of structural cells. This is potentially important, as , cellular crosstalk has been analyzed *in vitro* using conditioned media from one cell type to stimulate second cell type. This experimental paradigm, however, does not take into consideration cell-cell interactions between pulmonary epithelial and endothelial cells. Therefore, the specific objectives of this study were to assess the expression and release of inflammatory mediators, including alarmins in normal human bronchial airway epithelial (NHBE) cells after exposure to CSE as well as the expression of inflammatory mediators and adhesion molecules in human lung microvascular endothelial cells (HMVEC-L) after exposure to CSE-exposed NHBE cells. Although we hypothesized that acute exposure to CSE would elicit release of S100A8 and S100A9 from pulmonary epithelial cells and subsequently activate adjacent pulmonary endothelial cells, we observed an increase in select inflammatory mediators in response to CSE in NHBE cells including alarmins but this did not lead to the activation of endothelial cells

7.3 Methods

Reagents

Lipopolysaccharide ([LPS] 0111:B4) was purchased from Sigma-Aldrich and diluted to a concentration of 1ug/ml in BEBM.

Cell culture

NHBE and HMVEC-L cells were purchased from Lonza (Walkersville, Inc.). NHBE cells were cultured in T_{75} cell culture flasks in bronchial epithelial cell growth medium, which consists of bronchial epithelial cell growth basal medium (BEBM) supplemented with bovine pituitary extract [BPE], hydrocortisone, human epidermal growth factor [hEGF], epinephrine, transferrin, insulin, retinoic acid, triiodothyronine, and gentamicin/amphotericin-B). HMVEC-L cells were also cultured in T_{75} cell culture flasks in microvascular endothelial cell growth medium which consists of endothelial basal medium supplemented with human epidermal growth factor [hEGF], vascular endothelial growth factor [VEGF], R3-insulinlike growth factor-1 [R3-IGF-1], ascorbic acid, hydrocortisone, human fibroblast growth factor-beta [hFGF-β], fetal bovine serum [FBS], and gentamicin/amphotericin-B [GA]). Cells were maintained in humidified incubators at 37°C and 5% CO_2. The medium was changed every other day until cells reached around 80% confluency. Then, cells were detached using the subculture ReagentPack™ (Lonza). Cells were rinsed with 5 ml of room temperature HEPES buffered saline solution (HEPES-BSS) and then incubated with 2.5 ml of 0.025% Trypsin/EDTA solution at 37°C for 5 minutes. Once the cells were detached, trypsin was neutralized with 5 ml of Trypsin Neutralizing Solution. Cells were then centrifuged at 700g for 5 min, re-suspended in media and counted using a standard hemocytometer.

In order to mimic the lung–blood barrier, coculture models were established using the Transwell culture method. A coculture model consisted of NHBE cells cultured in the apical chamber of the Transwell insert and HMVEC-L cultured in the basolateral chamber. NHBE cells represent the pulmonary epithelium and HMVEC-L are a model for the cells lining the pulmonary capillaries.

NHBE cells were seeded in the apical layer of the insert (0.4 μm pore) of Transwell-12 Well Plate (Fisher scientific) at a density of 80,000 cells/well and grown to 80% confluency. HMVEC-L cells were cultured in 12-well plates at a density of 80 000 cells/well and grown to 80% confluency. Once they reached confluency, the cells were starved overnight with supplement-free media.

Preparation of Cigarette Smoke Extract

Research grade cigarettes (3R4F) with a filter were acquired from the Kentucky Tobacco Research Council (Lexington, KT). Each cigarette contains 0.73 mg of nicotine, 9.4 mg of tar, and 12.0 mg of CO, as described by the manufacturer. Cigarette smoke extract (CSE) was produced as previously described [26]. Briefly, CSE was prepared by bubbling smoke from a cigarette through 10 mL of BEBM and sterile-filtered with a 0.45- μm filter (Filtropur S0.45, membrane: PES, filtration surface: 5.3 cm^2); this preparation was used within 30 minutes. To ensure consistency in the CSE between experiments, an optical density was measured at 320nm wavelength immediately after preparation of the CSE; an optical density of 0.65 was considered to represent 100% (1 g/mL) CSE. All prepared CSE for our experiments were between 80-90% CSE, which was then diluted to 2% (0.02 g/mL) or 5% (0.05 g/mL) in BEBM.

Cell treatments

For the coculture experiment, NHBE cells cultured in the apical side of the transwell plate were exposed to 2% CSE, 5% CSE or 10% CSE; additional exposures included LPS with and without 2% CSE for 2h and 24h. 2h and 24h; these times were chosen to mimic short- and long-term exposure, respectively. LPS was chosen as a positive control, as it induces alarmins in NHBE cells [27]. Following exposures, the media was removed and 500ul of fresh BEBM was added to the

NHBE cells. The insert containing the cells was then moved to a 12-well plate containing HMVEC-L cells and cultured for an additional 24h as previously described [28].

LDH cytotoxicity assay

Lactate dehydrogenase (LDH) release was assessed in cell culture supernatant using the Pierce LDH Cytotoxicity Assay Kit as per manufacturer instructions (Thermo Fisher). Briefly, 50 µl of supernatant was transferred to a 96-well plate, 50 µl of reaction mixture was then added and incubated at room temperature for 30 mins. The reaction was stopped by adding Stop Solution. The absorbance at 490nm and 680nm was measured using a spectrophotometer (Infinite 200Pro). To determine LDH activity, the values at the 680nm absorbance value (background) were subtracted from the 490nm absorbance. To calculate % cytotoxicity, we subtracted the LDH activity of the control which is spontaneous LDH release (*i.e.,* water-treated) from the chemical-treated sample LDH activity (*i.e.,* CSE treatment or LPS). We then divided by the total LDH activity which is calculated as maximum LDH release (*i.e.,* cells treated with 10X Lysis Buffer) minus spontaneous LDH release. Finally, we multiplied this value by 100. This will gives us the cellular toxicity of CSE and LPS treatment.

RNA extraction

Following treatments, total RNA was extracted from NHBE and HMVEC-L cells with the Qiagen RNeasy Mini Kit (Toronto, ON, Canada) according to the manufacturer's instructions. Quantification of RNA was conducted using a Nanodrop 1000 spectrophotometer (infinite M200 pro, TECAN, CA).

Reverse transcription and quantitative real-time PCR

cDNA was synthesized by reverse transcription (RT) using iScript™ Reverse Transcription Supermix (BIO-RAD, Canada). The RT master mix consisted of 6 ul of iScript supermix, nuclease-free water and 10 ng of RNA template for a total of 20 µl. The mixture was incubated in a BioRad Thermal Cycler as follows: 5 minutes at 25ºC, 20 minutes at 46ºC to achieve full polymerase activity and 1 minute at 95ºC to inactivate the enzyme. Then, the mRNA levels of *IL-6*, *IL-8*, *S100A8* and *S100A9* were analyzed using this cDNA template and gene-specific primers (Table 1). For quantitative real-time PCR, 96-well reaction plates (Diamed, Mississauga, ON, Canada) were used with each condition containing cDNA template in a total volume of 3 µl sterile water, 0.5 µM of each forward and reverse primer and 5 µl iQ™ SYBR Green Supermix (BioRad, South San Francisco, CA, USA). The plates were sealed and cycled using a Thermal Cycler machine (BIO-RAD C1000 Touch TM, CA) that was initiated at 95 ºC for 3 minutes and followed by 39 cycles at 95 ºC for 10 s and annealing at 60 ºC for 45 s. Each condition was normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Fold change was determined through comparison of treated cells to the control using the $2^{-\Delta\Delta CT}$ method [29].

Enzyme-linked immunosorbent assay (ELISA)

Following treatment of NHBE cells, the supernatant was collected, and cell debris removed by centrifugation at 10000g for 3 min. The supernatant from each condition was then aliquoted and stored at -80ºC until analysis. Then the concentrations of IL-6 and S100A8 were determined by ELISA (R&D systems, Ottawa, ON, Canada) according to the manufacturer's instructions. Plates were read using a microplate reader (Infinite 200Pro) set to 450 nm with a wavelength correction

set to 570 nm. Data were analyzed by taking averages of the duplicate readings for each standard, control and sample and creating a standard curve.

Statistical analysis

Using GraphPad Prism 9 (Version 9.1.0) and SAS version 9.4, statistical analysis was performed for normally distributed data. First, we used a normality test (Shapiro-Wilk normality or Kolmogorov-Smirnove test) that showed p value > 0.05. Then, a one-way analysis of variance (ANOVA) and non-parametric tests were used followed by Dunnett's multiple comparisons test to compare all pairs to control. In all cases, a p value < 0.05 was considered statistically significant. Results are presented as mean ± standard deviation (SD).

7.4 Results

The expression of IL-8, S100A8 and S100A9 are increased in airway epithelial cells exposed to CSE

Our first objective was to evaluate the extent to which CSE could increase the expression of inflammatory mediators, including DAMPs. To avoid confounding issues related to cytotoxicity, we first evaluated cell viability in respond to CSE at the concentrations chosen in this study. Figure 1 shows that in response to increasing concentration of CSE (up to 10% CSE) as well as LPS, there was no significant induction in LDH release, with there being less than 5% cytotoxicity for all conditions. Therefore, we utilized 2%, 5% and 10% CSE for our studies, as these are commonly used percentages that yield an inflammatory response in primary pulmonary cells [26, 30].

We next evaluated the effects of CSE on the expression of key inflammatory markers, including alarmins. NHBE cells were exposed to 2% and 5% CSE for 3, 6 and 24h. Following exposure to CSE, there was a slight- but non-significant increase- in *IL-6* mRNA (Figure 2A). However, there was a significant increase in the expression of *IL-8* mRNA in NHBE cells in response to 5% CSE at 6 and 24 h (Figure 2B). We also observed a significant increase in mRNA expression of *S100A8* (Figure 2C) and *S100A9* (Figure 2D) in response to 2% CSE at 24h and in *S100A8* in response to 5% CSE at 24h. Interestingly, only S100A8 protein was significantly increased following exposure to 5% CSE for 6 and 24h (Figure 3B); the protein level of IL-6 was not significantly increased in the supernatant of NHBE cells exposed to CSE (Figure 3A). Thus, there is an increase in select inflammatory mediators in response to CSE in NHBE cells that occurs independent of alterations in cell cytotoxicity.

Short- and long-term exposure of NHBE cells to CSE does not activate HMVEC-L cells

To assess the effect of activated NHBE cells on the activation of HMVEC-L cells, we first exposed NHBE cells to 2%, 5%, 10% CSE, LPS or LPS+2% CSE for 2h and then cocultured them with HMVEC-L cells for 24h. Two hours was chosen to mimic a short-term exposure to CSE and allow the NHBE cells to secrete inflammatory mediators in the presence of HMVEC-L cells. mRNA and cell culture supernatants were isolated from NHBE cells following the 2h CSE exposure and an additional 24 coculture with HMVEC-L cells. There was no significant increase in the expression of *IL-6*, *IL-8*, *S100A8* or *S100A9* mRNA in NHBE cells in response to 2%, 5%, or 10% CSE for 2h (Figure 4A-D); there was also no change in IL-6 and S100A8 protein levels (Figure 5A and 5B). Furthermore, mRNA was isolated from HMVEC-L cells following their coculture with NHBE cells for 24h. There was no significant increase in the levels of the mRNA levels for *IL-6*,

VCAM-1 or *E-selectin* in HMVEC-L cells cocultured with NHBE cells that were exposed to different stimulus for 2h (Figure 6A-C).Therefore, exposure of epithelial cells to cigarette smoke for a short period of time does not lead to an increase in inflammatory cytokines from these cells and also does not lead to the activation of endothelial cells.

To mimic longer stimulus exposure, we next exposed NHBE cells to 2%, 5%, 10% CSE, LPS or LPS+2% CSE for 24h and then co-cultured them with HMVEC-L cells for 24h. mRNA and cell supernatant were isolated from NHBE cells following the 24h CSE exposure and an additional 24 coculture with HMVEC-L cells. There was no significant increase in the expression of *IL-6*, *IL-8*, *S100A8* or *S100A9* mRNA in NHBE cells in response to 2, 5, or 10% CSE for 24 hours (Figure 7A-D). However, there was significant increase in the expression of *IL-8*, *S100A8* and *S100A9* mRNA in NHBE cells exposed to 2% CSE with LPS (Figure 7B-D). The protein levels of IL-6 in the supernatant of NHBE cells were significantly increased following exposure to 10% CSE (Figure 8A) but no significant changes were observed in the levels of S100A8 (Figure 8B). Finally, mRNA was isolated from HMVEC-L cells following their coculture with NHBE cells for 24h. We observed a slight increase in the levels of IL-6; however, this did not reach statistical significance (Figure 9A). There was also no increase in the expression of the adhesion molecules VCAM-1 or E-selectin (Figure 9B and 9C). Therefore, exposure of epithelial cells to cigarette smoke for a longer period of time led to an increase in select inflammatory mediators in response to CSE but did not lead to the activation of endothelial cells under the experimental conditions tested.

7.5 Discussion

In a cell coculture model of CS exposure, this study demonstrated that exposure of NHBE cells to CSE leads to a significant increase in the expression of inflammatory mediator IL-8, as well alarmins S100A8 and S100A9 at the mRNA level and a significant increase in S100A8 at the protein level. However, when NHBE cells are exposed to CSE and then cocultured with HMVEC-L cells, they do not lead to the increase of inflammatory markers and adhesion molecules in HMVEC-L cells including IL-6, VCAM-1 or E-selectin.

The main aspect of this study was to study the expression and release of inflammatory mediators, including alarmins, in NHBE cells after exposure to CSE; as well as studying the expression of inflammatory mediators and adhesion molecules in HMVEC-L cells after exposure to CSE-exposed NHBE cells.

We demonstrated that exposure of NHBE cells to 5% CSE leads to a significant increase in the expression of IL-8 at 6h and 24h. These results are consistent with previous findings where there is increased levels of inflammatory mediators following exposure to CSE [31].

S100A8 and S100A9 are known to be expressed at high concentrations by granulocytes and during the early differentiation stage of monocytes but their expression are not well characterized in bronchial epithelial cells. One study by Henke et al. showed an increase in the expression of S100A8 and S100A9 in response to LPS in immortalized cell line human bronchial epithelial cells (16HBE14o-) and then validated those results in NHBE [27]. However the expression of S100A8 and S100A9 is not characterized in response to cigarette smoke in bronchial epithelial cells.

S100A8 and S100A9 are increased in bronchoalveolar lavage (BAL) fluid and serum of COPD patients as well as in the BAL of mouse models of CSE [32, 33]. A key aspect of our study was to

determine whether the cellular source of those specific alarmins were lung epithelial cells when exposed to CSE. We indeed showed that NHBE cells respond to CSE by increasing S100A8 and S100A9 mRNA expression. We observed a significant increase in the expression of S100A8 and S100A9 following exposure of cells to 2% and 5% CSE.

To gain insight on the role of activated lung epithelial cells on microvascular lung endothelial cells, we cocultured the cells together and used qRT-PCR and ELISA to evaluate the response of endothelial cells to activated epithelial cells. For the co-culture model, we looked at the effect of short term and longer term CSE exposure. Short term exposure to CSE did not lead to activation of NHBE or HMVEC-L cells. When looking at longer exposure to CSE, we observed that exposing NHBE cells to CSE alone did not increase expression of IL-8, S100A8 and S100A9 but it did in combination with LPS. This however, did not lead to the activation of HMVEC-L cells, as we did not observe a significant increase in the levels of IL-6, VCAM-1 or E-selectin from HMVEC-L cells.

Exposure of NHBE to CSE was previously shown by Nadia et al. as well in as in our previous experiment to significantly increase the expression of IL-6 and IL-8 at 3, 6 and 24h with the highest expression being reached at 24h[34]. In our study, we measured mRNA as well as protein levels at 26h in the short term CSE exposure and 48h in the longer term CSE exposure, this probably explains why we didn't observe significant differences in the mRNA and protein levels of inflammatory mediators.

Previous studies by Sharma et al. showed that CSE activates the lung endothelium and stimulates the expression of adhesion molecules including E-selectin, ICAM-1, and VCAM-1[35]. We did not observe an increase in the endothelial cells markers after 24h of exposure to activated NHBE cells. However, in the aforementioned study, the authors observed a transient increase in the

expression of E-selectin and VCAM-1 after direct exposure to CSE. An increase in the expression of E-selectin was observed after 5h of exposure to CSE and this increase was not seen at 24h. Similarly, VCAM-1 was significantly increased after 10h of CSE. In our study, HMVEC-L cells were indirectly exposed to CSE as they were exposed to NHBE cells that were previously exposed to CSE; we also quantified mRNA levels following 24h of exposure to the stimulus.

7.6 Strength and limitations

A novel aspect of this study was to mimic the lung–blood barrier by using a co-culture model that consisted of NHBE cells separated by a Transwell insert from HMVEC-L. We chose to use this model to consider cell-cell interactions between pulmonary epithelial and endothelial cells and to understand how CSE affects this interaction. Traditional single-cell culture usually lacks the ability to represent realistic cell-cell communications that occur in vivo situations.

We also used primary cells which are more physiologically relevant and reflective of the *in vivo* environment compared to immortalized cell lines. Primary cells and cell lines respond differently to stimuli; a study comparing cultured transformed lung epithelial cell lines and primary epithelial cells showed that primary airway epithelial cells including NHBE cells were more responsive to CSE than transformed cell lines with regard to the release of inflammatory cytokines such as interleukin (IL)-8 and IL-6 [31]. For the endothelial cells, we chose HMVEC-L which are primary cells that seemed ideal for our model because they are isolated from pulmonary microvessels.

A limitation to our experimental design is that we used submerged cultures to culture NHBE cells. Submerged culture conditions do not mimic many of the major *in vivo* features of airway epithelial cells such as mucociliary differentiation. Air-liquid interface (ALI) cultures, in contrast to submerged cell culture systems, allow polarization and differentiation of these cells. When

cultured at ALI, airway epithelial cells form a polarized, pseudostratified epithelium composed of ciliated and mucus-secreting cells. ALI cultures allow cells to be differentiated which mimics real situation in the human body. This could affect how the cells respond to the stimulus [36, 37].Another limitation is that we did not assess the function of endothelial cells. CSE has been shown to increase the adherence of monocytes to the endothelium and transendothelial migration. These events are important steps in pathogenesis of atherosclerosis [20]. Therefore, future experiments to test the function of adhesion molecules are needed.

7.7 Conclusion

In conclusion, this study demonstrated that cigarette smoke significantly increases the expression of alarmins in lung epithelial cells. It also considered the lung epithelium as the source of activation of lung endothelial cells when exposed to cigarette smoke as stimulus. We did not observe an activation of the endothelial cells, however, more experiments are needed to assess adherence of monocytes to the endothelium and transendothelial migration. Further studies are also needed to assess the mechanism by which alarmins are increased in the lung epithelium and how inhibition of alarmins secretion from lung epithelial cells can affect the activation of lung endothelial cells and subsequently inflammation in the lungs.

7.8 References

1. Buist, A.S., W.M. Vollmer, and M.A. McBurnie, *Worldwide burden of COPD in high- and low-income countries. Part I. The burden of obstructive lung disease (BOLD) initiative.* Int J Tuberc Lung Dis, 2008. **12**(7): p. 703-8.
2. Wang, Y., et al., *Role of inflammatory cells in airway remodeling in COPD.* Int J Chron Obstruct Pulmon Dis, 2018. **13**: p. 3341-3348.
3. Sin, D.D. and S.F. Man, *Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease.* Circulation, 2003. **107**(11): p. 1514-9.
4. Miller, J., et al., *Comorbidity, systemic inflammation and outcomes in the ECLIPSE cohort.* Respir Med, 2013. **107**(9): p. 1376-84.
5. Bonetti, P.O., L.O. Lerman, and A. Lerman, *Endothelial dysfunction: a marker of atherosclerotic risk.* Arterioscler Thromb Vasc Biol, 2003. **23**(2): p. 168-75.
6. Matsuzawa, Y. and A. Lerman, *Endothelial dysfunction and coronary artery disease: assessment, prognosis, and treatment.* Coron Artery Dis, 2014. **25**(8): p. 713-24.
7. Puchelle, E., et al., *Airway epithelial repair, regeneration, and remodeling after injury in chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2006. **3**(8): p. 726-33.
8. Rusznak, C., et al., *Effect of cigarette smoke on the permeability and IL-1beta and sICAM-1 release from cultured human bronchial epithelial cells of never-smokers, smokers, and patients with chronic obstructive pulmonary disease.* Am J Respir Cell Mol Biol, 2000. **23**(4): p. 530-6.
9. King, P.T., *Inflammation in chronic obstructive pulmonary disease and its role in cardiovascular disease and lung cancer.* Clin Transl Med, 2015. **4**(1): p. 68.
10. Tamimi, A., D. Serdarevic, and N.A. Hanania, *The effects of cigarette smoke on airway inflammation in asthma and COPD: Therapeutic implications.* Respir Med, 2012. **106**(3): p. 319-28.
11. D'Hulst A, I., et al., *Time course of cigarette smoke-induced pulmonary inflammation in mice.* Eur Respir J, 2005. **26**(2): p. 204-13.
12. Edwards, D., *Immunological effects of tobacco smoking in "healthy" smokers.* Copd, 2009. **6**(1): p. 48-58.
13. MacNee, W., *Pathogenesis of chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2005. **2**(4): p. 258-66; discussion 290-1.
14. Ning, W., et al., *Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease.* Proc Natl Acad Sci U S A, 2004. **101**(41): p. 14895-900.
15. Fels, A.O. and Z.A. Cohn, *The alveolar macrophage.* J Appl Physiol (1985), 1986. **60**(2): p. 353-69.
16. Lacy, P. and J.L. Stow, *Cytokine release from innate immune cells: association with diverse membrane trafficking pathways.* Blood, 2011. **118**(1): p. 9-18.
17. Blume, C., et al., *Cellular crosstalk between airway epithelial and endothelial cells regulates barrier functions during exposure to double-stranded RNA.* Immun Inflamm Dis, 2017. **5**(1): p. 45-56.

18. Orosz, Z., et al., *Cigarette smoke-induced proinflammatory alterations in the endothelial phenotype: role of NAD(P)H oxidase activation.* Am J Physiol Heart Circ Physiol, 2007. **292**(1): p. H130-9.
19. Overbeek, S.A., et al., *Cigarette smoke induces β2-integrin-dependent neutrophil migration across human endothelium.* Respir Res, 2011. **12**(1): p. 75.
20. Shen, Y., et al., *Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes.* Am J Physiol, 1996. **270**(5 Pt 2): p. H1624-33.
21. Newton, K. and V.M. Dixit, *Signaling in innate immunity and inflammation.* Cold Spring Harb Perspect Biol, 2012. **4**(3).
22. Chan, J.K., et al., *Alarmins: awaiting a clinical response.* J Clin Invest, 2012. **122**(8): p. 2711-9.
23. Roth, J., et al., *MRP8 and MRP14, S-100-like proteins associated with myeloid differentiation, are translocated to plasma membrane and intermediate filaments in a calcium-dependent manner.* Blood, 1993. **82**(6): p. 1875-83.
24. Edgeworth, J., et al., *Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells.* J Biol Chem, 1991. **266**(12): p. 7706-13.
25. Rahimi, F., et al., *FGF-2, IL-1beta and TGF-beta regulate fibroblast expression of S100A8.* Febs j, 2005. **272**(11): p. 2811-27.
26. Baglole, C.J., et al., *The aryl hydrocarbon receptor attenuates tobacco smoke-induced cyclooxygenase-2 and prostaglandin production in lung fibroblasts through regulation of the NF-kappaB family member RelB.* J Biol Chem, 2008. **283**(43): p. 28944-57.
27. Henke, M.O., et al., *Up-regulation of S100A8 and S100A9 protein in bronchial epithelial cells by lipopolysaccharide.* Exp Lung Res, 2006. **32**(8): p. 331-47.
28. Xu, H., et al., *Exosomal microRNA-21 derived from bronchial epithelial cells is involved in aberrant epithelium-fibroblast cross-talk in COPD induced by cigarette smoking.* Theranostics, 2018. **8**(19): p. 5419-5433.
29. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
30. Zago, M., et al., *Low levels of the AhR in chronic obstructive pulmonary disease (COPD)-derived lung cells increases COX-2 protein by altering mRNA stability.* PLoS One, 2017. **12**(7): p. e0180881.
31. Kode, A., S.R. Yang, and I. Rahman, *Differential effects of cigarette smoke on oxidative stress and proinflammatory cytokine release in primary human airway epithelial cells and in a variety of transformed alveolar epithelial cells.* Respir Res, 2006. **7**(1): p. 132.
32. Pouwels, S.D., et al., *A specific DAMP profile identifies susceptibility to smoke-induced airway inflammation.* Eur Respir J, 2014. **43**(4): p. 1183-6.
33. Merkel, D., et al., *Proteomic study of human bronchoalveolar lavage fluids from smokers with chronic obstructive pulmonary disease by combining surface-enhanced laser desorption/ionization-mass spectrometry profiling with mass spectrometric protein identification.* Proteomics, 2005. **5**(11): p. 2972-80.
34. Sharma, J., et al., *Lung endothelial cell platelet-activating factor production and inflammatory cell adherence are increased in response to cigarette smoke component exposure.* Am J Physiol Lung Cell Mol Physiol, 2012. **302**(1): p. L47-55.

35. Fizeșan, I., et al., *Responsiveness assessment of a 3D tetra-culture alveolar model exposed to diesel exhaust particulate matter.* Toxicol In Vitro, 2018. **53**: p. 67-79.
36. Ross, A.J., et al., *Transcriptional profiling of mucociliary differentiation in human airway epithelial cells.* Am J Respir Cell Mol Biol, 2007. **37**(2): p. 169-85.

7.9 Figures:

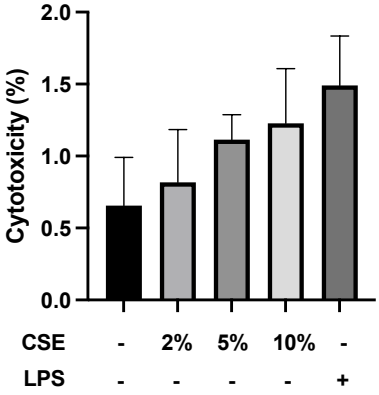


Figure 1. LDH release following exposure to CSE and LPS

Incubation of NHBE cells with 2%, 5%, 10% CSE and LPS for 24h did not significantly increase cellular cytotoxicity. Cytotoxicity was assessed using the Pierce LDH cytotoxicity assay kit. Results are expressed as the mean ± SEM of 4 independent experiments.

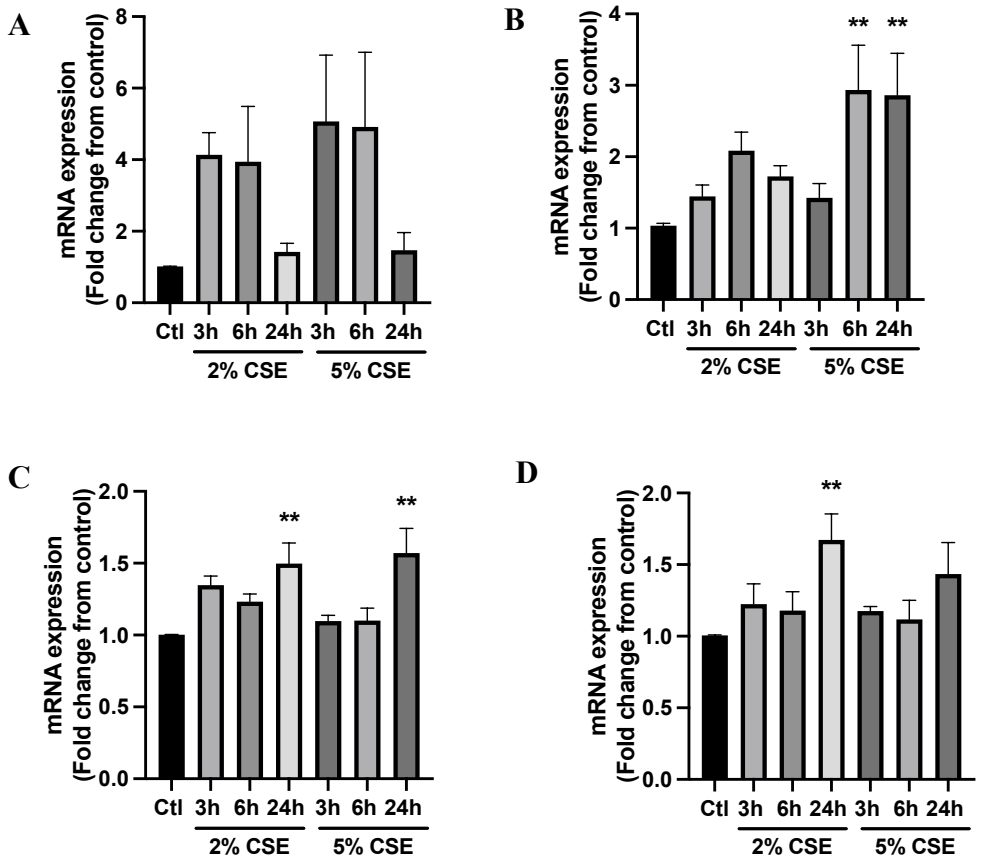


Figure 2. mRNA levels of inflammatory biomarkers IL-6(A), IL-8(B), S100A8(C), S100A9(D) following exposure of NHBE cells to CSE

IL-8 levels were significantly increased in NHBE cells in response to 5% CSE at 6 and 24 h. S100A8 and S100A9 levels were significantly increased in response to 2% CSE at 24h and in response to 5% CSE at 24h for S100A8. Cells were exposed to 2% CSE and 5% CSE for 3, 6 and 24h. IL-6, IL-8, S100A8 and S100A9 mRNA expression was measured by qRT-PCR. Gene expression was normalized to GAPDH**.** Results are expressed as the mean ± SEM of 6 independent experiments. **p= 0.001.

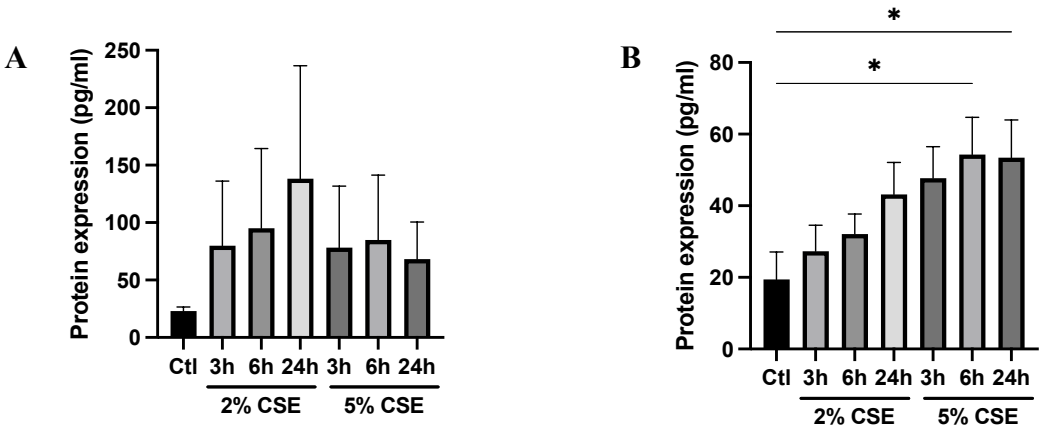


Figure 3. Protein levels of inflammatory biomarkers IL-6(A), and S100A8(B) following exposure of NHBE cells to CSE

No significant increase in IL-6 protein levels was observed in NHBE cells in response to CSE. S100A8 levels were significantly increased in NHBE cells following exposure to 5% CSE at 6 and 24h. Following exposure of NHBE cells to 2% and 5% CSE for 3, 6 and 24h, levels of IL-6 and S100A8 were measured in the supernatant by ELISA. Results are expressed as the mean ± SEM of 6 independent experiments. *$p < 0.05$

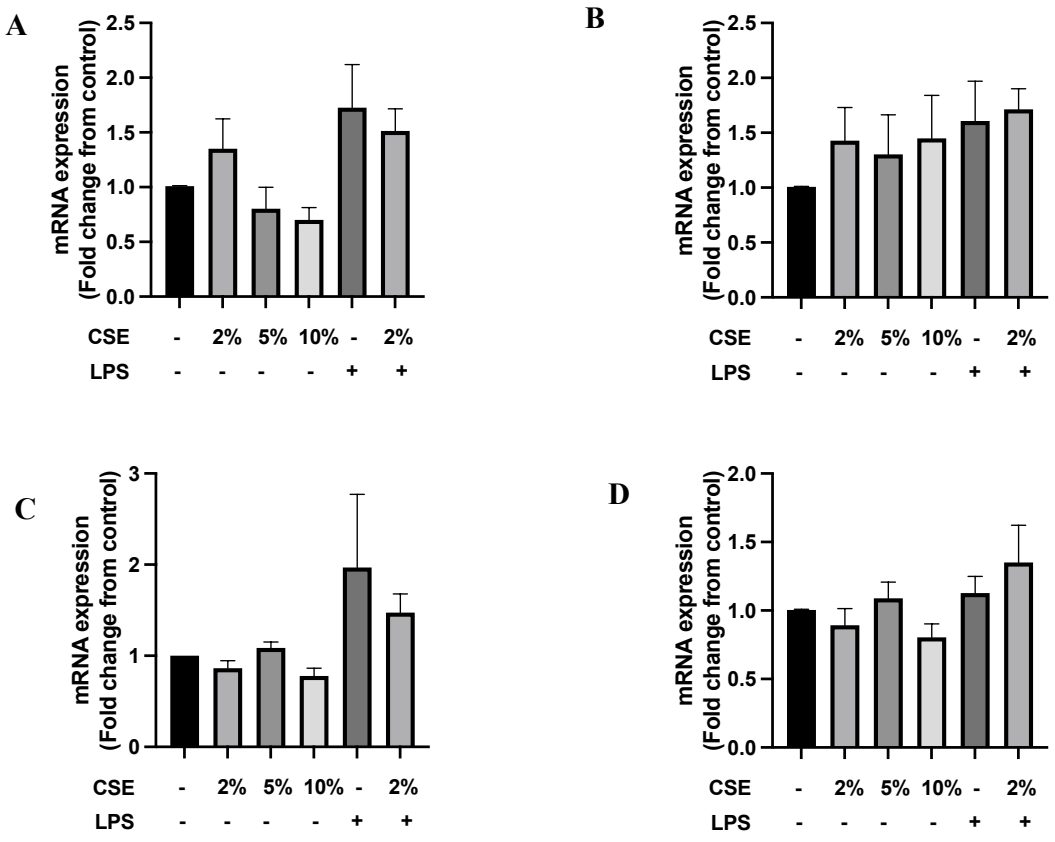


Figure 4. mRNA levels of inflammatory biomarkers IL-6(A), IL-8(B), S100A8(C), S100A9(D) following exposure of NHBE cells to CSE for 2h and then coculturing them with HMVEC-L cells for 24h

No significant increase in the expression of IL-6, IL-8, S100A8 or S100A9 was observed in NHBE cells in response to 2, 5, 10% CSE, LPS and LPS +2% CSE. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 2h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-coculture for 24h.

IL-6, IL-8, S100A8 and S100A9 mRNA expression was measured by qRT-PCR. Gene expression was normalized to GAPDH**.** Results are expressed as the mean ± SEM of 4 independent experiments.

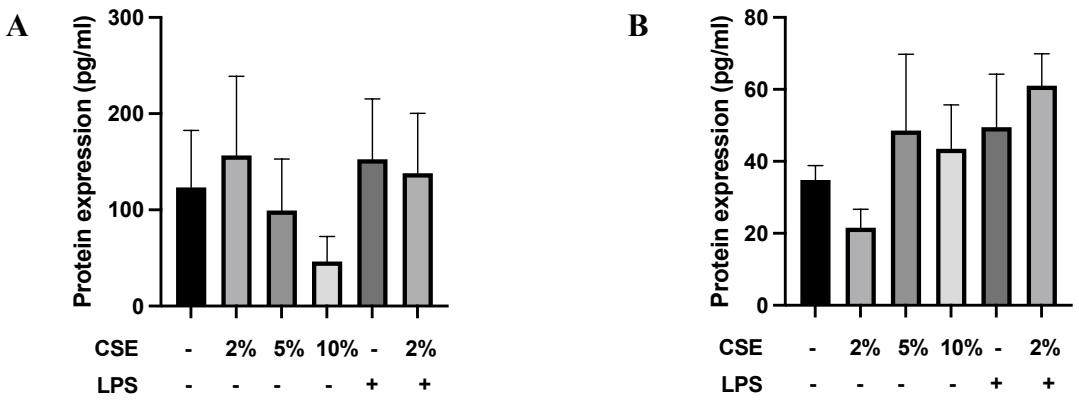


Figure 5. Protein levels of inflammatory biomarkers IL-6(A), and S100A8(B) following exposure of NHBE cells to CSE for 2h and then coculturing them with HMVEC-L cells for 24h

No significant increase in IL-6 and S100A8 protein levels was observed in NHBE cells in response to 2, 5, 10% CSE, LPS and LPS +2% CSE. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 2h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-coculture for 24h. Levels of IL-6 and S100A8 were then measured in the supernatant by ELISA. Results are expressed as the mean ± SEM of 4 independent experiments.

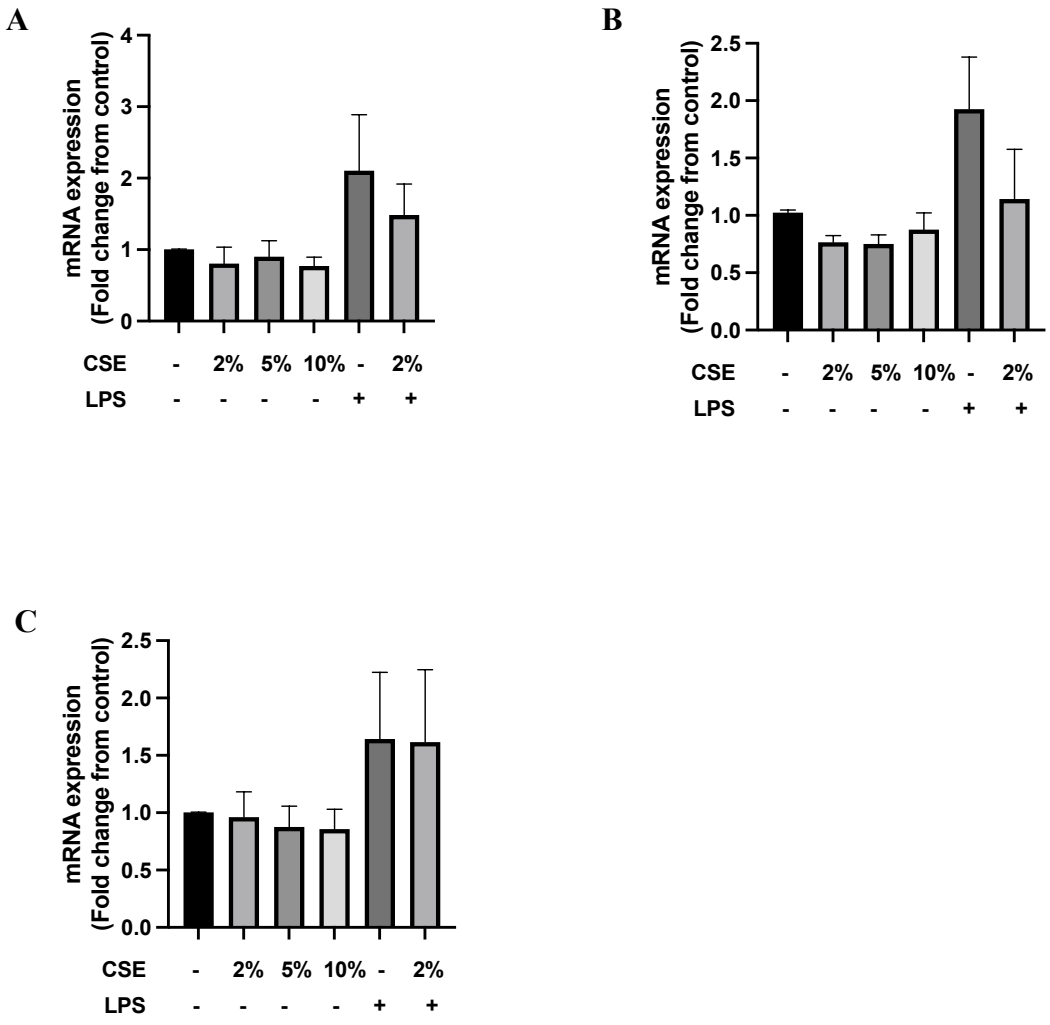


Figure 6. mRNA levels of inflammatory biomarker IL-6 (A) and adhesion molecules VCAM-1 (B) and E-selectin (C)

No significant increase was observed in the levels of IL-6, VCAM-1 or E-selectin when HMVEC-L cells were cocultured for 24h with NHBE cells that were exposed to different concentrations of CSE for 2h. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 2h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of

BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-coculture for 24h. IL-6, VCAM-1 and E-selectin mRNA expression was measured by qRT-PCR. Gene expression was normalized to GAPDH**.** Results are expressed as the mean ± SEM of 4 independent experiments.

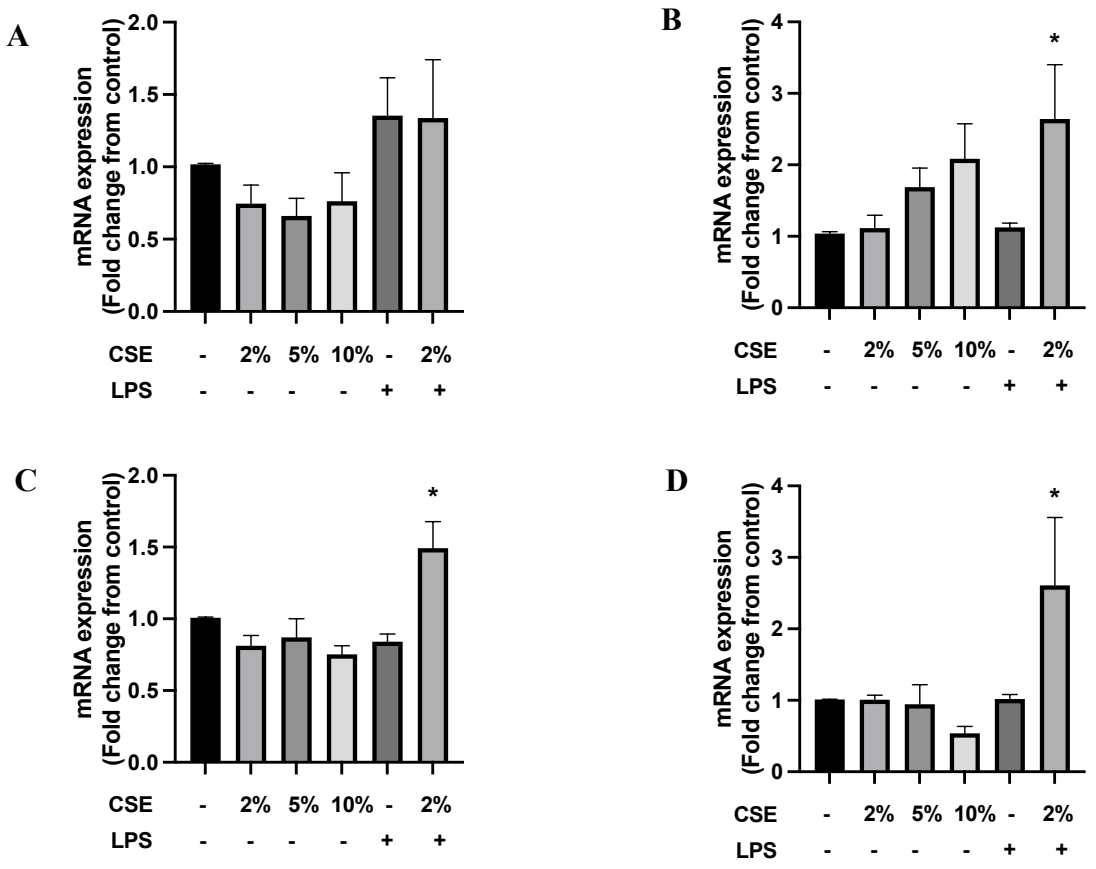


Figure 7. mRNA levels of inflammatory biomarkers IL-6(A), IL-8(B), S100A8(C), S100A9(D) following exposure of NHBE cells to CSE for 24h and then coculturing them with HMVEC-L cells for 24h

No significant increase in the expression of IL-6, IL-8, S100A8 or S100A9 was observed in NHBE cells in response to 2, 5, and 10% CSE. A significant increase was observed in the expression of IL-8, S100A8 and S100A9 when NHBE cells were exposed to 2% CSE as well as LPS. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 24h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-

coculture for 24h. IL-6, IL-8, S100A8 and S100A9 mRNA expression was measured by qRT-PCR. Gene expression was normalized to GAPDH**.** Results are expressed as the mean ± SEM of 4 independent experiments. *$p < 0.05$

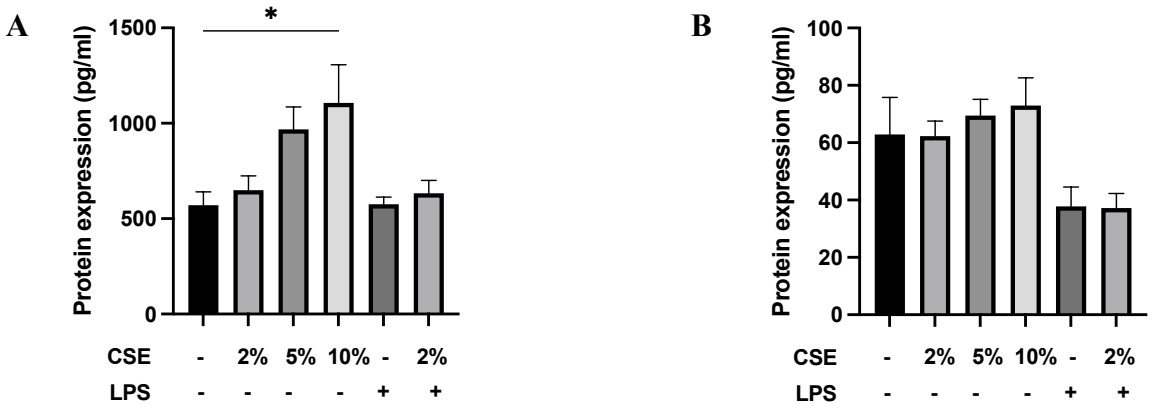


Figure 8. Protein levels of inflammatory biomarkers IL-6 (A), and S100A8 (B) following exposure of NHBE cells to CSE for 24h and then coculturing them with HMVEC-L cells for 24h

IL-6 levels were significantly increased in NHBE cells following exposure to 10% CSE. No significant increase was observed in S100A8 protein levels. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 24h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-coculture for 24h. Levels of IL-6 and S100A8 were then measured in the supernatant by ELISA. Results are expressed as the mean ± SEM of 4 independent experiments. *$p < 0.05$

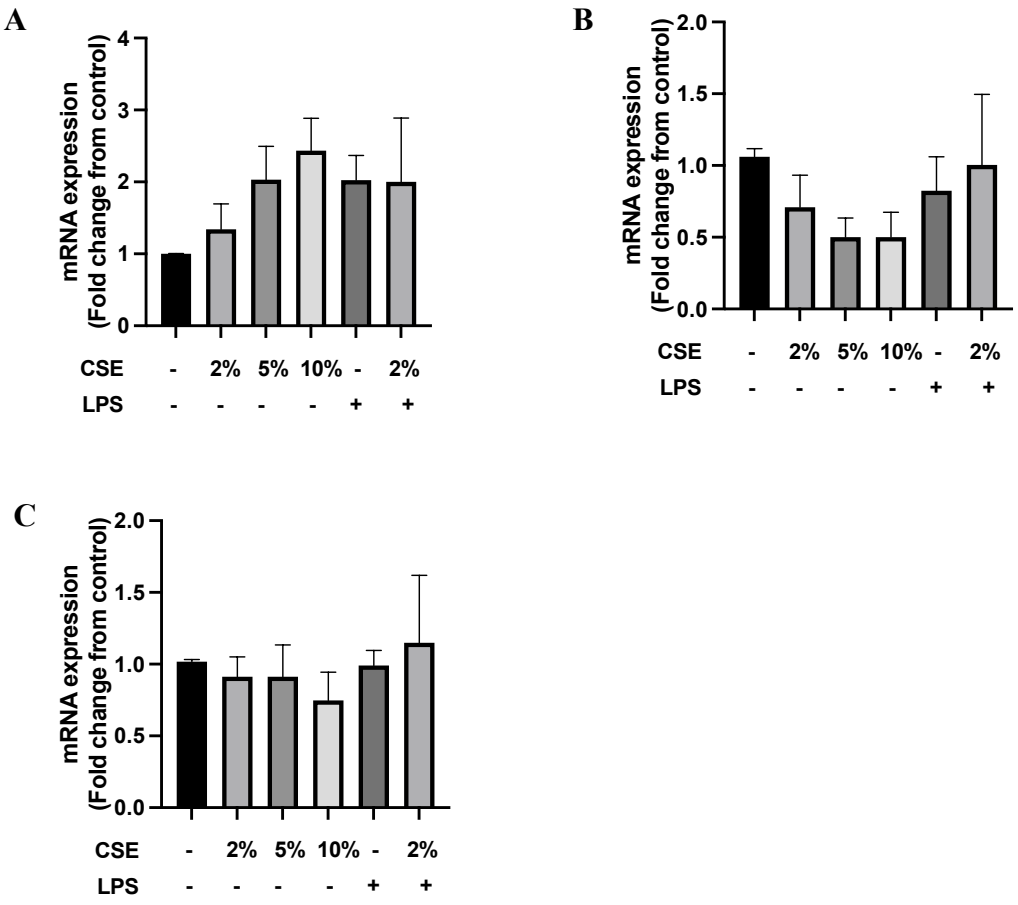


Figure 9. mRNA levels of inflammatory biomarker IL-6 (A) and adhesion molecules VCAM-1 (B) and E-selectin(C).

No significant increase was observed in the levels of IL-6, VCAM-1 or E-selectin when HMVEC-L cells were cocultured for 24h with NHBE cells that were exposed to different concentrations of CSE for 24h. NHBE cells cultured in the apical side of a transwell plate were exposed to 2% CSE, 5% CSE, 10% CSE as well as LPS and LPS+ 2% CSE for 24h. Following the exposure, the stimulus was stopped by removing the media and adding fresh 500 µl of BEBM to the NHBE cells. The insert is then moved to a 12 well plate where HMVEC-L were seeded. The cells are placed in co-coculture for 24h. IL-6, VCAM-1 and E-selectin mRNA

expression was measured by qRT-PCR. Gene expression was normalized to GAPDH**.** Results are expressed as the mean ± SEM of 4 independent experiments.

7.10 Tables

Table 1. Primer sequences used for qRT-PCR analysis

Gene	Forward Primer Sequence	Reverse Primer Sequence
GAPDH	AGC AAT GCC TCC TGC ACC ACC	CCG GAG GGG CCA TCC ACA GTC
IL-6	GTG TGA AAG CAG CAA AGA GG	TGC AGG AAC TGG ATC AGG
IL-8	GTG CAG TTT TGC CAA GGA GT	CTC TGC ACC CAG TTT TCC TT
S100A8	TCA GGA AAA AGG GTG CAG AC	ACG CCC ATC TTT ATC ACC AG
S100A9	AAA GAG CTG GTG CGA AAA GA	TCA GCT GCT TGT CTG CAT TT
VCAM-1	CATTGACTTGCAGCACCACA	GATGTGGTCCCCTCATTCGT
E-Selectin	TGTTTGGCACTGTGTGCAAG	TGGACTCAGTGGGAGCTTCA

qRT-PCR: Quantitative Reverse Transcription- Polymerase chain reaction; GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase; IL6: Interleukin-6; IL8: Interleukin-8; Vascular cell adhesion molecule-1 (VCAM-1)

Chapter 8: Summary of thesis findings, general discussion and overall conclusions

8.1 Summary of findings

This thesis examined the characteristics of comorbid COPD and CHF in a population sample and a clinical sample. It also examined the interaction and response of different lung cells following exposure to cigarette smoke; the main risk factor for COPD and CHF.

When considering the comorbidity of COPD and CHF in a large population-based multi-ethnic cohort MESA, several key findings were reported. As MESA is not a respiratory cohort, two definitions were used to define airflow obstruction: CLRD definition was used in the main cohort and defined as pre-BD FEV1/FVC ratio <0.7 and COPD definition was used in the subcohort and defined as post-BD FEV1/FVC ratio <0.7. As this is a population-based sample, and to capture early disease, a validated score that relies on clinical characteristics and echocardiography was used to define early HFpEF. First, it was found that in subjects sampled from the general population, there was a prevalence of 13.3% of undiagnosed HF in participants with CLRD, and a very similar prevalence of 12.7% in participants with COPD. In the main cohort, subjects in the CLRD with HF were significantly older and had a higher BMI and comorbidities including atrial fibrillation and obesity when compared to all the other groups including the CLRD without HF. Participants with CLRD and HF had lower pre-bronchodilator FEV1, lower pre-bronchodilator FVC values and lower pre-bronchodilator FEV1/FVC ratio when compared to those with only CLRD. Similar results were observed in the COPD subcohort. Percent emphysema and levels of NT-proBNP were not significantly different between subjects with CLRD/COPD with and those without HF. No significant association was demonstrated between percent emphysema, levels of NT-proBNP and severity of airflow obstruction and

having CLRD/COPD with HF when CLRD/COPD without HF was used as reference after fully adjusting for age, sex, race or ethnic group and smoking status as well as HF risk factors (hypertension, heart attack, obesity and diabetes) and minimally adjusting for age, sex, race or ethnic group, BMI and smoking status.

Even though we couldn't determine specific biomarkers (lung function or lung density) or blood biomarkers as prognostic biomarkers that could be used to distinguish COPD patient with CHF from those without CHF, we were still able to show the significantly high prevalence of CHF in COPD even in early undiagnosed disease. Both COPD and CHF represent important populations as they include individuals who are most likely to benefit from early intervention in order to prevent adverse events associated with either disease. This may have implication in primary care practice where these individuals are more likely to be seen. Although we have not found specific biomarkers to discriminate COPD patient with and without CHF, the results of this study should at least increase awareness about the diagnostic gap.

As MESA is a population-based cohort, data from this population captures early disease and early markers. To address the differences between early disease and diagnosed with more advanced disease, a clinical sample of COPD patients from a COPD specialized clinic at the Montreal Chest Institute of the McGill University Health Centre was selected. COPD diagnosis was already known as these patients were already followed up in the COPD clinic and had a diagnosis by spirometry with a post-bronchodilator FEV1/FVC <0.7. At baseline, each patient underwent a detailed and standardized cardiopulmonary evaluation that included: complete medical history and physical exam with special attention to signs and symptoms of COPD and CHF, clinical questionnaires (CAT, SF-36, mMRC), ECG, chest CT scan, complete pulmonary function test, echocardiogram and blood samples for measurements of serum biomarkers.

Several key findings were reported: in a specialized pulmonary COPD clinic, unrecognized CHF in COPD was still very common (prevalence 29.6%). Patients with COPD and a co-morbidity of CHF exhibited distinct characteristics: they were older, male and heavier smokers, most likely to have exacerbations in the past year and other cardiovascular comorbidities such as heart disease including coronary artery disease and arrythmias, hypertension and diabetes. In the 16 patients recognized as having abnormal echocardiography that qualify as HF, 6 were classified as having HFrEF (37.5%) and 10 were classified as having HFpEF (62.5%). These distinct features observed were more obvious in COPD with HFrEF compared to those with HFpEF as COPD patients with HFrEF tend to have more exacerbations that were complicated with a hospital admission and to be more symptomatic with a higher MRC and CAT score. In terms of blood biomarkers, troponin and eosinophils levels were higher in patients with COPD with HFrEF. In terms of association of biomarkers and adverse events, CAT score >10 was associated with a higher occurrence of exacerbations, and mMRC ≥ 2 and CAT ≥ 10 was associated with a significant increase in exacerbation rate. The study demonstrated that a high prevalence of undiagnosed CHF was present in COPD from a specialized COPD clinic. This finding is worrying since lack of optimal treatment for HF can negatively affect patient outcomes. Based on our study findings we cannot recommend using blood biomarkers, lung function and CT scan to discriminate stable COPD with HF from those without HF. However, clinician should be aware that there is still a large proportion of patient with undiagnosed HF and some clinical characteristics (male and heavy smoker, previous exacerbations and/or CVD), could help targeting stable COPD patients who are more likely to have concomitant HF, particularly those having COPD with HFrEF.

As cigarette smoking is the most important risk factor for the development of COPD and the inflammatory state in COPD is not confined to the lungs, a translational project was conducted to assess the effect of cigarette smoke on the interaction of different lung cells. NHBE cells were first exposed to CSE and then cocultured with HMVEC-L cells. The key finding of this study was the characterization of the expression of the novel inflammatory biomarkers alarmins. Cigarette smoke induced the expression of inflammatory markers including alarmins S100A8 and S100A9. Using a coculture model that consisted of NHBE cells and HMVEC-L, we showed that factors released from smoke-exposed lung epithelial cells do not activate lung endothelial cells.

8.2 Strengths and limitations

The three projects constituting this thesis had several strengths and limitations.

Study 1

To our knowledge, our analysis using MESA is the first study to investigate COPD with and without HF that has recruited its participants from the general population rather than more convenient sampling in clinical settings. The greatest strength of the MESA study is that it's a large community-based multi-ethnic cohort, it reflects events that are occurring in the population at large that have significant impacts on the health and wellbeing of people. Population-based sampling can better mirror prevalent COPD and HF populations at large. This population is more likely to reflect individual seen in primary care practice. The cohort also offers a good representation as it consists of men and women in almost equal proportion and individuals from different ethnic backgrounds. The cohort allows the detection and the characterization of early stages of disease. Along with respiratory questionnaires and spirometry tests, MESA has collected blood samples to allow studying biomarkers and genetic risks, and has also performed

CT scans to derive emphysema levels. A unique aspect of MESA exam 6 is that it has data from diagnostic tests for COPD as well as data on diagnostic tests for HF which allowed us to use the diagnostics test to classify participants as CLRD/COPD and HF which eliminated misclassification bias.

The limitations of MESA are that this cohort is not a COPD cohort and only a small proportion of the participants had post-bronchodilator spirometry performed. To address this limitation, airflow obstruction was defined using two definitions: CLRD applied to pre-bronchodilator values and COPD applied to post-bronchodilator values. Similarity in the results between both definitions was then confirmed. The limited sample size could pose a challenge for certain analyses. MESA is also a cohort that involves mostly mild COPD and we do not expect to see a large impact on the heart. Finally, while longitudinal data collection is ongoing, with the current status of the data collection, the long-term follow-up time will be of value for future analyses.

Study 2

The greatest strength of the clinical study is the extensive comprehensive cardiopulmonary evaluation that was performed on each patient to establish the diagnosis of CHF in COPD patients; which is something that has not been done in many other studies. This helped eliminate work-up bias and establish a true diagnosis. Another strength of the study is that the sample of COPD patients selected were representative of the COPD clinic: even though patients from the COPD clinic had more severe COPD disease than the study sample, when the comparison was made with respect to echocardiogram variables, no statistically significant differences could be demonstrated. The cohort also consists of men and women in almost equal proportion and contains patients with different levels in terms of the spectrum of disease severity. Another strength of the study is having a longitudinal 1-year follow-up with phone follow-ups every 3

months on every patient, this provides accurate and robust data on COPD exacerbation-like events and cardiovascular adverse events both moderate and severe.

The main limitation of the clinical study is the sample size. However, this will be mitigated as the project is still ongoing. One hundred COPD patients will be recruited for the project and all of them would complete an echocardiogram by the end of the summer, July-August 2022.

Study 3

The greatest strength of the translational project is the use of primary cells as they are more reflective of the *in vivo* environment of the lungs. The co-culture model we chose in this study allows the cell-cell interactions between lung epithelial and endothelial cells, this helped us understand how cigarette smoke could potentially affect this cross-talk.

The main limitation to the experimental design is the use of submerged cultures of epithelial cells instead of Air-liquid interface (ALI) cultures. ALI cultures of airway epithelial cells form a polarized, pseudostratified epithelium composed of ciliated and mucus-secreting cells. Therefore, the response we observed in airway epithelial cells in response to cigarette smoke does not necessarily mimic real response in the human body.

8.3 Overall conclusion

In conclusion, this thesis explored the characteristics of cardiac comorbidity in COPD, more specifically heart failure. It also explored the potential mechanisms that could be leading to cardiovascular comorbidity in COPD by using an *in vitro* model of cigarette smoke exposure.

In the population-based cohort MESA, a high prevalence of CHF in COPD was observed in early undiagnosed disease. Even though no specific lung function, lung structure or blood biomarkers were able to differentiate between COPD patient with HF from those without HF, specific

clinical characteristics were identified with some differences between the two groups. Future studies should be carried out in order to allow the continued follow-up of the participants with early features of COPD and those with features of COPD and CHF to track the development of disease from subclinical to clinic and to identify adverse events associated with the presence and progression of both diseases.

In the clinical sample of COPD patients from a COPD specialized clinic, a high prevalence of undiagnosed CHF was shown. The prevalence of undiagnosed CHF in the clinical sample was higher than the prevalence in the previously described population sample because subjects in the population have mostly mild COPD and we do not expect to see a large impact on the heart. Patients from the clinical sample have more severe COPD, are heavier smokers and have significantly lower lung function. We were able to identify some clinical characteristics that could help clinicians targeting stable COPD patients who are more likely to have concomitant CHF, particularly those with HFrEF. However, the majority of blood biomarkers quantified, lung function or CT scan abnormalities cannot be used to discriminate stable COPD without or with CHF. Our study provides evidence in favor of actively screening COPD patients with specific characteristics for CHF comorbidities. Clinicians should be aware of the diagnostic gap and that there is still a large proportion of patients with undiagnosed CHF. Clinicians should be targeting this subgroup of stable COPD patients with those specific characteristics as they are more likely to have concomitant CHF and actively screening them with an echocardiogram. Special attention should be given to recognize COPD patients with HFrEF as they have more risk of having an exacerbation.

Future studies with a similar design should be carried out to identify prognostic biomarkers that could be used to distinguish COPD patient with CHF from those without CHF.

In an *in vitro* model of cigarette smoke exposure, we were able to observe the effect of cigarette smoke on specific cells in the lungs. Cigarette smoke significantly increases the expression of inflammatory biomarkers including alarmins in lung epithelial cells. We considered the lung epithelium as the source of activation of lung endothelial cells when exposed to cigarette smoke as stimulus. However, we did not observe an activation of the endothelial cells. Future studies should be carried out to assess the function of adhesion molecules in endothelial cells following their exposure to smoke-exposed lung epithelial cells and assess adherence of monocytes to the endothelium. Further studies are also needed to assess the mechanism by which alarmins are increased in the lung epithelium and how inhibition of alarmins secretion from lung epithelial cells can affect the activation of lung endothelial cells and subsequently inflammation in the lungs. Elucidating the mechanisms by which lung epithelial and endothelial cells interact undert cigarette smoke conditions can help target the inflammation that leads to the association between COPD and CHF.

The overall course and burden of COPD can be aggravated by comorbidities manifesting in various systems from the lungs to those that are extra-pulmonary. Cardiovascular comorbidities, more specifically heart failure, negatively impacts patient outcomes. Diagnosing CHF in COPD patients is complicated by overlap in signs and symptoms, and diminished diagnostic value of additional investigations.

Successfully targeting COPD patients that are at risk for developing CHF could result in decreased morbidity, mortality, better quality of life for patients, as well as significantly fewer healthcare expenditures. Thus, it is important to consider the clinical translation of these findings. By identifying susceptible COPD patients using the clinical characteristics that were identified in this thesis and implementing evaluation and treatment of early cardiac dysfunction,

it may be possible to slow disease progression and reduce the likelihood of cardiac adverse events and mortality. Patients with COPD and suspected HF based on those characteristics must be considered to have left ventricular dysfunction until proven otherwise.

Further research will provide new information that may help identify new prognostic biomarkers that could be used to distinguish COPD patient with CHF from those without CHF. This would be highly useful in subjects in the early stages of disease who may benefit most from early diagnosis of CHF and preventive interventions.

Chapter 9: Reference list

1. Halbert, R.J., et al., *Global burden of COPD: systematic review and meta-analysis.* Eur Respir J, 2006. **28**(3): p. 523-32.
2. Kemp, S.V., M.I. Polkey, and P.L. Shah, *The epidemiology, etiology, clinical features, and natural history of emphysema.* Thorac Surg Clin, 2009. **19**(2): p. 149-58.
3. Ebert, R.V. and M.J. Terracio, *The bronchiolar epithelium in cigarette smokers. Observations with the scanning electron microscope.* Am Rev Respir Dis, 1975. **111**(1): p. 4-11.
4. Verra, F., et al., *Ciliary abnormalities in bronchial epithelium of smokers, ex-smokers, and nonsmokers.* Am J Respir Crit Care Med, 1995. **151**(3 Pt 1): p. 630-4.
5. Divo, M.J., et al., *Chronic Obstructive Pulmonary Disease (COPD) as a disease of early aging: Evidence from the EpiChron Cohort.* PLoS One, 2018. **13**(2): p. e0193143.
6. Guarascio, A.J., et al., *The clinical and economic burden of chronic obstructive pulmonary disease in the USA.* Clinicoecon Outcomes Res, 2013. **5**: p. 235-45.
7. Kessler, R., et al., *Symptom variability in patients with severe COPD: a pan-European cross-sectional study.* Eur Respir J, 2011. **37**(2): p. 264-72.
8. Miravitlles, M., et al., *Observational study to characterise 24-hour COPD symptoms and their relationship with patient-reported outcomes: results from the ASSESS study.* Respir Res, 2014. **15**(1): p. 122.
9. Miller, M.R., et al., *Standardisation of spirometry.* Eur Respir J, 2005. **26**(2): p. 319-38.
10. Fletcher, C., *Standardized Questionaries on Respiratory Symptoms.* BMJ 1960.
11. Jones, P.W., et al., *Development and first validation of the COPD Assessment Test.* Eur Respir J, 2009. **34**(3): p. 648-54.
12. Jones, P.W., M. Tabberer, and W.H. Chen, *Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores.* BMC Pulm Med, 2011. **11**: p. 42.
13. Vogelmeier, C.F., et al., *Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report. GOLD Executive Summary.* Am J Respir Crit Care Med, 2017. **195**(5): p. 557-582.
14. Lamprecht, B., et al., *COPD in never smokers: results from the population-based burden of obstructive lung disease study.* Chest, 2011. **139**(4): p. 752-763.
15. Thomsen, M., et al., *Characteristics and outcomes of chronic obstructive pulmonary disease in never smokers in Denmark: a prospective population study.* Lancet Respir Med, 2013. **1**(7): p. 543-50.
16. She, J., et al., *Chinese water-pipe smoking and the risk of COPD.* Chest, 2014. **146**(4): p. 924-931.
17. Tan, W.C., et al., *Marijuana and chronic obstructive lung disease: a population-based study.* Cmaj, 2009. **180**(8): p. 814-20.
18. Mercado, N., K. Ito, and P.J. Barnes, *Accelerated ageing of the lung in COPD: new concepts.* Thorax, 2015. **70**(5): p. 482-9.
19. Landis, S.H., et al., *Continuing to Confront COPD International Patient Survey: methods, COPD prevalence, and disease burden in 2012-2013.* Int J Chron Obstruct Pulmon Dis, 2014. **9**: p. 597-611.

20. Leung, C., et al., *The Prevalence of Chronic Obstructive Pulmonary Disease (COPD) and the Heterogeneity of Risk Factors in the Canadian Population: Results from the Canadian Obstructive Lung Disease (COLD) Study.* Int J Chron Obstruct Pulmon Dis, 2021. **16**: p. 305-320.
21. Tan, W.C., et al., *Characteristics of COPD in never-smokers and ever-smokers in the general population: results from the CanCOLD study.* Thorax, 2015. **70**(9): p. 822-9.
22. de Marco, R., et al., *Risk factors for chronic obstructive pulmonary disease in a European cohort of young adults.* Am J Respir Crit Care Med, 2011. **183**(7): p. 891-7.
23. Lange, P., et al., *Lung-Function Trajectories Leading to Chronic Obstructive Pulmonary Disease.* N Engl J Med, 2015. **373**(2): p. 111-22.
24. Lawlor, D.A., S. Ebrahim, and G. Davey Smith, *Association of birth weight with adult lung function: findings from the British Women's Heart and Health Study and a meta-analysis.* Thorax, 2005. **60**(10): p. 851-8.
25. Stoller, J.K. and L.S. Aboussouan, *Alpha1-antitrypsin deficiency.* Lancet, 2005. **365**(9478): p. 2225-36.
26. Marchetti, N., et al., *Association between occupational exposure and lung function, respiratory symptoms, and high-resolution computed tomography imaging in COPDGene.* Am J Respir Crit Care Med, 2014. **190**(7): p. 756-62.
27. Gershon, A.S., et al., *Lifetime risk of developing chronic obstructive pulmonary disease: a longitudinal population study.* Lancet, 2011. **378**(9795): p. 991-6.
28. Miller, J., et al., *Comorbidity, systemic inflammation and outcomes in the ECLIPSE cohort.* Respir Med, 2013. **107**(9): p. 1376-84.
29. Sin, D.D., et al., *Mortality in COPD: Role of comorbidities.* Eur Respir J, 2006. **28**(6): p. 1245-57.
30. Hillas, G., et al., *Managing comorbidities in COPD.* Int J Chron Obstruct Pulmon Dis, 2015. **10**: p. 95-109.
31. Smith, M.C. and J.P. Wrobel, *Epidemiology and clinical impact of major comorbidities in patients with COPD.* Int J Chron Obstruct Pulmon Dis, 2014. **9**: p. 871-88.
32. Yin, H.L., et al., *Prevalence of comorbidities in chronic obstructive pulmonary disease patients: A meta-analysis.* Medicine (Baltimore), 2017. **96**(19): p. e6836.
33. Kiri, V.A., et al., *Recent trends in lung cancer and its association with COPD: an analysis using the UK GP Research Database.* Prim Care Respir J, 2010. **19**(1): p. 57-61.
34. Diaz-Guzman, E., M. Khosravi, and D.M. Mannino, *Asthma, chronic obstructive pulmonary disease, and mortality in the U.S. population.* Copd, 2011. **8**(6): p. 400-7.
35. Cosentino, J., et al., *Analysis of Asthma-Chronic Obstructive Pulmonary Disease Overlap Syndrome Defined on the Basis of Bronchodilator Response and Degree of Emphysema.* Ann Am Thorac Soc, 2016. **13**(9): p. 1483-9.
36. Hardin, M., et al., *The clinical features of the overlap between COPD and asthma.* Respir Res, 2011. **12**(1): p. 127.
37. Menezes, A.M.B., et al., *Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma.* Chest, 2014. **145**(2): p. 297-304.
38. Gerhardsson de Verdier, M., et al., *Asthma and Chronic Obstructive Pulmonary Disease Overlap Syndrome: Doubled Costs Compared with Patients with Asthma Alone.* Value Health, 2015. **18**(6): p. 759-66.

39. van Boven, J.F., et al., *Comorbidome, Pattern, and Impact of Asthma-COPD Overlap Syndrome in Real Life.* Chest, 2016. **149**(4): p. 1011-20.
40. Putcha, N., et al., *Impact of co-morbidities on self-rated health in self-reported COPD: an analysis of NHANES 2001-2008.* Copd, 2013. **10**(3): p. 324-32.
41. Mannino, D.M., et al., *Prevalence and outcomes of diabetes, hypertension and cardiovascular disease in COPD.* Eur Respir J, 2008. **32**(4): p. 962-9.
42. Westerik, J.A., et al., *Associations between chronic comorbidity and exacerbation risk in primary care patients with COPD.* Respir Res, 2017. **18**(1): p. 31.
43. Divo, M., et al., *Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2012. **186**(2): p. 155-61.
44. Buch, P., et al., *Reduced lung function and risk of atrial fibrillation in the Copenhagen City Heart Study.* Eur Respir J, 2003. **21**(6): p. 1012-6.
45. Terzano, C., et al., *Atrial fibrillation in the acute, hypercapnic exacerbations of COPD.* Eur Rev Med Pharmacol Sci, 2014. **18**(19): p. 2908-17.
46. Mapel, D.W., D. Dedrick, and K. Davis, *Trends and cardiovascular co-morbidities of COPD patients in the Veterans Administration Medical System, 1991-1999.* Copd, 2005. **2**(1): p. 35-41.
47. Curkendall, S.M., et al., *Cardiovascular disease in patients with chronic obstructive pulmonary disease, Saskatchewan Canada cardiovascular disease in COPD patients.* Ann Epidemiol, 2006. **16**(1): p. 63-70.
48. Finkelstein, J., E. Cha, and S.M. Scharf, *Chronic obstructive pulmonary disease as an independent risk factor for cardiovascular morbidity.* Int J Chron Obstruct Pulmon Dis, 2009. **4**: p. 337-49.
49. McAllister, D.A., et al., *Diagnosis of myocardial infarction following hospitalisation for exacerbation of COPD.* Eur Respir J, 2012. **39**(5): p. 1097-103.
50. Donaldson, G.C., et al., *Increased risk of myocardial infarction and stroke following exacerbation of COPD.* Chest, 2010. **137**(5): p. 1091-7.
51. Kunisaki, K.M., et al., *Exacerbations of Chronic Obstructive Pulmonary Disease and Cardiac Events. A Post Hoc Cohort Analysis from the SUMMIT Randomized Clinical Trial.* Am J Respir Crit Care Med, 2018. **198**(1): p. 51-57.
52. Dransfield, M.T., et al., *β-Blocker Therapy and Clinical Outcomes in Patients with Moderate Chronic Obstructive Pulmonary Disease and Heightened Cardiovascular Risk. An Observational Substudy of SUMMIT.* Ann Am Thorac Soc, 2018. **15**(5): p. 608-614.
53. Lipworth, B., et al., *Beta-blockers in COPD: time for reappraisal.* Eur Respir J, 2016. **48**(3): p. 880-8.
54. Vestbo, J., et al., *Fluticasone furoate and vilanterol and survival in chronic obstructive pulmonary disease with heightened cardiovascular risk (SUMMIT): a double-blind randomised controlled trial.* Lancet, 2016. **387**(10030): p. 1817-26.
55. de Lucas-Ramos, P., et al., *Chronic obstructive pulmonary disease as a cardiovascular risk factor. Results of a case-control study (CONSISTE study).* Int J Chron Obstruct Pulmon Dis, 2012. **7**: p. 679-86.
56. Rutten, F.H., et al., *Heart failure and chronic obstructive pulmonary disease: An ignored combination?* Eur J Heart Fail, 2006. **8**(7): p. 706-11.

57. Bhowmik, A., et al., *Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations.* Thorax, 2000. **55**(2): p. 114-20.
58. Bucchioni, E., et al., *High levels of interleukin-6 in the exhaled breath condensate of patients with COPD.* Respir Med, 2003. **97**(12): p. 1299-302.
59. Crisan, L., et al., *Karma of Cardiovascular Disease Risk Factors for Prevention and Management of Major Cardiovascular Events in the Context of Acute Exacerbations of Chronic Obstructive Pulmonary Disease.* Front Cardiovasc Med, 2019. **6**: p. 79.
60. Watz, H., et al., *Decreasing cardiac chamber sizes and associated heart dysfunction in COPD: role of hyperinflation.* Chest, 2010. **138**(1): p. 32-8.
61. Barr, R.G., et al., *Percent emphysema, airflow obstruction, and impaired left ventricular filling.* N Engl J Med, 2010. **362**(3): p. 217-27.
62. Zangiabadi, A., C.G. De Pasquale, and D. Sajkov, *Pulmonary hypertension and right heart dysfunction in chronic lung disease.* Biomed Res Int, 2014. **2014**: p. 739674.
63. Louie, E.K., et al., *Doppler echocardiographic demonstration of the differential effects of right ventricular pressure and volume overload on left ventricular geometry and filling.* J Am Coll Cardiol, 1992. **19**(1): p. 84-90.
64. Sidney, S., et al., *COPD and incident cardiovascular disease hospitalizations and mortality: Kaiser Permanente Medical Care Program.* Chest, 2005. **128**(4): p. 2068-75.
65. Staszewsky, L., et al., *Clinical, neurohormonal, and inflammatory markers and overall prognostic role of chronic obstructive pulmonary disease in patients with heart failure: data from the Val-HeFT heart failure trial.* J Card Fail, 2007. **13**(10): p. 797-804.
66. Rutten, F.H., et al., *Recognising heart failure in elderly patients with stable chronic obstructive pulmonary disease in primary care: cross sectional diagnostic study.* Bmj, 2005. **331**(7529): p. 1379.
67. Rutten, F.H., et al., *Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease.* Eur Heart J, 2005. **26**(18): p. 1887-94.
68. Padeletti, M., S. Jelic, and T.H. LeJemtel, *Coexistent chronic obstructive pulmonary disease and heart failure in the elderly.* Int J Cardiol, 2008. **125**(2): p. 209-15.
69. Mascarenhas, J., A. Azevedo, and P. Bettencourt, *Coexisting chronic obstructive pulmonary disease and heart failure: implications for treatment, course and mortality.* Curr Opin Pulm Med, 2010. **16**(2): p. 106-11.
70. Hunt, S.A., *ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure).* J Am Coll Cardiol, 2005. **46**(6): p. e1-82.
71. Ponikowski, P., et al., *2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC.* Eur J Heart Fail, 2016. **18**(8): p. 891-975.
72. Braunwald, E., *Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities.* N Engl J Med, 1997. **337**(19): p. 1360-9.

73. Wang, T.J., et al., *Natural history of asymptomatic left ventricular systolic dysfunction in the community.* Circulation, 2003. **108**(8): p. 977-82.
74. Yusuf, S., et al., *Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions.* N Engl J Med, 1992. **327**(10): p. 685-91.
75. *Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017.* Lancet, 2018. **392**(10159): p. 1789-1858.
76. Bursi, F., et al., *Systolic and diastolic heart failure in the community.* Jama, 2006. **296**(18): p. 2209-16.
77. Benjamin, E.J., et al., *Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association.* Circulation, 2018. **137**(12): p. e67-e492.
78. Smeets, M., et al., *Burden of heart failure in Flemish general practices: a registry-based study in the Intego database.* BMJ Open, 2019. **9**(1): p. e022972.
79. van Riet, E.E., et al., *Epidemiology of heart failure: the prevalence of heart failure and ventricular dysfunction in older adults over time. A systematic review.* Eur J Heart Fail, 2016. **18**(3): p. 242-52.
80. Caruana, L., et al., *Do patients with suspected heart failure and preserved left ventricular systolic function suffer from "diastolic heart failure" or from misdiagnosis? A prospective descriptive study.* Bmj, 2000. **321**(7255): p. 215-8.
81. Hawkins, N.M., et al., *Heart failure and chronic obstructive pulmonary disease: diagnostic pitfalls and epidemiology.* Eur J Heart Fail, 2009. **11**(2): p. 130-9.
82. Roberts, E., et al., *The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting.* Bmj, 2015. **350**: p. h910.
83. Maisel, A., et al., *State of the art: using natriuretic peptide levels in clinical practice.* Eur J Heart Fail, 2008. **10**(9): p. 824-39.
84. Thomas, J.T., et al., *Utility of history, physical examination, electrocardiogram, and chest radiograph for differentiating normal from decreased systolic function in patients with heart failure.* Am J Med, 2002. **112**(6): p. 437-45.
85. Mant, J., et al., *Systematic review and individual patient data meta-analysis of diagnosis of heart failure, with modelling of implications of different diagnostic strategies in primary care.* Health Technol Assess, 2009. **13**(32): p. 1-207, iii.
86. Borlaug, B.A. and M.M. Redfield, *Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum.* Circulation, 2011. **123**(18): p. 2006-13; discussion 2014.
87. Niewoehner, D.E., J. Kleinerman, and D.B. Rice, *Pathologic changes in the peripheral airways of young cigarette smokers.* N Engl J Med, 1974. **291**(15): p. 755-8.
88. MacNee, W., *Pathogenesis of chronic obstructive pulmonary disease.* Proc Am Thorac Soc, 2005. **2**(4): p. 258-66; discussion 290-1.
89. Di Stefano, A., et al., *Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview.* Clin Exp Allergy, 2004. **34**(8): p. 1156-67.

90. Russell, R.E., et al., *Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease.* Am J Respir Cell Mol Biol, 2002. **26**(5): p. 602-9.
91. Di Stefano, A., et al., *Severity of airflow limitation is associated with severity of airway inflammation in smokers.* Am J Respir Crit Care Med, 1998. **158**(4): p. 1277-85.
92. Keatings, V.M., et al., *Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma.* Am J Respir Crit Care Med, 1996. **153**(2): p. 530-4.
93. Stănescu, D., et al., *Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils.* Thorax, 1996. **51**(3): p. 267-71.
94. Gao, W., et al., *Bronchial epithelial cells: The key effector cells in the pathogenesis of chronic obstructive pulmonary disease?* Respirology, 2015. **20**(5): p. 722-9.
95. Su, B., et al., *Inflammatory Markers and the Risk of Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(4): p. e0150586.
96. Duvoix, A., et al., *Blood fibrinogen as a biomarker of chronic obstructive pulmonary disease.* Thorax, 2013. **68**(7): p. 670-6.
97. Danesh, J., et al., *Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis.* Jama, 2005. **294**(14): p. 1799-809.
98. Hurst, J.R., et al., *Susceptibility to exacerbation in chronic obstructive pulmonary disease.* N Engl J Med, 2010. **363**(12): p. 1128-38.
99. Celli, B.R., et al., *Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease.* Am J Respir Crit Care Med, 2012. **185**(10): p. 1065-72.
100. Lomas, D.A., et al., *Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD.* Eur Respir J, 2009. **34**(1): p. 95-102.
101. Vestbo, J., et al., *Changes in forced expiratory volume in 1 second over time in COPD.* N Engl J Med, 2011. **365**(13): p. 1184-92.
102. Maisel, A.S., et al., *Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure.* N Engl J Med, 2002. **347**(3): p. 161-7.
103. Januzzi, J.L., et al., *NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study.* Eur Heart J, 2006. **27**(3): p. 330-7.
104. Doust, J.A., et al., *How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review.* Bmj, 2005. **330**(7492): p. 625.
105. Latini, R., et al., *Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure.* Circulation, 2007. **116**(11): p. 1242-9.
106. Anand, I.S., et al., *C-reactive protein in heart failure: prognostic value and the effect of valsartan.* Circulation, 2005. **112**(10): p. 1428-34.
107. Ridker, P.M., *C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus.* J Am Coll Cardiol, 2007. **49**(21): p. 2129-38.

108. Raymond, R.J., et al., *Elevated interleukin-6 levels in patients with asymptomatic left ventricular systolic dysfunction.* Am Heart J, 2001. **141**(3): p. 435-8.
109. Hill, J., et al., *Circulating surfactant protein-D and the risk of cardiovascular morbidity and mortality.* Eur Heart J, 2011. **32**(15): p. 1918-25.
110. Newton, K. and V.M. Dixit, *Signaling in innate immunity and inflammation.* Cold Spring Harb Perspect Biol, 2012. **4**(3).
111. Chan, J.K., et al., *Alarmins: awaiting a clinical response.* J Clin Invest, 2012. **122**(8): p. 2711-9.
112. Roth, J., et al., *MRP8 and MRP14, S-100-like proteins associated with myeloid differentiation, are translocated to plasma membrane and intermediate filaments in a calcium-dependent manner.* Blood, 1993. **82**(6): p. 1875-83.
113. Edgeworth, J., et al., *Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells.* J Biol Chem, 1991. **266**(12): p. 7706-13.
114. Rahimi, F., et al., *FGF-2, IL-1beta and TGF-beta regulate fibroblast expression of S100A8.* Febs j, 2005. **272**(11): p. 2811-27.
115. Xu, K., T. Yen, and C.L. Geczy, *Il-10 up-regulates macrophage expression of the S100 protein S100A8.* J Immunol, 2001. **166**(10): p. 6358-66.
116. Yen, T., et al., *Induction of the S100 chemotactic protein, CP-10, in murine microvascular endothelial cells by proinflammatory stimuli.* Blood, 1997. **90**(12): p. 4812-21.
117. Rashid, K., et al., *Lung cellular senescence is independent of aging in a mouse model of COPD/emphysema.* Sci Rep, 2018. **8**(1): p. 9023.
118. Pouwels, S.D., et al., Increased serum levels of LL37, HMGB1 and S100A9 during exacerbation in COPD patients. Eur Respir J, 2015. 45(5): p. 1482-5.
119. Wang, S., et al., S100A8/A9 in Inflammation. Front Immunol, 2018. 9: p. 1298.
120. Pouwels, S.D., et al., DAMPs activating innate and adaptive immune responses in COPD. Mucosal Immunol, 2014. 7(2): p. 215-26.
121. Morrow, D.A., et al., Myeloid-related protein 8/14 and the risk of cardiovascular death or myocardial infarction after an acute coronary syndrome in the Pravastatin or Atorvastatin Evaluation and Infection Therapy: Thrombolysis in Myocardial Infarction (PROVE IT-TIMI 22) trial. Am Heart J, 2008. 155(1): p. 49-55.
122. Ma, L.P., et al., S100A8/A9 complex as a new biomarker in prediction of mortality in elderly patients with severe heart failure. Int J Cardiol, 2012. 155(1): p. 26-32.
123. Schiopu, A. and O.S. Cotoi, S100A8 and S100A9: DAMPs at the crossroads between innate immunity, traditional risk factors, and cardiovascular disease. Mediators Inflamm, 2013. 2013: p. 828354.
124. Bhatt, S.P. and M.T. Dransfield, Chronic obstructive pulmonary disease and cardiovascular disease. Transl Res, 2013. 162(4): p. 237-51.
125. Basili, S., et al., Lipoprotein(a) serum levels in patients affected by chronic obstructive pulmonary disease. Atherosclerosis, 1999. 147(2): p. 249-52.
126. Maclay, J.D. and W. MacNee, Cardiovascular disease in COPD: mechanisms. Chest, 2013. 143(3): p. 798-807.
127. Szucs, B., et al., Molecular Characteristics and Treatment of Endothelial Dysfunction in Patients with COPD: A Review Article. Int J Mol Sci, 2019. 20(18).

128. Bonetti, P.O., L.O. Lerman, and A. Lerman, Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol, 2003. 23(2): p. 168-75.
129. Libby, P. and P. Theroux, Pathophysiology of coronary artery disease. Circulation, 2005. 111(25): p. 3481-8.
130. Hansson, G.K., Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med, 2005. 352(16): p. 1685-95.
131. Barnes, P.J. and B.R. Celli, Systemic manifestations and comorbidities of COPD. Eur Respir J, 2009. 33(5): p. 1165-85.
132. Brekke, P.H., et al., Troponin T elevation and long-term mortality after chronic obstructive pulmonary disease exacerbation. Eur Respir J, 2008. 31(3): p. 563-70.

Chapter 10: Supplementary material

10.1 Research Ethics Board (REB) approved study protocol for the COPD CHF clinical study

Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure

Principal investigators :
Dr. Jean Bourbeau (supervisor), MUHC Montreal Chest Institute, 1001 Boulevard Décarie, Montréal, QC, H4A 3J1
Dr. Michel White, Montreal Heart Institute, 5000 Rue Bélanger, Montréal, QC H1T 1C8

Collaborator :
Dr. Benjamin Smith RI-MUHC, 1001 Boulevard Décarie, Montréal, QC, H4A 3J1
Dr. Nadia Giannetti, MUHC Heart Failure clinic, 1001 Boulevard Décarie, Montréal, QC, H4A 3J1

Research team:
Raquel Farias PhD, research associate
Mira Abou Rjeili, PhD student

Broad goal

The broad goal of our research is to develop a strategy for early diagnosis of concomitant Chronic Obstructive Pulmonary Disease (COPD) and Chronic Heart Failure (CHF) in order to initiate prompt treatment to improve long term outcomes

Background

COPD and CHF are two highly prevalent conditions with a significant impact in the global burden of disease [1]. Although commonly studied as independent entities, both diseases are often concomitant; CHF is estimated to be present in 5 to 41% of patients with COPD and COPD is found in 10 to 40% of patients with CHF [2-4]. However, the majority of studies assessing the coexistence of COPD and CHF have been retrospective and lacked echocardiography and spirometry to confirm co morbid CHF or COPD, respectively [5, 6]. Furthermore, the landscape of CHF and COPD has changed significantly over the last decades. Patient populations are older and chronic co morbidities are frequent [2]. In addition a large proportion of patients with COPD with co morbid CHF syndrome now have preserved ejection fraction (pEF) [7]. However, the functional pulmonary abnormalities have not been well characterized in specialized HF clinics or in patients with HF-pEF. The presence of concomitant COPD and CHF is often overlooked. In primary care, two cross-sectional studies found that 20.5% of elderly patients with COPD have unrecognized CHF and that 27.6% of elderly CHF patients have undiagnosed COPD [8, 9]. In a cohort study involving tertiary care centers, it was estimated that 37.3% of CHF patients had spirometric of airway obstruction and that 17% of COPD patients had echocardiographic evidence

of left ventricular dysfunction [10]. Quite strikingly, only 6.5% of cardiologists and 12% of respirologists study had systematically evaluated their patients to confirm or rule out co morbid COPD or CHF prior to the latter study [10]. Results from studies assessing the coexistence of COPD and CHF highlight the importance of performing a comprehensive cardiopulmonary evaluation in every patient with a diagnosis of either condition. This includes chest imaging (x-ray, CT scan), electrocardiogram (EKG), echocardiogram and pulmonary function tests (PFTs)[3]. Unfortunately, this comprehensive evaluation is rarely performed in every day clinical practice. In an observational study assessing the prevalence of concomitant COPD and CHF in specialized cardiac and respiratory clinics, only 26.6% of COPD patients without confirmed CHF underwent diagnostic tests to rule out ventricular dysfunction and only 22.9% of CHF patients with no previous diagnosis of COPD underwent pulmonary function tests [11]. The coexistence of COPD and CHF highly impacts patient outcomes. Studies have consistently shown that COPD patients with concomitant CHF are less likely to receive β blockers, angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs)[12-15], even though these medications are safe and improve outcomes [16-18]. On the other hand, CHF patients with co morbid COPD are more frequently hospitalized and have increased mortality rates than those without COPD[12, 15]. In fact, COPD is a strong predictor of non-cardiovascular mortality in CHF [14, 19, 20]. Therefore, a prompt diagnosis and treatment of concomitant COPD and CHF could improve patient outcomes and reduce healthcare use. However, performing a comprehensive cardiopulmonary evaluation for all COPD and CHF patients could be costly and time consuming. A good alternative would be to screen patients at so that selected patients can be referred to a specialist for a more detailed assessment. In this regard, serum biomarkers could represent an attractive option for both, diagnostic and prognostic purposes [21]. Both, COPD and CHF are characterized by a chronic, sub-clinical pro-inflammatory state and several neuro-hormonal and trombo-inflammatory biomarkers have been characterized in both conditions [14, 22]. In terms of neuro-hormonal activation, Brain Natriuretic Peptide (BNP), its pro-hormone N-terminal (NT) proBNP are elevated in both COPD and CHF[14, 23]. When added to clinical information, NTproBNP levels improve diagnostic accuracy of CHF in patients with acute dyspnea [24]. Furthermore, NT-proBNP levels are useful for the detection of ventricular dysfunction in COPD patients [10]. In regards to markers of inflammation, levels of the cytokines interleukin (IL)-6 and IL-8, and levels of the pro-thrombotic mediators C-reactive protein (CRP), fibrinogen and troponin are elevated in both, COPD and CHF[25-28]. Addition of CRP, fibrinogen, IL-6, IL-8 and the lung-specific surfactant protein-D (SP-D) to established clinical factors improves risk stratification for all-cause mortality in COPD [26].

Rationale

Given the change in landscape for COPD and CHF in the recent years, it is of high relevance to better characterize these patient populations and screen them early for co morbidities. Therefore, a systematic approach for detecting COPD in patients with CHF and CHF in patients with COPD could lead to early diagnosis and prompt treatment of the co morbid condition, with the subsequent improvement in long term outcomes.

Hypothesis

Our central hypothesis is that concomitant COPD and CHF will be under diagnosed in specialized clinics.

Specific hypotheses

1.The coexistence of COPD and HF will be more frequent in HF-pEF than in HF with reduced ejection fraction (rEF).

2.Patients with HF-pEF will exhibit more significant abnormalities in pulmonary function tests (PFTs) such as lower FEV1, lower FEV1/CV, and a higher proportion of mixed syndrome (obstructive and restrictive). The magnitude of bronchial hyperactivity will be similar in HF-rEF compared with HF-pEF

3.Chronic administration of bronchodilators will be sub-optimal in HF patients treated in HF specialized clinics. Similarly, ACEi, ARBs and β-blockers will be under prescribed in COPD patients with CHF.

4.Patients with concomitant COPD and CHF will exhibit higher levels of neuro-hormonal and trombo-inflammatory biomarkers

Primary objective

To determine the prevalence of co-morbid COPD and CHF in specialized outpatient clinics.

Primary endpoint

Diagnosis of concomitant COPD and CHF:

a) Diagnosis of COPD in stable outpatients with confirmed CHF

b) Diagnosis of CHF in stable outpatients with confirmed COPD

Secondary objective

1) to characterize the pulmonary functional abnormalities in patients with CHF associated with reduced versus preserved ejection fraction;

2) to document treatment prescription and indications in COPD and CHF patient populations;

3) to characterize neuro-hormonal and inflammatory biomarker profiles in patients with COPD alone, CHF alone and in patients with concomitant COPD and CHF and to compare these profiles to those from a random sample of the populational study CanCOLD (including non-COPD, at risk for COPD, GOLD1 and GOLD2+), and

4) to determine whether a comprehensive cardiopulmonary risk assessment and measurements of neurohormonal and inflammatory biomarkers can serve as predictors for adverse outcomes in COPD and CHF.

Secondary endpoint

1) Characterization of pulmonary functional abnormalities in CHF patients- spirometry to be performed in all patients and chest CT scan for all patients with a history of smoking and/or abnormalities in spirometry.

2) Treatment prescription- hospital administrative databases

3) Quantification of neurohormonal and inflammatory biomarkers- serum levels of the NT-pro BNP, CRP, fibrinogen, IL-8 and SP-D

4) Assessment of cardiovascular risk and COPD risk,COPD exacerbations, cardiovascular events and acute CHF decompensations. Patient reported outcomes- health-related quality of life

Trial design

Primary endpoint, diagnosis of concomitant COPD and CHF

Patients will be recruited from the heart failure clinic at the MUHC and at the Montreal Heart institute and from the COPD clinic at the Montreal Chest Institute. Time points for recruitment and follow up visits can be found in figure 1. At baseline, patients will undergo a detailed and standardized cardiopulmonary evaluation that will include: complete medical history and physical exam with special attention to signs and symptoms of COPD and CHF; clinical questionnaires (CAT, SF-36); EKG; chest CT scan; post-bronchodilator spirometry; forced oscillation technique (FOT) and blood samples for measurements of serum biomarkers (table 1 and figure 2). Patients will undergo these tests during two visits. The diagnosis of COPD will be done based on GOLD criteria; a post-bronchodilator FEV1/FVC <0.07. Disease severity will be assessed by the percent predicted FEV1 value and classified as mild (FEV1 >80% predicted; GOLD1), moderate (50%≤FEVI<80% predicted; GOLD2), severe (30%≤FEVI<50% predicted; GOLD3) and very severe (FEV1<30% predicted; GOLD4) [29] in clinically stable patients with no evidence of pulmonary edema. Chest CT scan will be used to evaluate qualitative features of emphysema or airways disease in patients with a history of smoking and/or with abnormal spirometry. The diagnosis of CHF will be done based on the European Society of Cardiology (ESC) criteria; signs and symptoms of heart failure with objective evidence of structural or functional abnormality [30]. Echocardiography will be performed to confirm the diagnosis of CHF in COPD patients with clinical symptoms and in whom a cardiac structural or functional abnormality is suspected (i.e. cardiac murmur, abnormal EKG, cardiomegaly on chest X ray, elevated BNP or NTproBNP, among other). Once the diagnosis of COPD and CHF are done according to the established criteria, the prevalence rates and 95% confidence intervals (CI) of COPD, CHF and COPD with concomitant CHF will be calculated for each population.

Secondary endpoints

Characterization of pulmonary functional abnormalities in CHF patients- Qualitative evaluation of emphysema and/or airways disease in inspiratory/expiratory CT scan and pre and post-bronchodilator spirometry

Treatment prescription- data on medication and indications for COPD and CHF will be obtained from hospital administrative databases and from questionnaires at baseline visit.

Levels of neuro-hormonal and inflammatory biomarkers- In line with evidence-based good clinical practice, levels of the biomarkers NT-proBNP, troponin, CRP and fibrinogen will be quantified in patients to assess their neuro-hormonal and trombo-inflammatory state at baseline [10, 26, 31]. Additionally and based on previous evidence, levels of IL-8 and SP-D will be assessed at baseline to improve risk stratification for adverse events [26]. Levels of all the aforementioned

biomarkers will be compared among the following groups: 1) outpatients with COPD only; 2) outpatients with CHF only; 3) outpatients with both COPD and CHF; 4) COPD GOLD2+ subjects from CanCOLD and 5) non-COPD at risk subjects from CanCOLD. Differences between groups will be compared with linear mixed models with the different biomarkers as dependent variables to assess how each disease phenotype associates with the levels of each biomarker. Samples from this study will be stored as part of the Canadian Cohort Obstructive Lung Disease Study (CanCOLD) biobank. Subjects will have the option to have their blood specimens banked for future research in the CanCold Biobank.

Cardiovascular risk- Cardiovascular (CV) risk will be calculated for all patients with the Framingham risk score, according to the model proposed by D'Agostino [32, 33]. This is a validated method that uses a multivariable algorithm to predict cardiovascular events (including heart failure). The variables included in this algorithm are: age, sex, blood pressure, diabetic status, smoking status and serum levels of apolipoproteins A1 (ApoA1) and B (ApoB) [34]. Subjects' characteristics and clinical variables will be obtained from medical history and physical exam and from CanCOLD databases. Serum ApoA1 and ApoB levels will be measured from samples of patients or subjects at baseline by ELISA.

COPD risk- Individuals will be considered at risk for COPD if they have one or more of the following: history of smoking; exposure to smoke from home cooking or heating fuels; exposure to occupational dusts and chemicals; history of asthma and/or bronchial hyperreactivity and symptoms suggestive of chronic bronchitis [29].

COPD exacerbation-like events- Two different operational definitions will be used: i) 'symptom-based', requiring a change in at least one major symptom (dyspnea, sputum purulence, sputum volume), or ii) 'event-based', requiring change of at least one major symptom and use of antibiotics and/or systemic corticosteroids or health services such as hospital admissions. The purpose of using both definitions is to capture exacerbation-like respiratory events, with varying levels of severity (receiving treatment/hospital admission or not). Information regarding exacerbation-like events will be collected every 3 months during phone follow-ups over a 12 month period. Additionally, information on hospitalizations for COPD exacerbations will be confirmed with hospital databases.

Cardiovascular events and CHF decompensations- Prospective information on hospitalizations for cardiovascular adverse events and/or CHF decompensations will be collected every 3 months during phone follow-ups over a period of 12 months after the initial visit and verified with hospital databases. Data related to cardiovascular events or CHF decompensations prior to enrollment will be obtained from hospital administrative databases.

Data analysis- Poisson regression models will be used to estimate the association of cardiopulmonary risk and the levels of different biomarkers and the frequency of cardiovascular events (including heart failure decompensations) and COPD exacerbations during a 1-year period. Results of this regression analysis will be presented in terms of the estimated rate ratios (RR) with the corresponding 95% confidence interval (CI) and will be adjusted for sex, age and other important covariates. If conditional variances exceed conditional means in our data (overdispersion) we will use negative binomial regression models to estimate the association of biomarkers cardiopulmonary risk. Negative binomial regression can be used for over-dispersed count data, that is when the conditional variance exceeds the conditional mean. It can be considered

as a generalization of Poisson regression since it has the same mean structure as Poisson regression and it has an extra parameter to model the over-dispersion. If the conditional distribution of the outcome variable is over-dispersed, the confidence intervals for the Negative binomial regression are likely to be narrower as compared to those from a Poisson regression model.

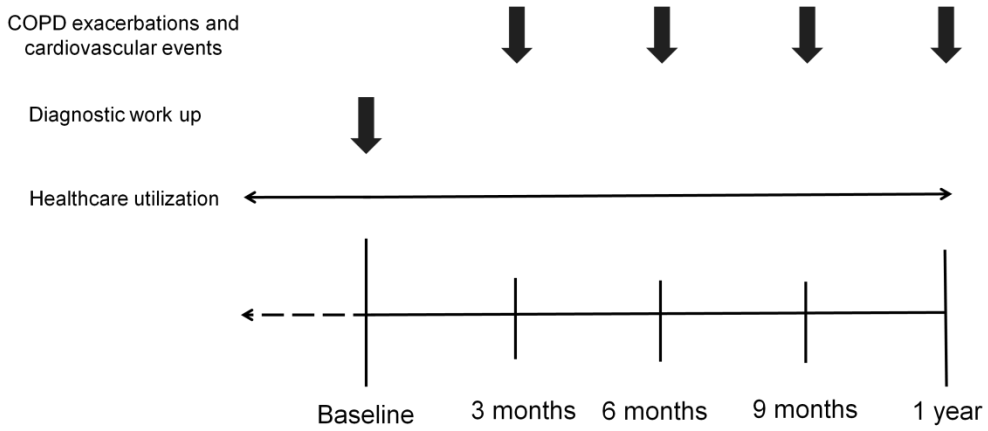
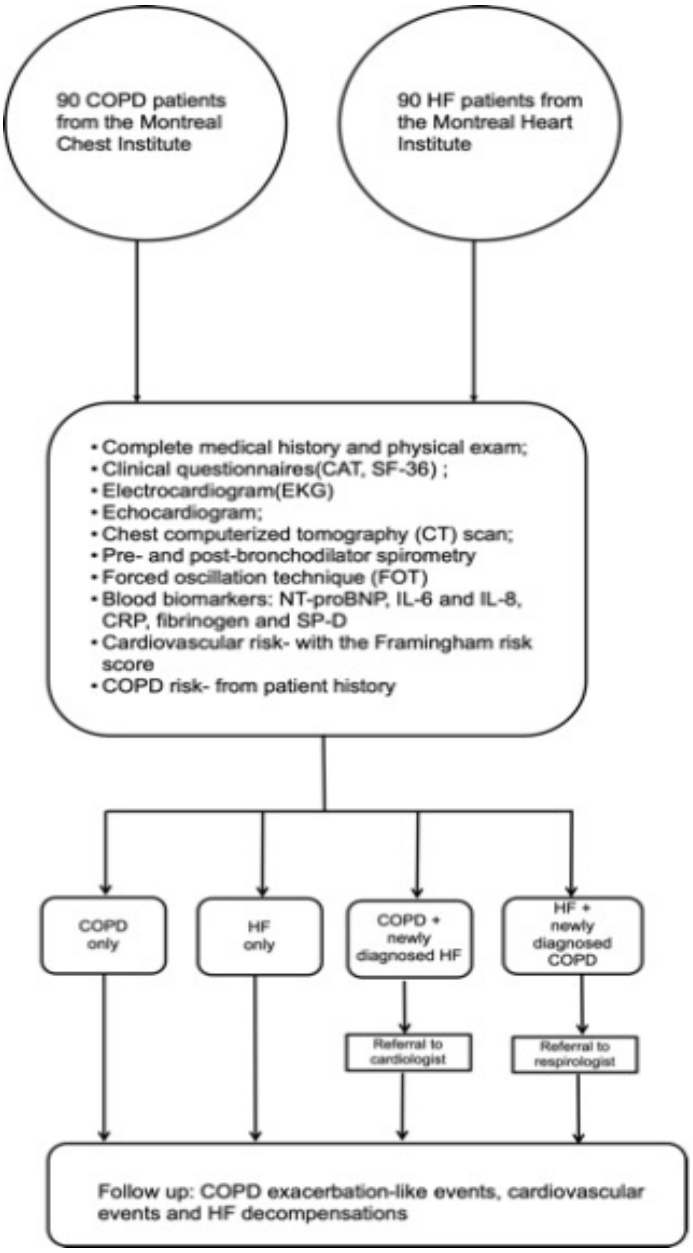


Figure 1. Patient recruitment and follow up visits

Table 1. Schedule of procedures for each visit

Study Procedure/Visit	Time estimated to be completed	Baseline V1 and V2 only if needed total of 5 hours	Visit 3 (3 months)	Visit 4 (6 months)	Visit 5 (9 months)	Visit 6 (12 months)
Complete physical exam and clinical questionnaires	60 mins	X				
EKG	30 mins	X				
Chest CT scan	60 mins	X				
Transthoracic echo	60 mins	X				
Complete PFT post bronchodilator	60 mins	X				
Forced oscillation technique	10-20 mins	X				
Blood draw	10 mins	X				
Phone follow-up (exacerbation-like events collection)			X	X	X	X

Figure 2. Diagnostic work up



Population

CHF outpatients followed at the Montreal Heart Institute (MHI) and at the Heart Failure clinic at the MUHC

COPD outpatients followed at the Montreal Chest Institute (MCI)

Non-COPD at risk and COPD GOLD 2+ patients from the population-based CanCOLD study[35]

Inclusion criteria CHF patients: 1) age ≥ 40y; 2) left HF confirmed by 2D echocardiogram with LVEF <45% or HF-pEF (symptoms and signs of HF with evidence of structural change within the myocardium by echocardiogram but with a LVEF ≥45%) and 3) NYHA class 2 or 3.

Inclusion criteria COPD patients: 1) age ≥ 40y; 2) COPD confirmed by post-bronchodilator FEV1/FVC <0.7; 3) Current or ex-smokers with a smoking history ≥ 10 pack-years, 4) GOLD1 to 4 confirmed by post-bronchodilator spirometry*.

* Patients with asthma COPD overlap (ACO) will also be included in the study 31. ACO will be defined as post bronchodilator airflow limitation that is not fully reversible, in symptomatic patients with risk factors for COPD and who have clinical features of both asthma and COPD.

Exclusion criteria, both COPD and CHF patients: 1) NYHA class 4; 2) unstable or advanced renal failure (GFR < 30ml/min); 3) heart failure caused by an active inflammatory condition such as sarcoidosis or any form of myocarditis;; 4) history of thoracotomy with pulmonary resection; 5) unstable or life-threatening cardiac arrhythmia; 6) respiratory failure that has required mechanical ventilation and/or admission to the ICU; 7) use of chronic home oxygen; 8) previous diagnosis of COPD for CHF patients and previous diagnosis of CHF for COPD patients.

Sample size

Aims 1a and 1b: Based on previous studies [8, 9], the prevalence of concomitant COPD and CHF is between 20-27%. Assuming a similar prevalence, we would need 90 subjects per group to provide 71% power to detect a 10% or greater increased proportion of subjects with co morbid COPD and CHF compared to those with COPD or CHF alone. The limits of a two-sided 95% confidence interval for the percentage of patients being co-morbid amongst one of the groups would be approximately 8.3% percentage points away from the estimate assuming a true proportion of 20%. Data will be stratified according to disease severity (GOLD) and the presence of ACO. Sensitivity analyses will be performed to assess the impact of ACO on co morbid CHF.

Aim 2: Based on estimates from previous studies[37-39] and assuming that the levels of serum biomarkers are normally distributed, we would need 32 subjects in each group (non-COPD non-CHF, COPD alone, CHF alone and COPD with CHF) to achieve a power of at least 80% to detect a medium effect size (0.5*SD) with a statistical significance of 0.05.

Aim 3: Assuming that the baseline proportion of subjects with higher levels of biomarkers is 40% and the incidence rate of COPD exacerbations/CHF decompensations is 0.5 per patient-year during 12 months follow-up in those with lower levels of biomarkers, we need about 151 subjects (alpha=0.05) to detect a rate ratio of 2 with 80% power.

Confidentiality

The principal investigator as well as research personnel will gather and record patient information in a paper copy of the case report form (CRF) and in an electronic research file. Research personnel from the RI-MUHC (principal investigator, research staff and collaborators) will have access the file. Only the information necessary to answer the scientific objectives of the project will be kept.

All the information gathered will remain strictly confidential within the limits provided by the law. In order to preserve patient's identity and the confidentiality of the information, the researcher that will analyze the data will only identify the patient by a code number. The key of the code relating the patient's name to the research file will be kept by the establishment, the MCI. The study's principal investigator, collaborators and research staff will have access to the study codes. The project's principal investigator will use the data gathered from this evaluation with the aim to answer the scientific objectives of the project described in this form. These data will be kept by the principal investigator for 7 years.

References

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* 2012;380(9859):2095-2128.
2. Chen W, Thomas J, Sadatsafavi M, FitzGerald JM. Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. *Lancet Respir Med.* 2015;3(8):631-639.
3. Hawkins NM, Petrie MC, Jhund PS, Chalmers GW, Dunn FG, McMurray JJ. Heart failure and chronic obstructive pulmonary disease: diagnostic pitfalls and epidemiology. *Eur J Heart Fail.* 2009;11(2):130-139.
4. Güder G, Brenner S, Störk S, Hoes A, Rutten FH. Chronic obstructive pulmonary disease in heart failure: accurate diagnosis and treatment. *Eur J Heart Fail.* 2014;16(12):1273-1282.
5. Hawkins NM, Virani S, Ceconi C. Heart failure and chronic obstructive pulmonary disease: the challenges facing physicians and health services. *Eur Heart J.* 2013;34(36):2795-2803.
6. Roversi S, Fabbri LM, Sin DD, Hawkins NM, Agusti A. Chronic Obstructive Pulmonary Disease and Cardiac Diseases: An Urgent Need for Integrated Care. *Am J Respir Crit Care Med.* 2016.
7. Rutten FH, Cramer MJ, Grobbee DE, et al. Unrecognized heart failure in elderly patients with stable chronic obstructive pulmonary disease. *Eur Heart J.* 2005;26(18):1887-1894.
8. Apostolovic S, Jankovic-Tomasevic R, Salinger-Martinovic S, et al. Frequency and significance of unrecognized chronic obstructive pulmonary disease in elderly patients with stable heart failure. *Aging Clin Exp Res.* 2011;23(5-6):337-342.
9. Macchia A, Rodriguez Moncalvo JJ, Kleinert M, et al. Unrecognised ventricular dysfunction in COPD. *Eur Respir J.* 2012;39(1):51-58.
10. Griffo R, Spanevello A, Temporelli PL, et al. Frequent coexistence of chronic heart failure and chronic obstructive pulmonary disease in respiratory and cardiac outpatients:

Evidence from SUSPIRIUM, a multicentre Italian survey. *Eur J Prev Cardiol.* 2017;24(6):567-576.
11. Fisher KA, Stefan MS, Darling C, Lessard D, Goldberg RJ. Impact of COPD on the mortality and treatment of patients hospitalized with acute decompensated heart failure: the Worcester Heart Failure Study. *Chest.* 2015;147(3):637-645.
12. Mentz RJ, Fiuzat M, Wojdyla DM, et al. Clinical characteristics and outcomes of hospitalized heart failure patients with systolic dysfunction and chronic obstructive pulmonary disease: findings from OPTIMIZE-HF. *Eur J Heart Fail.* 2012;14(4):395-403.
13. Staszewsky L, Wong M, Masson S, et al. Clinical, neurohormonal, and inflammatory markers and overall prognostic role of chronic obstructive pulmonary disease in patients with heart failure: data from the Val-HeFT heart failure trial. *J Card Fail.* 2007;13(10):797-804.
14. Tavazzi L, Swedberg K, Komajda M, et al. Clinical profiles and outcomes in patients with chronic heart failure and chronic obstructive pulmonary disease: an efficacy and safety analysis of SHIFT study. *Int J Cardiol.* 2013;170(2):182-188.
15. Salpeter S, Ormiston T, Salpeter E. Cardioselective beta-blockers for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev.* 2005(4):CD003566.
16. Sin DD, McAlister FA. The effects of beta-blockers on morbidity and mortality in a population-based cohort of 11,942 elderly patients with heart failure. *Am J Med.* 2002;113(8):650-656.
17. Rutten FH, Zuithoff NP, Hak E, Grobbee DE, Hoes AW. Beta-blockers may reduce mortality and risk of exacerbations in patients with chronic obstructive pulmonary disease. *Arch Intern Med.* 2010;170(10):880-887.
18. Rusinaru D, Saaidi I, Godard S, Mahjoub H, Battle C, Tribouilloy C. Impact of chronic obstructive pulmonary disease on long-term outcome of patients hospitalized for heart failure. *Am J Cardiol.* 2008;101(3):353-358.
19. Arnaudis B, Lairez O, Escamilla R, et al. Impact of chronic obstructive pulmonary disease severity on symptoms and prognosis in patients with systolic heart failure. *Clin Res Cardiol.* 2012;101(9):717-726.
20. Hurst JR. Precision Medicine in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med.* 2016;193(6):593-594.
21. Maclay JD, MacNee W. Cardiovascular disease in COPD: mechanisms. *Chest.* 2013;143(3):798-807.
22. Boeck L, Soriano JB, Brusse-Keizer M, et al. Prognostic assessment in COPD without lung function: the B-AE-D indices. *Eur Respir J.* 2016;47(6):1635-1644.
23. Calzetta L, Orlandi A, Page C, et al. Brain natriuretic peptide: Much more than a biomarker. *Int J Cardiol.* 2016;221:1031-1038.
24. Wright SP, Doughty RN, Pearl A, et al. Plasma amino-terminal pro-brain natriuretic peptide and accuracy of heart-failure diagnosis in primary care: a randomized, controlled trial. *J Am Coll Cardiol.* 2003;42(10):1793-1800.
25. McCullough PA, Hollander JE, Nowak RM, et al. Uncovering heart failure in patients with a history of pulmonary disease: rationale for the early use of B-type natriuretic peptide in the emergency department. *Acad Emerg Med.* 2003;10(3):198-204.
26. Bhatt SP, Dransfield MT. Chronic obstructive pulmonary disease and cardiovascular disease. *Transl Res.* 2013;162(4):237-251.

27. Celli BR, Locantore N, Yates J, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2012;185(10):1065-1072.
28. Tsutamoto T, Hisanaga T, Wada A, et al. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. *J Am Coll Cardiol.* 1998;31(2):391-398.
29. Harvey MG, Hancox RJ. Elevation of cardiac troponins in exacerbation of chronic obstructive pulmonary disease. *Emerg Med Australas.* 2004;16(3):212-215.
30. Bourbeau J, Tan WC, Benedetti A, et al. Canadian Cohort Obstructive Lung Disease (CanCOLD): Fulfilling the need for longitudinal observational studies in COPD. *COPD.* 2014;11(2):125-132.
31. Rodrigue C, Beauchesne MF, Mallette V, Lemière C, Larivée P, Blais L. Characterization of Asthma-Chronic Obstructive Pulmonary Disease Overlap Syndrome: A Qualitative Analysis. *COPD.* 2017;14(3):330-338.
32. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Global initiative for Chronic Obstructive Lung Disease (GOLD), 2016 Update. In:2016.
33. Dickstein K, Cohen-Solal A, Filippatos G, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail.* 2008;10(10):933-989.
34. Mannino DM, Tal-Singer R, Lomas DA, et al. Plasma Fibrinogen as a Biomarker for Mortality and Hospitalized Exacerbations in People with COPD. *Chronic Obstr Pulm Dis (Miami).* 2015;2(1):23-34.
35. D'Agostino RB, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.* 2008;117(6):743-753.
36. Lee HM, Lee J, Lee K, Luo Y, Sin DD, Wong ND. Relation between COPD severity and global cardiovascular risk in US adults. *Chest.* 2012;142(5):1118-1125.
37. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA.* 2009;302(18):1993-2000.
38. Boschetto P, Fucili A, Stendardo M, et al. Occurrence and impact of chronic obstructive pulmonary disease in elderly patients with stable heart failure. *Respirology.* 2013;18(1):125-130.
39. Xia J, Zhao J, Shang J, et al. Increased IL-33 expression in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol.* 2015;308(7):L619-627.
40. Pouwels SD, Nawijn MC, Bathoorn E, et al. Increased serum levels of LL37, HMGB1 and S100A9 during exacerbation in COPD patients. *Eur Respir J.* 2015;45(5):1482-1485.

10.2 Consent forms for COPD patients for the COPD CHF clinical study (in English and French)

Patient Information and Consent Form

Study title:	Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure
Principal investigators:	Dr. Jean Bourbeau (supervisor), MUHC Montreal Chest Institute. 1001 Boulevard Décarie, Montréal, QC, H4A 3J1 Dr. Michel White, Montreal Heart Institute. 5000 Rue Bélanger, Montréal, QC H1T 1C8 Raquel Farias PhD, research associate Mira Abou Rjeili, PhD student
Co-investigators:	Dr. Benjamin Smith RI-MUHC 1001 Boulevard Décarie, Montréal, QC, H4A 3J1 Dr. Nadia Giannetti, MUHC Heart Failure clinic 1001 Boulevard Décarie, Montréal, QC, H4A 3J1
MUHC study: code	MP-37-2019-4192
Study sponsor :	Dr. Jean Bourbeau Research Institute of McGill University Health Centre Funded by Novartis Canada

Introduction

You have been invited to participate in this study because you have Chronic Obstructive Pulmonary Disease (COPD). However, before accepting to participate in this project and before signing this information and consent form, please take the time to read, understand and carefully consider the information presented in this document.

This document may contain words that you do not understand. We invite you to ask all the questions you may have to the researcher in charge of the project or to other research staff participating in the study and ask them to explain you any word or information that is not clear.

Background

COPD is a respiratory disease that can affect your breathing and your daily life. Symptoms include shortness of breath, cough, sputum production and wheezing. Individuals that have COPD may experience exacerbations (sudden worsening of respiratory symptoms), which may need supplementary

medical attention. Patients with COPD often suffer from cardiac problems such as Congestive Heart Failure (CHF). Symptoms of CHF can be very similar to those of COPD, making it difficult for your treating physician to make a CHF diagnosis. However, it is very important to make the diagnosis of CHF so you can receive a prompt and appropriate treatment to prevent complications.

Our study objectives are:

- To identify the presence of CHF in patients with a diagnosis of COPD
- To determine if a blood test can help us better identify CHF patients at high risk for developing COPD
- To improve our knowledge of the characteristics of patients that have both, COPD and CHF

In order to carry out this research, 90 subjects with a diagnosis of CHF will be recruited from the heart failure clinic at the MUHC and at the Montreal Heart institute and 90 subjects with a diagnosis of COPD will be recruited from the COPD clinic at the Montreal Chest Institute.

Study procedures

As a participant in this evaluation, you are authorizing the people responsible for this research project to consult your medical record in order for them to analyse and document the number of consultations with a respirologist, ER visits and hospitalisations for the period covering 12 months preceding your enrolment in the study and 24 months after the last study visit.

Additionally, you will receive periodic phone calls every 3 months over a 12-month period during which you will be asked if you had any episodes of increased shortness of breath, cough or any other symptom aggravation, and whether you consulted a physician, went to the ER or were hospitalized during these episodes. During these follow-up calls, you will also be asked about any side effects and adverse events related to your medications.

During your participation in this research study, the study doctor or a member of the research team will conduct a series of tests and procedures. You might have already undergone some of these tests such as spirometry as part of your normal COPD care. Some other tests such as the electrocardiogram, echocardiogram and forced oscillation technique are additional to your regular care and will give us important information on possible cardiac problems. There will be two study visits each lasting about 2 and ½ hours. The study visits will be conducted at the Center of Innovative Medicine at the RI-MUHC. The table below contains a brief description of the tests and procedures that will be done throughout the study:

Procedure	Description
Medical history and physical exam	Your treating physician or resident will ask you about your symptoms and this will be followed by a physical exam
Questionnaires	We will ask you questions about your family medical history, the medicines you are using, occupational exposures, smoking history, quality of life, nutrition, how you feel in daily life and physical activities

Electrocardiogram (EKG)	An EKG is a test that records the electrical activity of your heart through small electrode patches that a technician attaches to the skin of your chest, arms, and legs.
Echocardiogram	An echocardiogram is a test that uses ultrasound to evaluate your heart muscle and heart valves.
Computed tomography (CT) scan	A registered CT technologist will perform the CT scan, which are many x-rays that measure the structure of your lungs.
Spirometry	Spirometry is a test used to assess how well your lungs work by measuring how much air you inhale, how much you exhale and how quickly you exhale. It is carried out both before and after administration of an inhaled bronchodilating drug, which are standard procedures for lung function testing.
Pulmonary Function tests	Pulmonary function tests (PFTs) are a group of tests that measure how well your lungs work. This includes how well you're able to breathe and how effective your lungs are able to bring oxygen to the rest of your body.
Forced Oscillation technique	Forced Oscillation technique (FOT) is a test used to assess your lung function using sound that you cannot hear.
Blood draw	Your blood will be drawn to be used for analysis of serum biomarkers. A biomarker also known as a biological marker refers to a measurable indicator of a biological state or condition. Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. You will have the option to have your blood samples banked for future research in the Canadian Cohort Obstructive Lung Disease Study (CanCold) Biobank. A separate consent form will have to be signed and this aspect of the research is optional.

The schedule of procedures for each visit is listed below:

Study Procedure/Visit	**Time estimated to be completed**	**Baseline V1 and V2 only if needed total of 5 hours**	**Visit 3 (3 months)**	**Visit 4 (6 months)**	**Visit 5 (9 months)**	**Visit 6 (12 months)**
Complete physical exam and clinical questionnaires	60 mins	X				
EKG	30 mins	X				
Chest CT scan	60 mins	X				
Transthoracic echo	60 mins	X				

Complete PFT post bronchodilator	60 mins	X				
Forced oscillation technique	10-20 mins	X				
Blood draw	10 mins	X				
Phone follow-up (exacerbation-like events and medication side effects and adverse event collection)			X	X	X	X

Participant Responsibilities

During your participation in this research project, you will continue to follow your regular medical treatment as prescribed by your doctor and/or healthcare professionals and you will have access to the care provided.
If you take any respiratory medication, we will ask you to withhold them before some of the tests (pulmonary function test and spirometry). However, if you feel you need to use your medications, you should use them as you would normally do. The study coordinator will provide you with instructions to let you know how many hours before each test you should withhold your respiratory medications. You will be able to take them once the tests are completed.

Risks related to the study

Questionnaires: It is possible that some questions may make you uncomfortable. You do not have to answer any questions that you do not feel comfortable with. Everything that you talk about with the research staff is completely confidential, meaning that you will not be identifiable.

Withholding (temporarily stopping) respiratory medications: As mentioned, we will ask you to withhold some of your respiratory medications (if you have any) before the tests. As a result, you could feel more short of breath. This is temporary and could be quickly relieved by using your usual rescue bronchodilators if it becomes too severe. If you feel the need to use your medications, you may use them as you would normally do. Please inform the study doctor(s) or the coordinator if you were unable to withhold your respiratory medication(s).

Breathing Tests (Spirometry and Full Pulmonary Function Tests "PFT"): Discomfort is unusual; however, some people experience headache and/or a sense of dizziness when performing these tests; these feelings are usually temporary. Spirometry is the standard test of lung function and has been performed in patients and normal subjects all over the world for the last 50 years. Spirometry and PFT procedures are very safe and do not involve needles. However, the breathing test (spirometry) involves maximum effort breathing out and this may cause you to feel dizzy or lightheaded. To reduce the risk of this, the breathing test is performed as you are seated in a chair. The personnel who administer the test are specially trained and certified in this procedure. If you already have doctor-diagnosed COPD, you will be asked to delay your bronchodilator medications until after the interview. However, if you feel you need these bronchodilator medications, you should use them as you would normally do.

Computed Tomography (or CT) scan: The CT scans will be used to measure the structure of your lungs. While there will be information obtained from these scans, the number of images that are generated are too small for an effective "clinical" assessment of your lungs. Therefore, unless there is something

dramatically wrong with your lungs that the study doctor believe requires further clinical investigation, you will not be informed of the results of this exam.

The radiation you will experience from the CT scan is comparable to the radiation one would experience annually from all natural sources of irradiation. As of now, there are no known side effects from this CT scanning procedure.

Some people who are afraid of confined spaces find the CT scan uncomfortable. The CT scanner is relatively narrow and you will always be able to see the room around you. Also, a microphone allows you to communicate with the radiology technician at all times, and you will be removed from the scanner if you become too uncomfortable.

Blood Tests: Drawing blood may cause some pain and carries a small risk of bleeding, light headedness, bruising, and/or infection (less than 1% of patients experience it) at the site where the blood is drawn. When taking blood tests we understand that some bruising may occur but this is not harmful and will disappear. The amount of blood that will be taken will not cause any symptoms or anemia (low red blood cell count).

Confidentiality: A possible risk associated with this study is a breach of confidentiality or use of your personal information by a third party. To limit this risk, we will take the steps to protect your confidentiality described in the Confidentiality section, below.

Advantages of participating in this research project

You may or may not benefit from your participation in this research project. However, the information gathered in this study will allow to increase medical knowledge in the field and to improve medical treatment.

The indirect benefits associated with participation in the study include:

- You may have undiagnosed CHF, which may be detected in this study. If so, you will be encouraged to see your physician for medical treatment.
- You will learn the current state of your heart function which, like knowing your blood pressure or blood sugar, is of value to your health.
- You may have the satisfaction of participating in an important study of lung health with wide public health implications.

Other possible treatments or interventions

You do not have to take part in this study to receive medical care for your condition. Your participation in this study will not involve changes to your medication. Throughout this evaluation, you will continue to follow your COPD treatment as prescribed by your doctor. Furthermore, you will continue with your regular follow-up visits at your clinic.

Voluntary participation and right to withdraw from the study

Your participation in this study is voluntary. Therefore, you may refuse to participate. You may also withdraw from the *ongoing* project at any time, without giving any reason, by informing a member of the study team. Your decision not to participate in the study, or to withdraw from it, will have no impact on the quality of care and services to which you are otherwise entitled. You will be informed in a timely manner if any information becomes available that may impact your willingness to continue participating

in this study.

The researcher or the Research Ethics Board may put an end to your participation without your consent. This may happen if new findings or information indicate that participation is no longer in your interest, if you do not follow study instructions, or if there are administrative reasons to terminate the project.

If you withdraw or are withdrawn from the study, you may also request that the data already collected about you be removed from the study. If you request that your data be removed and the information already collected about you can be identified as yours it will be destroyed. If the data has been anonymized or was always anonymous (i.e. does not contain any information that can be used to identify you), the data will continue to be used in the analysis of the study.

It is possible for us to obtain new information while you take part in the research project. This information could affect your health or your well-being or make you change your mind concerning your participation in the study. This information will be provided to you as soon as it is available and will be communicated to you in writing.

Confidentiality

During your participation in this project, the principal investigator as well as research personnel will gather and record your information in a paper copy of the case report form (CRF) and in an electronic research file. Research personnel from the RI-MUHC (principal investigator, research staff and collaborators) will have access to your personal file. Only the information necessary to answer the scientific objectives of the project will be kept. This information might include that contained in your medical file concerning your past and present health status, your lifestyle habits a well as results from tests, exams and procedures you have been subjected to before and during the project. Your file can also contain other information such as your name, sex, date of birth and ethnicity.

All the information gathered will remain strictly confidential within the limits provided by the law. In order to preserve your identity and the confidentiality of the information, the researcher that will analyze the data will only identify you by a code number. The key of the code relating your name to your research file will be kept by the establishment, the MCI. The study's principal investigator, collaborators and research staff will have access to the study codes. The project's principal investigator will use the data gathered from this evaluation with the aim to answer the scientific objectives of the project described in this form. These data will be kept by the principal investigator for 7 years.

Data gathered from this research can be published in specialized journals or be the object of scientific discussions, however, it will not be possible to identify you as an individual.

You will have the right to consult your research file to verify the information gathered and to rectify it if needed, as long as the researchers responsible for the project or the establishments involved retain this information. However, in order to preserve the scientific integrity of the project, you will only have access to certain information once your participation in the study has ended.

We will report to Novartis and to the relevant health authorities any side effects, adverse events and other relevant safety information related to Novartis marketed drugs. This might also include information contained in your medical file. However, as specified above, your personal identity will remain confidential since you will only be identified by a code number.

Study Funding

The study's principal investigator and the establishments have received monetary funding (money) from Novartis Canada, the sponsoring organism, for the realisation of this project.

Compensation

You will receive a total of 75$ for both visits as financial compensation for your participation in this study, this will cover the cost of parking and travel expenses.

Should you suffer any harm

Should you suffer harm of any kind following any procedure related to the research study, you will receive the appropriate care and services required by your state of health.

By agreeing to participate in this research project, you are not waiving any of your legal rights nor discharging the researcher, the sponsor or the institution, of their civil and professional responsibilities.

Incidental Findings

Material incidental findings are unexpected findings made in the course of the study that may have significant impacts on your current or future wellbeing or that of your family members. A material incidental finding concerning you in the course of this research will be communicated to you and to a health professional of your choice.

Resource person and contact information

If you have questions or if you have a problem you think may be related to your participation in this research study, or if you would like to withdraw, you may communicate with the researcher or with someone on the research team at the following number:

Dr. Jean Bourbeau:

514-934-1934 ext. 32185

Montréal Chest Institute

1001 Boulevard Décarie

Montreal QC

For any question concerning your rights as a research participant taking part in this study, or if you have comments, or wish to file a complaint, you may communicate with:

Stéphanie Urbain, the MUHC ombudsman and complaints commissioner (independent from researcher):

514 -934-1934 ext. 22223

MUHC Office of the Ombudsman
1650 Cedar Room E6.164 Montreal, Qc
H3G 1A4

Authorization to transmit results from the study

I authorize the study doctor to inform my treating physician that I am taking part in this study:

- Yes ☐ Name and contact information of treating physician: _____
- No ☐
- I do not have a treating physician/I am no longer being followed by my treating physician ☐

Authorization to deposit consent in medical records

A copy of the consent form will be deposited in my medical record. I understand that this document will be available to any person or organism that has access to my file.

Title of the research project:
Personalizing the approach for the diagnosis of patients with concomitant Chronic Obstructive Pulmonary Disease and Chronic Heart Failure

DECLARATION OF CONSENT

I have read and examined the consent document and the study has been explained to me. I have obtained satisfactory answers to my questions and I have had enough time to think about my decision to participate in this study.

I accept to take part in the study according to the conditions defined in this consent document of which I will receive a dated and signed copy.

Name of research participant (print)	**Signature of research participant**	**Date of consent dd mmm yyyy**	**Time of consent hh : mm**
			(if applicable)

I have explained the conditions of participation in the study to the participant, as they are indicated in this consent document and I have answered all of his/her questions. Dated and signed at:_____

Name of the person obtaining consent (print)	**Role in research study of the person obtaining consent**

Signature	**Date of consent dd mmm yyyy**	**Heure du consentement hh : mm**
		(if applicable)

Formulaire d'information et de consentement

Titre du projet de recherche : Une approche personnalisée pour le diagnostic des patients atteints de la Maladie Pulmonaire Obstructive Chronique (MPOC) avec Insuffisance Cardiaque (IC) concomitante

Chercheur responsable du projet de recherche : Dr. Jean Bourbeau, MUHC Institut thoracique de Montréal.
1001 Boulevard Décarie, Montréal, QC, H4A 3J1
Dr. Michel White, Institut de Cardiologie de Montréal.
5000 Rue Bélanger, Montréal, QC H1T 1C8
Raquel Farias PhD, associé de recherche
Mira Abou Rjeili, étudiante au doctorat

Co-chercheur(s) : Dr. Benjamin Smith RI-MUHC
1001 Boulevard Décarie, Montréal, QC, H4A 3J1
Dr. Nadia Giannetti, MUHC Heart Failure clinic
1001 Boulevard Décarie, Montréal, QC, H4A 3J1

Numéro de protocole : MP-37-2019-4192

Commanditaire : Dr. Jean Bourbeau
Institut de Recherche, Centre Universitaire de Santé McGill
Financé par Novartis Canada

Introduction

Vous avez été invité à participer à cette étude parce que vous avez une maladie pulmonaire obstructive chronique (MPOC). Cependant, avant d'accepter de participer à ce projet et de signer ce formulaire d'information et de consentement, veuillez prendre le temps de lire, de comprendre et de considérer attentivement les renseignements qui suivent.

Ce formulaire peut contenir des mots que vous ne comprenez pas. Nous vous invitons à poser toutes les questions que vous jugerez utiles au chercheur responsable du projet ou aux autres membres du personnel participant au projet de recherche et à leur demander de vous expliquer tout mot ou renseignement qui n'est pas clair.

Contexte

La maladie pulmonaire obstructive chronique (MPOC) est une maladie respiratoire qui peut affecter votre respiration et votre vie quotidienne. Les symptômes peuvent inclure l'essoufflement, la toux, des sécrétions et des sifflements. Plusieurs personnes atteintes de MPOC peuvent avoir des exacerbations (une aggravation soudaine des symptômes), qui peuvent nécessiter des soins médicaux supplémentaires.

Les patients atteints de la MPOC souffrent souvent de problèmes cardiaques tel que l'insuffisance cardiaque (IC). Les symptômes de l'IC peuvent être similaires à ceux de la MPOC, ce qui rend difficile pour votre médecin d'établir un diagnostic d'IC. Cependant, il est très important de faire le diagnostic de l'IC afin que vous puissiez recevoir un traitement rapide et adéquat pour prévenir des complications.

Objectifs de l'étude:

- Identifier la présence de l'IC chez les patients avec un diagnostic de MPOC
- Déterminer si un test sanguin peut nous aider à mieux identifier les patients atteints de la MPOC ayant un risque élevé de développer l'IC
- Pour améliorer notre connaissance des caractéristiques des patients qui ont à la fois, la MPOC et l'IC

Afin de réaliser cette recherche, 90 sujets ayant reçu un diagnostic d'IC seront recrutés à la clinique d'insuffisance cardiaque du CUSM et de l'Institut de cardiologie de Montréal et 90 sujets ayant un diagnostic de MPOC seront recrutés à la clinique de MPOC de l'Institut thoracique de Montréal.

Procédures de l'étude

En participant à cette évaluation, vous autorisez les personnes responsables de ce projet à consulter votre dossier hospitalier et cela afin d'analyser et de vérifier le nombre de consultations en pneumologie, de visites à l'urgence et d'hospitalisations au cours des 12 mois précédents votre entrée dans l'étude et 24 mois après votre dernière visite d'étude.

De plus, vous recevrez des appels téléphoniques périodiques tous les 3 mois pour une période de 12 mois au cours desquels vous serez demandé si vous avez eu des épisodes d'essoufflement accru, de toux ou toute autre aggravation de symptômes, et si vous avez consulté un médecin, visité l'urgence ou été hospitalisés pendant ces épisodes. Durant ces appels, nous allons vous poser des questions sur des effets secondaires ou effets indésirables reliés à vos médicaments.

Durant votre participation à cette étude de recherche, le médecin de l'étude ou un membre de l'équipe de recherche effectuera une série de tests et de procédures. Vous avez peut-être déjà subi certains de ces tests tels que la spirométrie dans le cadre de vos soins normaux de votre MPOC. Certains autres tests tels que l'électrocardiogramme, l'échocardiogramme et la technique d'oscillation forcée seront ajoutés à vos soins habituels et nous donneront des informations importantes sur des problèmes cardiaques possibles. Il y aura deux visites d'étude d'environ deux heures et demie chacune. Les visites d'étude se dérouleront au Centre de médecine innovatrice de l'IR-CUSM. Le tableau ci-dessous contient une brève description des tests et procédures qui seront effectués tout au long de l'étude:

Procédure	Description
Histoire médicale et examen physique	Votre médecin ou un résident vous posera des questions sur vos symptômes suivi d'un examen physique
Questionnaires	Des questions vous seront posées concernant vos antécédents médicaux, et familiaux; la présence de symptômes respiratoires, l'exposition à des facteurs de risques potentiels. Nous vous poserons aussi des questions sur votre occupation, votre utilisation du système de santé, votre médication, vos limites concernant vos activités, votre état de santé et votre alimentation.
Électrocardiogramme (ECG)	Un ECG est un test qui enregistre l'activité électrique de votre cœur à travers des électrodes qu'un technicien attache à la peau de votre poitrine, bras et jambes.

Échocardiogramme	Un échocardiogramme est un test qui utilise des ultrasons pour évaluer votre muscles cardiaques et valves cardiaques.
Tomodensitométrie (TDM)	Un technicien effectuera les scan pour mesurer la structures de vos poumons.
Spirométrie	La spirométrie est un test utilisé pour évaluer le fonctionnement de vos poumons en mesurant la quantité d'air que vous inspirez, la quantité d'air expiré et la vitesse à laquelle vous expirez. Il est effectué à la fois avant et après l'administration d'un médicament bronchodilatateur inhalé, ces procédures sont standard pour le test de la fonction pulmonaire.
Test de fonction pulmonaire	Les tests de la fonction pulmonaire (TFP) sont des groupes de tests qui mesurent le fonctionnement de vos poumons. Cela comprend votre capacité de respirer et l'efficacité de vos poumons à apporter de l'oxygène au reste de votre corps.
Technique d'oscillation forcée	La technique d'oscillation forcée (FOT) est une technique utilisée pour évaluer votre fonction pulmonaire en utilisant le son que vous ne pouvez pas entendre.
Prélèvement de sang	Votre sang sera prélevé et utilisé pour l'analyse des biomarqueurs dans votre sérum. Un biomarqueur, ou marqueur biologique, est un indicateur mesurable d'un état ou d'une condition biologique. Les biomarqueurs sont souvent mesurés et évalués pour examiner les processus biologiques normaux, les processus pathologiques ou les réponses pharmacologiques à une intervention thérapeutique. Vous aurez l'option d'entreposer vos échantillons de sang pour de futures études de recherche dans la biobanque de la cohorte canadienne pour la maladie pulmonaire obstructive (CanCold). Un formulaire de consentement séparé devra être signé et cet aspect de la recherche est optionnel.

Le calendrier des procédures pour chaque visite est indiqué ci-dessous:

Procédure	**Temps estimé pour être complété**	**Visite initiale V1 et V2 seulement si**	**Visite 3 (3 mois)**	**Visite 4 (6 mois)**	**Visite 5 (9 mois)**	**Visite 6 (12 mois)**

		requise totale de 5 heures				
Examen physique complet et questionnaires cliniques	60 mins	X				
Électrocardiogramme (ECG)	30 mins	X				
Échocardiogramme	60 mins	X				
Tomodensitométrie (TDM)	60 mins	X				
Test de fonction pulmonaire complets post-bronchodilatateur	60 mins	X				
Technique d'oscillation forcée	10-20 mins					
Prélèvement de sang	10 mins					
Suivi téléphonique (collection des épisodes d'exacerbation et des effets secondaires ou indésirables reliés à vos médicaments))			X	X	X	X

Responsabilités du participant

Durant votre participation à ce projet, vous continuerez de suivre votre traitement médical régulier tel que prescrit par votre médecin et l'équipe de soins et vous aurez accès aux soins fournis.
Si vous prenez des médicaments pour votre respiration, on vous demandera de ne pas les prendre avant certains tests (tests de fonction pulmonaire, spirométrie). Cependant, si vous ressentez le besoin de prendre vos médicaments, vous pourrez les utiliser comme vous faites d'habitude. La coordinatrice de l'étude vous fournira des instructions pour vous informer combien d'heures avant les tests vous devez arrêter vos médicaments respiratoires. Vous pourrez les prendre une fois les tests complétés.

Risques liés à l'étude

Questionnaires: Il est possible que certaines questions vous rendent mal à l'aise. Vous n'avez pas à répondre aux questions qui vous rendent mal à l'aise. Tout ce dont vous discutez avec le personnel de l'étude est entièrement confidentiel, ce qui signifie que vous ne pourrez pas être identifié.

Retenir (arrêt temporaire) des médicaments respiratoires : Tel que mentionné, on vous demandera d'arrêter vos médicaments respiratoires (si vous en avez) avant vos tests. Comme résultat, vous pourriez avoir plus d'essoufflement. Ceci est temporaire et pourrait être soulagé en utilisant votre bronchodilatateur de secours si l'essoufflement devient très sévère. Si vous ressentez le besoin d'utiliser vos médicaments, vous pouvez les utiliser normalement. Veuillez informer le médecin ou la coordinatrice de l'étude si vous n'êtes pas capable d'arrêter vos médicaments.

Tests respiratoires (Spirométrie et tests de fonction pulmonaire complets): L'inconfort est inhabituel; cependant, certaines personnes éprouvent un mal de tête et/ou une sensation d'étourdissement quand ils effectuent ces tests; ces sensations sont habituellement temporaires. La spirométrie est le test standard de fonction pulmonaire et elle a été effectuée chez des patients et des sujets en santé partout au monde au cours des 50 dernières années. La spirométrie et les procédures de PFT sont très sécuritaires et

n'impliquent pas d'aiguilles. Cependant, le test de respiration (spirométrie) implique un effort maximum d'expiration et ceci pourrait entrainer une sensation d'étourdissement ou de vertige. Pour réduire ce risque, le test de respiration est effectué en position assise, sur une chaise. Le personnel qui administre ce test est spécifiquement formé et certifié pour cette procédure. Si un médecin a déjà établi que vous avez une MPOC nous vous demanderons de prendre vos médicaments bronchodilatateurs seulement après l'entrevue. Cependant, si vous sentez que vous avez besoin de ces médicaments bronchodilatateurs, vous devriez les utiliser comme vous le faites habituellement.

Tomodensitométrie thoracique (TDM): Les TDMs seront utilisées pour mesurer la structure de vos poumons et de votre abdomen (une seule image) qui seront radiographiés. Malgré l'information obtenue de ces TDM le nombre d'images générées est trop petit pour un examen "clinique" efficace de vos poumons. Ainsi, à moins qu'il y ait une grave anomalie de vos poumons et que le médecin de l'étude croit qu'une investigation clinique soit requise, vous ne serez pas informé des résultats de cet examen.
La radiation que vous aller subir lors de la TDM est comparable à la radiation qu'une personne devrait subir annuellement de toutes les sources naturelles d'irradiation. Jusqu'à présent, il n'y aucun effet secondaire connu de cette procédure.
Certaines personnes qui ont peur des endroits étroits trouvent la cabine de fonction pulmonaire et la TDM inconfortables. La cabine de fonction pulmonaire est construite de plastique clair afin que vous puissiez voir au travers et le technicien vous permettra d'entrer et de sortir de la cabine selon vos besoins. Le scanner est relativement étroit et vous serez capable de voir la pièce autour de vous. De plus, un microphone vous permet de communiquer avec le technicien en radiologie à tout moment, et il vous permettra de sortir du scanner si vous devenez trop incomfortable.

Tests sanguins: Les prélèvements sanguins peuvent causer de la douleur et comportent un faible risque de saignement, d'étourdissement, d'ecchymoses, et/ou d'infection (moins de 1% des patients) au site de prélèvement. Lors des prises de sang nous comprenons que des ecchymoses (des bleus) peuvent survenir mais ceci n'est pas dommageable et disparaîtra. La quantité de sang qui sera prélevée ne causera aucun symptôme ou anémie (nombre de globules rouges diminués).

Confidentialité: Un risque potentiel associé à cette étude est une violation de la confidentialité ou l'utilisation de vos informations personnelles par un tiers. Pour limiter ce risque, nous prendrons les mesures nécessaires pour protéger votre confidentialité, cela est décrit dans la section Confidentialité ci-dessous.

Avantages de l'étude de recherche

Il se peut que votre participation à l'étude de recherche vous procure des avantages ou qu'elle ne vous en procure pas. Toutefois, les renseignements recueillis grâce à elle permettront peut-être d'augmenter les connaissances médicales dans ce domaine et d'améliorer le traitement des maladies.

Les bénéfices indirects associés à votre participation à cette étude sont:

- Vous avez peut-être une IC non-diagnostiquée qui pourrait être détectée au cours de cette étude. Dans ce cas, vous serez encouragé à voir votre médecin pour un traitement médical.
- Vous allez connaître l'état présent de votre fonction cardiaque qui, tout comme connaître votre pression sanguine ou votre taux de sucre, est important pour votre santé.
- Vous pourriez retirer de la satisfaction de votre participation à une étude importante sur la santé pulmonaire ayant d'importantes répercussions en santé publique.

Autres traitements possibles

Vous n'avez pas à prendre part à cette étude pour recevoir des soins médicaux pour votre maladie. Votre participation dans la présente étude n'entrainera pas de changements de vos médicaments. Au cours de ce projet d'évaluation, vous continuerez à prendre votre traitement pour votre MPOC tel que prescrit par votre médecin.

Participation volontaire et droit de retrait

Votre participation à cette étude est volontaire. Vous pouvez alors refuser de participer. Vous pouvez également vous retirer du projet en cours à tout moment, sans donner de raison, en informant un membre de l'équipe d'étude. Votre décision de ne pas participer à l'étude, ou de vous en retirer, n'aura aucune conséquence sur la qualité des soins et des services auxquels vous avez droit. Vous serez informé de toute information qui pourrait avoir un impact sur votre decision de continuer à participer à cette étude.

Le chercheur ou la comité d'éthique de la recherche peuvent mettre fin à votre participation sans votre consentement. Cela peut se produire si de nouvelles découvertes ou informations indiquent que votre participation n'est plus dans votre intérêt, si vous ne respectez pas les consignes du projet de recherche ou encore s'il existe des raisons administratives d'abandonner le projet.

Si vous vous retirez du projet ou êtes retiré du projet,vous pouvez demander que les données déjà recueillies à votre sujet soient retirées de l'étude. Si vous demandez que vos données soient supprimées et que les informations déjà collectées à votre sujet puissent être identifiées comme étant les vôtres, elles seront détruites. Si les données ont été rendues anonymes ou ont toujours été anonymes (c'est-à-dire qu'elles ne contiennent aucune information pouvant être utilisée pour vous identifier), les données continueront à être utilisées dans l'analyse de l'étude.

Il est possible que nous obtenions de nouvelles informations alors que vous prenez part à l'étude de recherche. Ces renseignements pourraient affecter votre santé ou votre bien-être ou vous amener à changer votre décision de participer à l'étude. Vous serez tenu au courant de toute nouvelle information dès qu'elle sera disponible, et elle vous sera communiquée par écrit.

Confidentialité

Durant votre participation à ce projet, le chercheur responsable et le personnel de recherche recueilleront et consigneront dans un dossier de recherche les renseignements vous concernant. Toutes les informations recueillies dans ce dossier seront sauvegardés dans un dossier électronique. Le personnel de recherche de l'IR-CUSM (chercheur principal, équipe de recherche et collaborateurs) auront accès à votre dossier. Seuls les renseignements nécessaires pour répondre aux objectifs scientifiques de ce projet seront recueillis.

Ces renseignements peuvent comprendre les informations contenues dans vos dossiers médicaux concernant votre état de santé passé et présent, vos habitudes de vie ainsi que les résultats de tous les tests, examens et procédures que vous aurez à subir durant ce projet. Votre dossier peut aussi comprendre d'autres renseignements tels que votre nom, votre sexe, votre date de naissance et votre origine ethnique.

Tous les renseignements recueillis demeureront strictement confidentiels dans les limites prévues par la loi. Afin de préserver votre identité et la confidentialité des renseignements, vous ne serez identifié que par un numéro de code pour le chercheur. La clé du code reliant votre nom à votre dossier de recherche sera conservée par l'établissement et par l'Institut thoracique de Montréal. Le chercheur principal,

collaborateurs ainsi que le personnel de recherche auront accès aux codes de l'étude. Le chercheur responsable du projet utilisera les données à des fins de recherche dans le but de répondre aux objectifs scientifiques du projet décrits dans le formulaire d'information et de consentement. Ces données seront conservées pendant 7 ans par le chercheur responsable.

Les données pourront être publiées dans des revues spécialisées ou faire l'objet de discussions scientifiques, mais il ne sera pas possible de vous identifier.

Vous avez le droit de consulter votre dossier de recherche pour vérifier les renseignements recueillis, et les faire rectifier au besoin, et ce, aussi longtemps que les chercheurs responsables du projet ou l'établissement détiennent ces informations. Cependant, afin de préserver l'intégrité scientifique du projet, vous pourriez n'avoir accès à certaines de ces informations qu'une fois votre participation terminée.

Nous allons communiquer à Novartis et aux autorités sanitaires des informations sur des effets secondaires ou indésirables ou toute autre information pertinente reliée à la sécurité des médicaments commercialisés par Novartis. Ceci peut inclure des informations de vos dossier médicaux. Pourtant, tel que spécifié précédemment, votre identité personnelle restera confidentielle et vous ne serez identifié que par un numéro de code.

Financement de l'étude de recherche

Le chercheur responsable de l'étude et l'établissement ont reçu du financement (de l'argent) de Novartis Canada, l'organisme subventionnaire, pour la réalisation du projet.

Compensation

Vous recevrez une compensation financière de 75 $ pour les deux visites pour votre participation à cette étude, qui couvrira le coût du stationnement et les frais de déplacement

En cas de prejudice

Si vous deviez subir quelque préjudice que ce soit par suite de toute procédure reliée à ce projet de recherche, vous recevrez tous les soins et services requis par votre état de santé.

En acceptant de participer à ce projet de recherche, vous ne renoncez à aucun de vos droits et vous ne libérez pas le médecin responsable de ce projet de recherche, le commanditaire et l'établissement de leur responsabilité civile et professionnelle.

Découvertes fortuites

Les découvertes fortuites matérielles sont les constatations faites dans le cadre de l'étude qui pourrait avoir des répercussions importantes sur votre bien-être actuel ou futur ou celle de vos membres de famille. Une découverte fortuite constater dans le cadre de cette recherche sera communiquée à vous et à un professionnel de la santé de votre choix.

Personnes-ressources et coordonnées

Si vous avez des questions ou éprouvez des problèmes en lien avec le projet de recherche, ou si vous souhaitez vous en retirer, vous pouvez communiquer avec le médecin responsable ou avec une personne de l'équipe de recherche au numéro suivant :

Dr. Jean Bourbeau:

514-934-1934 poste 32185

L'institut thoracique de Montréal

1001 Boulevard Décarie

Montreal QC

Pour toute question concernant vos droits en tant que participant à ce projet de recherche ou si vous avez des plaintes ou des commentaires à formuler, vous pouvez communiquer avec:

Stéphanie Urbain, le commissaire local aux plaintes et à la qualité des services du CUSM (indépendant du chercheur):

514 -934-1934 poste 22223

Bureau de la commissaire aux plaintes et à la qualité du CUSM
1650, av. Cedar, poste E6.164
Montréal, Qc
H3G 1A4

Autorisation de transmettre les résultats de l'étude- Page de signature

J'autorise le chercheur responsable de la présente recherche à informer mon médecin traitant que je prends part à cette étude.

- Oui ☐ Nom et coordonnées du médecin traitant : _____
- Non ☐
- Je n'ai pas de médecin traitant / Je ne suis plus suivi par mon médecin traitant ☐

Autorisation de déposer le consentement dans les dossiers médicaux

Une copie du document de consentement sera déposée dans mon dossier médical. Je comprends qu'elle sera à la disposition de toute personne ou de tout organisme qui a accès à mon dossier.

Titre du projet de recherche:

Une approche personnalisée pour le diagnostic des patients atteints de la Maladie Pulmonaire Obstructive Chronique (MPOC) avec Insuffisance Cardiaque (IC) concomitante

DÉCLARATION DE CONSENTEMENT

J'ai lu et examiné le document de consentement, et l'étude m'a été expliquée. J'ai obtenu des réponses satisfaisantes à mes questions et j'ai eu suffisamment de temps pour réfléchir à ma décision de participer à cette étude.

J'accepte de prendre part à cette étude conformément aux conditions définies dans ce document de consentement dont je recevrai une copie datée et signée.

Nom du participant à la recherche (en lettres moulées)	**Signature du participant à la recherche**	**Date du consentement jj mmm aaaa**	**Heure du consentement hh : mm**
			(s'il y a lieu)

J'ai expliqué au participant les conditions de sa participation à l'étude telles qu'elles sont indiquées dans ce document de consentement et j'ai répondu à toutes ses questions. Signé et daté à:_____

Nom de la personne qui obtient le consentement (en lettres moulées)	**Rôle dans l'étude de la personne qui obtient le consentement**

Signature	**Date du consentement jj mmm aaaa**	**Heure du consentement hh : mm**
		(s'il y a lieu)

10.3 COPD Assessment Test (CAT) for data collection from each participating patient in the COPD CHF clinical study (in English and French)

Your name:	Today's date:

How is your COPD? Take the COPD Assessment Test™ (CAT)

This questionnaire will help you and your healthcare professional measure the impact COPD (Chronic Obstructive Pulmonary Disease) is having on your wellbeing and daily life. Your answers, and test score, can be used by you and your healthcare professional to help improve the management of your COPD and get the greatest benefit from treatment.

For each item below, place a mark (X) in the box that best describes you currently. Be sure to only select one response for each question.

Example: I am very happy 0 X 2 3 4 5 I am very sad

			SCORE
I never cough	0 1 2 3 4 5	**I cough all the time**	
I have no phlegm (mucus) in my chest at all	0 1 2 3 4 5	**My chest is completely full of phlegm (mucus)**	
My chest does not feel tight at all	0 1 2 3 4 5	**My chest feels very tight**	
When I walk up a hill or one flight of stairs I am not breathless	0 1 2 3 4 5	**When I walk up a hill or one flight of stairs I am very breathless**	
I am not limited doing any activities at home	0 1 2 3 4 5	**I am very limited doing activities at home**	
I am confident leaving my home despite my lung condition	0 1 2 3 4 5	**I am not at all confident leaving my home because of my lung condition**	
I sleep soundly	0 1 2 3 4 5	**I don't sleep soundly because of my lung condition**	
I have lots of energy	0 1 2 3 4 5	**I have no energy at all**	
		TOTAL SCORE	

Last Updated: February 24, 2012

Nom:	Date:

COPD Assessment Test

Quel est l'état de votre BPCO? Répondez au questionnaire CAT (COPD Assessment Test™) pour évaluer votre BPCO

Ce questionnaire vous aidera, ainsi que votre médecin, à mesurer l'impact de la BPCO (BronchoPneumopathie Chronique Obstructive) sur votre bien-être et votre vie au quotidien. Vous pourrez, ainsi que votre médecin, utiliser les réponses et les scores du questionnaire pour mieux prendre en charge votre BPCO et obtenir le meilleur bénéfice de votre traitement.

Pour chaque élément ci-dessous, veuillez indiquer d'une croix (x) la case qui correspond le mieux à votre état actuel. Prenez soin de ne sélectionner qu'une seule réponse par question.

Exemple: Je suis très heureux (heureuse) 0 X 2 3 4 5 Je suis très triste

			POINTS
Je ne tousse jamais	0 1 2 3 4 5	**Je tousse tout le temps**	
Je n'ai pas du tout de glaires (mucus) dans les poumons	0 1 2 3 4 5	**J'ai les poumons entièrement encombrés de glaires (mucus)**	
Je n'ai pas du tout la poitrine oppressée	0 1 2 3 4 5	**J'ai la poitrine très oppressée**	
Quand je monte une côte ou une volée de marches, je ne suis pas essoufflé(e)	0 1 2 3 4 5	**Quand je monte une côte ou une volée de marches, je suis très essoufflé(e)**	
Je ne suis pas limité(e) dans mes activités chez moi	0 1 2 3 4 5	**Je suis très limité(e) dans mes activités chez moi**	
Je ne suis pas inquièt(e) quand je quitte la maison, en dépit de mes problèmes pulmonaires	0 1 2 3 4 5	**Je suis très inquièt(e) quand je quitte la maison, en raison de mes problèmes pulmonaires**	
Je dors bien	0 1 2 3 4 5	**Je dors mal à cause de mes problèmes pulmonaires**	
Je suis plein(e) d'énergie	0 1 2 3 4 5	**Je n'ai pas d'énergie du tout**	
		SCORE TOTAL	

Last Updated: February 24, 2012

10.4 Case report form (CRF) for data collection from each participating patient in the COPD CHF clinical study

COPD/ CHF Case Report Form (CRF)

Table of Content

Instructions

1 - Ensure all fields are completed.

2 - Ensure that everything is clearly written.

3 - If the information is not applicable, write *NI*A.

4 - Use a black or blue pen to complete questionnaires.

5 - Always use the following format to indicate date dd/mmm/yy.

6 - If a mistake is done, simply insert a line across the word, date and initialize it.
Example, ~~yes~~ no (PM 27/Jul/09).

7 - Never use liquid paper to correct an error.

Patient ID: _____

COPD/CHF study

Visit 1: Date of the Visit: _____/_____/_____
d d m m y y y y

Checklist

□ The participant read and understood the latest approved consent dated
_____/_____/_____ (dd/mmm/yy)

□ A copy of the consent form was given to the patient after.

□ The correct ID number was given to the subject.

□ General consent form was signed.
□ All questionnaires were verified and completed
□ All obligatory tests were performed

□ If subject was diagnosed with COPD according to the COPD CTS guidelines, do not tell the subject, simply tell them Principal Investigator will review their results and if they are diagnosed with COPD then a copy of their spirometry results will be sent to their doctor. Have the subject sign the release of information form.

□ Participant received $75 for participating in the study.

Comments**:**

_____

_____

_____

_____

_____

Patient ID: _____

Date of the Visit: _____/_____/_____
d d m m y y y y

SPIROMETRY QUESTIONNAIRE

Safety Questions

1. In the past three months have you had any surgery on your chest or abdomen? Yes ❑ No ❑

2. Have you had a heart attack within the past three months? Yes ❑ No ❑

3. Do you have a detached retina or have you had eye surgery within the past three months? Yes ❑ No ❑

4. Have you been hospitalized for any other heart problem within the past month? Yes ❑ No ❑

[If yes, continue with Question 4A; If no, skip to Question 5]

4.A. Have you been hospitalized for heart failure in the past month? Yes ❑ No ❑

4.B. Have you been hospitalized for angina in the past month? Yes ❑ No ❑

4. C. Have you been hospitalized for myocardial infarction in the past month? Yes ❑ No ❑

4. D. Have you been hospitalized for arrhythmia in the past month? Yes ❑ No ❑

5. Does the participant have a resting pulse of greater than 120 beats per minute? Yes ❑ No ❑

6. Are you currently taking medication for tuberculosis? Yes ❑ No ❑

7. Is there some other reason why this participant should not perform the spirometry maneuver? Yes ❑ No ❑

Patient ID: _____

If the answer to any of Questions 1 through 7 is "Yes", do NOT proceed with the test. Skip to the Spirometry Outcome section and mark Questions 11A and 11B as "No", and check the second box (data entry code '3') for Question 11C.

8. Have you had a respiratory infection (cold) in the last three weeks? Yes ❑ No ❑

9. Have you taken any medications for breathing in the last six hours? Yes ❑ No ❑

If yes, record name/type of medication(s) used.

_____

_____

If Question 9 is yes and medication used includes a short acting beta agonist, code Question 9A. 'Yes', else code Question 9A. 'No'.

9.A. Did participant use a short acting beta agonist, either alone or in combination with some other product, in the last six hours? Yes ❑ No ❑

Spirometry Outcome

10. A. Pre-bronchodilator test completed? Yes ❑ No ❑

10.B. Post-bronchodilator test completed? Yes ❑ No ❑

10.C. Unable to obtain satisfactory spirometry (check one)

The participant did not understand instructions ❑

The participant was medically excluded ❑

The participant was unable to physically cooperate ❑

The participant refused ❑

11. Were any adverse events related to the spirometry maneuver observed by the evaluator? Yes ❑ No ❑

If yes, please briefly describe event: _____

_____

_____

Patient ID: _____

12. If the participant had a condition that would affect the result of their spirometry test (e.g., kyphosis, dentures, missing limbs, etc.) note that condition here.

_____

_____

_____

Spirometry performed by: _____

At time: _____

SPIROMETRY WORKSHEET and FOT

Patient ID: _____

Height: _____ cm

Weight: _____kg

Pulse: _____beats/min

Pre - bronchodilator

Administer 2 puffs of Ventolin ❑

Start time: _____

Wait 15 minutes

End time: _____

Post - bronchodilator

Post-bronchodilator FOT:

Time of test: _____

Technician's signature: _____

Date: _____/_____ /_____

Patient ID: _____

Date of the Visit: _____/_____/_____
d d m m y y y y

CORE QUESTIONNAIRE

Demographics

1. What is the participant's sex? Male ❑ Female ❑

2. What is your race? _____

3. What is your date of birth? _____/_____/____ ____ ____ ____
d d m m y y y y

4. How many years of schooling have you completed? ___ ___

5. What is the highest level of schooling you have completed? Primary School ❑ Middle School ❑ High School ❑ Some College (Trade/Professional/Community) ❑ Four-Year College/University ❑ None ❑ Unknown ❑

Respiratory Symptoms and Disorders

These questions pertain mainly to your chest. Please answer yes or no if possible. If you are in doubt about whether your answer is yes or no, please answer no.

Cough

6. Do you usually cough when you don't have a cold? Yes ❑ No ❑

*[If **yes**, continue with Question 7A; If **no**, skip to Question 8]*

7A. Are there months in which you cough on most days? Yes ❑ No ❑

*[If **yes**, ask both Questions 7B & 7C; If **no**, skip to Question 8]*

Patient ID: _____

7B. Do you cough on most days for as much as three months each year? Yes ❑ No ❑

7C. For how many years have you had this cough? Less than 2 years ❑ 2-5 years ❑ More than 5 years ❑

Phlegm

8. Do you usually bring up phlegm from your chest, or do you usually have phlegm in your chest that is difficult to bring up when you don't have a cold? Yes ❑ No ❑

*[If **yes**, continue with Question 8A; If **no**, skip to Question 9]*

8A. Are there months in which you have this phlegm on most days? Yes ❑ No ❑

*[If **yes**, ask both Questions 8B & 8C; If **no**, skip to Question 9]*

8B. Do you bring up this phlegm on most days for as much as three months each year? Yes ❑ No ❑

8C. For how many years have you had this phlegm? Less than 2 years ❑ 2-5 years ❑ More than 5 years ❑

Wheezing/Whistling

9. Have you had wheezing or whistling in your chest at any time in the last 12 months? Yes ❑ No ❑

*[If **yes**, ask both Questions 9A & 9B; If **no**, skip to Question 10]*

9A. In the last 12 months, have you had this wheezing or whistling only when you have a cold? Yes ❑ No ❑

9B. In the last 12 months, have you ever had an attack of wheezing or whistling that has made you feel short of breath? Yes ❑ No ❑

Breathlessness

10. Are you unable to walk due to a condition other than shortness of breath? Yes ❑ No ❑

Patient ID: _____

[*If **yes** to Question 10, please describe this condition on the line below and then skip to Question 12. If **no**, go directly to Question 11.]*

Nature of condition(s): _____

11. Are you troubled by shortness of breath when hurrying on the level or walking up a slight hill? Yes ❑ No ❑

*[If yes, ask Question 11A through 11D; If **no**, skip to Question 12]*

11A. Do you have to walk slower than people of your age on level ground because of shortness of breath? Yes ❑ No ❑ Does not apply ❑

11B. Do you ever have to stop for breath when walking at your own pace on level ground? Yes ❑ No ❑ Does not apply ❑

11C. Do you ever have to stop for breath after walking about 100 yards (or after a few minutes) on level ground? Yes ❑ No ❑ Does not apply ❑

11D. Are you too short of breath to leave the house or short of breath on dressing or undressing? Yes ❑ No ❑ Does not apply ❑

12. Has a doctor or other health care provider ever told you that you have emphysema? Yes ❑ No ❑

13. Has a doctor or other health care provider ever told you that you have asthma, asthmatic bronchitis or allergic bronchitis? Yes ❑ No ❑

*[If **yes**, ask Question 13A. If **no**, skip to Question 14]*

13A. Do you still have asthma, asthmatic bronchitis or allergic bronchitis? Yes ❑ No ❑

14. Has a doctor or other health care provider ever told you that you have chronic bronchitis? Yes ❑ No ❑

*[If **yes**, ask Question 14A. If **no**, skip to Question 15]*

14A. Do you still have chronic bronchitis? Yes ❑ No ❑

15. Has a doctor or other health care provider ever told you that you have chronic obstructive pulmonary disease (COPD)? Yes ❑ No ❑

Management Section

Now I am going to ask you about medicines that you may be taking to help with your breathing and your heart. I want to know about medicines that you take on a regular basis and medicines that you may take only for the relief of symptoms. I would like you to tell me each medicine that you take, what form do you take it in, and how often you take it each month.

16. In the past 12 months, have you taken any medications for your breathing and/or heart condition or your blood pressure (including medications for nasal congestion)?

Yes ❑

No ❑

If participant does not take any medications to help their breathing or heart, skip to Question 17

16A.Medication Name (not entered)							
16B.Medication Code							
16C.Formulation	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑	Pills ❑ Inhaler ❑ Nebulizer ❑ Liquid ❑ Suppository ❑ Injection ❑ Other ❑
16D.Is the Medicine taken on most days, or just when you have symptoms, or both? *(If 'most days' ask Q16E, if 'symptoms', ask Q16F, if 'both', ask both Q16E and Q16F.)*	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑	Most Days ❑ Symptoms ❑ Both ❑
16E. When you are taking the medication, how many days a week do you take it?	_______ days	_______ days	_______ days	_______ days	_______ days	_______ days	_______ days
16F. When you are taking the medication, how many months in the past 12 months have you taken it?	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑	0-3 ❑ 4-6 ❑ 7-9 ❑ 10-12 ❑

Patient ID: _____

17. Please tell me about any other products that you take or things you do to help your breathing or heart that you have not already told me about.

Medicine or Activity	***Code***

18. Has a doctor or other health care provider ever had you blow into a machine or device in order to measure your lungs (i.e., a spirometer or peakflow meter)? Yes ❑ No ❑

*[If **yes**, ask Question 18A. If **no**, skip to Question 19]*

18A. Have you used such a machine in the past 12 months? Yes ❑ No ❑

19. Have you ever had a period when you had breathing problems that got so bad that they interfered with your usual daily activities or caused you to miss work? Yes ❑ No ❑

*[If **yes**, ask Question 19A. If **no**, skip to Question 20]*

19A. How many such episodes have you had in the past 12 months? ____ ____ ____ episodes

[If 19A >0, ask Questions 19B and 19C, else skip to Question 20]

19B. For how many of these episodes did you need to see a doctor or other health care provider in the past 12 months? _____ _____ episodes

19C. For how many of these episodes were you hospitalized overnight in the past 12 months? _____ _____ episodes

[If 19C >0, ask Question 19C1, else skip to Question 20]

19C1. All together, for how many total days were you hospitalized overnight for breathing problems in the past 12 months? ____ ____ ____ days

Patient ID: _____

Tobacco Smoking

Now I am going to ask you about smoking. First I will ask about cigarettes.

20. Have you ever smoked cigarettes? Yes ❑ No ❑

("Yes," means more than 20 packs of cigarettes in a lifetime or more than 1 cigarette each day for a year)

*[if **yes**, ask questions 20A through 20D; otherwise, skip to Question 22)*

20A. How old were you when you first started regular cigarette smoking? _____ _____ years old

20B. If you have stopped smoking, how old were you when you last stopped? (If the participant has not stopped smoking, record as code '99'.) _____ _____ years old

20C. On average over the entire time that you smoke(d), about how many cigarettes per day do (did) you smoke? ____ ____ ____ cigarettes/day

20D. On average over the entire time that you smoke(d), do (did) you primarily smoke manufactured or hand-rolled cigarettes? Manufactured ❑ Hand-rolled ❑

[If the participant currently smokes cigarettes (Question 20B is '99'), then ask Questions 21A and 21B. Otherwise, skip to Question 22]

21A. In the last year, how many times have you quit smoking for at least 24 hours? ____ ____ ____ times

21B. Are you seriously thinking of quitting smoking? Yes, within the next 30 days ❑ Yes, within the next 6 months ❑ No, not thinking of quitting ❑

22. Have you ever smoked a pipe or cigar? Yes ❑ No ❑

*[If **yes**, ask question 22A. If **no**, proceed to question 23]*

22A. Do you now smoke a pipe or cigar? Yes ❑ No ❑

*[If the participant has never smoked (answered "no" to **both** Questions 20 and 22), then skip to Question 25. Otherwise, proceed to Question 23]*

Patient ID: _____

23. Has a doctor or other health care provider ever advised you to quit smoking? Yes ❑ No ❑

*[If **yes**, ask Questions 23A and 23B. If **no**, skip directly to Question 24]*

23A. Have you received medical advice to stop smoking within the past 12 months? Yes ❑ No ❑

23B. Have you used any medication (prescription or non-prescription), including a nicotine patch, to help you stop smoking? Yes ❑ No ❑

*[If **yes**, ask Question 23B1, then ask Question 24. If **no**, skip directly to Question 24]*

23B1. What kind of medication did you take to help you stop smoking?

Nicotine Replacement ❑
Buproprion ❑
(Common commercial names: Wellbutrin, Zyban)
Tofranil ❑
Varenicline (Champix) ❑
Other ❑

24. Have you used or done anything else to help you stop smoking? Yes ❑ No ❑

*[If **yes**, ask Question 24A, otherwise skip to Question 25]*

24A. What did you do?

Hypnosis ❑
Acupuncture ❑
Biofeedback ❑
Other ❑

Occupational Exposure

25. Have you ever worked for a year or more in a dusty job? Yes ❑ No ❑

*[If **yes**, ask Question 25A, otherwise skip to Question 26]*

25A. For how many years have you worked in dusty jobs? _____ _____ years

Patient ID: _____

Additional Co-morbidities

26. Has a doctor or other health care provider ever told you that you had:

26A. Heart disease	Yes ❑ No ❑
26B. Hypertension	Yes ❑ No ❑
26C. Diabetes	Yes ❑ No ❑
26D. Lung cancer	Yes ❑ No ❑
26E. Stroke	Yes ❑ No ❑
26F. Tuberculosis	Yes ❑ No ❑

*[If **yes** to 26F, then ask 26F1; otherwise, skip to Question 27]*

26F1. Are you currently taking medicine for tuberculosis?	Yes ❑ No ❑

*[If **no** to 26F1, then ask 26F2; otherwise, skip to Question 27]*

26F2. Have you ever taken medicine for tuberculosis?	Yes ❑ No ❑
27. Have you ever had an operation on your chest in which a part of your lung was removed?	Yes ❑ No ❑
28. Were you hospitalized as a child for breathing problems <u>prior to</u> the age of 10?	Yes ❑ No ❑
29. In the past 12 months did you get a flu shot?	Yes ❑ No ❑
30. Has a doctor or other health care professional told your father, mother, sister or brother that they had a diagnosis of emphysema, chronic bronchitis or COPD?	Yes ❑ No ❑
31. Has anyone living in your home (besides yourself) smoked a cigarette, pipe or cigar in your home during the past two weeks?	Yes ❑ No ❑

Patient ID: _____

Cardiovascular Symptoms and Disorders

These questions pertain mainly to your heart. Please answer yes or no if possible. If you are in doubt about whether your answer is yes or no, please answer no.

32. How would you rate your angina (chest pain, tightness or discomfort)?

Grade	**Description**	
Grade I	Ordinary physical activity does not cause angina, such as walking and climbing stairs. Angina with strenuous or rapid or prolonged exertion at work or recreation	❑
Grade II	Slight limitation of ordinary activity. Walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, or in cold, or in wind, or under emotional stress, or only during the few hours after awakening. Walking more than two blocks on the level and climbing more than one flight of ordinary stairs at a normal pace and in normal conditions	❑
Grade III	Marked limitation of ordinary physical activity. Walking one or two blocks on the level and climbing one flight of stairs in normal conditions and at normal pace	❑
Grade IV	Inability to carry on any physical activity without discomfort, anginal syndrome may be present at rest	❑

33. What is the number of pillows you use while sleeping? _____pillows

(orthopnea)

34. Do you experience attacks of severe shortness of breath and coughing at night? (Paroxysmal nocturnal dyspnea) Yes ❑ No ❑

35. Do you notice swelling in your ankles and/or abdomen? Yes ❑ No ❑

36. Do you feel like your heart has skipped a beat or added an extra beat or that your heart is racing or pounding? (palpitations) Yes ❑ No ❑

37. Do you experience fainting or syncope? Yes ❑ No ❑

Patient ID: _____

38. How does physical activity affect your fatigue or dyspnea? (NYHA classification of heart failure)

Class	Description	
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).	❑
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).	❑
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.	❑
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.	❑

Patient ID: _____

POT/MARIJUANA SMOKING QUESTIONNAIRE

Pot/marijuana Smoking

Now I am going to ask you about recreational smoking other than cigarettes.

1. Have you ever smoked pot/marijuana? Yes ❑
   No ❑

If the answer is Yes, ask the following questions:

1A. How old were you when you first started smoking old pot/marijuana? _____years

1B. Have you smoked pot/marijuana in the past year? Yes ❑
No ❑

1C. If you have stopped pot/marijuana, how old were you when you last stopped? (If the participant has not stopped smoking, record as code '99'.) _____years

1D. On average over the entire time that you smoke(d), about how many joints per week do (did) you smoke? ____joints/week

1E. In an average week how many days do (did) you smoke pot/marijuana? ____no. of days per week

1F. How many years have you smoked pot/marijuana? _____no. of years

Completed by: _____

COPD/ CHF Case Report Form (CRF)

Table of Content

Instructions

1 - Ensure all fields are completed.

2 - Ensure that everything is clearly written.

3 - If the information is not applicable, write *NI*A.

4 - Use a black or blue pen to complete questionnaires.

5 - Always use the following format to indicate date dd/mmm/yy.

6 - If a mistake is done, simply insert a line across the word, date and initialize it.
Example, ~~yes~~ no (PM 27/Jul/09).

7 - Never use liquid paper to correct an error.

Patient ID: _____

COPD/CHF study

Visit 1: Date of the Visit: _____/_____/_____
d d m m y y y y

Checklist

□ The participant read and understood the latest approved consent dated
_____/_____/_____ (dd/mmm/yy)

□ A copy of the consent form was given to the patient.

□ The correct ID number was given to the subject.

□ General consent form was signed.
□ All questionnaires were verified and completed
□ All obligatory tests were performed

□ If subject was diagnosed with COPD according to the COPD CTS guidelines, do not tell the subject, simply tell them Principal Investigator will review their results and if they are diagnosed with COPD then a copy of their spirometry results will be sent to their doctor. Have the subject sign the release of information form.

□ Participant received $75 for participating in the study.

Comments**:**

_____

_____

_____

_____

_____

Patient ID: _____

Date of the Visit: _____/_____/_____
d d m m y y y y

QUESTIONNAIRE DE SPIROMÉTRIE

Questions de sécurité

1. Au cours des derniers trois mois, avez-vous subi une chirurgie à la poitrine ou à l'abdomen?	Oui ❑ Non ❑
2. Avez-vous subi une crise cardiaque au cours des derniers trois mois?	Oui ❑ Non ❑
3. Souffrez-vous de décollement rétinien ou avez-vous subi une chirurgie à l'oeil au cours des derniers trois mois?	Oui ❑ Non ❑
4. Avez-vous été hospitalisé pour tout autre problème cardiaque au cours du dernier mois?	Oui ❑ Non ❑
[Si oui, passez à la question 4A; Si non, passez à la question 5]	
4.A. Avez-vous été hospitalisé pour une insuffisance cardiaque au cours du dernier mois?	Oui ❑ Non❑
4.B. Avez-vous été hospitalisé pour angine au cours du dernier mois?	Oui ❑ Non❑
4. C. Avez-vous été hospitalisé pour un infarctus du myocarde au cours du dernier mois?	Oui ❑ Non❑
4. D. Avez-vous été hospitalisé pour arythmie au cours du dernier mois?	Oui ❑ Non❑
5. Le participant a-t-il un pouls de plus de 120 battements/minute au repos ?	Oui ❑ Non ❑
6. Prenez-vous présentement des médicaments pour la tuberculose?	Oui ❑ Non ❑
7. Y-a-t-il une autre raison pour laquelle ce participant ne devrait pas effectuer le tests de spirométrie	Oui ❑ Non ❑

*Si vous obtenez la réponse "Oui"à toute question de 1 à 8, ne procédez **PAS** au test. Passez à la section 'Résultats de la spirométrie' et indiquez 'Non ' aux questions 11A et 11B et cochez la seconde boîte (code d'entrée de données '3') à la question 11C.*

Patient ID: _____

8. Avez-vous eu une infection respiratoire (rhume) au cours des dernières trois semaines ? Oui ❑ Non ❑

9.Avez-vous pris des médicaments pour faciliter votre respiration au cours des dernières six heures?

*Si **Oui**, veuillez inscrire les noms et types de médicaments utilisés*

_____

_____

_____

Si la réponse à la question 9 est 'Oui' et qu'un béta agoniste à courte durée d'action fut utilisé, codez la Question 9A. 'Oui'. Sinon, codez la Question 9A. 'Non'.

9A. *Le participant a-t-il utilisé un beta-agoniste à courte durée d'action seul ou combiné à un autre produit, au cours des dernières six heures?* Oui ❑ Non ❑

Résultats de la spirométrie

10A. Test pré-bronchodilatateur complété? Oui ❑ Non ❑

10B. Test post-bronchodilatateur complété? Oui ❑ Non ❑

10C. Incapable d'obtenir une spirométrie satisfaisante (cochez une seule boîte)

Le participant ne comprenait pas les directives ❑
Le participant fut exclu pour des raisons médicales ❑
Le participant était physiquement incapable de coopérer ❑
Le participant a refusé ❑

11. L'évaluateur a-t-il observé des événements indésirables reliés au test de spirométrie ? Oui ❑ Non ❑

Si Oui, veuillez décrire brièvement ces événements:

_____

_____

_____

Patient ID: _____

12.Si le participant exhibait une condition pouvant affecter les résultats de sa spirométrie (ex :., cyphose, dentiers, membre manquant, etc.), veuillez décrire la condition ci-dessous.

_____

_____

_____

Spirométrie interprété par: _____

Heure: _____

SPIROMETRY WORKSHEET and FOT

Patient ID: _____

Height: _____ cm

Weight: _____kg

Pulse: _____beats/min

Pre - bronchodilator

Administer 2 puffs of Ventolin ❑

Start time: _____

Wait 15 minutes

End time: _____

Post - bronchodilator

Post-bronchodilator FOT:

Time of test: _____

Technician's signature: _____

Date: _____/_____ /_____

Patient ID: _____

Date of the Visit: _____/_____/_____
d d m m y y y y

QUESTIONNAIRE PRINCIPAL

Données sociodémographiques

1. Quel est le s*exe du participant?* Masculin❑ Féminin ❑

2. Quelle est votre race? _____

3. Quelle est votre date de naissance? ____ ____/____ ____/____ ____ ____ ____
j j m m a a a a

4. Combien d'années d'études avez-vous complété? ___ ___

5. Quel est le plus <u>haut niveau</u> d'études que vous ayez complété?
École primaire ❑
École intermédiaire ❑
École secondaire ❑
Cégep ❑
Université ❑
Aucun ❑
Ne sait pas ❑

Symptômes et problèmes respiratoires

Ces questions ont rapport à votre thorax. Veuillez répondre '**Oui** ' ou '**Non**', si possible. Dans le doute, veuillez répondre '**Non**'.

<u>Toux</u>

6. Avez-vous l'<u>habitude</u> de tousser sans avoir de rhume? Oui ❑ Non ❑

*[Si **Oui**, poursuivez avec la question 7A; si **Non**, passez à la question 8]*

7A. Y-a-t-il des **<u>mois</u>** où vous toussez **<u>presqu'à tous les jours</u>**? Oui ❑ Non ❑

*[Si **Oui**, posez les questions 7B & 7C; Si **Non**, sautez à la question 8]*

7B. Toussez-vous la **<u>plupart des jours</u>** pendant au moins **<u>trois</u>** <u>mois à chaque année</u>? Oui ❑ Non ❑

Patient ID: _____

7C. Depuis combien **d'années** avez-vous cette toux? Moins de 2 ans ❑
2-5 ans ❑
Plus de 5 ans ❑

Expectorations

8. Ramenez-vous **habituellement** des crachats qui viennent de votre **poitrine** ou avez-vous habituellement des sécrétions à la poitrine qui sont difficiles à ramener quand vous n'avez pas de rhume? Oui ❑ Non ❑
*[Si **Oui**, poursuivez avec la Question 8A; si **Non**, sautez à la Question 9]*

8A. Y-a-t-il des mois où vous avez ces sécrétions la plupart des jours? Oui ❑ Non ❑

*[Si **Oui**, posez les Questions 8B & 8C; si **Non**, sautez à la Question 9]*

8B. Ramenez-vous ces crachats la **plupart des jours** pendant au moins **trois mois à chaque année**? Oui ❑ Non ❑

8C. Depuis combien d'**années** produisez-vous ces crachats? Moins de 2 ans ❑
2-5 ans ❑
Plus de 5 ans ❑

Cillements / sifflements

9. Avez-vous eu des **cillements** ou **sifflements** à la poitrine au cours des **derniers 12 mois**? Oui ❑ Non ❑

*[Si **Oui**, posez les Questions 9A & 9B; Si **Non**, sautez à la Question 10]*

9A. Au cours des **derniers 12 mois**, avez-vous eu ces cillements ou sifflements seulement lorsque vous aviez un rhume? Oui ❑ Non ❑

9B. Au cours des **derniers 12 mois**, avez-vous déjà eu une crise de sifflements (cillements) qui vous ait **essoufflé** ? Oui ❑ Non ❑

Essoufflement

10. Êtes-vous incapable de marcher à cause d'une condition autre que l'essoufflement? Oui ❑ Non ❑

Patient ID: _____

[*si **Oui** à la Question 10, veuillez décrire cette condition à la ligne ci-dessous et sautez ensuite à la question 12. Si **Non**, sautez directement à la question 11.*
Nature de la condition: _____

11. Devenez-vous essoufflé quand vous vous dépêchez sur un terrain plat ou quand vous montez une pente légère? Oui ❑ Non ❑

*[Si **Oui**, posez les questions 11A à 11D; si **Non**, sautez à la question 12]*

11A. Devez-vous marcher plus lentement que les gens de votre âge sur un terrain plat parce que vous devenez essoufflé? Oui ❑ Non ❑ Sans objet ❑

11B. Vous arrive-t-il de vous arrêter pour reprendre votre souffle quand vous marchez à votre rythme sur un terrain plat? Oui ❑ Non ❑ Sans objet ❑

11C. Vous arrive-t-il de vous arrêter pour reprendre votre souffle après avoir marché environ 100 mètres (ou après quelques minutes) sur un terrain plat? Oui ❑ Non ❑ Sans objet ❑

11D. Êtes-vous trop essoufflé pour quitter la maison ou devenez-vous essoufflé en vous habillant ou en vous déshabillant? Oui ❑ Non ❑ Sans objet ❑

12. Est-ce qu'un médecin ou autre professionnel de la santé vous a déjà dit que vous souffrez d'emphysème? Oui ❑ Non ❑

13. Est-ce qu'un médecin ou autre professionnel de la santé vous a déjà dit que vous êtes atteint d'asthme, de bronchite asthmatique ou de bronchite allergique? Oui ❑ Non ❑
*[Si **Oui**, posez la question 13A. Si **Non**, sautez à la question 14]*

13A. Souffrez-vous toujours d'asthme, de bronchite asthmatique ou de bronchite allergique? Oui ❑ Non ❑

14. Est-ce qu'un médecin ou autre professionnel de la santé vous a déjà dit que vous souffrez de bronchite chronique? Oui ❑ Non ❑

*[Si **Oui**, posez la question 14A. Si **Non**, sautez à la question 15]*

14A. Souffrez-vous toujours de bronchite chronique? Oui ❑ Non ❑

15. Est-ce qu'un médecin ou autre professionnel de la santé vous a déjà dit que vous avez une maladie pulmonaire obstructive chronique (MPOC)? Oui ❑ Non ❑

Patient ID: ___________

Section gestion

Je vais maintenant vous poser des questions sur les médicaments que vous prenez peut-être pour améliorer votre respiration, votre cœur et votre tension artérielle J'aimerais savoir plus sur les médicaments que vous prenez régulièrement et ceux que vous prenez seulement pour soulager certains symptômes. J'aimerais que vous m'informiez pour chaque médicament que vous prenez, sous quelle forme vous le prenez et à quelle fréquence vous le prenez à chaque mois.

16. Au cours des 12 derniers mois, avez-vous pris des médicaments pour votre respiration et / ou une maladie cardiaque ou votre tension artérielle (incluant les médicaments pour congestion nasale.) Oui❑ Non❑

Si le participant ne prend aucun médicament pour sa respiration, sautez à la question 17.

16A.Médicament Nom **(**Non inscrit)							
16B.Médicament Code	___ ___ ___	___ ___ ___	___ ___ ___	___ ___ ___	___ ___ ___	___ ___ ___	___ ___ ___
16C.Forme	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐	Comprimés ☐ Inhalateur ☐ Nébuliseur ☐ Liquide ☐ Suppositoire ☐ Injection ☐ Autre ☐

16D.Prenez-vous ce médicament la plupart des jours ou seulement en cas de symptômes, ou les deux? *(Si « la plupart des jours » posez la question 16E; si « symptômes » posez la question 16F; si «les deux » posez les questions 16E et 16F.*	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐	Plupart des jours☐ Symptômes ☐ Les deux ☐

16E. Lorsque vous prenez ce médicament, pendant combien de jours par semaine le prenez-vous?	_____ jours	_____ jours	_____ jours	_____ jours	_____ jours	_____ jours	_____ jours
16F. Lorsque vous prenez ce médicament, pendant combien de mois, au cours des derniers 12 mois, l'avez-vous pris?	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐	0-3 ☐ 4-6 ☐ 7-9 ☐ 10-12 ☐

Patient ID: _____

17. S.V.P. dites-moi quel autre produit vous prenez ou choses que vous faites pour vous aider à mieux respirer ou pour votre cœur et dont vous ne m'avez pas encore parlé.

Médicament ou activité	Code
	____ ____ ____
	____ ____ ____
	____ ____ ____
	____ ____ ____

18. Est-ce qu'un médecin ou professionnel de la santé vous a déjà fait souffler dans une machine ou un appareil pour mesurer votre capacité pulmonaire (spiromètre ou débitmètre de pointe)? Oui ❑ Non ❑

*[Si **Oui**, posez la question 18A. Si **Non**, sautez à la question 19]*

18A. Avez-vous utilisé un tel appareil au cours des 12 derniers mois? Oui ❑ Non ❑

19. Avez-vous déjà vécu une période où vos problèmes respiratoires étaient si sévères qu'ils vous empêchaient de vaquer à vos activités journalières ou vous empêchaient de travailler? Oui ❑ Non ❑

*[Si **Oui**, posez la question 19A. Si **Non**, sautez à la question 20]*

19A. Combien de tels épisodes avez-vous eu au cours des 12 derniers mois? ____ ____ ____ épisodes

*[Si 19A >0, posez les questions 19b et 19C, si **Non** sauter à la question 20]*

19B. Pour combien de ces épisodes avez-vous dû consulter un médecin ou autre professionnel de la santé au cours des 12 derniers mois? _____ _____ épisodes

19C. Pour combien de ces épisodes avez-vous passé la nuit à l'hôpital au cours des 12 derniers mois? _____ _____ épisodes

*[Si 19C >0, posez la question 19C, si**Non** sautez à la question 20]*

19C1. En tout, au cours des 12 derniers mois, combien de nuits avez-vous passé à l'hôpital à cause de vos problèmes respiratoires? ____ ____ ____ jours

Patient ID: _____

Tabagisme

Je vais maintenant vous poser des questions sur le tabagisme. Premièrement je vais vous interroger au sujet de la cigarette.

20. Avez-vous déjà fumé la cigarette? Oui ❑ Non ❑

*("**Oui**," veut dire plus de 20 paquets de cigarettes au cours de la vie ou plus d'une cigarette par jour pendant un an)*

*[Si **Oui**, posez les questions 20A à 20D; si **Non**, sautez à la question 22)*

20A. Quel âge aviez-vous lorsque vous avez commencé à fumer la cigarette? _____ _____ ans

20B. Si vous avez cessé de fumer, quel âge aviez-vous lorsque vous avez cessez? (Si le participant fume toujours inscrire 99'.) _____ _____ ans

20C. En moyenne, pour toute la période de temps où vous fumez (ou fumiez), combien de cigarettes, par jour fumez (ou fumiez) vous? ____ ____ ____ cigarettes/jour

20D. En moyenne, pour toute la période de temps où vous fumez (ou fumiez), fumez (ou fumiez) vous principalement des cigarettes commerciales ou des rouleuses? Commerciales ❑ Rouleuses ❑

[Si le participant fume présentement la cigarette (Question 20B est '99'), posez les questions 21A et 21B. Sinon, sautez à la question 22]

21A. Au cours de la dernière année, combien de fois avez-vous cessé de fumer pour au moins 24 heures? ____ ____ ____ fois

21B. Envisagez-vous sérieusement de cesser de fumer? **Oui**, dans les prochains 30 jours ❑ **Oui**, dans les prochains 6 mois ❑ **Non**, ne pense pas arrêter ❑

22. Avez-vous déjà fumé la pipe ou le cigare? Oui ❑ Non ❑

*[Si **Oui**, posez la question 22A. Si **Non**, passez à la question 23]*

22A. Fumez-vous présentement la pipe ou le cigare? Oui ❑ Non ❑

Patient ID: _____

*[Si le participant n'a jamais fumé (réponse "**Non**" aux questions 20 et 22), sautez à la question 25. Si **Non**, procédez avec la question 23.]*

23. Est-ce qu'un médecin ou professionnel de la santé vous a déjà recommandé d'arrêter de fumer? Oui ❑ Non ❑

*[Si **Oui**, posez la question 23A et 23B. Si **Non**, sautez directement à la question 24]*

23A. Avez-vous reçu un avis médical pour cesser de fumer au cours des 12 derniers mois? Oui ❑ Non ❑

23B. Avez-vous utilisé des médicaments (avec ou sans-ordonnance), incluant le timbre de nicotine, pour vous aider à cesser de fumer? Oui ❑ Non ❑

*[Si **Oui**, posez la question 23B1, ensuite posez la question 24. Si **Non**, sautez directement à la question 24]*

23B1. Quel type de médicament avez-vous pris pour vous aider à cesser de fumer?
Substitution de nicotine ❑
Buproprion ❑
(Common commercial names: Wellbutrin, Zyban)
Tofranil ❑
Champix ❑
Autre ❑

24. Avez-vous utilisé ou fait autre chose pour vous aider à cesser de fumer? Oui ❑ Non ❑

*[Si **Oui**, posez la question 24A; si **Non**; sautez à la question 25]*

24A. Qu'avez-vous fait?
Hypnose ❑
Acupuncture ❑
Rétroaction biologique ❑
Autre ❑

Exposition professionnelle

25. Avez-vous déjà travaillé, pendant un an ou plus, dans un travail poussiéreux? Oui ❑ Non ❑

*[Si **Oui**, posez la question 25A, si **Non** sautez à la question 26]*

25A. Pendant combien d'années avez-vous travaillé dans un travail poussiéreux? _____ _____ ans

Patient ID :_____

Co-morbidités additionnelles

26. Est-ce qu'un médecin ou autre professionnel de la santé vous a déjà dit que vous souffrez de :

26A. Maladies cardiaques	Oui ❑ Non ❑
26B. Hypertension artérielle	Oui ❑ Non ❑
26C. Diabète	Oui ❑ Non ❑
26D. Cancer du poumon	Oui ❑ Non ❑
26E. Accident cérébrovasculaire	Oui ❑ Non ❑
26F. Tuberculose	Oui ❑ Non ❑

*[Si **Oui** à 26F, posez la question 26F1; si **Non**, sautez à la question 27]*

26F1. Prenez-vous présentement des médicaments pour la tuberculose?	Oui ❑ Non ❑

*[Si **Non** à 26F1, posez la question 26F2; si **Non**, sautez à la question 27]*

26F2. Avez-vous déjà pris des médicaments pour la tuberculose?	Oui ❑ Non ❑
27. Avez-vous déjà subi une chirurgie à la poitrine au cours de laquelle on a extrait une partie de votre poumon?	Oui ❑ Non ❑
28. Dans votre enfance et <u>avant</u> l'âge de 10 ans, avez-vous été hospitalisé pour des problèmes respiratoires?	Oui ❑ Non ❑
29. Au cours des 12 derniers mois, avez-vous été vacciné contre la grippe?	Oui ❑ Non ❑
30. Est-ce qu'un médecin ou autre professionnel de la santé a déjà dit à votre père, mère, soeur ou frère qu'ils souffraient d'emphysème de bronchite chronique ou de MPOC?	Oui ❑ Non ❑
31. À part vous-même, est-ce quelqu'un demeurant à votre domicile, à déjà fumé la cigarette, la pipe ou le cigare à la maison au cours des deux dernières semaines?	Oui ❑ Non ❑

Patient ID: _____

Symptômes et troubles cardiovasculaires

Ces questions ont rapport à votre cœur. Veuillez répondre '**Oui** ' ou '**Non**', si possible. Dans le doute, veuillez répondre '**Non**'.

32. Comment évalueriez-vous votre angine (douleur à la poitrine, oppression ou malaise)?

Grade	**Description**	
Grade I	L'activité physique ordinaire, comme marcher et monter les escaliers, ne cause pas d'angine. Angine de poitrine avec effort intense, rapide ou prolongé au travail ou pendant les loisirs.	❑
Grade II	Légère limitation de l'activité ordinaire. Marcher ou monter les escaliers rapidement, marcher à une montée, monter ou marcher après un repas, quand il fait froid, quand il y a du vent, ou être soumis à un stress émotionnel, ou seulement pendant les quelques heures qui suivent le réveil. Marcher plus que deux blocs au niveau et monter plus d'un escalier à un rythme ordinaire et dans des conditions normales	❑
Grade III	Limite marquée de l'activité physique ordinaire. Marcher un ou deux blocs au niveau et monter un escalier dans des conditions normales et à un rythme normal	❑
Grade IV	Incapable d'exercer une activité physique sans gêne, syndrome angineux peut être présent au repos	❑

33. Quel est le nombre d'oreillers que vous utilisez quand vous dormez? _____oreillers (orthopnea)

34. Avez-vous des crises d'essoufflement grave et de la toux la nuit? Oui ❑ Non ❑

(Paroxysmal nocturnal dyspnea)

35. Avez-vous remarqué un gonflement de vos chevilles et / ou de votre abdomen? Oui ❑ Non ❑

36. Avez-vous l'impression que votre cœur a sauté un battement ou ajouté un battement ou que votre cœur bat trop vite? (palpitations) Oui ❑ Non ❑

37. Est-ce que vous vous évanouissez? (syncope) Oui ❑ Non ❑

Patient ID: _____

38. Comment l'activité physique affecte-t-elle votre fatigue ou votre essoufflement? ? (NYHA classification of heart failure)

Class	**Description**	
I	Aucune limitation de l'activité physique. L'activité physique ordinaire ne provoque pas de fatigue excessive, de palpitations ou de dyspnée (essoufflement).	❑
II	Légère limitation de l'activité physique. Confortable au repos. L'activité physique ordinaire entraîne une fatigue, des palpitations, ou la dyspnée (essoufflement).	❑
III	Limite marquée de l'activité physique. Confortable au repos. Une activité moindre que la normale entraîne une fatigue, des palpitations ou la dyspnée.	❑
IV	Incapable de mener une activité physique sans malaise. Symptômes d'insuffisance cardiaque au repos. Sensation de malaise quand une activité physique est effectuée.	❑

Patient ID: _____

QUESTIONS RELIÉES À LA CONSOMATION DE POT/MARIJUANA

Consommation de pot/marijuana

Je vais maintenant vous poser quelques questions sur l'utilisation d'autres substances qui se fument, à l'exception de la cigarette.

1. Avez-vous déjà fumé de la marijuana? Oui ❑ Non ❑

Si la réponse est Oui, poser les questions suivantes :

1A. Quel âge aviez-vous lorsque vous avez fumé de la marijuana pour la première fois? _____ _____ ans

1B. Avez-vous fumée de la marijuana au cours de la dernière année des douze derniers mois? Oui ❑ Non ❑

1C. Si vous avez cessé de fumer de la marijuana quel âge aviez-vous lorsque vous avez cessé? _____ _____ ans
(Si le participant n'a pas cessé de fumer, enregistrer le code « 99 »)

1D. Combien de joints fumez-vous ou fumiez-vous en moyenne par semaine? ____ ____ ___ joints/semaine

1E. Dans une semaine moyenne, quel est le nombre de jours au cours desquels vous fumez ou fumiez de la marijuana? _____ no. de jours par a semaine.

1F. Pendant combien d'années avez-vous fumé de la marijuana? ____ _____ no. d'années

Completed by: _____